ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΤΡΟΦΙΜΩΝ & ΔΙΑΤΡΟΦΗΣ ΤΟΥ ΑΝΘΡΩΠΟΥ
ΕΡΓΑΣΤΗΡΙΟ ΜΗΧΑΝΙΚΗΣ ΤΡΟΦΙΜΩΝ, ΕΠΕΞΕΡΓΑΣΙΑΣ & ΣΥΝΤΗΡΗΣΗΣ ΓΕΩΡΓΙΚΩΝ ΠΡΟΪΟΝΤΩΝ

Μάκρο και μίνι/νάνο-γαλακτώματα με τη χρήση διαφόρων γαλακτωματοποιητών και σταθεροποιητών και παραγωγή νέων γεύσεων από εκχυλίσματα φυτών και καρπών

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΟΛΓΑ Ι. ΚΑΛΤΣΑ

Επιβλέπων Καθηγητής: Γιαννιώτης Σταύρος

ΑΘΗΝΑ
ΟΚΤΩΒΡΙΟΣ 2015

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο

ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΕΚΠΑΙΔΕΥΣΗ ΚΑΙ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗ
επένδυση στην κοινωνία της γνώσης
ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ & ΘΡΗΣΚΕΥΜΑΤΩΝ, ΠΟΛΙΤΙΣΜΟΥ & ΑΘΛΗΤΙΣΜΟΥ
ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ
Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

ΕΣΠΑ 2007-2013
πρόγραμμα για την ανάπτυξη
ΕΥΡΩΠΑΪΚΟ ΚΟΙΝΩΝΙΚΟ ΤΑΜΕΙΟ

ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΤΜΗΜΑ ΕΠΙΣΤΗΜΗΣ ΤΡΟΦΙΜΩΝ & ΔΙΑΤΡΟΦΗΣ ΤΟΥ ΑΝΘΡΩΠΟΥ
ΕΡΓΑΣΤΗΡΙΟ ΜΗΧΑΝΙΚΗΣ ΤΡΟΦΙΜΩΝ, ΕΠΕΞΕΡΓΑΣΙΑΣ &
ΣΥΝΤΗΡΗΣΗΣ ΓΕΩΡΓΙΚΩΝ ΠΡΟΪΟΝΤΩΝ

Μάκρο και μίνι/νάνο-γαλακτώματα με τη χρήση διαφόρων γαλακτωματοποιητών και σταθεροποιητών και παραγωγή νέων γεύσεων από εκχυλίσματα φυτών και καρπών

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΟΛΓΑ Ι. ΚΑΛΤΣΑ

Επιβλέπων Καθηγητής: Γιαννιώτης Σταύρος

ΑΘΗΝΑ
ΟΚΤΩΒΡΙΟΣ 2015

AGRICULTURAL UNIVERSITY OF ATHENS
DEPARTMENT OF FOOD SCIENCE & HUMAN NUTRITION
LABORATORY OF FOOD PROCESS ENGINEERING

Macro and mini/nano-emulsions production containing different emulsifiers and stabilizers. Action of plant and fruit ingredients for the development of new flavors in final products.

Ph.D. Thesis

OLGA I. KALTSA

Supervising Professor: Yanniotis Stavros

ATHENS
OCTOBER 2015

Μάκρο και μίνι/νάνο-γαλακτώματα με τη χρήση διαφόρων γαλακτωματοποιητών και σταθεροποιητών και παραγωγή νέων γεύσεων από εκχυλίσματα φυτών και καρπών

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΟΛΓΑ Ι. ΚΑΛΤΣΑ

Μέλη Εξεταστικής Επιτροπής

Επιβλέπων Καθηγητής

Σταύρος Γιαννιώτης, Καθηγητής, Εργαστήριο Μηχανικής Τροφίμων, Επεξεργασίας & Συντήρησης Γεωργικών Προϊόντων, ΓΠΑ

Μέλη Τριμελούς Επιτροπής

Dr. Camille Michon, Καθηγήτρια, Unit for Food Process Engineering**,** AgroParisTech/CNAM/INRA

Δρ Ιωάννα Μαντάλα, Επίκουρη Καθηγήτρια, Εργαστήριο Μηχανικής Τροφίμων, Επεξεργασίας & Συντήρησης Γεωργικών Προϊόντων, ΓΠΑ

Μέλη Επταμελούς Επιτροπής

Δρ Βάιος Καραθάνος, Καθηγητής Food Engineering and Physical Chemistry, Department of Nutrition, Harokopion University of Athens.

Δρ Μόσχος Πολυσίου, Καθηγητής, Εργαστήριο Γενικής Χημείας, ΓΠΑ.

Δρ Μαρία Καψοκεφάλου, Αναπληρώτρια Καθηγήτρια, Εργαστήριο Χημείας & Ανάλυσης Τροφίμων, ΓΠΑ.

Δρ Βασιλική Ευαγγελίου, Επίκουρη Καθηγήτρια, Εργαστήριο Χημείας & Ανάλυσης Τροφίμων, ΓΠΑ.

ΣΥΝΤΟΜΗ ΠΕΡΙΛΗΨΗ

Η παρούσα διατριβή εξετάζει τη δυνατότητα παρασκευής νανογαλακτωμάτων με βάση το ελαιόλαδο χρησιμοποιώντας ως γαλακτωματοποιητές πρωτεΐνες ζωικής (πρωτείνες ορού γάλακτος) ή φυτικής προέλευσης (πρωτεΐνη αρακά). Επίσης μελετήθηκαν οι ιδιότητες των γαλακτωμάτων που προέκυψαν παρουσία νέων βιολειτουργικών σταθεροποιητών (κόμμι τριγωνέλλας) προς αντικατάσταση κοινών εμπορικών κόμμεων (ξανθάνη, γκουάρ, χαρουπιού). Στα πλέον σταθερότερα δείγματα της κατηγορίας των επικαλύψεων σαλατών που προέκυψαν ενσωματώθηκαν βιολειτουργικά συστατικά όπως ο Κρόκος Κοζάνης και ο χυμός από ρόδι και μελετήθηκε η επίδρασή τους στα οργανοληπτικά χαρακτηριστικά, την οξείδωση όπως επίσης και η επιπτώση της μακράς αποθήκευσης στα τελικά προϊόντα.

Παρότι κατέστη δυνατή η παρασκευή γαλακτωμάτων μεγέθους υπομικρού σε τιμές pH 7 με τη χρήση πρωτεϊνών ορού γάλακτος, αυτό δεν ήταν εφικτό στην περίπτωση των όξινων γαλακτωμάτων, γεγονός που μπορεί να αποδοθεί στις υποδεέστερες γαλακτωματοποιητικές ιδιότητες των πρωτεϊνών. Το γεγονός αυτό ενισχύεται και από το γεγονός ότι δεν παρατηρήθηκαν συνθήκες υπερεπεξεργασίας των γαλακτωμάτων ξανθάνης για διάφορες τιμές έντασης και χρόνου επεξεργασίας με υπερήχους, καθώς η αύξηση και των δύο παραμέτρων οδήγησε σε συνεχή μείωση της μέσης διαμέτρου των λιποσφαιρίων. Το κόμμι τριγωνέλλας με την υψηλότερη περιεκτικότητα σε γαλακτομαννάνη παρουσίασε παρόμοιες τιμές σταθερότητας με τις υπόλοιπες γαλακτομαννάνες και μπορεί επομένως να χρησιμοποιηθεί με επιτυχία για την αντικατάστασή κοινών σταθεροποιητών αυτού του τύπου.

Η μείωση της περιεχόμενης πρωτεϊνης ορού γάλακτος είχε ως αποτέλεσμα την παραγωγή σταθερών γαλακτωμάτων τα οποία συγκρίθηκαν με αυτά που περιείχαν πρωτεΐνη αρακά, ενώ παράλληλα χρησιμοποιήθηκε μέθοδος ανακυκλοφορίας νερού ψύξης για την επί μακρόν επεξεργασία των προγαλακτωμάτων. Ωστόσο, παρά τους υψηλούς χρόνους επεξεργασίας με υπερήχους η παραγωγή νανογαλακτωματων δεν κατέστη εφικτή γεγονός που μπορεί να οφείλεται στην περιορισμένη ποσότητα πρωτεϊνης και το υψηλό ιξώδες των γαλακτωμάτων παρουσία σταθεροποιητή. Νανογαλακτώματα διαμέτρου έως 200 nm περίπου παρελήφθησαν με την ίδια διάταξη επεξεργασίας απουσία σταθεροποιητών και παρουσία υψηλής ποσότητας πρωτεΐνης, τα οποία χαρακτηρίζονταν από υψηλότερη σταθερότητα σε σύγκριση με

τα ομόλογα μάκρο-γαλακτώματα εξαιτίας της μείωσης του μεγέθους των λιποσφαιρίων και της ενίσχυσης των ιξωδοελαστικών τους ιδιοτήτων.

Η αποθήκευση και το είδος επεξεργασίας δεν είχε σημαντική επίδραση στο αρωματικό προφίλ των μοντέλων γαλακτωμάτων που περιείχαν τις βέλτιστες συγκεντρώσεις Κρόκου Κοζάνης και χυμού από ρόδι, ενώ δε βρέθηκε ευεργετική επίδραση αυτών των συστατικών όσων αφορά τα προϊόντα δευτερογενούς οξείδωσης στις μελετούμενες συγκετρώσεις.

Τέλος, η προσθήκη του Κρόκου Κοζάνης βελτίωσε γευστικά τα παραγόμενα τελικά προϊόντα σε σχέση με τα δείγματα αναφοράς και με χυμό από ρόδι. Ωστόσο, η αποθήκευση είχε ως αποτέλεσμα την υποβάθμιση του χρώματός τους λόγω της αποδόμησης των κροκινών.

Επιστημονική περιοχή: Μηχανική Τροφίμων, Νανοτεχνολογία Τροφίμων

Λέξεις κλειδιά: νανογαλακτώματα, υπέρηχοι, κόμμι τριγωνέλλας, Κρόκος Κοζάνης, χυμός ρόδι, άρωμα, γεύση.

SHORT ABSTRACT

This thesis examines the feasibility of preparing olive oil nanoemulsions by using emulsifiers of animal (whey proteins) or vegetable (pea protein) origin. The properties of emulsions in the presence of new biofunctional stabilizers (fenugreek gum) were also studied in order to replace common commercial gums (xanthan, guar, or carob). The most stable salad dresing model emulsions were selected in order to incorporate biofunctional ingredients such as saffron and pomegranate juice and evaluate their influence on the organoleptic characteristics and antioxidant properties, as well as the impact of long storage on the finished products.

Although it was possible to obtain submicron emulsions under neutral pH values using whey protein, this was not possible in the case of the acidic emulsions, which may be attributed to the inferior emulsifying properties of proteins. This is reinforced by the fact that no overprocessing conditions were observed in xanthan emulsions for various ultrasonic intensity and time treatments, as the increase of both parameters led to continuous decrease in the average diameter of the oil droplets. The fenugreek gum with the highest content of galactomannan showed similar stability values with other galactomannans and can therefore be successfully used to replace common stabilizers of this type.

Reducing the content of whey protein resulted in the production of stable emulsions which were compared with those containing pea protein, while a recirculating cooling water method was used allowing long-term treatment of the preemulsion. Despite high processing duration, it was not feasible to produce nanoemulsions and this may be due to the limited amount of protein and the high viscosity of the emulsions in the presence of a stabilizer. Nanoemulsions with diameter of about 200 nm were obtained by the same processing device in the absence of stabilizers and the presence of high amount of protein, which were characterized by higher stability compared to their macro-emulsions counterparts, attributed to oil droplet size reduction and enhanced viscoelastic properties.

Both storage and emulsification treatment had no significant effect on the flavor profile emulsion models containing optimal concentrations of saffron and pomegranate juice, while the contribution of these ingredients with regards to secondary oxidation products could not be considered beneficial.

Finally, the addition of saffron improved the taste of finished products compared to control and pomegranate juice samples. However, one major drawback during storage is color degradation due to the degradation of its main colorants, crocins.

Scientific field: Food Engineering, Food Nanotechnology

Keywords: nanoemulsions, ultrasound, fenugreek gum, saffron, pomegranate juice, aroma, flavor.

Macro and mini/nano-emulsions production containing different emulsifiers and stabilizers. Action of plant and fruit ingredients for the development of new flavors in final products.

ISBN

«Η έγκριση της παρούσης Διαδακτορικής Διατριβής από το Γεωπονικό Πανεπιστήμιο Αθηνών δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέα (Ν. 5343/1932, άρθρο 202, παρ.2)».

Η εκπόνηση της διδακτορικής διατριβής έτυχε χορήγηση υποτροφίας από το Πρόγ ραμμα Ηράκλειτος ΙΙ: Ενίσχυση του ανθρώπινου ερευνητικού δυναμικού μέσω της υλοποίησης διδακτορικής διατριβής».

Η παρούσα έρευνα έχει συγχρηματοδοτηθεί από την Ευρωπαϊκή Ένωση (Ευρωπαϊκό Κοινωνικό Ταμείο - ΕΚΤ) και από εθνικούς πόρους μέσω του Επιχειρησιακού Προγ- ράμματος «Εκπαίδευση και Δια Βίου Μάθηση» του Εθνικού Στρατηγικού Πλαισίου Αναφοράς (ΕΣΠΑ) - Ερευνητικό Χρηματοδοτούμενο Έργο: Ηράκλειτος ΙΙ . Επένδυση στην κοινωνία της γνώσης μέσω του Ευρωπαϊκού Κοινωνικού Ταμείου.

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο

ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΕΚΠΑΙΔΕΥΣΗ ΚΑΙ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗ
επένδυση στην κοινωνία της γνώσης
ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ & ΘΡΗΣΚΕΥΜΑΤΩΝ, ΠΟΛΙΤΙΣΜΟΥ & ΑΘΛΗΤΙΣΜΟΥ
ΕΙΔΙΚΗ ΥΠΗΡΕΣΙΑ ΔΙΑΧΕΙΡΙΣΗΣ

ΕΣΠΑ 2007-2013
πρόγραμμα για την ανάπτυξη
ΕΥΡΩΠΑΪΚΟ ΚΟΙΝΩΝΙΚΟ ΤΑΜΕΙΟ

Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

In loving memory of my father Ioannis & to my family

ACKNOWLEDGEMENTS

Conducting a Ph.D. project can be both painfull and enjoyable experience. It is accompanied by hardships, frustration, encouragement and trust with so many people's kind help. Though it will not be enough, I would like to give many thanks to the following people.

I would like to express my special appreciation and thanks to my supervisor Professor Dr Stavros Yianniotis, for being a great mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on both research have been priceless. I would also like to thank my committee members, Assistant Professor Dr Ioanna Mandala, and Professor Dr Camille Michon for serving as my committee members even at hardship. I also want to thank you for letting my defense be an enjoyable moment, and for your usefull comments and suggestions, thanks to you.

I would like to express my gratidude to Dr Elke Scholten for accepting me in the laboratory of Food Physics in Wageningen University (the Netherlands) and her support throughout the experiments conducted there, as well as for her help when reviewing and submitting one of the publications (Kaltsa et al., 2014b).

I would also like to thank assistant Professor Dr Vasiliki Evangeliou for the help she offered me on surface tension measurements and chemical analysis of some samples, as well as for her friendly discussions and support during my research.

The thesis would not have come to a successful completion, without the help I received from the staff of the National Technical University of Athens (NTUA), especially Dr Evangelia Pavlatou and Dr Anastasia Georgiadou for the measurements on droplet size conducted at the Department of Chemical Engineering.

Many thanks to Mr Kostas Karavasilis of Unilever-Knorr Hellas and the staff of the company for helping me with NMR size measurements.

I gratefully acknowledge the funding sources that made my Ph.D. work possible and especially Dr Ioanna Mandala who was the major contributor concerning the project proposal. I was funded by the European Union (European Social Fund, ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) Research Funding Program: Heracleitus II, Investing in knowledge society through the European Social

Fund. Many thanks to my colleagues, Manolis Anagnostaras, Stella Protonotariou, Kleopatra Tsatsaragkou and Evi Paximada, Maria Georgiadou and Antonis Drakos have all extended their support in a very special way, and I gained a lot from them, through their personal and scholarly interactions, their suggestions at various points of my research programme. Many thanks to the students: Ioanna Gkatsi and Neolea Spiliopoulou for their help during the experiments.

I would like to thank my family for all their love and encouragement. For my parents, who raised me and supported me in all my pursuits, as well as my cousin Matina Chasioti, being the only PhD student of the family at the same time, for her support and encouragement.

Last, but not least, I am grateful to my partner Konstantinos for all his patience and support during the last two years of my research. Your encouragement was valuable for me.

Olga Kaltsa,

Athens, 2015

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abs: Absorbance

BS: Backscattering

CD: Conjugated Dienes

d_{32}: Sauter mean diameter

d_{43}: De Brouckere mean diameter

d_{50}: median diameter

D_{50}: volume weighted cimulative mean diameter

dBS: Backscattering variation

FGB: Fenugreek Gum type B

FGF: Fenugreek Gum Fenulife ®

G': Storage modulous

G": Loss modulous

GG: Guar Gum

HS: High Speed

LBG: Locust Bean Gum

NMR: Nuclear Magnetic Resonanance

OHC: Oil Holding Capacity

p-Av: p-Anisidine value

PDI: Polydispersity Index

PPI: Pea Protein Isolate

RS: Resistant Starch

WHC: Water Holding Capacity

WPC: Whey Protein Concentrate

WPI: Whey Protein Isolate

US: ultrasonic/ultrasound/ultrasonication

XG: Xanthan Gum

ABSTRACT

The present thesis examines the effect of different protein emulsifiers (whey and pea protein) and stabilizers (galactomannans and xanthan gum) on the properties of o/w emulsions, attempting to create stable emulsions containing up to 20 %wt olive oil, whose droplets fall preferably within the nanoscale by using ultrasonic technology homogenization. As a further step, bioactive ingredients such as pomegranate juice and saffron would be incorporated in the most stable model emulsion formulations to prepare salad dressing or mayonnaise type products with new flavors.

Chapter 1 contains a literature review on emulsions, focusing on the techniques for nanoemulsion formation, emulsion destabilization phenomena, the regulatory status governing the manufacture of nanoparticles in general and toxicity issues related with their exposure. Moreover, the properties of the materials used for the preparation of emulsions in this thesis are discussed, along with the techniques used for the evaluation of their properties.

The first experimental part, **Chapter 2**, suggests a preliminary study on the stabilizing properties of different hydrocolloids used in the food industry (xanthan, guar and locust bean gum) of model emulsions containing milk whey protein at neutral pH (7), where proteins demonstrate superior emulsifying properties. Two different emulsification energy inputs (single- and two-stage, methods A and B, respectively) were tested. Ultrasonication energy input duplication from 11 kJ to 25 kJ (method B) resulted in stable emulsions production at 0.5 wt% concentration of any of the stabilizers used, whereas lower concentrations resulted in unstable samples due to depletion flocculation phenomena, regardless of emulsification energy input used. Higher energy input and increased stabilizer concentration resulted in sub-micron oil-droplets emulsions (D_{50} = 0.615 μm) with narrower particle size distribution, decreased viscosity and higher stability against coalescence/flocculation phenomena. DSC experiments revealed no presence of bulk oil formation, suggesting stability for XG 0.5%wt emulsions prepared by both methods. Reduced enthalpy values found when method B was applied suggesting structural modifications produced by extensive ultrasonication. Change of ultrasonication conditions results in significant changes of oil droplet size and stability of the produced emulsions.

In **Chapter 3**, the most stable formulations (0.25 and 0.5 %wt gum) were also studied at acidic pH 3.8, typical for this type of emulsified products. A new type of

galactomannan gum (fenugreek gum) was introduced due to its health related benefits, and its stabilizing properties were compared to that of the common stabilizers used in the previous chapter. Ultrasonication method B used in Chapter 2 was used, as it was able to produce submicron emulsions. Comparison between emulsions prepared with high speed (HS) and ultrasonication method (US) were made in order to examine the effectiveness of the methods. Results from this study revealed that fenugreek gum with increased galactomannan content (type FGF) can be successively replace commercially used galactomannans, especially locust bean gum. Ultrasonication though, was not able to substantially increase the stability of the emulsions, due to extensive stabilizer degradation observed during processing, which can result up to almost 98 % loss of viscosity. Despite process intensification (sample volume was decreased by 60 %, compared to emulsions at pH 7), the production of submicron emulsions was not achieved. This was probably observed due to decreased emulsifying properties of the protein near the isoelectric point or recoalescence during ultrasonic emulsification. Finally, stable coarse emulsions were obtained by decreasing the amount of WPI at 1 %wt level in the presence of 0.5 %wt XG.

In **Chapter 4** an investigation regarding the effect of sonication parameters was conducted in one of the systems used in the previous Chapter (3) in order to examine whether the reason for obtaining macro- instead of submicron sized emulsions was due to overprocessing leading to re-coalescence of the droplets, and thus propose a more suitable sonication method. The sample containing 0.25 %wt XG was chosen in the specific study, because it exhibited similar droplet size as the other ultrasonicated samples containing 0.5 %wt galactomannan, studied in Chapter 3. Ultrasonic treatment duration changed from 1 to 4 min at constant amplitude of 70 %. And amplitude intervals of 40, 60, 80, and 100 % were chosen, for a constant time of 1 min. Similarly, time and amplitude conditions were used to treat solutions of XG of 1 %wt and evaluate their influence on viscosity and how that was related to the stability of the emulsion. Increase of sonication time led to significant oil droplet size decrease from 1.14 to 0.89 μm (median droplet diameter). The viscosity of emulsions and XG solutions was highly influenced and considerably decreased with sonication time applied. At those conditions, the backscattering increased from 58.9 to 72.7 % after 10 days of storage, meaning that more stable emulsions, thinner and of smaller oil droplet size. Amplitude increase had a similar effect, but the droplet sizes and creaming were always greater than those noticed by changing the sonication time.

However, the rate of viscosity, droplet size, and stability change was greater by increasing the amplitude rather than by changing the sonication time. Results from this study suggest that no recoalescence was observed for up to 4 min of sonication (70% amplitude) as evidenced by the examined average diameters (De Brouckere, Sauter and median) and the optimum sonication level for this system regarding stability would be 70 % amplitude/3 min.

In **Chapter 5**, the possibility to obtain submicron sized emulsions was investigated by using a more intensive sonication method (lower processing volume and cooling water recirculation), which allowed extended processing of the most stable coarse emulsion formulation derived from Chapter 4 (1 %wt WPI, 20 %wt olive oil and 0.5 %wt XG). Additionally, the role of olive oil fraction (5-20 %wt) on oil droplet size was studied and comparisons were made with homologue emulsions containing pea protein as an alternative plant derived emulsifier. It was revealed that the formation of submicron emulsions cannot be achieved when processing highly viscous samples even after extended sonication (8 min) or lower oil fractions being used. Coarse pea protein emulsions were more viscous and exhibited higher stability compared to the ones containing whey protein, and their stability was less affected by ultrasonication processing. Pea protein emulsions containing the highest oil content (20 %wt), coarse or sonicated for 1 min, were the most stable among all samples examined and could be therefore used for the preparation of salad dressing type products.

In **Chapter 6**, the possibility of obtaining nanoemulsions in less viscous systems containing initially the emulsifier and oil phases was investigated. The same processing conditions were studied as in Chapter 5, although sonication time was extended up to 12 min in order to obtain emulsions with smaller droplets and less polydisperse. Increased amounts of WPI (10 %wt) and near neutral pH conditions were used in order to facilitate the formation of submicron droplets. Oil fraction increase led to increased average droplet size and polydispersity index values of nanoemulsions. The formation of nanoemulsions with the lowest polydispersity was achieved at the highest sonication time level. Fenugreek gum was incorporated afterwards in the formulation to produce dressing model emulsions, instead of XG, in order to avoid the formation of electrostatic WPI-XG complexes as seen in Chapter 3. Comparisons were made between nanoemulsion formulations and coarse ones. Results obtained showed that nanoemulsion models had increased viscoelastic

properties leading to significantly increased stability regarding flocculation, while phase separation was hindered in formulations prepared with the lowest oil content (2.5 %wt) after long term storage.

In **Chapter 7**, the most stable and suitable emulsion formulations obtained so far were chosen in order to incorporate pomegranate juice and saffron powder and their effect on aroma profile and oxidative stability during storage was evaluated. For this purpose the most stable emulsions prepared so far were chosen (coarse and 1 min sonicated pea protein emulsions containing 20 % olive oil). Nanoemulsions produced in Chapter 6 could not be consumed due to legislation restrictions regarding nanoparticles and not yet approved fenugreek gum for use in food products. The optimum levels of saffron and pomegranate juice powder levels were set at 100 mg/kg and 5 %wt respectively, as shown by consumer preference test in a commercial emulsified product. The vast majority of aroma/odor descriptors were not affected by emulsification method used or storage. Results obtained from p-Av analysis regarding the formation of rancid oxidation compounds correlate well with those obtained by sensory evaluation (QDA) revealing no significant differences among coarse or sonicated emulsions regarding rancidity. However a deeper investigation would be necessary in order to fully elucidate the role of saffron and pomegranate juice powder in food matrices due to ambuiguities found regarding their antioxidant properties.

Finally in **Chapter 8**, resistant starch (RS) and seasonings were added in the final formulation of pea protein emulsions investigated in Chapter 7 and the physical stability and properties of the final products were evaluated during long term ambient storage (6 months). All salad dressings exhibited remarkable stability and no phase separation was observed. However, samples prepared by HS (high speed) homogenization with added pomegranate juice powder were the most stable ones regarding flocculation-coalescence phenomena attributed to slightly enhanced viscoelastic properties. Storage had a detrimental effect on the color properties of saffron dressings most, probably due to the degradation (photo-oxidation) of crocins. Saffron addition enhanced the preference of dressing in terms of taste, while sonication ameliorated the color likeness of pomegranate emulsions.

INTRODUCTION

The food industry is one of many sectors that heavily rely on the use of emulsions, emulsifiers and stabilizers. Emulsions are dispersion systems covering a wide range of daily consumed products, including mayonnaise, dressings, sauces, margarines, creams, batters, drinks and beverages for example. On the mean time, emulsions provide exceptional means for the production of nutraceuticals and functional foods.

Within the last decade there has been a significant shift of the scientific and industrial interest towards the developement a new category of emulsions, the so-called nanoemulsions (also known as mini, submicron or micro-emulsions) a type of colloidal dispersion characterized by very small droplet diameters, within the range between 10-500 nm. The main drive for this shift is associated to several advantages that this category of emulsions posses: remarkable kinetic stability, increased bioavailability upon consumption, use as delivery systems of highly sensitive compounds or flavors and possible optical transparency.

Nevertheless, the vast majority of nanoemulsions referred on literature are manufactured with the addition of small molecular weight emulsifiers (i.e. sorbitan esters, lactic acid esters), the use of which is often restricted due to toxicity issues or inferior organoleptic properties involved. Proteins on the other hand are natural occuring polymers and no maximum allowance levels exist concerning their incorporation in food systems. Eventhough, the emulsifying properties of milk proteins have been extensively studied, a new trend towards the use of proteins of plant origin has been developed corresponding to consumer needs derived from nutritional, religious or ethical reasons and limited resources.

With regards to hydrocolloids used as stabilizers (i.e. guar, locust bean and xanthan gum), financial implication such as price increase and fluctuations generated by increased demand for non-food applications -mainly as lubricants in the drilling sector- lead to the quest for new sources of natural occurring stabilizers. Among them, fenugreek gum derived from *Trigonella foenum graecum* seeds, a species of the Leguminosae family, seems very promising attributed to its chemical relativeness to commercially used galactommanans, accompanied by several health benefits related to its consumption. Yet, fenugreek gum is less explored compared to the rest of the galactomannans or other types of commercially available gums. Additionally, only a

few studies dealing with the formation of submicron or nanoemulsions have been reported in the presence of stabilizers, whereas the vast majority of nanoemulsions reported focuses on simple systems formatted solely by emulsier and oil phases, instead of product models.

Ultrasonic emulsification technology is strongly acknowledged for its implementation and sanitization easiness along with its cost effectiveness regarding large scale food applications, whereas microfluidizers are more suitable for pharmaceutical ones.

Finally, consumer demand over healthier and functional food has increased. Olive oil, pomegranate juice and saffron (*Crocus sativus*) are ingredients known for their health promoting properties and numerous *in vitro* and *in vivo* studies have been conducted in this field. Nevertheless, their effect on emulsified products is only merely studied in the case of pomegranate juice and scarce information can be retrieved for saffron considering their sensory and antioxidant properties.

To sum up, the overall objectives of this thesis could be summarized to the followings:

- To explore the potential for nanoemulsion formation by using relatively high amounts of olive oil (~20%) in the presence of whey protein emulsifiers and commercial stabilizers.
- To compare the stabilizing properties of different fenugreek fractions with other hydrocolloids used for the manufacture of emulsified products such as salad dressings and mayonnaise.
- To incorporate plant derived proteins in salad dressing model emulsions.
- To incorporate optimum levels of pomegranate juice and saffron on the most stable emulsion formulations produced within the previous sections and explore their effect on aroma and antioxidant properties and assess the properties of final products (salad dressings) during long term storage.

CHAPTER 1

LITERATURE REVIEW

1.1 Emulsions in food science and industry

The term “emulsion” is derived from the Latin verb “mulgeo” or ”mulgere”, which means to milk out, initially used to describe the process of obtaining a milky liquid after crushing almonds in water, in the early’s of 17^{th} century.

Emulsions are systems of high importance in food science and industry since a great deal of natural or processed foods are in an emulsified state. These foods include milk and milk beverages, cream liquers, salad dressings, mayonnaise, vinegraite and sauces, butter, margarine, cake batters, ice cream, etc. One last example of unconventional/non-traditional food emulsion is the so-called “emulsion-type” sausages or “meat emulsions” (McClements, 2005).

1.2 Definitions and classification of emulsions

Emulsions are heterogenous mixtures of two immiscible liquids (most usually oil and water), in the form of sphearical droplets of one into the other. The liquid which exists as droplets is referred as *dispersed phase* or *internal phase*, whereas the surrounding liquid suggests the *external* or commonly called *continuous phase*.

The diameter of the droplets in most cases of food emulsions lies within the colloidal region (0.1-100 μm). Emulsions can be conveniently classified in two main categories according to the state of the dispersed and continuous phase or their droplet size (McClements, 2005).

A system which consists of oil droplets dispersed in the aqueous phase is called *oil-in-water emulsion* (O/W), whereas the inverse system (water droplets dispersed in oil) is referred as *water-in oil-emulsion* (W/O). Typical examples of O/W emulsions include foodstuffs such as milk, mayonnaise, salad dressings, soups and sauces (Ford et al., 1997), while margarine, butter and spreads suggest the most common examples of W/O emulsions. Multiple (or double) emulsions, oil-in-water-in-oil (O/W/O), or water-in-oil-in-water (W/O/W) may also be formed under certain circumstances (co-existence of hydrophilic and lipophilic surfactants). These types of emulsions, O/W/O for example are comprised of one or many small droplets of oil into larger water droplets which are finally dispersed in the oil continuous phase (Dickinson and McClements, 1995). W/O/W on the other hand is the most commonly abundant multiple emulsion type (Jiménez-Colmenero, 2013).

O/W/O systems can be used in order to develop and create healthier and functional foods, since their multi-compartmentalization exhibits several advantages over conventional O/W emulsions. They can be used as delivery systems for bioactive lipids and for encapsulation, protection and controlled release of hydrophilic components (McClements et al., 2007).

Double emulsions offer a means of preparing micro- and nano-capsulates (in solid or semi-solid form) containing hydrophilic and lipophilic compounds. Multiple emulsions may offer some advantages for food applications, since it has been found to be a potentially useful strategy for producing low calorie and reduced fat products, masking flavors, preventing oxidation, and improving sensory characteristics of foods, or controlling the release of and protecting labile ingredients during eating and digestion (Dickinson, 2011, McClements et al., 2007).

Regarding droplet diameter size as the criterion of classification, emulsions can be distinguished in conventional (micrometer-size droplets) emulsions –elsewhere reported as macro-emulsions-, nano-emulsions (submicrometer size droplets) and micro-emulsions.

The term *"nanoemulsion"* was initially introduced by Nakajima (1993), and has been ever since adopted in order to replace synonyms such as *miniemulsion, submicron emulsion, ultrafine,* or *fine dispersed* emulsion. Although there is a general agreement that the size of nanoemulsions' droplets lays within the submicrometer scale (<1 μm), there has been no agreement established for a specific range that could distinguish them from conventional macro-emulsions. Most commonly sizes stated in literature referring to nanoemulsions range between 20 up to 100, 200 or 500 nm. For instance, according to several authors nanoemulsions are metastable (thermodynamically unstable or kinetically stable) submicron oil-in-water dispersions with droplet diameter in the range of 50–200 nm (r < 100 nm), both on a number- and volume-weighted basis (Tadros et al., 2004; Mason et al., 2006; McClements, 2007). Other authors extend their range between 100-500 nm, (Bruschi, 2015). The appearance of nanoemulsions may vary from milky (turbid) to optically transparent/translucent (bluish) depending on the size of droplets formed (Fig. 1.1). For the creation of optically transparent nanoemulsions it is essential that the entire droplet distribution is below 80 nm (Wooster et al., 2008).

Figure 1.1 Appearance of emulsions as affected by their radii: (A) nanoemulsion and (B) macroemulsion (reprinted from: Mason et al., 2006).

In contrast to nanoemulsions, microemulsions are considered thermodynamically stable isotropic systems, typically composed of oil and water stabilized by an interfacial film of surfactant and sometimes in conjunction with a co-surfactant. Their particle size varies between 5-100 nm (Rao and McClements, 2012). Microemulsions, also referred as *"mesophases"*, or *"swollen micelles",* have been the object of debate whether or not they are considered emulsions in the classical sense. Unlike nanoemulsions which require high shear methods to form (*bottom-down approach*), microemulsions are spontaneously self-assembled thermodynamic phases according to the *bottom-up* approach, (Mason et al., 2006). The term microemulsion has been well established and used by the scientific community inappropriately in spite of its misleading word components, since their size does not fall within the micrometer scale. The above misconception throughout the scientific literature was eventually identified and discussed in the first issue of Emulsions and Microemulsions journal (Solans and Aramaki, 2008). Other reviews published later on aimed to clarify similarities among nanoemulsions and microemulsions in terms of composition, structure, manufacture, properties and stability (Anton and Vandamme, 2011; McClements, 2012).

Table 1.1 Key differences between emulsions, nanoemulsions and microemulsions.

Emulsions	Nanoemulsions	Microemulsions
Thermodynamically unstable	Kinetically stable	Thermodynamically stable
Appearance: cloudy/milky	Appearance: cloudy/milky or translucent	Appearance: translucent
Form only after application of large input energy	Form only after application of high/extreme shear or chemical energy input	Form spontaneously

1.3 Nanoemulsion formation techniques

Since nanoemulsions are considered thermodynamically unstable/non-equilibrium systems, their formation could not be achieved spontaneously. Therefore, energy input generated by mechanical devices or from the chemical potential of compounds is required. Methods for nanoemulsion fabrication are classified into two categories according to energy input requirements: i) High energy approaches and ii) Low energy approaches.

1.3.1 High energy approaches

High energy approaches for nanoemulsion fabrication include High Pressure Homogenization (HPH), Microfluidization and Ultrasonic Emulsification (USE), Colloid mills and Microporous membranes.

1.3.1.1 High pressure homogenizers

Homogenizers are widely used for the production of dairy and food emulsions for more than 100 years. The first milk homogenizer was presented to the public by August Gaulin (French Patent no. 295.596) during the World Fair in Paris in 1890 (Jahnke, 1998). In the early 1990s a new generation of homogenizers was developed which differed in terms of homogenization chamber geometry, which was able to attain up to 15-fold higher pressures involved compared to classical homogenizers (Paquin, 1999).

High-pressure homogenizers essentially consist of a high pressure pump and a subsequent homogenizing nozzle (relief valve) (Fig. 1.2). The task of the pump is to compress the crude (coarse/premix) emulsion, initially prepared by a high speed

mixer, to the homogenization pressure and push it through the homogenization valve. The relief valve basically consists of two parts: a fixed (static) valve seat and a valve (adjustable), which form a radial adjustable gap. Homogenization is completed in the area between the valve and the seat, where the emulsion experiences a combination of intense disruptive forces that causes the larger droplets to break down to smaller ones (Stang et al., 2001). A number of different mechanisms have been proposed in order to explain the droplet disruption taking place in or near the gap: elongation, laminar shear, turbulent inertia, turbulent viscous and cavitation (Walstra and Smoulders 1998; Phipps 1985). Cavitation occurs in high pressure homogenizers as a result of the static pressure distribution over the valve gap. Theoretical as well as experimental and computational approaches of pressure profiles have shown the existence of a sub-atmospheric region inside and around the valve gap zones. The extreme loss of pressure in these areas to pressures below the vaporization pressure of water causes the creation of small bubbles in the flow. High turbulence and pressure fluctuation will cause bubble deformation and eventually implosion and generation of shockwaves, which lead to further droplet fragmentation. Detrimental effects of cavitation on the overall performance of the high pressure homogenizers include high-frequency noise, apparatus wear-off, as well as droplet coalescence (Håkansson et al., 2010).

High pressure systems can be classified according to the geometry of the nozzle. In this sense, four types of nozzles are the most frequently used:

i. the “standard nozzle”,
ii. the “microfluidizer”,
iii. the “jet disperser” and
iv. the “orifice plate” (Fig. 1.2)

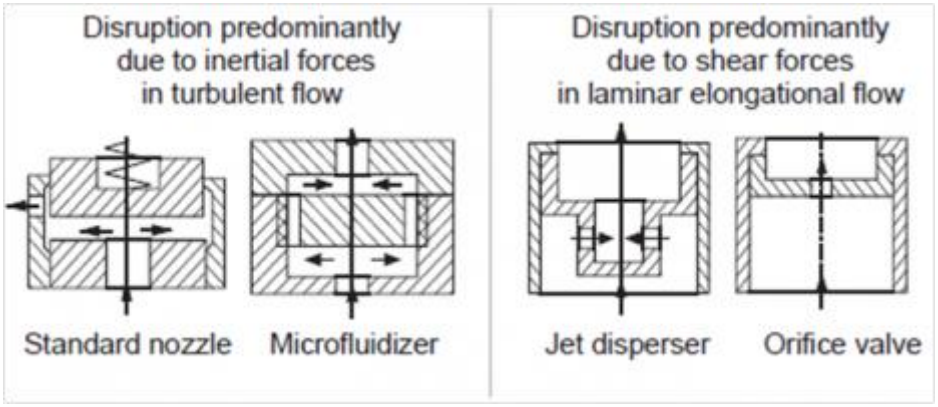


Figure 1.2 High pressure homogenization nozzles classified by mechanism of droplet disruption (reprinted from: Stang et al., 2001).

Typically high pressure homogenizers operate in a range of pressures between 50 and 150 MPa. Higher pressures between 500-700 MPa were feasible to achieve with more recently developed equipment (e.g. microfluidizers and jet dispersers) (Solans et al., 2005). Homogenization conducted within the ranges of 200-400 MPa or higher is also referred as Ultra High Pressure (UHPH) (Cortes-Munoz et al., 2009; Dumay et al., 2013).

The *standard nozzle* represents the most commonly used type for industrial applications (Stang, 2001).

In contrast to the standard nozzle, the Microfluidizer is highly capable of creating ultrafine emulsions, used either as primary or secondary homogenization. In other words, this technique can be used in order to further reduce the droplet size of a crude premix (macroemulsion) by flowing through different channels, or to create emulsions by directly inserting the oil in the first channel and the aqueous phase into the other (Dickinson and Stainsby, 1988). The operation of this device is based on the submerjed jet principle. According to this principle, two different crude emulsion jets are accelerated at high velocities when flowing into two opposite channels. The opposite jet streams recombine later in the reaction chamber by colliding at 180^{o}, hence extreme shearing action is generated.

Emulsification by high pressure treatment is the result of a dynamic equilibrium between droplet breakup generated by deformation and recoalescence promoted by droplet collisions (Niknafs et al., 2011). The effect of stabilizing agent concentration, the applied pressure and the number of cycles on the droplet size of the resulting

emulsion has been studied. In general, a decrease of droplet diameter is observed by increasing the pressure and number of passes (Donsi et al., 2011; Qian and McClements, 2011). A linear relationship has been identified to relate the mean droplet diameter of nanoemulsions and pressure applied when plotting the data in log-log scale, attributed to the increase of the magnitude of the disruptive forces generated within the chamber, in the presence of sufficient emulsifier. Increasing the number of passes from 1-8 resulted in less dramatic droplet size decrease, compared to that observed upon increase of pressure from 4 to 14 kbar, thus nanoemulsions with an average diameter of ~ 150 nm were obtained in the presence of 2 % lactoglobulin at the highest levels of pressure and passes used (Qian and McClements., 2011). Slightly smaller droplet diameters were reported (~110 nm) for nanoemulsions prepared in the presence of 3 % Tween 20 in a Microfluidizer at 150 MPa (Lee and Norton, 2013). The smallest droplet size to our knowledge is that reported for lemongrass oil – alginate nanoemulsions with an average diameter of 6-7 nm, produced by a Microfluidizer (150 MPa, 3 cycles) (Salvia-Trujillo et al., 2013). Slightly higher diameters were reported by Rao and McClements (2011) who produced lemon oil-sucrose palmitate nanoemulsions of 15 nm with the same geometry (62 MPa, 3 cycles).

Comparative studies regarding the efficiency of different high pressure devices are also available. Similar efficiency in terms of droplet size reduction has been identified between the conventional HPH and Microfluidizer, resulting in w/o nanoemulsion formation ranging from 100 down to 50 nm, after several (five) passes at 50 MPa pressure. Partly contradictory findings have been reported by others (Lee and Norton, 2013), who concluded that after the first pass the Microfluidizer was able to produce smaller droplet sizes and narrower droplet distributions that the HPH, while after several passes (5) the same result was obtained for both chamber geometries used.

High pressure homogenizers can be easily scaled-up for industrial applications, whereas the Microfluidizer, due to the appreciably low production rate, is usually used in the pharmaceutical area (Lee et al., 2014). Pharmaceutical nanoemulsions created by Microfluizers are used mainly as drug delivery systems and parenteral (injectable) feeding.

Similarly to the Microfluidizer, in the *jet disperser* two or more jets of premix emulsion introduced in opposing canals collide with each other but not in the same way (Urban, 2006). The diameter of the canals (bores) is about 0.3 – 0.5 mm wide.

The simplest form of high pressure homogenization nozzle is the *orifice plate*. The diameter of the bore is similar to the dimensions of the jet disperser and the inlet head diameter of the orifice plate is typically 10-60 mm (Stang et al., 2001). A more recently modification of this system with double or multiple orifices was recently evaluated regarding droplet breakup and prevention of re-coalescence efficiency (Finke et al., 2014). It was concluded that the double orifice geometry with a small (80 μm) primary orifice and a larger (120 μm) secondary orifice in a large distance (5169 μm) was more efficient compared to a triple orifice system in terms of break up due to higher energy density. The laminar elongation flow occurring ahead of the bores is the main cause of droplet disruption observed in jet dispersers and orifice plates. In contrast to the radial standard nozzle, the rest of the geometries do not contain moving parts, therefore they can tolerate very high pressures up to 300 - 400 MPa (Jafari et al., 2008).

1.3.1.2 Ultrasonication

Ultrasonic emulsification was first reported in 1927 by Wood and Loomis (Wood and Loomis, 1927) and the first patent was filed in Switzerland in 1944 (Swiss Patent 394.390, 1944). However the technological gap at the time did not allow the wide use of this technique, hence the use of power ultrasonics for the generation of nanoemulsions and food applications is a more recent development.

Ultrasounds are oscillating sound pressure waves with a frequency greater than 16 kHz. Most devices though operate at frequencies above 18 kHz in order to avoid audibility which is inconvenient for users. Emulsification can be achieved by applying ultrasounds below 5 MHz due to the decrease in homogenization efficiency observed when increasing frequency. Additionally, only powerful ultrasound (16-100 kHz) has the ability to interact with matter, producing physicochemical changes, while frequencies above 1 MHz do not affect the structure of materials subjected to ultrasonication and are used for diagnostic and analytical purposes (Mason, 1997; Canselier, 2002). For the above reasons commercially available devices operate within the range of 20 – 50 kHz.

Ultrasounds generation is feasible by mechanical (whistle, siren) or electrical means (magnetostrictive and reverse piezoelectric effect transducers). Piezoelectric transducers are the most popular type for industrial applications because of scalability easiness in comparison to magnetostrictive ones (Patist and Bates, 2008).

Piezoelectric transducers convert the electrical power into mechanical. This is based on the reverse piezoelectric phenomenon: a high frequency electric field is converted into mechanical vibrations of the same frequency when passing through a material such as quartz, that expands and contrasts in response to electrical voltage.

In batch ultrasonic emulsification, the energy input is provided via fixed transducers at the walls of a vessel or through the sonotrodes, elsewhere referred sonicator probes. The tip of the probe is immersed in the liquid, generating vibrations and causing cavitation (Abismail et al., 1999). Continuous operation is also possible in which the fluid circulates through a small reactor that includes a probe or several transducers located inside the tubing assembly (Jafari et al., 2008).

Ultrasonic emulsification is believed to occur through two mechanisms, cavitation and high shear. The phenomenon of cavitation is known since 1895, observed in a submarine propeller that became pitted and eroded after a short period of operation, as a concequence of collapsing bubbles due to hydrodynamic cavitation that generated intense pressure and temperature gradients in the nearby vicinity (Leong, 2011).

According to Li and Fogler (1978) the mechanism of ultrasound emulsification comprises of two separate stages (Fig. 1.3). The first one involves the generation of primary droplets, due to the eruption of the oil phase into water caused by the interfacial waves of the acoustic field. During the second stage the primary droplets deform and eventually implode during the high-pressure cycle of the sound wave. This phenomenon is termed cavitation. Cavitational collapse produces intense local heating (~5000 K), high pressures (~1000 Atm) (Suslick, 1998), turbulence and shear forces resulting in violently imploding bubbles and liquid microjet streams. Both bubble implosion and microjets lead to further breaking of the primary formed oil droplets into nano-sized ones.

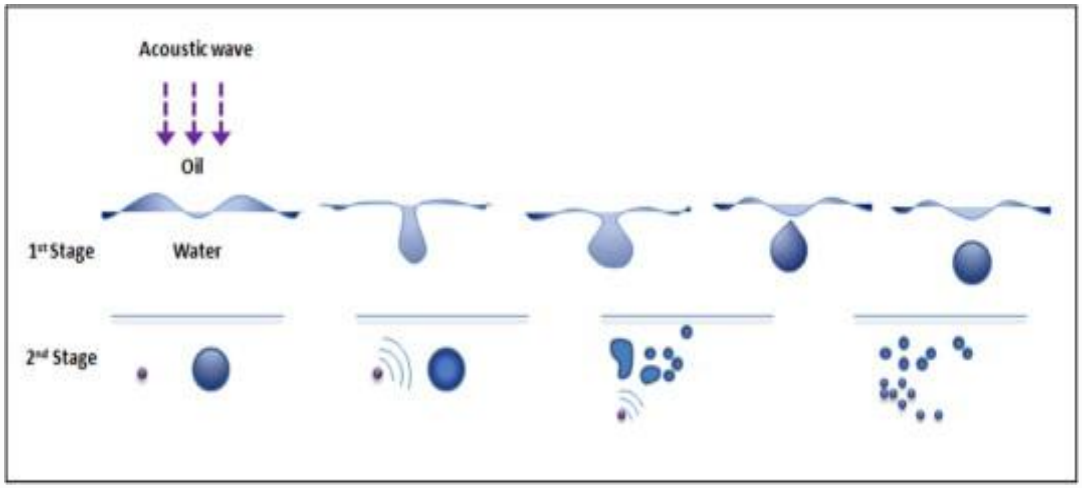


Figure 1.3 Two-stage ultrasound emulsification: droplet formation and break-up (reprinted from: Sivakumar et al., 2014).

The advantages of ultrasonics to prepare emulsions over conventional methods include (Chen et al., 2012):

- Lower energy consumption
- More stable emulsions
- Little or no surfactant is required
- Sub-micrometer emulsions with very narrow droplet size distributions can be formed up.

Despite advantages referred, the use of ultrasonics is also related to several drawbacks that should be taken into consideration. In some cases, ultrasound can also cause demulsification (Canselier, 2002; Jafari et al., 2008). Additionally, the deterioration of oils has also been referred. For instance, ultrasonication (20 or 50 kHz for 1h) caused flavor and composition deterioration in sunflower oil. Off-flavors, such as rancid derived from heptanal or metallic odors, were developed in insonated oil (Chemat et al., 2004). Therefore process optimization should be sought when selecting ultrasound processing to avoid undesirable implications (Juliano et al., 2014). Ultrasonication has been reported to cause degradation of polysaccharides used as emulsion stabilizers (Tiwari et al., 2010). In ultrasonic emulsification, polymer chain scission is more related to mechanical shearing phenomena, rather than chemical effects related to OH and H radicals, since their generation is prominent in the frequency range from 200 to 600 kHz (Koda et al., 2011). Burnt off-flavor aromas were also observed in sonicated milk (Marchesini et al., 2012). Apart from the physicochemical properties of the system components (emulsifying agent type and

concentration, internal phase volume fraction) and volume processed, the efficiency of ultrasonic emulsification for a given geometry generally depends on parameters such as irradiation temperature, duration, power/intensity, frequency and immersion depth of the probe (Behrend et al., 2000).

Ultrasonic power or intensity has been considered as one of the important factors. In many studies, only the input electric power is given as a measure of the ultrasonic power (nominal). However, quite few factors acting individually or in combination may influence the amount of power transferred (effective) to the processed liquid during ultrasonic irradiation: the amplitude of ultrasonic waves, external pressure, temperature and viscosity of the liquid (Raso et al., 1999).

Several methods have been proposed for estimating the quantity of the effective ultrasonic power used in a process including chemical dosimetry such as Fricke dosimetry (Fe^{+2} oxidation) or iodine release from potassium iodide during sonication, known as the "Weissler reaction". However it is rarely used because of their difficulty in finding a standard reaction which can be performed in all type of solvents (Koda et al., 2003).

On the other hand, calorimetric power measurement is feasible by recording the heat generated into a liquid by recording the increase in temperature over time. This method is very reliable and has been used in several researches (Mason et al., 1996; Kimura et al., 1996).

Energy dissipation by calorimetry, in terms of power (P), is estimated under adiabatic conditions, using water as a liquid, according to the following equation:

$$P = mc_p \, dT/dt \qquad \text{Eq. 1.1}$$

Where, m is the mass of the water (kg), c_p is the heat capacity of water (4.2 J/g ^oC) and dT/dt is the temperature variation over time (Kimura, 1996; Raso et al., 1999).
Energy dissipation into the sample is preferably expressed in terms of energy density or power density. Power density (W/mL) for different applied powers (% of maximal power) is calculated by dividing the absolute power dissipated (P), determined by Eq. 1.2, with the total volume (V) of the sonicated sample (mL):
Power density =P/V (Kimura et al., 1996). Alternatively, the term ultrasonic intensity can be used estimated from the following equation:

$$UI = 4P / \pi D^2 \qquad \text{Eq. 1.2}$$

, where UI is ultrasonic intensity (W/cm2), P is power (W) and D is diameter of ultrasound probe (cm) (Tiwari et al., 2010).

Increased temperatures facilitate droplet disruption by reducing the emulsion viscosity, interfacial tension and Laplace pressure, but an optimization is essential to avoid detrimental effects on the emulsifying properties of emulsifiers (particularly biopolymers) and extreme increase of the collision frequency leading to a higher re-coalescence (Jafari et al., 2007). The existence of an optimum preparation temperature level at 45^{o}C generating whey stabilized nanoemulsions with the smallest diameter (225 nm) is also reported (Chalothorn and Warisnoicharoen, 2012).

As a rule of thumb it is observed that as the irradiation power and time increase, the mean droplet diameter decreases (Li and Fogler, 1978; Abismail et al., 1999; Kentish et al., 2008; Leong et al., 2009; Tang et al., 2013). This is because of the fact that as the irradiation power increases, the pressure amplitude of the applied sound pressure increases, which will increase the cavitation phenomena (number of events and the cavity collapse intensity) (Leong et al., 2009).

However, the over-processing effect may also occur under high shear conditions leading to coalescence of droplets and increase of the average diameters (Jafari et al., 2006; Kentish et al., 2008). For instance, the decrease of droplet diameter and preparation of flaxseed o/w nanoemulsions (< 200 nm) was feasible when increasing the nominal power up to 200 W, whereas further power increase had detrimental effect on size. This was associated with secondary Bjerknes forces which increase in intensity and increase the proximity of the droplets when increasing the applied power (Kentish et al., 2008).

Increasing the frequency of ultrasound waves also contributes to droplet size reduction. Submicron oleic acid/water emulsions formation in the absence of emulsifier has been reported with decreasing droplet diameters to less than 400 nm with increasing the frequency from 40 kHz up to 1 MHz (Kamogawa et al., 2004).

1.3.1.3 Membrane emulsification

Membrane emulsification (ME) was firstly reported by Nakashima and Shimizu (1996), who developed the Shirasu porous glass (SPG) membrane. This relatively novel method has received increasing attention within the last 20 years, mainly due to advantages such as low energy consumption, enhanced control of the droplet size and distribution and process mildness.

The principle of the method is based on forcing the dispersed phase-to-be into the continuous phase through a membrane characterized by a uniform pore size distribution. The dispersed phase is pressed perpendicular towards the membrane, while the continuous phase flows tangential to the membrane (Fig. 1.4) (Leal-Calderon et al., 2007). The preparation of vitamin E loaded nanoemulsions with very small diameters has (~78 nm) been recently reported (Laouni et al., 2012) by optimum combination of surfactant mixture (Tween 80/Brij 35).

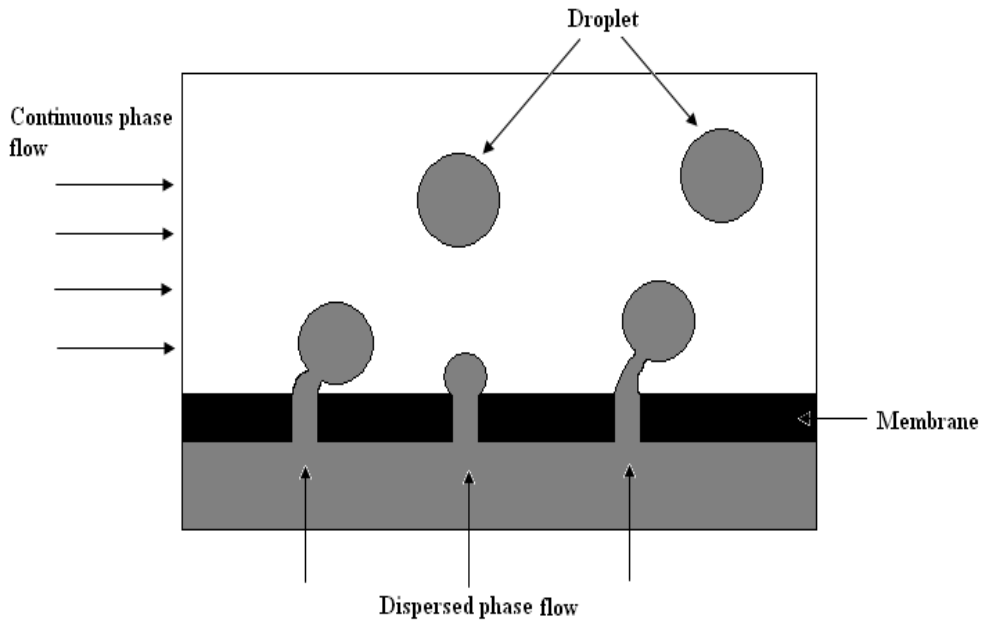


Figure 1.4 Schematic diagram of a cross-flow membrane emulsification process (reprinted from: Pathak, 2012).

1.3.2 Low energy approaches

Despite high energy approaches being quite straight forward for the formation of nanoemulsions, as higher energy inputs usually to the formation of smaller droplets, still they are not considered cost-effective methods. Only a small percentage of the total energy available -approximately 0.1 %- is finally used for emulsification (Tadros et al., 2004), while the rest is dissipated mainly as heat, a route which is generally unfavorable for industrial applications. Despite this major drawback, nanoemulsions may also be formed by employing/manipulating the physicochemical properties of the system (surfactants, co-surfactants and excipients), generally referred to as low-energy emulsification methods. The two commonly reported low-energy emulsification methods are the phase inversion method and the spontaneous emulsification (Anton et al., 2008).

1.3.2.1 Phase inversion emulsification

Phase inversion is often used for the industrial preparation of cosmetics and its main advantage is the production of concentrated, monodisperse emulsions consisting of fine droplets (<1μm) (Leal-Calderon et al., 2007). This type of emulsification uses the chemical energy released during the emulsification procedure owed to the occurring phase transitions. Phase inversion occurs when altering the structure of the emulsion in that way so that the dispersed phase may become the continuous phase and the continuous phase the dispersed phase or vice versa. This phenomenon may be induced by altering numerous variables such as temperature, the concentration of oil or water, pressure, salinity and the use of a co-surfactant (Morales et al., 2003). Phase inversion pathways can be further segregated into *catastrophic* or *transitional* (Tadros et al., 2004).

- *Catastrophic phase inversion (CPI)*

Catastrophic inversion can be generated by the increase of the effective volume fraction of the dispersed phase, either by continuous addition of the dispersed phase or by continuous stirring (Tadros et al., 2004). This technique begins with the creation of a so-called "abnormal" w/o emulsion, containing a hydrophilic surfactant. An abnormal emulsion is characterized by high instability, is formed if the emulsifier is more soluble in the dispersed phase and can be therefore maintained in this state for a short period of time provided a vigorous stirring is offered (Salager et al., 2004). Then water is added dropwise to the oil phase where the surfactant is been dissolved. The increasing addition of water causes an increase of the coalescence in the emulsion up to the point that the rate of water droplet formation is greater than that of droplet coalescence and the abnormal w/o emulsion switches irreversibly to o/w emulsion (Sajjadi, 2006).

- *Transitional phase inversion (TPI)*

The term transitional phase inversion (TPI) was first introduced to describe emulsion inversion caused by changes in the affinity of surfactant(s) by altering the water/oil ratio (Salager, 1979). The most commonly used transitional methods for the preparation of nanoemulsions are the Emulsion Inversion Point (EIP) and the Phase Inversion Temperature (PIT).

- *Emulsion Inversion Point (EIP)*

The affinity of a surfactant toward a phase is conventionally measured by its hydrophile–lipophile balance (HLB). The HLB of a surfactant measures the degree to which it is hydrophilic or lipophilic (Griffin, 1949). Altering the HLB results in the surfactant being attracted more towards the oil or water phase depending on the direction of change. This method is also referred to as emulsion inversion point (EIP) or phase inversion composition (PIC) and occurs under constant temperature conditions (Sajjadi et al., 2004).

- *Phase Inversion Temperature (PIT)*

Transitional inversion can also be induced by other parameters that affect the HLB number of the system, e.g. temperature and/or electrolyte concentration. The phase inversion temperature method (PIT) was first described by Shinoda and Shaito (1968), who used a specific non-ionic polyethoxylene-type surfactant, which presented a temperature-dependant affinity towards water or oil. The surfactant spontaneous curvature is affected by temperature due to changes in hydration of the poly(oxyethylene) chains. More specifically, the increase of temperature increases the lipophilic character of this type of surfactant by means of dehydration. Initially, mixtures containing oil, water and the surfactant are prepared at their PIT. The PIT represents the point where the affinities of the surfactant towards water or oil are equal (Lehnert et al., 1994). If these solutions undergo rapid heating, then w/o emulsions are obtained, whereas cooling promotes the formation of o/w emulsions. After the preparation of emulsions close to the PIT region, cooling is applied leading to a point of zero spontaneous curvature characterized by minimum interfacial tension (10^{-2}–10^{-5} mN m^{-1}), thus the formation of fine dispersed oil droplets is promoted (Kunieda and Friberg, 1981). Fast cooling or heated is considered essential in order to stabilize the emulsions, since the newly formed fine droplets are very susceptible to recoalescence and coarse polydisperse emulsions are formed otherwise (Morales et al., 2003).

1.3.2.2 Spontaneous emulsification

The term spontaneous emulsification was introduced in by Ruschak and Miller (1972) about a study on an ethanol-toluene-water system. Spontaneous emulsification

occurs under isothermal conditions in the absence of external energy supply, when mixing two immiscible liquids with very low interfacial tension. Ultrafine droplets are formed at the boundary between an aqueous and organic phase with a specific composition. The most famous example of spontaneous emulsification is the so-called "Ouzo effect". This phenomenon is observed in several aperitifs like greek originated ouzo and french pastis that are ethanol extracts of anis seeds. Upon dilution with water, these aperitifs which mainly contain ethanol, water and trans-anethole (oil), become slightly cloudy. Anethole is soluble in ethanol and almost insoluble in water, while ethanol is water soluble. Therefore, oil is mainly surrounded by ethanol molecules. Its significant is high since it offers the feasibility to create fine-sized dispersed particles with no added surfactants, dispersing agents or mechanical stirring (Vitale and Katz, 2003). The main source of required energy for spontaneous emulsification is the interfacial turbulence generated by interfacial tension gradients due to the diffusion of a solute between phases. The liquid interfaces are subject to capillary waves generated by thermal fluctuations. As the interfacial tension is decreased their amplitude increases, and the break-up of droplets is feasible when under sufficient amplitude conditions.

Three kinds of methods have been identified for the creation of nanoemulsions by spontaneous emulsification. The first involves a simple mixing of three components, oil, water and water-miscible solvent. According to another method, nanoemulsions may also form by bringing in contact a system containing oil, a hydrophobic surfactant and a water-miscible solvent with aqueous phase. One last method is that of adding oil and a hydrophilic surfactant mixture into an aqueous phase. Nanoemulsions are formed due to the rapid diffusion of the hydrophilic surfactant towards the aqueous phase which occurs with concurrent inclusion of oil (Fig 1.5). This method is of particular interest for food application, since it does not require a solvent. Food grade nanoemulsions (d_{32} <200 nm) by using a system of medium chain triglycerides and Tween 80 at high surfactant-to-oil ratio (SOR=2) (Komaiko and McClements, 2015). Analogous systems have been tested for vitamin D or E loaded nanoemulsions serving as delivery systems in functional food and beverages (Gutoff et al., 2015).

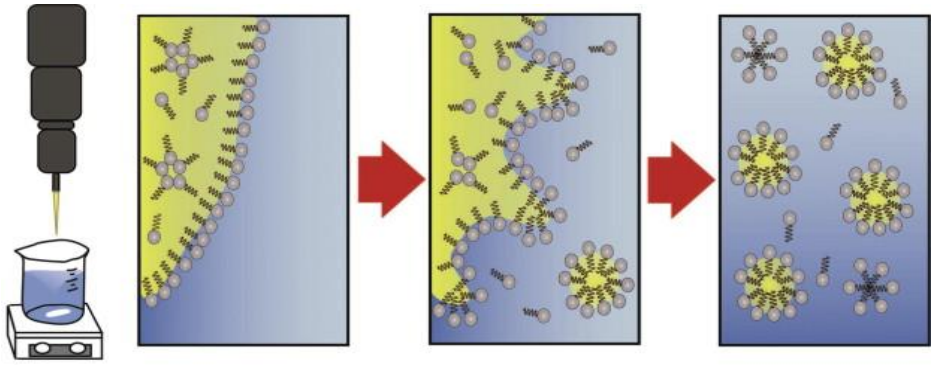


Figure 1.5 Schematic representation of the spontaneous emulsification method. (Reprinted from: Komaiko and McClements, 2015).

1.4 Emulsion destabilization phenomena

Emulsion stability is defined as the ability of the emulsion to resist changes in its properties over time. As, emulsion are considered thermodynamically unstable systems, changes will eventually occur. Hence, a stable emulsion is characterized by low destabilization rates, rather than being totally unchanged. The physical destabilization of emulsions and nanoemulsions includes phenomena such as creaming, sedimentation, flocculation, coalescence and Ostwald ripening (Fig. 1.6). Phase inversion can be also observed, but it's out of the scope of this review. Chemical stability in terms of oxidation is also of high importance when preparing emulsified food products. Typically, nanoemulsions are less susceptible to the majority of destabilization phenomena (creaming, flocculation and coalescence) in comparison to macroemulsions, being Ostwald ripening their major destabilization mechanism. Nanoemulsions on the contrary are reported to exhibit higher oxidation rates (Tabibiazar et al., 2015).

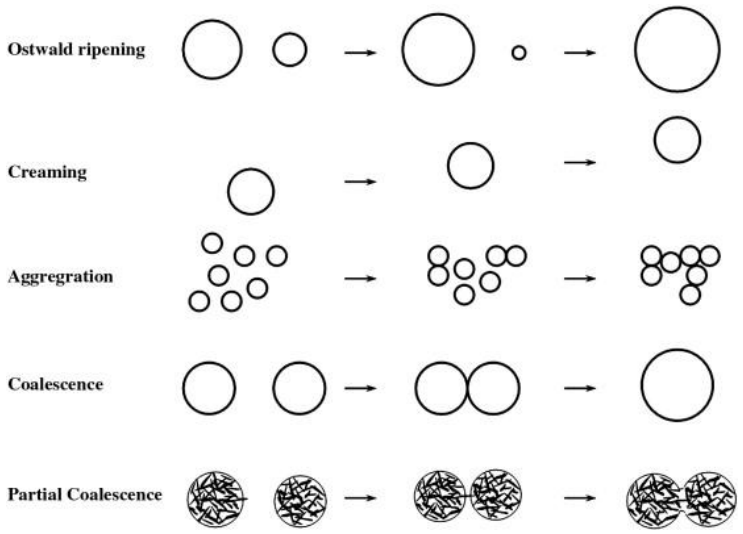


Figure 1.6 Schematic representation of potential instability mechanisms in oil-in-water emulsions, (reprinted from: Walstra, 2003).

1.4.1 Creaming and sedimentation

These types of destabilization are both regarded as gravitational separation of the emulsion droplets due to density difference between the dispersed and the continuous phase. Taking into account that the density of the oils usually incorporated in food o/w emulsions have lower densities than water, oil droplets accumulate on the top, which is referred as *creaming*. By definition, water droplets contained in w/o emulsions will move downward and finally accumulate on the bottom of the dispersion, this phenomenon is known as *sedimentation* (McClements, 2005).

Stoke's law (Eq. 1.3) can provide a prediction of the creaming rate (creaming velocity) in dilute non flocculated emulsions (Dickinson, 1992):

$$U = -2gr\,2(\rho_w - \rho_o)\,/9\eta_c \qquad \text{Eq. 1.3}$$

,where U is the creaming velocity of the droplet, ρ_w is the aqueous phase density, ρ_o is oil phase density, r is radius of the droplet, η_c is viscosity of continuous phase, and g is the acceleration due to gravity. The equation can be modified to include flocculated emulsions by substituting the radius and of the droplet and the density of the oil phase by those of the generated flocs, provided they are spherical. The radius and the density of the flocs depend on the number of the droplets per floc and the internal packing of the droplets (McClements, 2004). However, Stokes law is valid under dilute conditions ($\varphi < 2\%$), hence emulsified products such as salad dressings and mayonnaise are not considered dilute, since their oil fraction (φ) is generally

much higher. In this case the modified Stokes law applies (Robins, 1991). The creaming velocity of droplets in concentrated emulsion is lower compared to dilute emulsions, due to hydrodynamic interactions between droplets.

1.4.2 Droplet aggregation

Emulsion droplet movement due to Brownian motion, gravitational force, or mechanical agitation can result in droplet collision. Depending on the intermolecular forces developed between the droplets, this can lead to droplet aggregation. Two main types of aggregation can be distinguished: *flocculation* and *coalescence*.

Flocculation occurs when intermolecular forces are sufficient to keep the droplets apart at a small equilibrium distance so that droplets keep their physical properties. It has been shown that the presence of non-absorbing colloidal high molecular weight polymers (xanthan, dextran, ets.) or surfactant micelles in the continuous phase induces droplet flocculation by increasing the attractive osmotic forces between droplets owed to polymer particle exclusion from the intervening space. These attractive forces increase when increasing the concentration of the colloidal particles and when they become higher than the repulsive forces between droplets, then flocculation is observed. This type of weak and reversible droplet aggregation is known as *depletion flocculation* due to the polymer depleted zone observed caused by osmotic gradients (McClements, 2005). Typically, a minimum concentration of polymer is required to induce depletion flocculation (critical flocculation concentration, CFC) depending on the molecular weight of the polymer and droplet diameter. The critical concentration level increases as the molecular weight of the polymer decreases or the emulsion droplet size decreases (Dickinson, 1997). Apart from microscopical observation, the critical concentration depletion flocculation onset can be illustrated by the height of the cream layer in a series of emulsions prepared with increasing polymer concentrations. In the absence of polymer or at very low polymer concentration, stable emulsions can be formed for a short period of time, whereas higher amounts of non-adsorbing polysaccharides causes them to flocculate and fast creaming is observed (McClements, 1999).

Although it is usually regarded as a destabilization phenomenon, flocculation may also hinder creaming under certain conditions. In dilute emulsions, droplet flocs do not majorly interact with each other, while in the mean time the average hydrodynamic diameter of the floc is higher than that of single droplets, so that they

tend to separate faster and the creaming velocity is enhanced. On the other hand, flocculation can produce re-stabilization by conferring increased apparent emulsion viscosity as a result of the interconnected three-dimensional or strong gel network formation, preventing the movement of individual droplets (depletion stabilization) (Robins, 2000).

In contrast to depletion flocculation, *bridging flocculation* is caused when an adsorbing polymer is added to a suspension of particles and a single polymer chains becomes attached to two or more particles. In protein, food emulsions bridging flocculation can be developed if the amount of the emulsifier available is not sufficient to cover the newly formed interface during homogenization. It may also occur after emulsification via cross-linking of adsorbed protein molecules on different droplet due to thermal or high shear treatment or through complexation with multivalent ions or charged surfactant. It is well established that bridging flocculation produces flocs due to higher attraction energy (often of electrostatic origin) which can withstand high-shear, hence it is irreversible. Several direct or indirect methods have been proposed for monitoring the emulsion flocculation, the most promising of them being ultrasound spectroscopy, diffusion wave spectroscopy, front face fluorescence spectroscopy and nuclear magnetic resonance (Dickinson, 2010).

Coalescence suggests the irreversible collision of droplets upon rupture of the interfacial membrane resulting in formation of larger oil droplets and eventually oil layer formation (oiling-off) appearing at the top of the emulsion (Marucci, 1969). Membrane rupture is caused by the flattening of the surface of the droplets when moving in close proximity to each other. An intermediate phenomenon combining features of droplet aggregation and coalescence (also referred to as *'true'* coalescence for differentiation reasons) is that of *partial coalescence* (clumping). Partial coalescence is possible if the droplets contain solid particles such as crystals of oil molecules. Once present in such droplets or globules preferably, these crystals are able to form a network in the inside, which eventually protrudes them into the water phase. Upon collision with other globules, the crystals penetrate the surface of the other globule and create a contact surface, thereby making an oil-oil contact, while the initial globules remain visible (Fredick et al., 2010).

1.4.3 Ostwald ripening

Ostwald ripening is the phenomenon of gradual growth of larger droplets at the expense of smaller ones due to diffusion of dispersed phase material through the intervening dispersion medium under the influence of the Laplace pressure difference. The pressure gradient between the inside and the outside of the drop is described in Eq. 1.4.

$$\Delta P = 2\sigma / r, \qquad \text{Eq. 1.4}$$

Where, ΔP is the Laplace pressure difference, σ is the interfacial tension, and r is the droplet radius. The Ostwald ripening rate "ω", can be calculated according to the LSW (Lifshitz–Slezov–Wagner) theory from Eq. 1.5, which establishes a linear relationship between the cube of the droplet radius r^3 and storage time t:

$$\omega = \frac{dr3}{dt} = \frac{8\, C\infty\, \gamma\, Vm\, D}{9\, \rho\, R\, T} \qquad \text{Eq. 1.5}$$

Where, C^{∞} is the bulk phase solubility, γ is the interfacial tension between the dispersed phase and continuous phase, V_m is the molar volume of the dispersed phase, D is the diffusion coefficient of the dispersed droplets in the continuous phases, ρ is the density of the oil, R is the gas constant and T is the absolute temperature (Henry et al., 2009):.

In order for this to occur a relative solubility of the dispersed material (oil) in the continuous phase is required (Dickinson and Golding, 1998). Ostwald ripening is the main mechanism for the destabilization of foams, whereas it is considered unimportant for oil-in-water emulsions because triaglycerol molecules are very insoluble in water and the viscosity of the oil is high. However, Ostwald ripening is mostly important for food grade nanoemulsions containing essential oils (d-limonen, orange oil, thyme oil) as flavorants, fragrance carriers or antimicrobial agents. This is because, low molar volume oils (200–350 $cm^3\, mol^{-1}$) exhibit relatively high solubility in water (Sagalowicz and Leser, 2010). Strategies developed towards inhibiting or retarding Ostwald ripening include the addition of insoluble oils (corn or olive oil), rather than the essential oil alone, incorporation of thickening agents, interfacial membrane thickness enhancement, and lay-by-layer droplet coating

(electrostatic or via enzymatic crosslinking) (Rao and McClements, 2011; Chang et al., 2012; Zeeb et al., 2012).

1.5 Potential applications of nanoemulsion in the food industry-*Why "nanos" anyway?*

The application of nanotechnology in the food sector has received a great attention from the scientific community driven by the increasing consumer demand for safer, healthier and health promoting food products, as well as industry demands for products with enhanced organoleptic and stability properties. The fabrication of *safer* foodstuffs is reflected in the use of nanoemulsions as delivery systems of compounds possessing antimicrobial and bactericidal activity on enteric and other pathogens. Microbial spoilage and growth inhibition could be achieved by either incorporating nanoemulsions in edible films (smart packaging) (Otoni et al., 2014), by directly adding them in a food formulation (Donsi et al., 2010) or immersing a food in it (Barghava et al., 2015). Essential oils extracted from plant materials are one type of ingredients used against pathogenic microorganisms involved in foodborne illnesses (Calo et al., 2015). Nevertheless, their application in food is restrained by flavor considerations, as the effective dosage for microbial inhibition may not be organoleptically accepted. Potential essential oil nanoemulsion applications include ingredients such as lemon myrtile oil, d-limonene, trans-cynamaldeyhyde, thyme oil, eucalyptus oil, clove bud oil, oregano oil, essential oils of the Asteraceae family (Acevedo-Fani et al., 2015).

The characterization 'healthier' in the basis of nanoemulsions refers to the ability of creating healthier food products with lower fat content (dressings, spreads, icecream), without compromising any sensory attributes. Products such as low fat nanostructured spreads and mayonnaise are still in development stages, but they are expected to be commercially available in the near future (Cushen et al., 2012).

"Health promoting" properties are related to the ability to encapsulate numerous compounds (minerals, vitamins, phytosterols, lutein, fatty acids, lycopene and antioxidants) that demonstrate significant health benefits when consumed (Champagne and Fustier, 2007). Unfortunately, most of these compounds exhibit low solubility and bioavailability in aqueous-based foods. Therefore the use of nanoemulsions as carriers is essential for addressing delivery and bioavailability efficacy issues. Much of the latest research elaborated in the nutraceutical sector

focuses in the encapsulation of vitamin E, vitamin D derivatives and β-carotene (pro-vitamin A) and phytochemicals such as lycopene, lutein, curcumin and phenolics (quercetin, resveratrol, epigalocatechin gallate). Microemulsions enveloping vitamin E were characterized by higher eminent sustaining release efficiency (up to ~87 %) in comparison to the bulk form (Feng et al., 2009). The bioaccessability of translucent vitamin D_3 enriched nanoemulsions was also proved to be higher when low chain triaglycerides were used in comparison to medium chain ones. The same observation was noticed for β-carotene nanoemulsions making them suitable for incorporation in functional beverages (Qian et al., 2012). The bioavailability of carotenoids encapsulated within nanoemulsions has been reported to be increased when compared to non-encapsulated carotenoids (Ribeiro et al., 2006).

1.6 Nanoemulsion toxicity issues

Nanotechnology is a new scientific sector that involves the manipulation of matter at the atomic and molecular level. The nanoscale size of the materials or devices formed with various top-down or bottom-up procedures offers them unique properties which can be further translated in applications to several sectors such as medicine, cosmetics, textiles, agriculture, electronics and the food industry.

Despite the advantages they have been acknowledged with for having tremendously small dimensions, this represents only one side of the coin, since elements at the nanoscale behave differently than they do in their bulk form. This is attributed to the larger area they occupy in comparison to the same amount of material in a larger lump and also to the fact that their phenomena are interpreted by quantum physics. Therefore, there's an ongoing concern that some nanomaterials/particles could be toxic towards humans, animals or plants. This is becoming an issue of increasing interest throughout the scientific community and human community in general, since the available information through research conducted on the field of nanomaterial toxicity is limited (Kong et al., 2011). The general status of this situation characterized by an enormous number of studies focused on nanoparticles at the expense of studies regarding possible toxicity or safety issues is depicted in Fig. 1.7. Very recently, the trust and willingness of consumers to buy a functional juice containing nanoencapsulated vitamin D was assessed (Roosen et al., 2015). The authors concluded that nanotechnology raises concerns in consumers regarding the

product itself or its packaging, although it was not established if this is generated from the lack of awareness of nanotechnology in the general public or lack of awareness of nanotechnology uses in the food industry. Another research revealed that consumers are more hesitant in buying foods with nanotechnology packaging and even more hesitant towards nanotechnology foods (Siegrist et al., 2007).

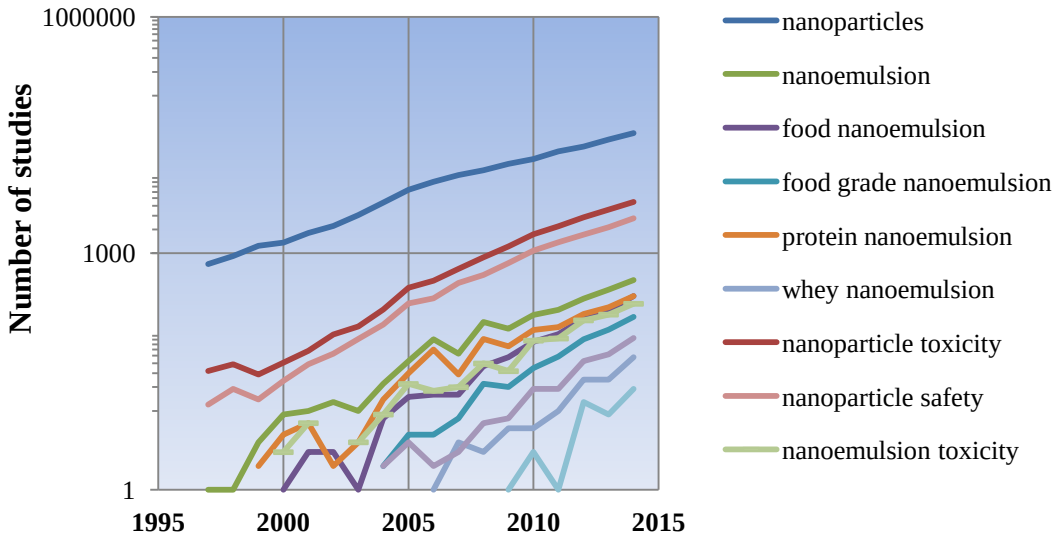


Figure 1.7 Number of related studies conducted in the field of nanotechnology from 1997 to 2014 according to keyword search used in sciencedirect.com.

To address related toxicity concerns of nanotechnologies in general, Nanotoxicology was developed as the subdiscipline science of the toxicology that is concerned with studying and evaluating the effects of human, animal and environmental exposure to nanomaterials by *in vitro* and *in vivo* means (Fereira et al., 2013).

Nanoparticles may enter the human body through physical exposure which can be dermal uptake, inhalation and ingestion, or medical administration (rectal, injection) and can reach the bloodstream and affect multiple body organs (Zeliger, 2011). A number of characteristics of the nanoparticles like size, shape, surface charge, chemical properties, solubility and degree of agglomeration may determine their effect on biological systems in general and possible toxicity which can be expressed as inflammation, stimulation or suppression of the immune system, genotoxicity or cellular proliferation, DNA breakdown, and gene expression disrupt leading to oxidative stress (Fereira et al., 2013).

Titanium oxide (TiO_2) nanoparticles as well as other metal oxides (silica, silver, ferric) and metal nanoparticles such as gold and silver are of the most well documented ones within the context of nanotoxicity. More specifically, titanium oxide is labeled as food additive (E 171), used as a white colorant typically in various products (confectionery, sauces, and creams). In the form of nanoparticles, it acts as an anticaking agent in powdered products improving handling and enhances color and durability. TiO_2 nanocomposites, can also be used as oxygen sensors in packaging films. Nevertheless, manufactures are not obliged to indicate the particle size of this additive (Smolkova et al., 2015). Numerous *in vivo* studies suggest that exposure on TiO_2 nanoparticles can be harmful and cause damages by accumulating in organs, while *in vitro* it has been demonstrated that they can be cyto-, neuro- and genotoxic, lead to cell apoptosis, increase inflammation and oxidative stress, alter gene expression and enzyme activity (Cloete et al., 2010). Carbon nanotubes (CNTs) have also been identified to exhibit reversible *in vivo* toxicity in mice (Bai et al., 2010). However, studies addressing their cytotoxicity show diverging results, depending on material type. For instance, while CNTs reduced cell viability to less than 50 % after 24 h incubation, the treatment with another form of them (single-walled CNTs) did not reveal any cytotoxicity (Wörle-Knirsch et al., 2006).

With respect to nanoparticles or lipid nanoparticles that may be present in foodstuffs, the research carried out currently considering their toxicity is very limited (Card et al., 2011). More specifically, in a recent review published by Card et al. (2011), a total of 30 studies (21 *in vivo* and 9 *in vitro*) were identified as toxicology studies investigating the exposure, by oral administration or relevant *in vitro* assays, to nanomaterials with potential food-related applications. However, according to the authors the vast majority of the *in vivo* studies were lacking some critical components of study design such as limited duration of exposure, restricted number of physicochemical properties evaluated, non report of the oral gavage dose volume etc. On the other hand, 7 out of the 9 in vitro studies focused on cytotoxity, whereas two of them evaluated genotoxicity.

Another category of food-grade nanoparticles involves polysaccharide nanocrystals derived from chitin, chitosan, cellulose (nanocrystalline cellulose, NCC) or starch as well as cellulose nanofibers. Acute toxicity studies for chitosan nanoparticles showed that they are not toxic (Zhang et al., 2011). Findings on NCC particles in aquatic species have reported low environmental ecotoxicity (Kovacs et

al., 2010). No evidence of inflammatory effects or cytotoxicity on mouse and human macrophages was observed due to exposure to nanofibrillated cellulose (NFC) (Vartiainen et al., 2011). On the contrary, clear genotoxic effects depending on the material source have been shown for cellulosic nanofibers in plant, human and animal cells (de Lima et al., 2012). Considering starch nanocrystals, there have been no scientific data so far regarding their toxicity.

Potential toxicity of lipid nanoparticles could be associated with increased bioavailability of bioactive compounds that are toxic at high levels, direct absorption, interference with normal gastrointestinal function and compositional (high levels of specific surfactants or solvents used for their production) (McClements, 2013).

With respect to food-grade nanoemulsions, their possible *in vitro* cytotoxicity was recently investigated in cancer-derived (Caco-2) cells which mimic the exposure in the small intestine and hepatic (HepG2) cells from the liver. The size of nanoemulsions prepared with Tween 20, whey protein isolate or modified starch ranged between 155-172 nm and their effect was compared to micron-sized emulsions (5.7-10.3 μm). It was finally concluded that nanoemulsions were not significantly more toxic than their macroemulsion counterparts, with regards to small intestine epithelium. Nanoemulsions formed with modified starch and WPI though, showed increased cytotoxicity on HepG2 cells, hence more *in vivo* investigation is required, since there has been no evidence that nanoemulsions can reach the liver tissues by permeating the epithelium layers lining the digestive tract (Yu and Huang, 2013).

Adverse results have been reported for nanoemulsions prepared with food-grade proteins and surfactants intended as drug release systems (He et al., 2011). According to this research nanoemulsions prepared with whey, soy or β-lactoglobulin proteins demonstrated higher Caco-2 cell viability in comparison to traditional emulsifiers such as egg phosphatidylcholine, and Tween 80 at 3 mg/ml total emulsifier concentration level, indicating significant cytotoxicity.

A concentration dependant compositional cytotoxicity of food-grade microemulsions owed to polysorbate (Tween 80) has also been observed *in vitro*. Exposure of Caco-2 cells to low microemulsion concentration (0.015 %) did not cause significant cytotoxicity as evidenced by high cell viability (> 90 %), whereas a two-fold increase led to decrease at levels < 90 %, exhibiting concentration dependency. At the same study, the encapsulation of β-carotene did not ameliorate cell viability as stated in similar studies (Roohinejad et al., 2015).

Furthermore Tween 20/glycerol monooleate based nanoemulsions (~130 nm in diameter) intended for resveratrol delivery in food and nutraceutical applications were also evaluated regarding cytotoxicity in Caco-2 cells. Results demonstrated that the used blank formulation (no added resveratrol) did not affect cell viability at any dilution level (1:10 up to 1:100) (Sessa et al., 2014).

All of these findings provide a better insight on the potential of nanoemulsions and food-grade nanoparticles in general for human consumption. However, it is obvious that due to the concentration and material-specific dependency and various types of toxicities, there is a great need for more systematic research and *in vivo* studies to ensure the safety of nanoemulsions in food stuffs.

1.7 Current status of nanotechnology legislation in EU

The European Union had recently formed a group to study the implications of nanotechnology, called the Scientific Committee on Emerging and Newly Identified Health Risks. The deliberation of the Commitee resulted in the publication of a list of risks related with nanoparticles, in the so-called opinion of "Scientific basis for the definition of the term nanomaterial". On the basis of the Committee's opinion, in 2011, the European Commission released a specific recommendation (EU, 2011) on the definition of a nanomaterial:

> ***Nanomaterial*** *means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range* ***1 nm–100 nm.***
>
> ***In specific cases*** *and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between* ***1% and 50%***

Nevertheless, the Commission's definition is not considered legally binding, until it is incorporated into legislation, and guidance on the legislation should be developed dealing with requirements issues. The Commission's definition aims to

provide the means for identification of nanomaterials by certain specifications on the size range, rather than classifying them as inherently hazardous.

Currently, the nanomaterials and nanotechnologies in EU fall under the regulations of horizontal and vertical legislation. However, the initial scope of the existing horizontal legislation (General Product Safety Directive - GPSD, Evaluation, Regulation on Authorisation and Restriction of Chemicals-REACH, Regulation on Classification, Labelling and Packaging-CLP, Biocidal Product Regulation-BPR) was broad and did not specifically aim to enclose the attributes of nanotechnologies (Cushen et al., 2012).

GPSD provides the definition of a safe product (excluding ecosafety), covering all types of products on the market as well as those offered to consumers during a service. Nanomaterials are regulated by REACH and CLP because they are covered by the definition of a chemical "substance" in both Regulations. The general obligations in REACH and CLP therefore apply as for any other substance, although there are no provisions referring explicitly to nanomaterials. REACH provides an over-arching legislation applicable to the manufacture, placing on the market and use of substances on their own, in preparations or in articles. Nanomaterials that fulfill the criteria for classification as hazardous under Regulation (EC) No 1272/2008 on classification, labeling and packaging (CLP) of substances and mixtures must be classified and labeled.

With regards to the horizontal regulation on biocidal products, BPR was recently revised, being the first regulation that merely includes the EU's recommended definition on nanomaterials. In the context of BPR the 50% threshold as stated in the definition document is not declared in the text of the regulation, thus leaving space for deviations (Bleeker et al., 2013). A key feature of the EU biocidal products Regulation is the provisions regarding nanomaterials, which apply to products and substances that meet the criteria defined within the regulation, based on the definition of nanomaterials recommended by the Commission.

Vertical legislation, which was introduced within the last few years, specifically regulates nanotechnologies and industrial sectors that utilize them. Revisions made on the regulation for cosmetics (EC No 1223/2009) were able to provide a first vertical regulation with a legal definition for nanomaterials. Within the revised regulation it is declared that nanomaterials used for the production of cosmetics should be assessed

with regards to toxicity safety before allowed to commercialization. Their presence should also be included in the list of ingredients indicated with the word "*nano"* in brackets (Cushen et al., 2012).

The Regulation on Novel Foods (EC No 258/97) contains indirectly and *inter alia* a legal definition of nanomaterials. According to the Regulation novel foods are defined as *"foods and food ingredients that have not been used for human consumption to a significant degree within the Community"*. As a consequence, food products containing nanomaterials are regarded as novel foods, rising safety assessment and authorization requirements before commercialization. Nevertheless, an agreement has yet to be reached between the European Council and the European Parliament on the proposed amendments for the existing Regulation (Coles and Frewer, 2013).

Another vertical regulation relates to food additives. The Food Additives Directive, (EC No 1333/2008), entered into force in 2010, represents the first piece of legislation referring exlusively on nanotechnologies. As mentioned in article 12, if an already approved starting material or additive undergoes any changes in particle size for example through nanotechnology, shall be considered a different additive requiring *de novo* safety evaluation and authorization.

Food contact materials (FCM) are reulated under the Active and Intelligent Materials and Articles Intended to Come in Contact with Food Regulation (EC No 450/2009). The regulation states that whenever quantity limitations exist by legislation for a substance contained in a food, these limits cannot be exceeded regardless of the source i.e. originally included in the food formulation or as a consequence of migration of the substance from the FCM. Any substances released into the food from the material, must be stated in the ingredients list. The same approach is used with the regulation on plastic materials and in this sense, active substances also require safety assessment by EFSA and Community authorization in order to be included in a positive list and used under certain constraints (Cushen et al., 2012).

1.8 Food polymers used as emulsifiers and stabilizers

The chemical composition and functional properties of the main polymeric emulsifiers and stabilizers used for the preparation of mayonnaise and salad dressings as well as those used in this thesis is discussed in brief in the following sections.

1.8.1 Polysaccharides

1.8.1.1 Xanthan gum

Xanthan gum is a unique anionic exocellualar polysaccharide discovered in the United States during 1950s, secreted during the anaerobic fermentation of sugars by the bacterium *Xanthomonas campestris* (Richardson and Ross-Murphy, 1987).

It is accepted as a safe food additive in the USA, Canada, Europe, and many other countries, with E number E415. The official definition of the EU food regulation for E415 is: "Xanthan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate with natural strains of *Xanthomonas campestris*, purified by recovery with ethanol or propane-2-ol, dried and milled. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid and pyruvic acid, and is prepared as the sodium, potassium or calcium salt. Its solutions are neutral. The molecular weight must be approximately 1 MDa and the color must be cream". The heteropolysaccharide's primary structure consists of repeated pentasaccharide units: two glucose units linked with two mannose units and one glucuronic acid in the molar ratio 2.8:2:2. In more detail, the main chain of xanthan consists of a β (1–4)-linked glucose backbone with orderly distributed trisaccharide side chains on every second glycosyl residue. The branched trisaccharide chain is composed of a glucuronic acid unit linked between two D-mannose units (Li and Feke, 2015).

Two different conformational structures have been indentified for xanthan gums: an *ordered double stranded helical* conformation in the presence of salts and at low temperatures, and a *disordered single stranded random coil,* induced by heating above the transition temperature. Factors affecting the transition temperature include salt concentration, the molecular weight and the acetyl and pyruvate content of the xanthan molecules (Capron et al., 1998).

Xanthan gum is known to exhibit synergistic interaction with galactommannans resulting in solutions with increased viscosity or gelation. However, the actual mechanism which leads to gel formation is still under debate and several models have

been proposed to explain it. The presence of acetyl moieties on the xanthan chains along with the branching degree of the galactomannan both suggest significant parameters for gel formation, whereas the increase of galactose content hinders gel formation (Sandolo et al., 2010).

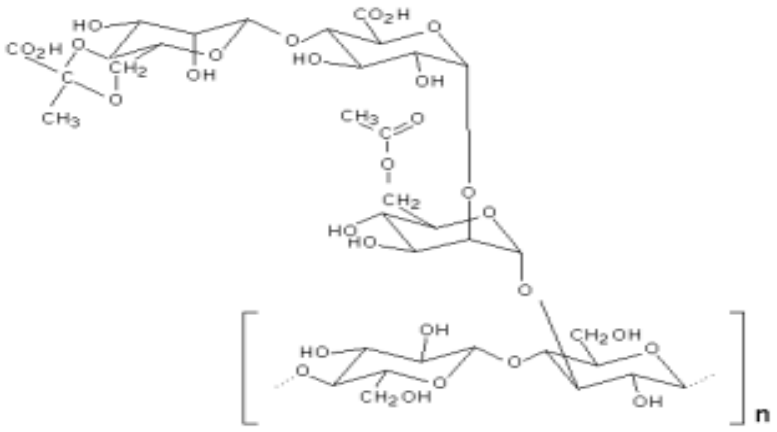


Figure 1.8 Chemical structure of Xanthan gum.

1.8.1.2 Galactomannans

Galactomannans are neutral polysaccharides, composed of a linear mannan backbone bearing side chains of a single galactose unit. A large group of galactomannans is produced from the seeds of the Leguminoseae family, which embraces full size trees as well as herbaceous plants.

The germ comprises about 25 % of the seed and is rich in proteinaceous constituents (nearly 48 % of the dry mass), mainly composed of reserve proteins, such as globulins (40%), while the remainder are functional proteins (enzymes, inhibitors, etc.) (Rakhmanberdyeva et al., 2005).

Generally, galactomannans are composed of a mannose backbone (main chain), with galactose residues (side chains). The mannose/galactose ratio (M/G) is characteristic for each galactomannan. There are several galactomannans industrially employed, namely tara gum (M/G ratio of 3:1), guar gum (M/G ratio of ~2:1), locust bean gum (M/G ratio of 4:1) and fenugreek gum with a M/G ratio of ~1:1(Butt et al., 2007).

Due to their high water binding capacity, galactomannans can form highly viscous solutions at low concentrations without forming gels on their own, with the

exception of locust bean gum which can form gels at high concentrations and under subzero temperature conditions (Wu et al., 2009).

- *Guar gum*

Guar gum is a natural non-ionic polysaccharide obtained from the endosperm of the guar bean (*Cyamopsis tetragonolobus*), a plant which primarily grows in the region of India and Pakistan. It is a water-soluble galactomannan comprised of a linear backbone of β-1, 4 linked D-mannopyranosyl units and randomly, in pairs and triplets arranged α-1,6 linkedD-galactopyranosyl units as side chains. Typically, the M/G ratio varies between 1.6 and 1.8 depending on the natural source (Cheng et al., 2002).

The high molecular weight of the galactomannan (1000–2000 kg mol^{-1}) and the interactions of the unbranched parts of the backbone, also referred as "hyperentanglements", are the main reason for the high viscosity of aqueous solutions observed even at at low concentrations (Wientjes et al., 2000).

Guar gum is also considered a source of soluble dietary fiber and may be used for enrichment purposes in food products. Consumption levels of 20 g per day have been assessed to be safe (Slavin and Greenberg, 2003) and in Europe, the food additive code E412 has been assigned for guar gum. Usage levels fall under the *quantum satis* status regarding many food products, with the exception of use in jelly mini-cups or dehydrated foods which rehydrate on ingestion.

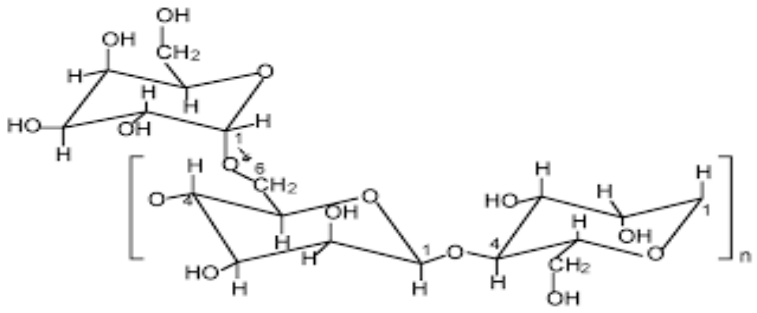


Figure 1.9 Chemical structure of Guar gum.

- *Locust bean gum*

Locust bean gum is a white to creamy white powder obtained after milling of seed endosperm of fruit pod of the carob tree, a member of legume family, botanically known as *Ceratonia siliqua* L. which is found in Mediterranean regions. Locust bean

gum is extracted from the seed endosperm of the carob tree. Hence, locust bean gum is also known as carob gum (Barak and Mudgil, 2014).

Locust bean gum generally has an average mannose to galactose ratio of about 3.5 which is the highest among the commercially available galactomannan such as guar gum (1.8) and tara gum (3.0). The degree of galactose substitution on mannose chain affects water solubility of the galactomannan. This is the reason for the guar gum being cold water soluble whereas LBG shows low solubility at ambient temperature and requires heat treatment for maximum solubility and maximum water binding capacity (Maier et al., 1993).

The solubility of locust bean gum in water can reach approximately 70–85% when heated to 80 °C/30 min (Dakia et al., 2008). Solubility differences compared to guar gum may be due to the high molecular weight of galactomannan component and lower galactose residues as side chain, which can be solubilized at higher temperature. Longer galactose side chains yield stronger synergistic interactions with other polymers and greater functionality (Higiro et al., 2006).

It is also a source of soluble fiber and can be utilized for the development of dietary fiber enriched food products. Its consumption in routine diet reduces the risk of heart diseases, diabetes and digestive disorders in human beings. In food industry it is a food additive, coded as E-410 in the European Union.

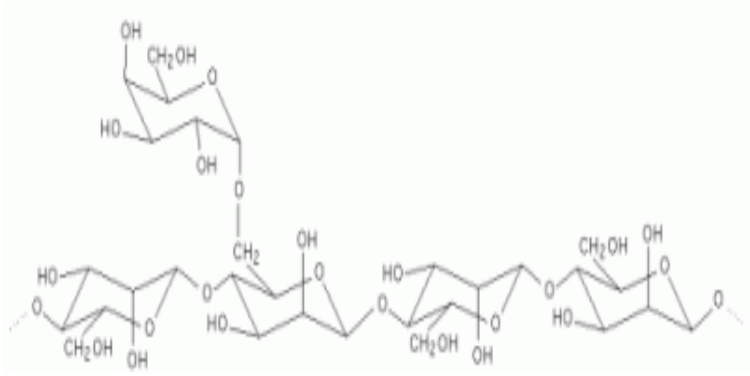


Figure 1.10 Chemical structure of Locust bean gum.

- *Fenugreek gum*

Fenugreek gum (Lat. *fenum Graecum* = Greek hay) is a soluble fiber extracted from the seeds of fenugreek (*Trigonella foenum-graecum)*, a legume plant grown

worldwide, mainly in India. The storage polysaccharide found in the seed endosperm is a galactomannan, similar to locust bean, guar and tara gum, but more heavily substituted. More specifically, the molar ratio of galactose to mannose of fenugreek gum is 1:1. In comparison to other types of gum like guar, fenugreek gum has higher water solubility due to higher galactose content. Reported value of the molecular weight is 1.4 million compared to 1.2 and 1.3 for locust bean and guar gum respectively. In addition to these properties, recent research suggests fenugreek gum may also be surface active. This galactomannan is less exploited in the food industry as compared to other ones, such as guar and locust bean. Solution properties of fenugreek gum are typical of random coil polymer. Fenugreek gum is a non gelling galactomannan and shows resistance against freeze-thaw treatments, whereas little synergistic interaction is observed with other gums (Biliaderis and Izydorczyk, 2007). Fenugreek and guar galactomannans were reported to be more effective emulsion stabilizers than LBG (Huang et al., 2001).

Fenugreek gum is a type of soluble dietary fiber and can be incorporated in bread making also. Though it is enlisted as GRAS (Generally Recognised As Safe) in the US by the FDA, it has not been yet approved as a food additive in the EU.

1.8.1.3 Resistant starch

Resistant starch (RS) represents the fraction of starch that cannot be digested by a-amylase in the small intestine of healthy individuals, whereas it may be digested in the large intestine (Englyst et al., 1992).

Depending on its botanical origin and process employed to form it, RS can be divided into four categories: RS1 is physically inaccessible for reasons such as starch entrapment in a protein matrix or a plant cell wall (e.g. in seeds and unprocessed whole grain); RS2 is raw granular starch which cannot be absorbed in the small intestine (e.g. those from potato and green banana); RS3 is retrograded starch, mainly retrograded amylose formed during cooking and cooling processes; and RS4 is chemically modified starch which is cross-linked by chemical agents and insusceptible to digestion and absorption in the small intestine (Chung, et al., 2011).

As an emerging functional food ingredient, resistant starch has been shown to have equivalent and/or superior impacts on human health similar to conventional fiber. Resistant starch has been introduced to human nutrition and the food industry in recent years as an increasingly important functional food ingredient. Unlike some

carbohydrates and digestible starches, resistant starch resists enzymatic hydrolysis in the upper gastrointestinal tract, resulting in little or no direct glucose absorption. In addition, there is increased microbial fermentation and production of short-chain fatty acids (SCFAs) in the large intestine, a typical phenomenon of fiber consumption (Chung et al., 2011).

Compared to traditional fibers, such as whole grains, bran or fruit fibers (Pérez-Alvarez, 2008), RS possesses the advantage of affecting the sensory properties of the final products less, which is very positive for consumer acceptability. Resistant starch provides many technological properties, such as better appearance, texture, and mouthfeel than conventional fibres (Charalampopoulos et al., 2002).

1.8.2 Proteins

1.8.2.1 Whey proteins

Whey protein is a mixture of globular proteins isolated from whey, the liquid material created as a by-product of cheese production resulting from the coagulation of milk. In cow milk, whey proteins represent the 20 % of total milk proteins, while the main fraction (80%) is caseins. Whey consists of a mixture of globular protein molecules comprising β-lactoglobulin (β-LG~50% wt/wt), α-lactalbumin (α-LA~20% wt/wt), immunoglobulins (IgG; ~10% wt/wt), and bovine serum albumin (BSA~6%, wt/wt) and other minor protein or peptide components (lactoferrin, lactoperoxidase, lysozyme) and growth factors (Walstra and Jenness, 1984).

The functional properties of whey proteins in foods include solubility, dispersibility, heat stability, network formation (gels and edible films), and surface activity (emulsions and foams) (Foegeding et al., 2002). β-Lactoglobulin (β-LG) is the most abundant protein in whey, and the functionality of a commercial whey form is mainly reflecting the functionality of this protein. β-LG is a globular, amphiphilic protein with the ability to adsorb at the water/oil interface. β-LG contributes in this way to dressing formation by lowering the interfacial tension, and also by stabilizing the film formation at the interphase (Damodaran, 1996). β-LG unfolds at the interphase and forms intermolecular associations, either by hydrophobic interactions or S–S bridges. α-lactalbumin (α-LA), represents the second most abundant protein in whey, which is also reported to exhibit emulsifying and stabilizing properties. Together with β-LG it participates in the S–S bridging and film formation at the

droplet interphase (Leman, 1999). Different whey products are categorized based on their protein concentration. The most typical commercial form of whey proteins are whey protein concentrates (WPCs) and whey protein isolates (WPIs). Protein concentration in WPCs may vary between 20 to 89 % and they also contain low levels of fat, and carbohydrates in the form of lactose. WPIs are processed to remove the fat and lactose and contain at least 90 % protein (Onwulata and Huth, 2009). For the production of WPC and WPI, whey may be subjected to several treatments to recover whey protein in a more concentrated form. The afore-mentioned membrane-based separation technologies include ultrafiltration (UF) to concentrate proteins, or diafiltration (DF) to remove most lactose, minerals and low molecular weight components (Foegeding et al., 2011).

1.8.2.1 Pea proteins

Pea proteins are obtained by extraction from the yellow pea seeds of Pisum sativum. Dry pea seeds contain approximately 20-25 % crude protein, with a well-balanced amino acid profile, especially a high content in lysine, which is considered a *limiting* amino-acid (Schneider and Lacampagne, 2000).

The majority of pea proteins are storage globulins, mainly legumin and vicilin/convicilin, which represent the 65-80 % of total buffered extractable protein (Koyoro and Powers, 1987). Salt-soluble globulins are composed of two fractions, namely legumin and vicilin/convicilin (Tzitzikas, Vincken, de Groot, Gruppen, and Visser, 2006). Legumin is classified as 11S globulin and is an hexameric oligomer with a molecular weight of 330–410 kDa constituted of six polypeptides of ≈60 kDa, with one basic L_β (19–22 kDa) and one acidic subunit L_α (38–40 kDa) linked via S–S (disulfide) bond. Vicilin is considered a 7S globulin, a trimeric protein with a molecular weight of ~170 kDa. It does not contain any cysteine residues, hence the formation of disulfide bonds is not feasible. Convicilin is a third major storage protein that has a subunit molecular weight of 71 kDa and in its native form of 290 kDa (Croy et al., 1980).

Vicilin exhibits superior emulsifying properties than legumin (Kimura et al., 2008). Pea proteins suggest a promising substitute for soybean proteins which represent the most consumed plant proteins globally due to the similarities of legumin and vicilin to soy glycinin and conglycinin (USDA, 2009). However, the use of pea

proteins in foodstuffs is limited due to partial loss of functionality caused by ph shifts and drying during wet fractionation, as well as limited information on their structural and functional properties (Adebiyi and Aluko, 2011).

1.8.3 Food bioactive ingredients exhibiting aroma and taste modifying properties

1.8.3.1 Saffron

Saffron, the world's most expensive spice is derived from the dried stigmas of the purple saffron crocus (*Crocus sativus* L.) that belongs to Iridaceae family and is the only plant whose product is sold by gram. The oldest evidence for utilization of saffron dates back nearly 3000 years to the Achaemenian Persian dynasty, while the Greek historian Polien at 2 B.C. reports that that saffron was used at levels of 1 g per day in the court kitchen. Saffron has a long history of use as a spice attributed to its remarkable odor, taste and odor. Nowadays, it has been greatly used in confectionary, alcoholic and non alcoholic drinks and dairy products (cheese, butter, icecream) for its taste and color improving properties. In the US it is permitted as a colorant for use in sausages, margarines and shortenings (Kafi et al., 2006).

Chemical analyses on saffron composition have revealed that it consists of approximately 10% moisture, 12% protein, 5% fat, 5% minerals, 5% crude fiber, and 63% sugars (starch, reducing sugars, pentosans, gums, pectin, and dextrins) (% w/w). Vitamins such as riboflavin and thiamine vitamins have also been identified in traces in saffron. The concentration of all chemical constituents though, presents great variation depending on growing conditions and region of origin (Melnyk et al., 2010).

Crocin (mono-glycosyl or di-glycosyl polyene esters), crocetin (a natural carotenoid dicarboxylic acid precursor of crocin), picrocrocin (monoterpene glycoside precursor of safranal and product of zeaxanthin degradation), and safranal are the major bioactive compounds of saffron (Fig. 1.11). These compounds contribute to the sensory attributes of saffron (color, taste, and aroma, respectively), as well as to the health-promoting properties (Melnyk et al., 2010).

The major compound responsible for its characteristically pleasant aroma is safranal, (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) (Tarantilis et al., 1998), a volatile oil which is obtained from the hydrolysis of picrocrocin (4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) by β-glucosidase, producing 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde

also known as HTCC. HTCC is later transformed to safranal by dehydration during the drying process. Therefore, safranal is not primarily present in fresh stigmas, which means that its concentration in saffron will vary depending on drying and storage conditions (Maggi et al., 2010).

The color of saffron is derived from the presence of various water soluble glicosilated esters of a dicarboxilic acid named crocetin (commonly known as crocins) which exhibit yellow and red hues, while the bitter taste of saffron is attributed mainly to picrocrocin and to a lesser extent to kaempferols (Licón et al., 2012a,b).

The world's saffron sectors have classified saffron by its aroma strength, flavour strength, and colouring strength using the ISO Normative 3632/TS (ISO, 2011). The norm establishes quality specifications for dried saffron obtained from the pistils of *Crocus sativus* L. flowers. It applies to saffron in the form of filaments, cut filaments or powder, which can be classified into three categories based on different physical and chemical parameters. The maximum allowed moisture and volatile matter in saffron threads is 12 % and 10 % in saffron powder and applies in all quality categories. Both the price of saffron and consumer acceptance are dictated by the coloring strength of its aqueous extract, defined as $E_{440\ nm}$. According to ISO 3632/TS requirements for commercial category I, coloring strength values should be higher of 190, whereas a minimum of 100 has been established as a minimum requirement in order to belong to category III (Maggi et al., 2010).

Beneficial health effects of saffron consumption in model animals include lower risk of diseases including metabolic disorders (gastric disorder), premenstrual syndrome, depression, insomnia and anxiety, cardiovascular disease, as well as many types of cancers (Melnyk et al., 2010; Bhandari et al., 2015).

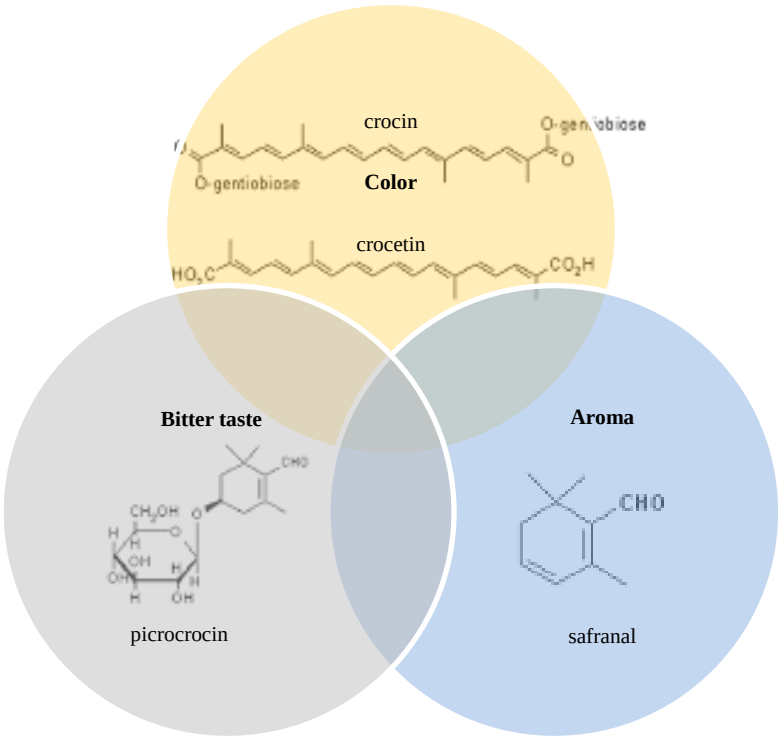


Figure 1.11 Chemical structures of crocin, crocetin, picrocrocin and safranal (Reprinted from: Melnyk et al., 2010).

1.8.3.2 Pomegranate juice

Pomegranate (*Punica granatum* L.) is one of the oldest fruits presenting health promoting effects. Its origin sets back in the Middle East, following a wide spread to Mediterranean, China and tropical/subtropical regions around the world. It is estimated that over 1000 cultivars of pomegranate exist, broadly classified into two varieties: ornamental and edible pomegranates. The edible one consists of two subcategories, namely sweet and sour types, according to the taste of the resulting juice (Li et al., 2015).

Worldwide interest has been observed regarding the whole pomegranate fruits and their products (juice, jelly, jam etc.) by both consumers and researchers because their consumption has been related to several health benefits, including prevention and treatment of cancer, cardiovascular disease, diabetes, Alzheimer's, arthritis and colitis (Li et al., 2015).

The edible part of the fruit (pomegranate arils), is rich in sugars, organic acids, vitamins, minerals and polyphenols (Tehranifar et al., 2010). Polyphenols are the major antioxidant and health functional factor found in pomegranate aril and juice.

The major polyphenol compounds are ellagitannin (punicalagin), gallic acid, ellagic acid, anthocyanins (especially the 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin) catechins, caffeic acid, and quercetin (Du et al., 1975).

Apart from aril polyphenols, tannins abundant in the pomegranate peel are also known to exhibit preventing properties against chronic diseases (Fischer et al., 2011). Pomegranate juice has higher contents of antioxidant compounds than the fresh fruit resulting in higher antioxidant capacity than squeezed arils. This is due to the migration of phenolic compounds from the rind during the pressing of fruits (Nuncio-Jáuregui et al., 2015). Therefore, whole-fruit squeezing tends to be superior to aril juicing methods in pomegranate processing (Turfan et al., 2011).

Pomegranate juice incorporation has been shown to enhance the taste and flavor attributes in products such as olive oil marinades, chicken meat (Topuz et al., 2014; Bazargani-Gilani et al., 2015). The aromatic profile of fresh pomegranate juice is attributed to the predominance of terpenes, while furans are only present in processed juices. The characteristic flavor of pomegranate juice is related to the presence of several compounds including esters, alcohols, and terpenes (Nuncio-Jáuregui et al., 2015).

1.9 Characterization methods of dispersions and emulsions

1.9.1 Particle size

Currently, a number of methods are available to provide information on droplets size profile in emulsions. Most commonly used techniques include laser scattering, ultrasonic attenuation spectroscopy, nuclear magnetic resonance and microscopy-image analysis. In the following paragraphs, the methods used in this thesis for estimation of droplets characteristics are discussed.

1.9.1.2 Light scattering techniques

Light scattering technologies used in the field of particle characterization include two main techniques: static light scattering (SLS, the measurement of scattering intensities due to light–particle interaction at various spatial locations) and dynamic light scattering (DLS, the measurement of scattering due to light–particle interaction as a function of time) (Xu, 2015).

- *Static Light Scattering (SLS)*

In SLS technique (also known as laser diffraction), a high intensity monochromatic light, usually a laser, passes through a dilute solution containing the particles. The laser beam is scattered by the particles in many directions and detectors are used to measure the scattering intensity at one or many angles. The scattering pattern (intensity of scattered light *vs* scattering angle) is related to the particle size profile of the dispersion, which can be analyzed using the Mie theory of light scattering to calculate the particle size distribution, assuming a volume equivalent sphere model. Mie theory applies when the particles are of similar or not significantly higher diameter than the wavelength of the beam. On the contrary, the Fraunhofer theory is used for larger particles.

Mie theory requires knowledge of the optical properties (refractive index and imaginary component) of both the sample being measured, along with the refractive index of the dispersant. Usually the optical properties of the dispersant are relatively easy to find from published data, and many modern instruments will have in-built databases that include common dispersants. For samples where the optical properties are not known, the user can either measure them or estimate them using an iterative approach based upon the goodness of fit between the modeled data and the actual data collected for the sample.

To prevent multiple scattering effects the emulsions must be highly diluted prior to analysis. Commercially available SLS devices allow the determination of particle diameters ranging from 0.1 up to 1000 μm. The software accompanying the devices may provide several diameters, such as $D_{(4,3)}$ (volume weighted mean diameter), $D_{(3,2)}$ (surface weighted mean diameter), $D_{(0.5)}$ (volume median diameter, for which 50% of the distribution is above and 50% is below), $D_{(0.1)}$ or $D_{(0.9)}$ (for which 10% or 90% of the volume distribution is below this value, respectively).

- *Dynamic Light Scattering*

Dynamic light scattering (DLS), initially referred to as Quasi-Elastic Light Scattering (QELS) or Photon Correlation Spectroscopy or (PCS), is the most popular and common technique that is used to determine the size and size distribution of macromolecules, polymers and submicron sized particles typically dissolved in suspensions (Merkus, 2009). Devices of the latest technology can provide measurement of particles with size smaller than 1 nm up to several microns which is difficult to achieve with other techniques (Malvern, 2008).

The ability of the method to estimate particle size is based on the Brownian motion – random thermal, translational, or rotational (diffusion) movement- of the particles in suspensions. When a monochromatic light beam (typically a laser), is shined onto a solution with particles in Brownian motion, it causes a Doppler Shift when the light hits the moving particle, changing the wavelength (typically red light at 633 nm or near-infrared at 830 nm for biomolecular applications) of the incoming light. In other words, due to the constant movement of the particles, the intensity of the scattered light changes temporarily with time. These time-dependant variations are related to the size of the particles, which can be detected and recorded as Intensity *vs* time profiles. The auto-correlator of the device generates afterwards a correlation function, which is an exponentially decaying time equation. The decay constant can be used to calculate the diffusion coefficient of the particle, which provides an estimation of the hydrodynamic radius (R_h) of the droplet using the Stokes–Einstein equation:

$$R_h = \frac{k\,T}{6\,\pi\,\eta\,D} \qquad \text{Eq. 1.6}$$

Where, k is the Boltzmann constant, T the absolute temperature, η the viscosity of the continuous phase and D is the diffusion coefficient (Goddeeris et al., 2006).

The main components of a DLS device are: a laser source accompanied by laser delivering optics, a sample holder, optics for collecting of scattering light, a detector and an auto-correlator. The monochromatic, vertically polarized, and coherent laser light emitted from a He-Ne laser is used as a light source. Light delivering optics consist of apertures and collimators that focus the beam in a small area of the sample. The appropriately diluted sample is placed in a cuvette (glass or polysterene) which is placed inside the sample holder. The scattered light collecting optics collect the light at a specific angle, usually placed at 90° to the source and consist of lenses and apertures. Commonly used detectors are photomultiplier tubes (PMT) and photodiodes (PD) (Schärtl, 2007).

The accuracy of particle size distribution measurement by DLS may be affected by several parameters including the concentration of the suspension, the composition of solvents, dust and other additives (e.g. ions or free radicals). Other parameters that the equipment software uses for calculations include the solvent viscosity, the refractive index and sample temperature. It should also be noted that aggregates or

agglomerates of smaller particles are usually identified or counted as one large particle with the DLS method (Schärtl, 2007).

1.9.1.3 Nuclear Magnetic Resonance (NMR)

Pulsed field gradient NMR (Pfg-NMR) can be used to measure droplet sizes in the range between 50 nm and 20 μm. Pfg-NMR is a non-invasive technique that has the advantage of requiring little sample preparation. NMR spectroscopy was initially developed for the assessment of water droplet distribution in food emulsions such as margarine or spreads. A modification of Pfg-NMR, the so-called low-field NMR, was later introduced to expand droplet size measurements in o/w emulsions (Goudappel et al., 2001).

The measurement principle for water and oil droplet size profiles is based on the diffusion of the liquids (Duynhoven et al., 2002). In o/w emulsions two types of diffusion can be observed, which contribute to the NMR measurements. The first is the self-diffusion of individual molecules inside the oil droplets and the second one, the diffusion of oil droplets.

Oil molecules move (diffuse) inside the oil droplet during the self-diffusion process, and their movement is restricted when they reach the droplet interface (wall). The diffusion path of oil molecules is measured in time and the size of the droplet can be obtained by using the diffusion decay curves. For the calculation of the size distribution profile, two equal field-gradient pulses are applied within a standard spin-echo pulse sequence. The molecular diffusion of the oil molecules causes the reduction of the echo intensity until the molecules reach the droplet wall (Shahidi and Zhong, 2005).

During the second process a systematic error concerning the droplet size profile derived from calculations by means of the molecule diffusion is introduced due to the movement of small oil droplets within the continuous phase. This means that the total NMR magnetization intensity decay signal is expressed as the product of droplet and molecule decay (Goudappel et al., 2001).

From a practical point of view the technique is very user-friendly and its main advantage is that unlike conventional laser scattering techniques it requires minimum sample preparation with no dilution and the possibility of full automatic measurement and data processing. In addition, it lacks of typical problems associated with common available techniques (droplet clustering, discrimination between oil droplets from

particles of different origin). However, the Pfg-NMR has not been established as a standard method for o/w emulsions, owed to high magnetic field strengths required to obtain sufficient chemical shift resolution, which significantly elevates the cost of the equipment (Goudappel et al., 2001; Kiokas et al., 2004).

1.9.2 Rheology

Rheology is the study of the deformation and flow of the materials, a term invented by Bingham and accepted by the American Society of Rheology in 1929. The materials under investigation can range from low-viscosity fluids to semisolids and gels to hard, solid-like food products.

The importance of the flow properties of emulsions lays on both technical and consumer acceptance issues. Considering technical matters, numerous industrial processes and handling (pumping, filling, mixing, and packing) of emulsified products require information regarding the flow properties to estimate pump transfer rates, energy consumption and mixing efficiency. Consumers' buying intention is also driven by several rheological properties, the most important of them being creaminess, consistency, body as well as pourability easiness (Barnes, 1994).

In total, rheology characterization is very important during the development of an emulsified food product, because it determines its stability after processing (McClements, 2005). The investigation of most emulsion destabilization phenomena like creaming and sedimentation, flocculation, Ostwald ripening or phase inversion can be elaborated by using various rheological techniques. The bulk rheology of emulsified systems can be assessed using steady state (shear stress as a function of shear rate), constant stress or oscillatory methods (Tadros, 1994). Most commonly used rheology techniques in food emulsions or dispersion include flow and small amplitude oscillatory tests.

1.9.2.1 Flow measurements

Depending on the operation principle of the rheometer used (stress-controlled or strain-contolled), during a flow test the fluid sample is being subjected to different values of shear rates (γ), while the resulting values of shear stress (τ) are measured, or inversely. Stress-controlled rheometers provide an advantage, since they can operate in both controlled stress (torque) as well as controlled strain (rotational speed) mode (Brookfield, 2013). The resulting flow curves can be used to characterize the

dispersion regarding Newtonian (no shear dependency of viscosity) or non-Newtonian behavior (pseudoplastic or dilatant). Usually, the flow curves begin with increasing shear rates (or stress), although it is also possible to conduct experiments starting at high shear rates which gradually decrease. Performing, both upward and downward measurements can provide information on the time dependant flow behavior of the sample derived from the surface area in between the two flows (Brummer, 2006).

For the steady shear measurements, the Power law, the Carreau, Herschel–Bulkley or the Casson model has been widely used to describe the flow properties of mayonnaise and salad dressings (Batista et al., 2006).

1.9.2.2 Small amplitude oscillatory measurements

The viscoelastic properties of emulsions can be investigated using oscillatory (dynamic) measurements. Small amplitude oscillatory measurements are very useful and popular methods for several reasons including:

- Non-destructive character allowing investigation of the molecular strucrure of a sample.
- The simultaneous estimation of two quantities (G' and G") provides a check on experimental errors.
- In most cases it is faster compared to others like creep or relaxation tests.

Dynamic oscillatory measurements are often employed to provide information about the soft-solid rigidity (viscoelastic behavior) and yield stress (gel strength) of food stuffs resulting from, for example, flocculation and interaction of dispersed particles or droplets, or cross-linking and entanglement of dissolved polymers. It is important to emphasize that the applied strains (or stresses) used in these experiments are very small, in order to assure that the theory behind the test is applicable. This is achieved by performing preliminary tests to retrieve the linear viscoelastic region, meaning the range within which the stress is proportional to the applied strain (Gunasekaran and Mehmet, 2000).

In an oscillatory shear experiment a sample, which is subjected to a sinusoidal strain with amplitude γ_o at an angular frequency ω (rad/s) will respond with a gradual approach to a steady sinusoidal stress σ:

$$\gamma(t) = \gamma_o \sin(\omega t) \qquad \text{Eq. 1.7}$$

$$\sigma(t) = \gamma_o (G'(\omega) \sin(\omega t) + G''(\omega) \cos(\omega t)) \quad \text{Eq. 1.8}$$

From this type of experiment the storage modulus G′, the loss modulus G″, the dynamic viscosity $\eta' = G''/\omega$ and loss tangent, $\tan\delta = G''/G'$, (measure of damping) can be determined.

The storage modulus (G') expresses the capacity of a material to store or recover elastic energy during a deformation cycle and describes the solid-like (elastic) characteristic of the viscoelastic system. This means that after the deformation load is removed, this energy is completely available, now acting as a reformation process to compensate the deformation. The loss modulus (G") expresses the energy lost as viscous dissipation, describing its liquid-like (viscous) behavior. The viscous dissipation refers to generated heat due to frictional forces acting between molecules, particles or greater domain of the superstructure. When G' values obtained are much higher than G" ones, the deformation will be essentially elastic and the material will behave more as a solid. The opposite observation means that the energy used to deform a material is viscously dissipated and the material behaves like a liquid. The loss tangent (or damping factor, tanδ), represents the ratio of the energy lost to that stored per deformation cycle (Rao, 2007).

1.9.3 Emulsion physical stability

The simplest method to determine emulsion stability is visual observation for the calculation of the separation percentage caused by serum formation in the bottom. However, even optically homogenous samples that do not exhibit gravitational separation can still destabilize by aggregation phenomena. Nowadays, multiple light scattering (MLS) is the most widely used technique to monitor the dispersion state of a product, identifying and quantifying destabilization phenomena. It works on concentrated dispersions (up to 40 % total solids) without dilution.

The multiple light scattering device allows the optical characterization of any type of dispersion by using a mobile reading head composed of a transmitting NIR diode (λ=850 nm) and two synchronous detectors analyzing the transmitted (T) and backscattered (BS) light with acquisitions every 40 μm. Emulsion or dispersion samples are inserted in a borosilicate tube and scanned from bottom to 80 mm tube height in order to obtain back scattering and transmission intensity *vs.* tube height

profiles. When light is sent through the sample, it is backscattered by the particles / droplets. The backscattering or transmission intensity is directly proportional to the size and volume fraction of the dispersed phase. Therefore, local changes in concentration (creaming and sedimentation) and global changes in size (flocculation, coalescence) can be detected and monitored (Mengual et al., 1999a,b).

1.9.4 Surface tension

Surface or interfacial tension measurements can be used as a mean for emulsifier screening regarding their ability to stabilize emulsions or foams. The ability to reduce surface tension is one of the requirements for an emulsifier, along with the ability to stabilize an emulsion. The du Noüy ring method is a technique by which the surface tension of a liquid can be measured. In this method, a platinum wired ring is slowly lifted from the surface of a liquid. The force, F, required to raise the ring from the liquid's surface is measured and related to the liquid's surface tension, γ:

$$F = 4 \cdot \pi \cdot R \cdot \gamma \cdot \beta \qquad \text{Eq. 1.9}$$

Where, R is the radius of the ring, and β is a correction factor that depends on the dimensions and the density of the ring (McClements, 2005).

1.9.5 Optical Microscopy

Optical microscopy is a technique often used to observe the structure and phenomena taking part in emulsions. The four basic elements of a microscope are an illumination source, mechanical stage for samples, optical system and detector. The illumination source of visual light interacts with the sample and the optical system with lenses collects and focuses the light into the eye and/or camera. Images obtained can be also used for droplet size estimations by using image analysis software.

1.9.6 Emulsion oxidation

The quality of emulsified products like mayonnaise or dressings can be negatively affected through auto-oxidation of the unsaturated and polyunsaturated fats in the oil (Depree and Savage, 2001). The available methods to monitor lipid oxidation in foods can be classified into five groups based on what they measure: the absorption of oxygen, the loss of initial substrates, the formation of free radicals and

the formation of primary and secondary oxidation products. Primary and secondary oxidation product monitoring is very important for food emulsions because it is related with aroma and taste deterioration caused by the formation of unpleasant volatile compounds (Shahidi and Zhong, 2005).

1.9.6.1 Conjugated diene hydroperoxides

The measurement of conjugated dienes is a simple, fast method for monitoring the formation of primary oxidation products in fats and oils, as it requires small amounts of samples and no chemical reagents are needed. Despite its lower specificity and sensitivity, it has been found to correlate well with other peroxide value methods.

The principle of the method is based on the spectrophotometric detection of the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced, due to the rearrangement of the double bonds. Almost immediately after peroxides are formed, the non-conjugated double bonds that are present in natural unsaturated lipids are converted to conjugated double bonds. Conjugated double bonds (conjugated dienes) absorb ultraviolet light strongly at around 232 nm (Shahidi and Zhong, 2005).

1.9.6.1 p-Anisidine value (p-Av)

The p-Av is a reliable indicator of oxidative rancidity in fats and oils and fatty foods, exhibiting good correlation with the concentration of total volatile substances, as well as flavor scores has been found.

The p-anisidine value (p-Av) method measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides. The method is based on the color formation from the reaction with p-methoxyaniline (anisidine). Under acidic conditions p-anisidine reaction with the aldehydic compounds results in formation of yellowish products that absorb at 350 nm. The sensitivity of the method is higher regarding unsaturated rather than saturated aldehydes, due to the strongest absorbance caused by the colored products from unsaturated aldehydes at 350 nm. The color is quantified and converted to p-Av. The p-Av is defined as the absorbance of a solution resulting from the reaction of 1 g of fat in isooctane solution (100 ml) with p-anisidine (Shahidi and Zhong, 2005).

1.9.7 Sensory evaluation

The field of sensory evaluation has been developed rapidly during the last 5 decades owed to the simultaneous growth of the food processing industries. Sensory evaluation has been defined as *"a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing"* (Stone and Sidel, 1992). It is considered a valuable tool for the food industry as well as for the scientific community for the development of new products, since it can reduce uncertainty and risks in decision making, ensures cost-efficient delivery of new products with high consumer acceptability. Moreover, instruments are unable to measure the likeness of a product, while sometimes their detection thresholds of some compounds (e.g. odorants) are higher that those of human observers.

Sensory evaluation includes three testing categories depending of the goal and the criteria for the selection of assessors:

- Discrimination tests, identifying differences among products.
- Descriptive, quantifying differences among products.
- Affective or consumer or hedonic tests, quantifying product likeness or preference (Lawless and Heymann, 2010).

1.9.7.1 Quantitative Descriptive Analysis

Descriptive sensory tests involve the simultaneous detection (discrimination) and description of the qualitative and quantitative sensory attributes of a consumer product by trained panels of judges (Meilgaard et al., 1991).

Quantitative Descriptive Analysis (QDA®) was developed during the 1970s in response to the need to deal with poor statistical treatment on data performed by other descriptive tests. The method uses statistical techniques for the determination of appropriate panelists, descriptors and procedures used while evaluating a specific product. The objective of descriptive analysis is to provide quantitative descriptions of products based on the perceptions of a group of trained group of up to 15 panelists (Stone and Sidel, 1992).

Initial screening of participants involves demographic, background and dietary criteria retrieved by questionnaires attempting to identify individuals who are not users of the product to be examined or dislike it. This is because it has been shown

that frequent consumers of the product are more sensitive to product differences and thus more discriminating (Muray et al., 2001). Within the screening process individuals are required to participate in a series of discrimination tests to assess subject sensitivity to product differences. Suitable discrimination techniques, such as the duo-trio test or the triangle test provide objective results on the ability of subjects to perceive product differences. Only persons who are able to identify more than the 2/3 of samples within the discrimination tests are qualified for participation in the final QDA panel (Meilgaard et al., 1991). Taking into account that approximately 30% of the initial participants fail to the tests, the number of volunteers recruited for screening should be 1.5 times the number of those required for the final QDA (Stone and Sidel, 1995).

Once the appropriateness of the panelists in terms of discrimination has been ensured, panel members deliberate to develop a common descriptor vocabulary and are trained in several sessions with a total duration of approximately 10-15 hours to understand the meaning of the attributes. The training of QD panel requires the use of products and references to stimulate the generation of terminology, especially in the case that panelists are confused or descriptors cause disagreements (Stone and Sidel, 2004). Panelists quantitate the intensity based on their own approach using a 15 cm scale divided in 9 intervals of ascending intensity. Measures are subjected afterwards to statistical analysis by means of analysis of variance (ANOVA), Principal Components Analysis (PCA) or similar methods (Meilgaard et al., 2001).

1.9.7.2 Consumer test

Affective tests are used to assess consumer likeness or preference to products and depending on the purpose they can be subdivided into qualitative or quantitative. The simplest (qualitative) approach to assess consumer acceptability of a product would be to offer it to people along with other ones and see if the majority of individuals demonstrate a clear preference for that. However, this type of test only provides basic knowledge concerning product likeness or preference, whereas it offers no detailed information to one major question: *"How much do the consumers like the product"?* To address the issue of likeness magnitude the method of hedonic scale was introduced in the 50's. The method is based on a 9-point likeness scale, in which each point represents an equal psychological change/category that can be used as a number to conduct statistical analysis. The scale is balanced including equal numbers

of negative and positive categories with intervals of equal size, ranging from 1 (dislike extremely) up to 9 (like extremely) with a centered neutral point.

Whichever type of test is used, care needs to be taken to ensure the sample of testers is representative of the target population expected to buy the product. The main disadvantage of the method would be the difficulty to retrieve a representative sample of consumers (Lawless and Heymann, 2010). From a statistical point, hedonic tests should have a minimum of 30 consumers (Stone and Sidel, 2004).

CHAPTER 2

ULTRASONIC ENERGY INPUT INFLUENCE ON THE PROPERTIES OF WHEY PROTEIN EMULSIONS CONTAINING COMMON STABILIZERS AT PH 7.

2.1 Introduction

High intensity ultrasonication (HIUS) with a frequency range between 16 and 100 kHz, and 10 and 1000 W/cm^2 of power, is a technology that has various applications on the food industry. It can be used in processes such as cooking, freezing, drying, degassing, filtration and emulsification in order to assist or even replace conventional methods (Mason et al., 1996; Chemat et al., 2011).

Ultrasound devices can generate very stable emulsions that require little surfactant (Povey and Mason, 1998; Abismaıl et al., 1999), or even produce emulsions with small droplet sizes directly from separate oil and water phases (McClements, 2008). Ultrasound generators are capable of producing emulsions in the sub-micron range or form translucent nano-emulsions with average droplet sizes up to 40 nm (Jafari et al., 2007; Leong et al., 2009; Nerina et al., 2010) and are more efficient in producing smaller droplets in comparison to rotor-stator systems with droplet size as small as 0.2μm (Jafari et al., 2008). However, a comparison of the average droplet diameter versus power consumption using different emulsifying machines showed that the smallest droplet diameters were obtained when using the high pressure homogenizers (Tadros, 2009). Micro-fluidization has been found to be more power efficient than ultrasound, but it is considered less practicable with respect to production cost as well as equipment contamination (Abismail et al., 1999).

Considering that this technology is increasingly being up-scaled as its use is both time and power saving, thus making it a cost-effective emulsion formation technique (Behrend et al., 2001; Patist and Bates, 2008; Vilkhu eta l., 2008) the interest in its improvement is high.

On the other hand, emulsifiers use in food industry is shifted towards food-grade ones, among them to proteins from different sources. Whey protein concentrates (WPCs) are widely used in food industry because they are considered high functional and nutritional ingredients. Their functionality is related to their protein content, mainly β-lactoglobulin (β-lg) and α-lactalbumin (α-la) (Tosi et al., 2007).

When HIUS emulsification is used, whey proteins can result in emulsions of different particle size (Gordon and Pilosof, 2010) or modify the viscosity of the continuous phase (Zisu et al., 2010). However, a better control of the process should be achieved as the effects of high-intensity ultrasound on the structural and functional

properties of both food proteins and/or other hydrocolloids used as stabilizers in the mix need to be further studied.

Hydrocolloids commonly used in the production of emulsions when low fat food products such as light mayonnaise are produced can be xanthan, locust bean and guar gum (Dickinson, 2009; Bortnowska et al., 2006). Xanthan gum is a poly-anionic polysaccharide mainly considered a non-gelling agent that presents weak-gel shear-thinning properties and it is used in order to control the viscosity. It interacts with whey proteins and the nature of those interactions depends on proteins' iso-electric point (Ip). Thus, above Ip, as in this study, an electrostatic repulsion of protein-polysaccharide occurs because both components are negatively charged. Apart from xanthan, the presence of locust bean gum is reported to be detrimental to protein-protein or protein-polysaccharide interactions for the development of protein gels (Goh et al., 2009). A range of textures from soft to rigid can be formed by changing gel condition formation based on whey protein-locust bean gum interactions (Rocha et al., 2009; van den Berg et al., 2009) that may also find applications in emulsions/gel-like products. Furthermore, xanthan or locust bean gum successfully substitute starch in low fat emulsions (Dolz et al., 2007). On the other hand guar gum can change significantly the viscosity of emulsion containing whey proteins inducing their shear-thinning behaviour (Erçelebi and Ibanoğlu, 2009) or can reduce coalescence of oil droplets in heated emulsions (Ye and Singh, 2006). However, due to its lately high price locust bean gum is being routed as guar gum credible replacer (Byrne, 2012).

Stability is related to both droplet size and viscosity change due to ultrasonication. The knowledge regarding the impact of HIUS application on both droplet size and stability using different polysaccharide/protein blends should increase.

The main objective of this study was the investigation of HIUS effect on WPC o/w model-emulsions production at pH 7, where proteins have better emulsifying properties , hence smaller droplets are formed compared to a pH near their isolectric point ($4 < pH < 6$) (Charoen et al., 2011). A formula containing reduced extra virgin olive oil content was selected for nutritional reasons and health benefits related to its composition in combination with the fact that no oxidation or hydrolysis effects are observed when ultrasounds (20kHz, 120s) are applied (Jiménez et al., 2007).

Additionally, in comparison to other edible oils that are primarily used in salad dressings such as corn and sunflower oil, olive oil presented the highest absence of off-flavoring after sonication which is related to the composition of the unsaturated fatty acids (Chemat et al., 2004).

2.2 Materials and methods

2.2.1 Materials

Whey protein concentrate (WPC) Lacprodan 80 was kindly provided by Arla (Arla Foods Ingredients, Amba-Denmark). The composition of WPC powder in protein as stated by the manufacturer was protein 78±2%wt, fat maximum amount 8 %wt, ash 2.74 %wt and lactose 7±2 %wt. Quantification of this whey protein by means of Reversed Phase-HPLC can be found in Panaras et al. (2011). Xanthan gum (XG), guar gum (GG) and locust bean gum (LBG) were obtained from Sigma (St. Louis, MO, USA). Extra virgin olive oil Altis (Elais Unilever, Athens, Greece) was purchased from a local store. Phosphate buffer solution powder (pH 7.0 +/- 0.2) and sodium azide were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland).

2.2.2 Emulsion preparation

Whey protein phosphate buffer stock solution 20 %wt was prepared by agitation with a magnetic stirrer for 60 min. Solutions were left overnight at 5 ^{o}C to ensure complete hydration. A few drops of sodium azide 0.02 %wt were added to the whey solution as an antibacterial agent to prevent from spoiling during storage. Xanthan gum solution 1 %wt was prepared by hot stirring in a water bath at 90 ^{o}C for 90 min. Locust bean gum solutions 1 %wt were prepared under heating and agitation at 60 ^{o}C for 60 min. Guar gum solutions 1%wt were also prepared with agitation at ambient temperature for 60 min. Coarse emulsions of total 100g (20 %wt olive oil, 3 %wt WPC) and gums (0.1-0.5 %wt) were prepared in two stages. First, the WPC stock solution and olive oil were mixed for 4 min at 6.500 rpm using an Ultraturrax T25 device (IKA Werke, Staufen, Germany). Afterwards, appropriate weights of gum solutions were added and the mixing continued for another 4 min at the same speed. The final emulsions (100 mL) were prepared in a glass beaker (60 mm internal diameter) covered by ice to prevent the temperature rise above 50 ^{o}C using an ultrasound device model Sonopuls 3200, equipped with a 13 mm diameter VS 70T probe (Bandelin Gmbh &Co, Berlin, Germany) operating at a frequency of 20 kHz

and varying amplitudes and times (method A: single-stage and method B: two-stage). The probe was immersed 1 cm below the surface of the emulsion. The sonication device operates by controlling amplitude (100 % amplitude corresponding to 170 μm_{ss} for the specific probe used) or power (150 W maximum nominal power). It also has the ability to display or monitor the energy release (kJ) in the sample during sonication. In method A, 70 % amplitude of sonication for 2 min was used, while in method B 70 % amplitude for 3 min followed by 90 % amplitude of sonication for 1 min (4 min in total).

In our case the ultrasonication time was limited to 4 minutes after preliminary experiments, considering also that the energy applied to the system and temperature rise were high. Furthermore, a total sonication time of 5 minutes at a frequency of 20-24 KHz was found to produce optimum results in a 15 % o/w emulsion, when the dispersed phase was flaxseed oil (Kentish et al., 2008). Another appoach is the reduction of amplitude (e.g. 20 %) and significant increase in sonication time (e.g. 20 min), operating at 20 kHz, applied in protein solutions (Arzeni et al., 2012). However, sonication time depends on equipment geometry and sample volume used (Gogate et al., 2003; Leong et al., 2009) and comparisons are difficult to be made.

The pH of the final emulsions was adjusted to 7.0 with a few drops of HCl 1M. Experimental data of energy release and temperature rise in the samples during emulsification are given in Table 2.1.

Table 2.1 Experimental parameters of ultrasonication according to method applied.

Gum content (%wt)	**Method A**		**Method B**	
	Energy (kJ)	**Temperature (^oC)**	**Energy (kJ)**	**Temperature (^oC)**
0.1	11.84 ±0.14	45.10 ±1.95	np*	np*
0.25	11.68 ± 0.52	43.05 ±1.99	25.66 ±0.53	31.42 ±0.71
0.5	11.52 ±0.34	45.80 ±1.36	25.77 ±0.35	33.05 ±2.37

*Emulsions not further prepared with method B due to very high instability as seen in method A.

2.2.3 Particle size estimation

Oil droplet size measurements were performed on a Bruker Minispec NMR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with a Bruker ND 2176 probe at 20^{o}C. All samples were previously stored at 5 ^{o}C and measured about 24h after preparation. Measurements were carried out in duplicate and results are demonstrated as the median diameter D_{50} and cumulative diameters $D_{97.5}$ and $D_{2.5}$, representing the 50, 97.5 and 2.5% of droplets being smaller than each value respectively.

2.2.4 Apparent viscosity

Viscosity flow curves were obtained using a controlled stress SR-5 rheometer (Universal Stress Rheometer/Rheometrics Scientific, Inc., NJ) with plate-plate geometry and 0.5 mm gap. The diameter of the upper plate was 20 mm. The temperature was constant (25 ±0.1^{o}C) by circulating water from a constant temperature circulator. Steady-state flow curves were obtained at shear rates between 0.01 and 100 s^{-1} approximately to avoid slippage in the case of final emulsions prepared with both sonication methods and up to 1000 s^{-1} in the case of 1 %wt gum solutions that had been subjected to various sonication treatments (70% amplitude for 1, 2 (method A) or 3 min and as in method B). Viscosity measurements were performed only in emulsions containing 0.5 %wt of various gums that were the most stable. In the case of gum solutions, a higher concentration of 1 %wt instead of 0.5 %wt was selected in order to better fit the precision of the rheometer geometry, since it is more accurate at relatively high viscosity values. The viscosity of 1 %wt gum solutions was calculated at a low shear rate (10 s^{-1}), mean values of at least two differently prepared formulations are reported and viscosity flow curves are demonstrated.

2.2.5 Light microscopy

Emulsion structure was observed in freshly prepared samples with a conventional optical microscope (Kruss Optronik, Germany) with a 10 x magnification and several micrographs were obtained per sample from the center area of each slide. The emulsions were not subjected to dilution in order not to disturb their initial structure. The micrographs were recorded using a camera (SONY, Hyper HAD, CCD-Iris) connected to a computer.

2.2.6 Emulsion stability

Emulsion stability was evaluated with a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France) during 10-day storage at 5 ^{o}C. Emulsion samples of about 6 ml were put in a glass tube and scanned from bottom to 80 mm tube height on a daily basis in order to obtain back scattering and transmission profiles that allow the calculation of instability due to creaming, sedimentation, coalescence as well as other phenomena. The procedure was replicated at least three times. Emulsion stability due to clarification at the bottom of the tube is expressed as % Serum. Briefly, the height of serum phase separation was defined as the height at 50 % loss of the initial BS ($H_{50\%}$) and it was expressed as a percentage of the total height of emulsion measured with a ruler (H_T) (Jourdain et al., 2008; Zinoviadou et al., 2012), elsewhere reported as creaming index:

$$\% \text{ Serum} = 100 \text{ x } (H_{50\%} / H_{T)} \quad \text{Eq. 2.1}$$

The initial BS intensity (t = 0) is a value related to the size and density of particles in emulsions; BS enhances as the increase of the number of particles, and decreases as a function of the size of individual droplets and/or presence of flocs (Palazolo et al. 2004, 2005). It has to be noted though that according to Mie's law, the BS intensity decreases when the droplet size increases only for particles with diameter larger than 0.5 μm (Mengual et al., 1999a,b; Buron et al., 2004; Pizzino et al., 2009).

Emulsion stability regarding coalescence-flocculation phenomena was evaluated from BS values at the middle of the sample and the variation of back scattering (dBS) with time during cold storage was calculated according to Eq. (2.2) (Huck-Iriart et al., 2011):

$$dBS = BS_0 - BS_{10} \quad \text{Eq. 2.2}$$

Where, dBS: back scattering change of cold stored samples (%), BS_0, back scattering of freshly prepared emulsions (%) and BS_{10}, back scattering of emulsions after 10 days of cold storage (%).

2.2.7 Thermal analysis of XG emulsions

Thermographs of freshly made emulsions samples (15±1mg in a hermetically sealed aluminum pan) were obtained with a DSC Q100 (TA Instruments, DE, USA). The

DSC was calibrated using mercury, distilled water and indium. An empty pan was used as reference. Samples were cooled from 40 to -40 ^{o}C and heated to 40 ^{o}C, with a rate 5 ^{o}C/min. The cooling/heating cycle was repeated 2 times within the experiment in order to investigate the influence of continuous and dispersed phase crystallization on the stability of emulsions. Enthalpy changes (ΔH), as well as onset (T_{on}) and off-set (T_{off}) temperatures of crystallization were determined from the measured peak areas in the DSC thermographs of the samples containing 0.5 %wt XG during the 3 heat-cool cycles with the TA Universal Analysis 2000, v.4.2E software.

2.2.8 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and F-test was applied in order to compare the mean values of selected properties (% Serum on day 10 of cold storage, dBS, D_{50}, and apparent viscosity of gum solutions at shear rate 10 s^{-1}) at 95 % level of confidence.

2.3 Results and discussion

2.3.1 Emulsion droplet size

During the emulsification process two different phenomena, droplet disruption and re-coalescence occur at the same time, the kinetics of each one individually can affect the final droplet size of emulsions (Walstra, 2003; Jafari et al., 2007). As a consequence, concerning ultrasonic emulsification, an optimum energy input should be established to avoid over-processing regarding droplet size increase (Kentish et al., 2008).

Particle size parameters of the emulsions prepared with both methods are shown in Table 2.2. Formulations prepared with low energy input (method A) exhibited an oil droplet median diameter D_{50} higher than 1 μm in all cases (1.01-1.32 μm).

Method B was able to further decrease the average diameter in all formulations, thus producing finer droplets with a D_{50} between 0.615-0.876 μm. Also, a drastic reduction of $D_{97.5}$ was observed in all formulations containing the same type of stabilizer at the same concentration. As a result the $D_{97.5}$ values ranged between 2.64-4.23 μm and 1.87 - 2.94 μm for method A and B accordingly. Thus, method B was more effective upon decreasing the size of bigger droplets resulting in narrower droplet size distributions.

This pronounced efficacy of method B was strongly related not only to increased duration (4 min), but also because a second stage at higher pressure amplitude (90% for 1 min) was involved. An increase in the applied pressure amplitude led to intensive cavitation due to an increased number of bubbles generated by pronounced breaking of liquid threads. It is also known that an increase in amplitude and duration increase the energy release in the system that leads to subsequent temperature rise. These phenomena facilitate the dispersion of each phase into another by means of interfacial tension, viscosity and Laplace pressure decrease. Nevertheless, even though the temperature rise was grater in method A (~43-45 ^{o}C) and viscosity decrease of emulsions and gum solutions lower (Fig. 2.1, 2.2 and 2.3), still the total energy applied on the system as a consequence of limited duration and amplitude was not enough to create fine dispersions as in method B. Thereby smaller droplets were produced, while at lower amplitude a wider droplet distribution was observed. Same observations were noticed by (Canselier et al., 2002; McClements, 2005; Seekkuarachchi et al., 2006; Gaikwad and Pandit, 2008).

It has been found that the addition of gum in the emulsion during homogenization enhanced the adsorption of the protein molecules onto the droplet surface due to increased protein/surface contact and led to reduced droplet size (Ye et al., 2004), but this is only merely in accordance with our findings. As shown in Table 2.2 the D_{50} diameters of emulsions prepared with method B decrease upon stabilizer concentration increase. The opposite phenomenon was observed in the case of method A, even though the differences of LBG emulsions were less evident ($p>0.05$). This controversy could be mainly attributed to the higher viscosities of the continuous phase during emulsification owned to decreased fragmentation of the hydrocolloids when the low energy input method was used. Tzoumaki et al. (2011) have also observed an increase in the average droplet size of ultrasonically prepared WPI emulsions when chitin nanocrystals' concentration was increased above 0.5 % attributed to high viscosity of the continuous phase. Concerning the stabilizer type, LBG was more efficient in decreasing the droplet size of emulsions prepared by both methods, while XG formulations contained bigger droplets especially those prepared at low energy input.

In general, sonication energy input increase was more efficient in reducing the droplet size than the stabilizers used, hence samples prepared with method B had lower droplet size and improved stability, regardless of composition. These findings

are also in accordance to a recent study (Delmas et al., 2011), in which it was found that the sonication power was significant in reducing the oil droplet size, whereas the continuous phase viscosity, surfactant characteristics and volume fraction had much lower influence on the final droplet size. Viscosity matters will be analysed in more details in next section.

Table 2.2 Particle size parameters of emulsions containing 0.25 and 0.5 %wt stabilizers prepared by two ultrasonication methods.

	Method A			Method B		
	D_{50} (µm)	**$D_{97.5}$ (µm)**	**$D_{2.5}$ (µm)**	**D_{50} (µm)**	**$D_{97.5}$ (µm)**	**$D_{2.5}$ (µm)**
XG 0.25%	1.11^{f}±0.15	4.23±0.28	0.28±0.04	0.83^{cd}±0.04	2.94±0.36	0.25±0.03
XG 0.5%	1.32^{g}±0.1	2.64±0.16	0.71±0.13	0.79^{bc}±0.08	2.53±0.19	0.27±0.05
GG 0.25%	1.09^{ef}±0.06	3.57±0.42	0.38±0.03	0.84^{cd}±0.09	2.24±0.03	0.37±0.10
GG 0.5%	1.33^{g}±0.14	4.21±0.11	0.21±0.06	0.77^{b}±0.07	1.87±0.14	0.56±0.03
LBG 0.25%	1.02^{e}±0.14	3.39±0.24	0.34±0.04	0.88^{d}±0.05	2.66±0.20	0.23±0.05
LBG 0.5%	1.08^{e}±0.11	3.43±0.19	0.39±0.08	0.61^{a}±0.05	2.22±0.12	0.38±0.04

Results presented as average out of two measurements of two repeated formulations.

Mean values of the D_{50} followed by the same letters are not significantly different ($p>0.05$).

2.3.2 Apparent viscosity of 0.5%wt emulsions and 1%wt gum solutions

Viscosity as a function of shear rate of emulsions containing 0.5 %wt XG, GG and LBG prepared with both sonication methods is presented in Fig. 2.1. The flow curves of all formulations exhibited a typical shear-thinning behavior over the shear rate tested. XG samples presented higher viscosity values compared to emulsions containing galactomannans (XG>>LBG≥GG).

Samples that underwent an elongated and more intensive sonication (method B) presented lower viscosity values, thus emulsification method affected the flow behavior of emulsions. Viscosity values followed the same order accordingly to the hydrocolloids used, regardless of the sonication energy input. Xanthan produced the thickest samples, i.e. higher viscosity values that were still greater than the ones of samples containing LBG or GG after extended sonication.

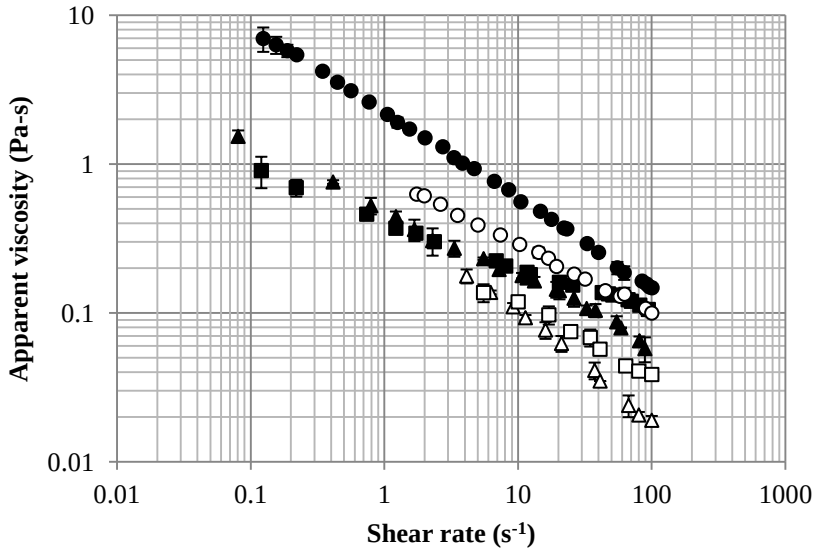


Figure 2.1 Apparent viscosity of emulsions containing 0.5 %wt stabilizers prepared with method A; (●) XG, (▲) GG and (■) LBG and prepared with method B; (○) XG, (Δ) GG and (□) LBG.

Generally, the emulsion viscosity is influenced by the continuous phase viscosity, interfacial film viscosity and droplet size. Concerning droplet size and viscosity Taylor equation describes their connection as follows:

$$r \sim \gamma / (\eta_c \times \gamma') \qquad \text{Eq. 2.3}$$

Where, r is the droplet radius, γ is the interfacial tension, η_c is the continuous phase viscosity and γ' is the shear rate.

In their original form, the galactomannans used have different molecular weight and viscosities (LBG>GG). Furthermore, they do not interact directly at the droplet interface, because they are neither charged macromolecules nor hydrophobic substances that could create interfacial films around oil droplets. On the other hand, xanthan gum, is a poly-anionic hetero-polysaccharide exhibiting elastic behavior. Its molecular weight and viscosity is very high, comparable to those of galacatomannans used. Thus, Taylor equation may be not applicable in these type of emulsions, which are highly concentrated, so gums' viscosity after sonication is hardly related to droplet size.

A significant reduction of gum viscosity due to depolymerization by increasing sonication energy input was also found in this study. Regarding viscosity reduction it is known that sonication results in pressure fluctuations, which propagate through the material resulting in the formation of microscopic bubbles that collapse within a few milliseconds (cavitation). As a result, extreme effects in the vicinity of these bubbles, where biopolymers exist, can occur. These include heating, high pressure and high shear rates (Tiwari et al., 2010; Wang et al., 2008; Xu and Pao; 2009) and can lead to cleavage of polysaccharides mainly due to glycosidic linkages breakage and structure changes (e.g. xanthan shift in ordered structure) (Tiwari et al., 2010).

Furthermore the chemical effects due to OH and H radicals are more prominent and influence the degradation of polymers in aqueous solutions at high frequency sonication (200 to 600 kHz) than at low frequency (Koda et al., 2003; Mark et al., 2008). Although there are many reports on ultrasonic degradation of polysaccharides (Lorimer et al., 1995; Tayal and Khan, 2000; Wasikiewicz et al., 2005; Goodwin et al., 2011), these findings focus on rheological properties as affected by molecular weight reduction, thus information are provided in terms of gum tailoring. Therefore, there are only limited data to our knowledge relating the effect of gum viscosity decrease on emulsion stability (Vinod et al., 2011; Karaman et al., 2012; Ansari et al., 2012). In Fig. 2.2 viscosity flow curves of 1 %wt gum solutions are demonstrated and Fig. 2.3 summarizes the effect of different sonication treatments on the apparent viscosity values at 10 s^{-1} rate. XG solutions are characterized by higher viscosity values compared to GG and LBG (XG>>LBG>GG) in all cases of untreated or sonicated gum solutions, hence the viscosity of final emulsions is mainly affected by

the final viscosity of the concurrently sonicated polysaccharide aqueous phase. As demonstrated by the flow curves in Fig. 2.2 galactomannan solutions (GG and LBG) presented a viscosity reduction when sonication time and intensity (method B) increased. Their viscosity was then reduced and the newtonian-like behavior was enhanced, especially after 3 min of sonication at 70% amplitude. It should also be noted that a more intensive treatment (method B) was not able to further decrease the viscosity of the galactomannan solutions, thus their viscosity reached a limiting value. On the contrary, XG solutions maintain their initial shear-thinning behavior and higher viscosity even after 4 min of sonication (method B).

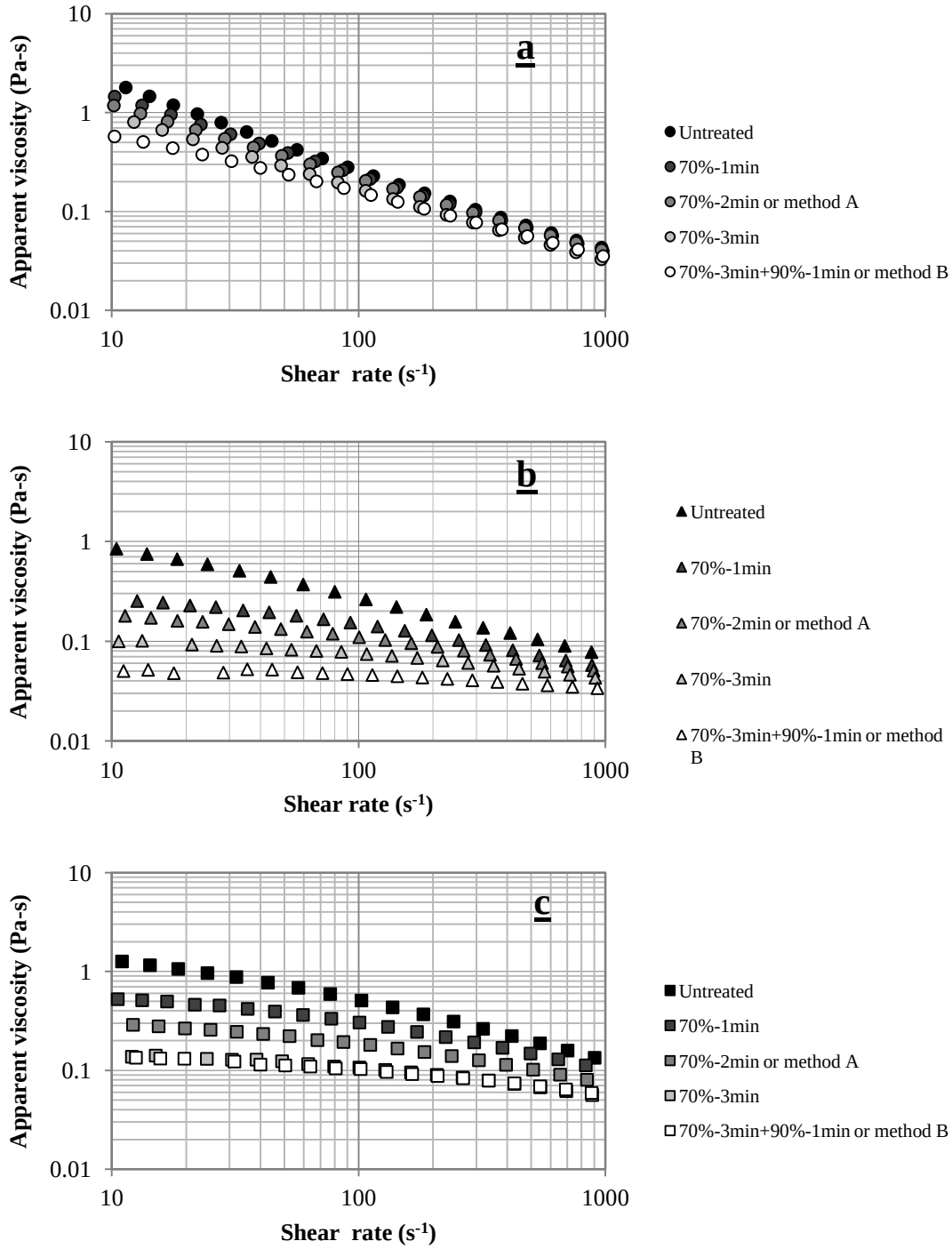


Figure 2.2 Apparent viscosity flow curves of 1 %wt gum solutions (a) XG, (b) GG and (c) LBG, as affected by different sonication treatments.

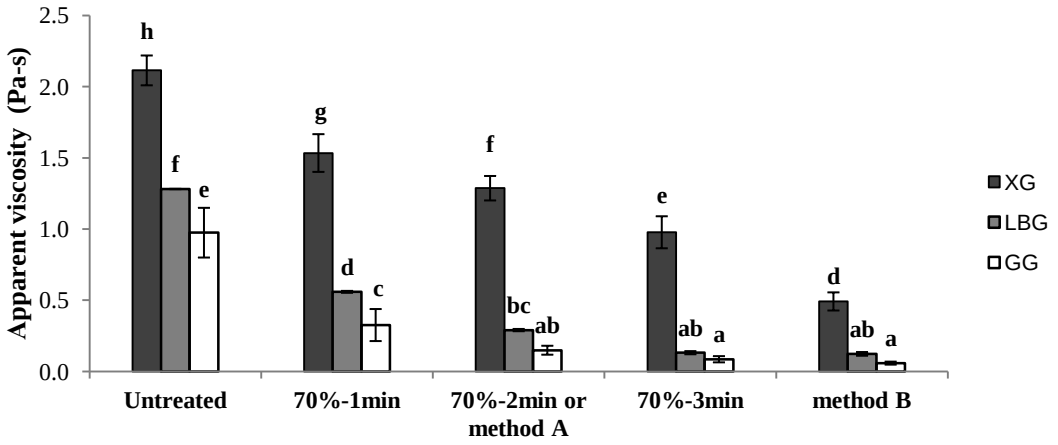


Figure 2.3 Apparent viscosity values at 10 s^{-1} rate of 1 %wt gum solutions as affected by different sonication treatments.

[a-h] Mean values followed by the same letters are not significantly different ($p>0.05$).

Our findings are in accordance with those of Tiwari et al (2010) who also showed that xanthan was more resilient against degradation and maintained its shear thinning behavior compared to guar by increasing ultrasonic intensity. This was shown by means of the consistency index k, which is a measure of viscosity (0.699 and 0.021 for XG and GG accordingly) and the flow behavior index n (0.594 and 0.932 for XG and GG accordingly), for which values near unity indicate a Newtonian behavior.

In total, when comparing untreated gum solutions with those that underwent the most intensive sonication (method B) there was a reduction of XG, LBG and GG viscosity by a factor of 4, 10 an 17 accordingly, thus GG was the most sensitive against ultrasonic depolymerization. Nevertheless, the viscosity decrease of the polymer, enhanced by temperature increase during sonication, improved the stability against coalescence of 0.5 %wt GG and LBG emulsions produced with method B (Fig. 2.4d, f) as a result of significant droplet size reduction. In the case of 0.5%wt XG emulsions, coalescence was not affected by sonication method, assuming that the droplet size reduction in emulsions prepared with method B was able to counterbalance the polymer viscosity decrease.

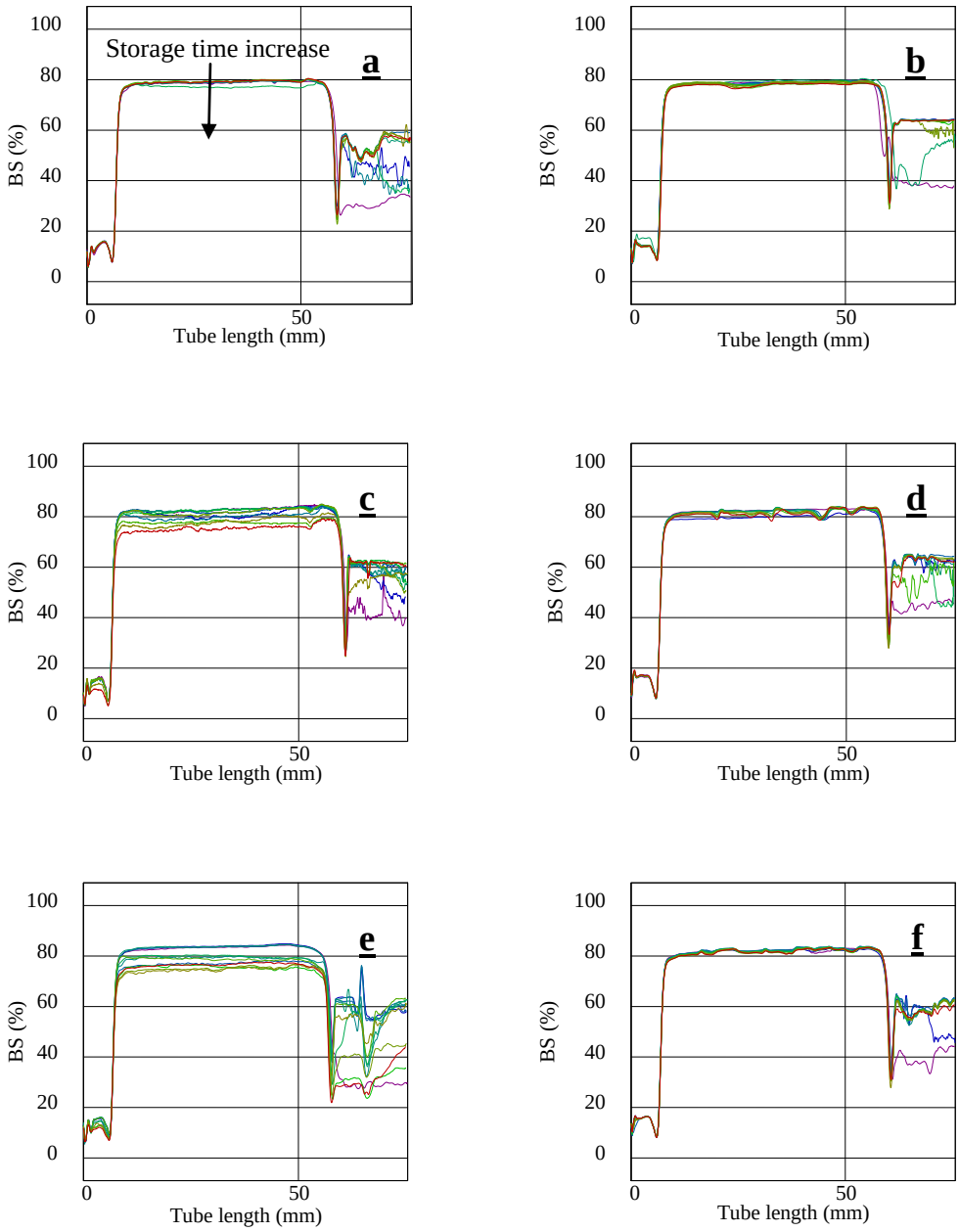
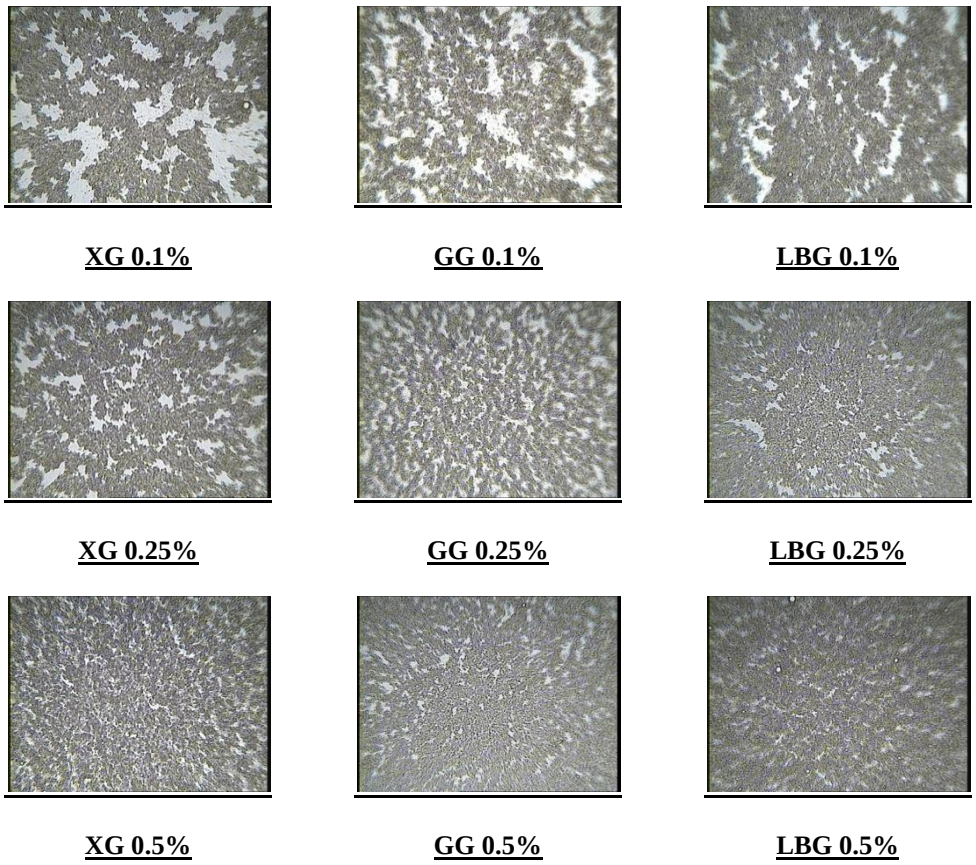


Figure 2.4 Back scattering (BS), as a function of the tube length for samples stored at 5^{o}C (arrow denotes time: day 0 to day 10) in emulsions containing 0.5 %wt gums, prepared with method A containing, (a) XG, (c) GG, (e) LBG and prepared with method B B containing, (b) XG, (d) GG, (f) LBG.

2.3.3 Light microscopy

In Fig. 2.5 micrographs of all emulsions formulations prepared with both ultrasonic emulsification techniques are depicted. Emulsions containing 0.1 %wt gums had a loose structure, forming extended gaps in the aqueous phase. These gaps were formed due to depletion flocculation phenomenon, since reversible phase

separation was observed upon gentle shaking. Thus, the weak flocks can easily be broken down to smaller sizes or even to single droplets (Diftis et al., 2005). The gaps formed seem to be more extended in the case of XG, which is typical due to its high molecular weight and repulsive interactions with proteins, in comparison to GG and LBG emulsions of the same concentration. This is in accordance to the high serum values found in samples containing 0.1%wt XG, whereas samples containing GG and LBG exhibit better dispersion, resulting in lower % Serum values (Fig. 2.6a). Emulsions containing 0.25%wt of polysaccharide prepared with both methods showed a lower degree of depletion flocculation than samples containing 0.1 %wt of stabilizers (method A), thus exhibited lower values of serum percentage. Furthermore, samples with 0.5%wt had an even more homogenous structure and therefore stable emulsions were formed in terms of serum layer absence (Fig. 2.7).



XG 0.1% **GG 0.1%** **LBG 0.1%**

XG 0.25% **GG 0.25%** **LBG 0.25%**

XG 0.5% **GG 0.5%** **LBG 0.5%**

(a)

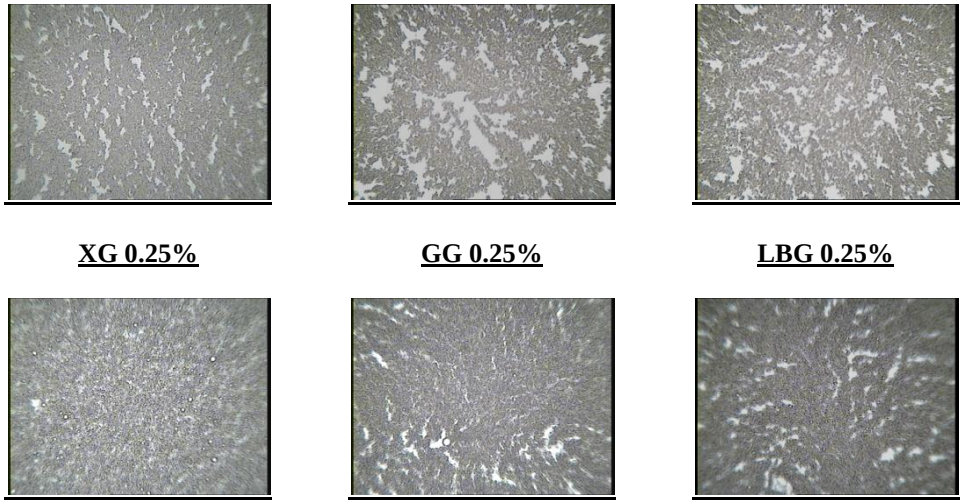


Figure 2.5 Light microscopy pictures of emulsions containing XG, GG and LBG prepared with two ultrasound techniques, using, (a) method A, (b) method B.

2.3.4. Emulsion stability

Creaming, flocculation, coalescence and partial coalescence are examples of natural instability during storage. The presence of hydrocolloids in an emulsion and their concentration strongly influence emulsion stability. It is known that the addition of XG up to a critical concentration (~0.2 %wt) promotes extensive flocculation leading to creaming or coalescence, whereas at higher concentrations (~0.5 %wt) little or no flocculation occurs (Ye et al., 2004; Chanamai et al., 1998; Sun et al., 2007). This is because even though the droplets are aggregated, their motion is limited, mainly because of the high viscosity of the continuous phase or the formation of a gel-network by the polysaccharides (McClements, 2000).

Fig. 2.6 demonstrates the serum percentage values of emulsions prepared with both methods as affected by storage time, which represents the instability of the samples containing 0.1 and 0.25 %wt XG, GG and LBG.

Formulations containing 0.1 %wt of XG presented the highest instability after 10 days of storage (% Serum = 59.6 %), whereas LBG the lowest one (34.5 %) (Fig. 2.6a). Due to their high instability emulsions at 0.1%wt gum concentration were not subjected to the two-stage emulsification or further tested. Instability of the emulsions containing 0.25 %wt started later and proceeded at a much lower rate than those containing 0.1 %wt of polysaccharide. Emulsions containing 0.25 %wt of GG and

LBG produced by method B presented generally similar % Serum values to those produced with the single-stage ($p>0.05$) (Fig. 2.6b). Even though the D_{50} was considerably reduced in these emulsions by higher energy input, a delay in creaming did not occur and destabilization was observed after 2 days of storage, while those prepared with method A started to cream on day 3 (Fig. 2.6b). Thus, creaming was not reduced by applying method B for GG and LBG emulsions, suggesting that the reduction of the droplet size could not counterbalance the aqueous phase viscosity reduction. On the contrary, there is a statistically significant difference for the emulsion of XG between the two methods with method B producing a more stable emulsion since the D_{50} was decreased at 0.83 nm and the viscosity of the XG was less affected by sonication treatment compared to that of the galactomannans (Fig. 2.3). As a general trend the % Serum values of 0.25 %wt emulsions follows the order GG≥LBG>XG. Similar findings about the influence of XG and LBG on emulsion stability have been reported (Huang et al., 2001; Makri et al., 2006). Concluding, at low gum concentrations creaming depended on energy input and destabilization occurred despite the significant reduction of the droplet size in GG and LBG emulsions owned to significant viscosity reduction.

In Fig. 2.4 and Table 2.3, back scattering (BS) profiles and variation values (dBS) for emulsions containing even higher gum concentration (0.5 %wt) are shown.

Table 2.3 Back-scattering variation (dBS, %) of emulsions containing 0.5 %wt stabilizer stabilizers during cold storage.

	Method A			**Method B**		
	BS_0	**BS_{10}**	**dBS**	**BS_0**	**BS_{10}**	**dBS**
XG	81.02±1.22	79.19±0.71	1.30[a]±0.69	79.64±0.49	78.58±0.38	1.06[a]±0.40
GG	82.40±0.26	73.94±0.91	8.65[b]±0.86	82.64±0.66	80.88±0.95	1.3[a]±0.04
LBG	83.01±0.50	74.14±0.28	8.99[b]±1.10	83.35±0.88	82.45±0.96	0.90[a]±0.22

Results presented as average out of three measurements.
[a-b] Mean values of dBS followed by the same letters are not significantly different ($p>0.05$).

These emulsions, regardless of the preparation method, remained stable during cold storage and even much longer (BS profiles obtained up to 30 days, data not

shown). This means that neither serum layer was observed nor BS peak near the zone of 50 mm, indicative for the creaming of droplets (Fig. 2.3). However, oil droplets aggregated as it can be seen by the decrease in BS values, since BS reduction is strongly affected by droplet size (Mengual et al., 1999).

The reduction of oil droplet size can cause a decrease in the attractive forces acting between the droplets resulting in emulsions less susceptible to coalescence (Gu et al., 2005; McClements et al., 2004).

Formulations containing 0.5 %wt GG and LBG prepared with method A appear more susceptible to coalescence/flocculation phenomena compared to XG. They exhibit greater differences of back scattering between preparation day (BS_0) and the last day of cold storage (BS_{10}) and they are additionally characterized by lower viscosity values that can favor the mobility of the droplets. Emulsions prepared with method B were more stable in terms of coalescence. Only a slight reduction of BS values (dBS= 0.90-1.06 %) (Table 2.3) was observed in all formulations despite the significant reduction of the continuous phase viscosity. Concluding, ultrasonication energy input increase (method B) resulted in stability improvement regardless of the composition when gum concentration in emulsions was 0.5 %wt.

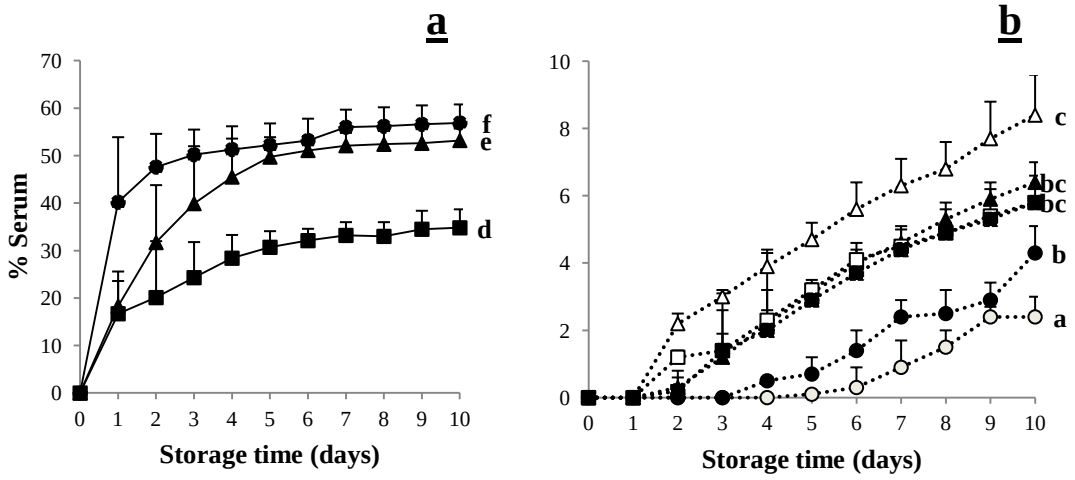


Figure 2.6 Stability (serum layer formation) of emulsions during storage at 5 ^{o}C using (a) method A containing 0.1 %wt stabilizers (●) XG, (▲) GG and (■) LBG, solid lines (b) containing 0.25 %wt stabilizers prepared with method A; **(**○**)** XG, (Δ) GG and (■)(□) LBG, dotted lines.
[a-f] Mean values on day 10 followed by the same letters are not significantly different (p>0.05).

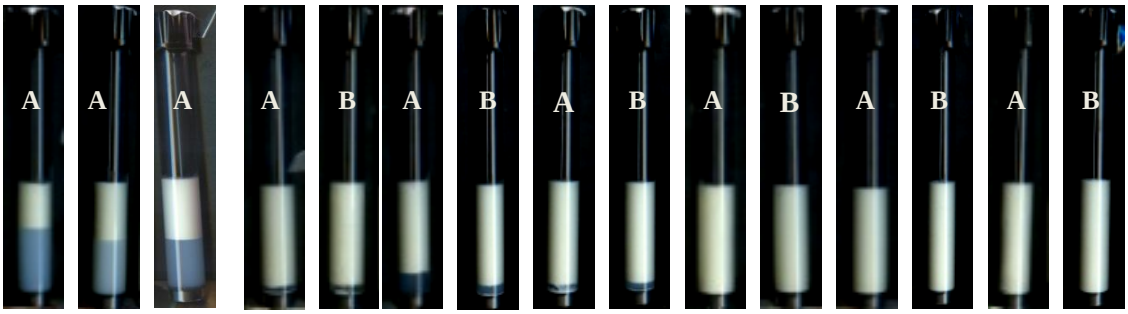


Figure 2.7 Emulsion prepared by ultrasonic emulsification methods A and B, containing various stabilizers at different concentrations. Serum separation on 10th day of cold storage in samples containing 0.1 and 0.25 %wt stabilizers can be seen.

2.3.5. Thermal analysis of XG emulsions

As it is shown in Fig. 2.4 (a, b) emulsions containing 0.5 wt% XG were the most stable during cold storage, since only a small reduction in BS occurred and no serum layer formation was observed. In other words, flocculation/coalescence phenomena of oil droplets were the only instability phenomena detected during cold storage. Nevertheless, the term coalescence involves two different instability mechanisms. The first one, also referred as "true coalescence", is caused by extended contact of oil droplets that locally causes high Laplace pressures and breakage of the droplet emulsifier film layers with the subsequent creation of a bigger droplet. Secondly, the presence of solid particles in the dispersed phase can lead to "partial coalescence" that is encouraged when emulsions are subjected to temperature fluctuations (Vanapalli et al., 2002). Upon cooling or cold storage, crystalline regions associated with saturated triacylglycerols (TAGs) of the dispersed phase are formed. These fat crystals can penetrate into another droplet to cause collision, which can lead to "true" coalescence upon reheating of the emulsions (Walstra, 2003; Van Bookel and Walstra, 2002; McClements, 2005).

This DSC cyclic heat-cool method was conducted in order to detect mainly the crystallization phenomena of the dispersed phase that could have a possible contribution on partial coalescence. A preliminary test using only olive oil was performed with a simple temperature ramp (Fig. 8a) within the same ranges (-40 to

40°C). The cooling line shapes of olive oil (extra virgin) were quite similar to those reported by Chiavaro et al. (2008), even though the major peak at around -38°C associated with the highly unsaturated TAGs was not clearly obtained within this temperature range (a much clearer thermograph was obtained when cooling at -50°C, data not shown). In our case another minor exothermic event with an onset temperature at -14.68 °C was observed, that is attributed to the crystallization of more saturated TAG fractions of the extra virgin olive oil (Chiavaro et al., 2007; Tan and Che Man, 2000).

Fig. 2.8 demonstrates cooling shape lines of bulk olive oil (a) and samples of freshly prepared emulsions containing 0.5wt% XG (b). The shape-line of method A has been slightly annotated for presentation reasons.

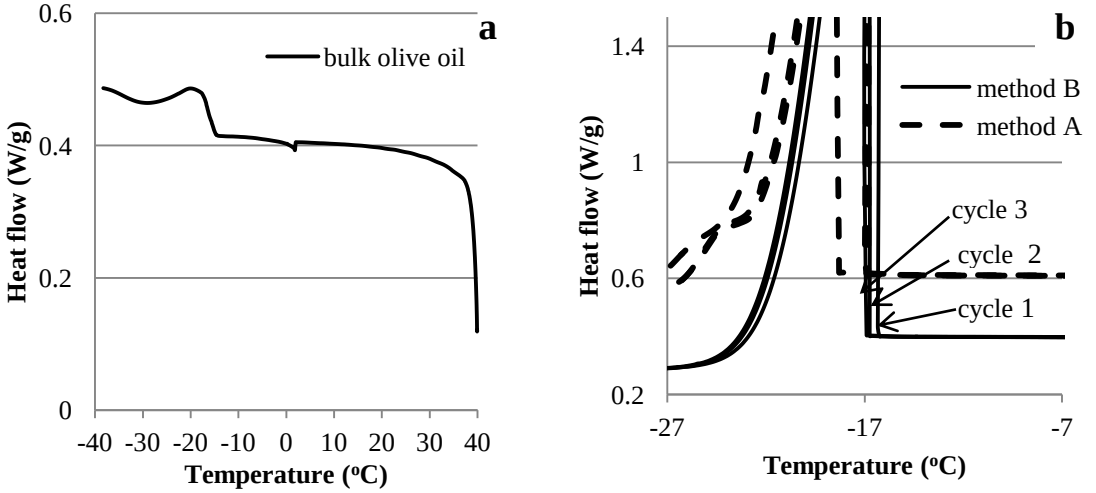


Figure 2.8. DSC cooling thermographs of (a) bulk olive oil and (b) 0.5 %wt XG emulsions.

In Fig. 2.8b it can be seen that no crystallization of the dispersed phase occurred upon cooling for emulsions prepared with both methods (characteristic peaks were not observed), hence the olive oil remained well emulsified and no bulk oil derived by coalescence was detected during the procedure.

In Table 2.4 it can be noticed that the emulsification method influenced the thermal properties of the emulsions.

Table 2.4. Thermal properties of emulsions containing 0.5 %wt XG prepared with two ultrasonication methods.

	T max (^{o} C)			**ΔH** (J/g)		
	Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3
Method A						
XG 0.25%	-16.93^{ef}	-17.10^{f}	-15.62^{cde}	252.9^{b*}	253.95^{b*}	253.95^{b*}
	±0.16	±1.93	±0.57	±35.78	±37.41	±36.56
XG 0.5%	-15.34 ^{bcde}	-17.00^{ef}	-16.53^{def}	294.40^{b*}	291.30^{b*}	292.10^{b*}
	±0.05	±0.04	±0.09	±0.71	±0.28	±0.10
Method B						
XG 0.25%	-12.63^{ab}	-14.23^{abcd}	-14.25^{cde}	240.00^{a*}	240.65^{a*}	240.45^{a*}
	±1.93	±1.19	±2.31	±0.57	±0.07	±0.07
XG 0.5%	-12.97^{a}	-13.07^{a}	-14.21^{abc}	237.80^{a*}	237.97^{a*}	237.77^{a*}
	±0.95	±0.44	±0.58	±1.71	±1.62	±2.03

Results presented as average out of two measurements.

Mean values concerning followed by the same letters are not significantly different (p=0.05).

The aqueous phase crystallization of samples prepared with method A was characterized by more suppressed initiation temperatures (T_{onset}) and increased enthalpy values. Another interesting aspect during the freeze-thaw cycles is that the crystallization enthalpies of the samples remained practically the same, assuming that the amount of freezable water was not affected by the process.

Still, the explanation of the influence of the ultrasonic treatment on the aqueous phase thermal properties remains complex, considering synchronous phenomena occurring such as xanthan gum depolymerization (Milas et al., 1986). Ultrasonication is known to alter the functional properties of WPCs proteins in a less detrimental way. It has been shown that sonication treatment may enhance protein stability against aggregation (Chandrapala et al., 2011), increase solubility and decrease the freezing point of WPC solutions (Krešic et al., 2008). Furthermore, the WPC, unlike the isolates (WPIs), contains important quantities of fat and lactose and therefore cannot be considered a pure substance, hence a deeper knowledge on the overall crystallization phenomena encountered should be achieved.

2.4 Conclusions

Olive oil micron-sized emulsions (D_{50}= 1.3 μm) or sub-micron (D_{50}= 0.615 μm) were formulated using whey protein, three kinds of hydrocolloids and two different emulsification energy inputs. Stable emulsions were only formed at 0.5 %wt stabilizer content. High energy input during ultrasonic emulsification resulted in emulsions of smaller droplet size and decreased viscosity. Nevertheless, these emulsions were more stable against coalescence/flocculation phenomena even though the viscosity of the continuous phase was greatly affected by sonication. Galactomannans'solutions viscosity was greatly influenced by sonication treatment intensity comparing to XG (XG>>LBG≥GG), influencing both creaming and coalescence/flocculation phenomena.

CHAPTER 3

STABILITY PROPERTIES OF DIFFERENT HYDROCOLLOIDS IN EMULSIONS PREPARED BY HIGH-SHEAR AND ULTRASONIC METHODS AT PH 3.8.

3.1 Introduction

Within the last few years numerous polysaccharide extracts have been proposed as possible thickening and stabilizing agents including mesquite gum, cordial gum, lepidium perfoliatum gum, psylium gum, angum gum, peach gum, durian seed gum etc (Lopez-Franco et al., 2013; Haq et al., 2013; Soleimanpour et al., 2013, Alftren et al., 2012; Gharibzahedi et al., 2013; Jindal et al., 2013; Qian et al., 2011; Ghorbani Gorji et al., 2014).

This increased interest arises from the fact that commonly used food polysaccharides like guar gum are used in non-food applications (well drilling, textile, paper, paint, cement, cosmetic, food, pharmaceutical etc), mainly in petroleum refining and pharmaceuticals (Vaughn et al., 2013; Prajapati et al., 2013). Along with the decrease of global production this has resulted in price fluctuations, price increase and severe supply shortage issues have arisen (Bahamdan et al., 2006; Barati et al., 2011; Anon, 2012).

Fenugreek (*Trigonella foenum-graecum L.*) is a legume grown annually in India, Ethiopia, Egypt and Turkey in northern Africa, the Mediterranean, Western Asia, northern India and Canada. The seeds are generally used as a spice but also as a remedy for their medicinal properties. They contain 45% to 60% carbohydrate (mainly galactomannan), 6 % to 10 % lipid (mainly polyunsaturated fatty acids), and 20 % to 30 % protein (4-hydroxyisoleucine being one of the major amino acids) (Rao et al., 1996; Raghuram et al., 1994). In addition, the seeds contain 5 % to 6 % saponins and 2 % to 3 % alkaloids.

As a spice, fenugreek not only provides a characteristic flavor like artificial maple syrup or rum in products, but adds nutritive value to foodstuffs as well (Shankaracharya et al., 1973). Fenugreek galactomannan consists of a (1 → 4)-β-D-mannan backbone to which single α-D-galactopyranosyl groups are attached at the O-6 position of the D-mannopyranosyl residues and the galactose/mannose ratio ranges between 1.00:1.02-1.14 (Brummer et al., 2003). Different components abundant in fenugreek plant parts (seed and leaves) have been shown to demonstrate cholesterol lowering, anti-inflammatory and anti-diabetic properties (Acharya et al., 2008; Im and Maliakel, 2008).

The antidiabetic properties of fenugreek gum were initially attributed to its high fiber content that slowed down the absorption of glucose and lipids in the intestine. Its

hypoglycemic effect has been demonstrated in both animals (streptozotocin-induced diabetic rats, alloxan-diabetic dogs and type II diabetic subjects with type 1 and type 2 diabetes mellitus) and humans (Ribes et al., 1986; Amin et al., 1988; Madar et al., 1988; Sharma and Raghuram, 1990; Hannan et al., 2007). The addition of FG caused a dose response reduction in glucose liberated from breads (Roberts et al., 2012). More recently it was revealed that other minor seed components such as 4-hydroxy-isoleucine and steroidal saponins, may also act synergistically in inhibiting glucose absorption and promoting pancreatic functions (Hamden et al., 2010).

According to another research, the optimal galactomannan content to decrease the rate of glucose uptake in the small intestine was establisted at 0.35% (wt/wt), since higher concentrations of fiber did not further influence the diffusion of glucose (Srichamroen et al., 2009).

The use of fenugreek gum has been increased in the food industry within the last years, owned to being a thickening agent as well asan emulsifier in many food products (Işıklı and Karababa, 2005). Despite related health benefits, one of the major drawbacks of using it in food products is its unpleasant flavor. Fenugreek consumption often results in unpleasant taste in several animal derived foods, as well as in a strong odor in human's sweat or urine (odour of "maple syrup" urine disease) (Bartley et al., 1981; Korman et al., 2001; Mazza et al., 2002; Sewell et al., 1999). Additionally, it has been found that supplementation with fenugreek flour up to 20% in biscuits had a negative effect on their sensory characteristics. More specifically, the sensory score regarding the taste of the biscuits was reduced from 7.25 to 2.78 (Hooda and Jood, 2005).

The aim of this work was to compare the stabilizing properties of different fenugreek gums with commonly used polysaccharides, namely guar and locust bean gum. Emulsions were prepared with two different methods, high speed and ultrasonic (US) homogenization and the stability of the prepared emulsions was compared. Considering that US technology is increasingly being up-scaled as its use is both time and power saving, thus making it a cost-effective emulsion formation technique, the interest in its improvement is high. Despite the above advantages referred a major restriction on US technology arises due to polymer degradation occurring during sonication. Even though the effect of sonication on rheological properties has been studied for several common stabilizers such as galactommanans and xanthan (Tiwari

et al., 2010), starches (Jambrak et al., 2010; Chung et al., 2002) or carrageenan (Lii et al., 1999) there have been no data concerning fenugreek gum to our best knowledge.

3.2 Materials and methods

3.2.1 Materials

Whey protein isolate (WPI) Lacprodan DI-9224 was kindly provided by Arla (Arla Foods Ingredients, Amba-Denmark). The composition of WPI powder in protein as stated by the manufacturer was protein 92 ± 2 %, and maximum amounts of fat 0.2 %, ash 4.5 % and lactose 0.2 %. Fenugreek gum type B (FGB) and Fenulife® (FGF) were kind gifts from Airgreen (Air Green Co., Ltd, Japan) and Frutarom Ltd (Israel) respectively. Xanthan gum (XG) Guar gum (GG) and locust bean gum (LBG) were bought from Sigma (St. Louis, MO, USA). Virgin olive oil Altis (Elais Unilever, Greece) was purchased from a local store. Citric acid, phosphate and sodium azide were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland).

3.2.2 Gum characterization

The moisture content of gum powders (dry/wet basis) was determined by AACC 44-16 Official method (105 ^{o}C, 2 hours). Total nitrogen contents were determined by Dumas method using a flash EA 1112 NC analyzer (Thermo Fisher Scientific Inc., Waltham, Ma, USA) and 6.25 was used as protein conversion factor (Koocheki et al., 2009).

For oil-holding capacity (OHC) determination, 5 g of olive oil was mixed with 0.25 g gum. The mixture was centrifuged at 4000 rpm for 5 min after vortexing for 30 s and left undisturbed for 5 min. The oil layer was carefully separated from the top of the tube using a syringe. The difference between the weight of oil added and the weight of oil separated at the top of the tube was expressed in g of oil/ 100 g of gum to give oil-holding capacity.

3.2.3 Emulsion preparation

Whey protein stock solution 10 %wt at pH 3.8 (typical of salad dressing and mayonnaise type products) was prepared by mixing appropriate solutions of citric acid (0.1 M) and phosphate (Na_2HPO_4 0.2 M) aqueous solutions followed by agitation with a magnetic stirrer for 90 min in ambient conditions. Gum solutions 1 %wt were prepared by hot stirring in a water bath at 90 ^{o}C for 90 min. Thereafter, solutions were

kept overnight at 5 ^{o}C to ensure complete hydration. Sodium azide 0.02 %wt was added to the aqueous solutions as an antibacterial agent. Coarse emulsions contained 2.7 %wt WPI, 20 %wt olive oil and 0.25 or 0.5 %wt of different galactommanans solutions. A total of 50 g were prepared by mixing appropriate amounts of the aqueous stock solutions and oil with a high-shear device Ultraturrax T25 device (IKA Werke, Staufen, Germany) at 13.500 rpm for 2 min. Small adjustments on emulsion pH were made with a few drops of HCl 1M if required. 40 ml of the coarse emulsion were placed in a glass beaker (38 mm internal diameter) covered by ice to prevent overheating. Secondary emulsions were produced following a batch-type sonication approach. The ultrasonic tip of a 13 mm diameter cylindrical titanium probe (VS 70T) was immersed in the centre of the glass beaker at a repeatable depth of 1 cm and different sonication treatments were applied generated from an ultrasonic device (Sonopuls 3200, Bandelin Gmbh&Co, Berlin, Germany) operating at constant frequency of 20 kHz. The sonication device operates by controlling either amplitude (100 % amplitude corresponding to 170 μm_{ss} for the specific probe used) or power (150 W nominal maximum power). It also has the ability to display or monitor the energy input (kJ) in the sample during sonication. The selection of emulsification conditions was based on our previous findings (Kaltsa et al., 2013). The application of 70%-3 min+90%-1 min sonication treatment (referred as method B in Chapter 2) was shown to result in emulsion formation with sub-micrometer size (D_{50} ~ 600-800 nm) at 0.5% galactomannan gum concentration (pH 7.0). The volume of sample processed was reduced though to 40 ml, which theoretically could lead to further droplet size reduction, preferably within the nanoscale range.

3.2.4 Light microscopy

Emulsion structure was observed in freshly prepared samples with a conventional optical microscope (Kruss Optronik, Germany) with a 40x magnification and several micrographs were obtained per sample from the center area of each slide. The emulsions were only slightly diluted with the same buffer (pH 3.8) in order not to disturb their initial structure. Several photos were taken from random sample positions. The micrographs were recorded using a camera (SONY, Hyper HAD, CCD-Iris) connected to a computer.

3.2.5 Droplet size evaluation

Oil droplet size measurements were performed on a Bruker Minispec NMR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with a Bruker ND 2176 probe at 20 ^{o}C. Measurements were performed in freshly prepared samples. Droplet size measurements were carried out in duplicate and results are expressed by the mean volume weighted diameter $D_{50.}$

3.2.6 Viscosity measurements

Viscosity measurements of different gums were conducted with a controlled stress SR-5 rheometer (Universal Stress Rheometer/Rheometrics Scientific, Inc., NJ) with plate-plate geometry and 0.25 mm gap. The diameter of the upper plate was 20 mm. The temperature was constant (25 ± 0.2 ^{o}C) by circulating water from a constant temperature circulator. Viscosity measurements were performed only in emulsions containing 0.5%wt of various gums that were the most stable. A hybrid rheometer DHR (TA Instruments, New Castle, US) equipped with plate-plate geometry (upper plate diameter 50 mm, gap 1 mm) was used to obtain flow curves from 0.1 up to 1000 s^{-1} at 25 ± 0.01^{o}C by circulating water from a peltier. The total measurement time was 900 s. All the flow curves of 1 %wt gum solutions and 0.5 %wt emulsions were analyzed using the Power law model, model as follows:

$$\tau = k\,\gamma^{n} \qquad \text{Eq. 3.1}$$

Where τ is the shear stress (Pa), k is the consistency coefficient (Pa s^{n}), γ is the shear rate (s^{-1}) and n is the flow behavior index (dimensionless). Mean values of at least three different preparations are demonstrated. Fitting the Herchel-Buskley model resulted in negative yield stress values for HS emulsions and untreated solutions and was therefore not appropriate for analysis, hence these results were omitted (Feys et al., 2007; Emadzadeh et al., 2011).

3.2.7 Emulsion stability

Emulsion stability was evaluated by obtaining daily acquisitions of back scattering profiles with a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France) during 10 days of storage at 5^{o}C. The procedure was replicated at

least three times. Emulsion stability due to clarification at the bottom of the tube is expressed as % Serum and was calculated as in Chapter 2.

3.2.8 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and the Fisher's LSD was applied in order to compare the mean values of emulsions and gum solutions properties at 95 % level of confidence.

3.3 Results and discussion

3.3.1 Oil holding capacity

In the present study the oil-holding capacity (OHC) of the different galactomannans and XG, as seen in Fig. 3.1, varied between 70.97 for FGB up to 138.02 g/100g for LBG. Values from 43.7 up to 128.8 (g oil/100 g gum) have been reported for Durian seed gum (Mirhosseini and Amid, 2012) and for karaya gum (81–114 g oil/100 g) (Galla and Dubasi, 2010). The mechanism of fat/oil absorption capacity was explained by Kinsella (1979) as a physical entrapment of oil by the non-polar chains of protein, but it could also be due to the presence of non-polar amino acids (Lazos, 1992). Among fenugreek fractions FGB presented higher OHC than FGF. FGB and GG contained the same amount of protein (3.92 %) (Table 3.1), but the OHC of GG was higher (~77.6 %). LBG having the greatest protein content presented the highest OHC, although high oil holding capacity was observed for XG which contained almost half the protein of LBG. Results obtained in this study indicate that the OHC of the various gums in not correlated to their protein content. This can be explained by other factors such as the surface area, porosity and capillary attraction of the fiber that can affect oil absorption capacity as well (Chau et al., 1997; Chau and Huang, 2003).

Table 3.1 Partial chemical composition, odor intensity and oil holding capacity of various galactommanans and xanthan gum.

Gum type	Galactomannan content* (%wt)	Protein (%wt wet basis)	Protein (%wt dry basis)	Moisture (%wt w.b)	Odor intensity**	OHC (oil g/ 100 g gum powder)
FGB	61.2	3.92 ±0.10	4.26 ±0.15	7.95 ±0.2	+++	70.97^{d} ±3.17
FGF	83.2	2.96 ±0.01	3.17 ±0.02	6.73 ±0.24	-	76.52^{c} ± 2.84
GG	np***	3.92 ±0.03	4.37 ±0.05	10.25 ±0.03	-	77.59^{c} ±1.89
LBG	np***	6.43 ±0.00	7.31 ±0.00	11.99 ±0.14	-	138.02^{a} ±3.3
XG	-***	3.47 ±0.04	3.91 ±0.06	11.2 ±0.13	-	99.7^{b} ±4.34

*As stated by the manufacturer/supplier
**Where (+++) intense odor and (-) odorless gum powder
*** np, not provided information, (-) does not contain galactomamann.
[a-d] Mean values with different superscripts are considered significantly different ($p<0.05$).

3.3.2 Droplet size

Table 3.2 demonstrates the effect of gum concentration and emulsification method on the volume mean diameter (D_{50}) of emulsions containing various gums.

The size of the emulsion droplets was affected by gum concentration. The decrease in droplet size observed in most cases of HS or emulsions (with the exception of FGB and FGF emulsions) when increasing gum concentration has been attributed to the viscosity increase of the continuous phase and consequent droplet immobilization, hence living more time for the protein to adsorb and stabilize the droplet (Kiosseoglou and Doxastakis, 1988; Makri and Doxastakis, 2006a; Tsaliki et al., 2004).

According to the Stokes-Einstein equation the droplet radius is reversely related to the viscosity of the medium. However, this is valid for dilute monodisperse droplets, a fact that is not always evident. Moreover, the droplet deformation can be described by Weber number ($We=G\eta r/2\gamma$). It increases with increase in the Weber number, which is proportional to the external stress $G\eta$ (where G is the velocity gradient and η is the viscosity). Additionally, a reduction in droplet size is achieved

by reducing the interfacial tension γ (Tadros et al., 2004). However, in the case of XG emulsions the inverse phenomenon was observed, upon increase of gum concentration from 0.25 to 0.5 %wt. This finding is also in agreement to those reported by (Chivero et al., 2015) who reported smaller average diameters (d_{32}) by GG incorporation, compared to XG emulsions. Thus, an optimum viscosity increase can be beneficial towards droplet size decrease.

Finer oil droplets were formed when sonication was applied, resulting in micron-sized particles at 0.5 %wt gum, whereas for HS emulsions D_{50} ranged between 3.37- 9.76 μm. Concentration impact was observed for most emulsions prepared with both techniques, with the exception of FGB and XG. The viscosity of FGB type is low compared to all other ones and is extremely reduced by ultrasonication (Figure 2), Thus, concentration effects on droplet size, using US, are hardly shown. On the other hand the increase of concentration in XG emulsions resulted in formation of bigger droplets. This is most probably associated to the increased viscosity of XG. Similar observations have been reported for emulsions prepared with different HPMCs varying in molecular weight and viscosity (Camino and Pilosof, 2011). In general, an optimum of gum concentration is beneficial in terms of reducing the average droplet size, since it could enhance protein adsorbtion and prevent recoalescence during US homogenization, provided that the mixing efficiency and ultrasonic wave propagation is not hindered by high viscosity. For instance, higher average diameters were obtained in emulsions when the concentration of chitosan exceeded 0.5 %wt (Tzoumaki et al., 2012) or when pectin concentration was 2 %wt, resulting also in polydispersed emulsions (Tawakatsu et al., 2001).

In our previous findings the smallest droplet size (D_{50} = 0.85 μm) was observed in model emulsions prepared with LBG at 0.5% wt at pH 7 after 4 min of sonication (Chapter 2). Ultrasonication intensity increase, at these experimental conditions, was more efficient in decreasing the polydispersity of the emulsions rather than remarkably minimizing the size of the smaller droplets in the system.

In this study, the minimum droplet size was found to be around 1.40 μm, which is greater than that found in the previous chapter, even though the volume size of the sample processed was reduced by 60 %. A possible explanation is that the emulsifying ability of whey protein was lower at pH ~ 4. At neutral pH values, oil droplets possess a negative charge and are strongly repelled. On the contrary at pH values near pI the surface charge diminishes and repulsive forces are weakened,

leading to enhanced attraction of the droplets. It is the charge and magnitude of the ionisable groups of protein on oil droplet that affect the formation and stability of emulsions, as a highly charged interface induces the electrostatic repulsion between droplets (Guzey and McClements, 2007). pH has also a significant effect on the secondary structure of whey proteins due to changes in ionization form of the amino-acid side groups (Onwulata and Huth, 2009). At pH near 4 up to 5 b-lactoglobulin, the most abundant protein of whey, undergoes the dimer to octamer transition. This is considered a rather rigid structure, resulting in slow surface denaturation around the droplets (Das and Kinsella, 1989).

Another interesting aspect considering droplet size is the residual protein content of the gums. Fenugreek like all other galatomannans do not carry any electrostatic charge to interact with the positevely charged WPI on droplet interface. However, proteinaceous moieties at concentration as low as 0.1 %wt in fenugreek gums may impart an amphiphilic property to the gum which enables it to act somehow like an emulsifier (Brummer et al., 2003; Youssef et al., 2009). The same researchers also reported that reducing protein content reduced the surface activity of purified fenugreek gum compared to unpurified gum. Gaonkar (1991) also found that purified guar or locust bean gum showed no surface activity. This would justify the fact that FGB of higher protein content resulted in lower droplet size than FGF. However, GG and LBG that also had high protein content did not result in low droplet size emulsions. Comparing also FGB and GG of approximately the same protein content, FGB resulted in lower droplet size ($p<0.05$) in all cases. Despite this, the role of the proteins of the gums can be altered due to their denaturation. Specifically, in our case, it is most likely that these protein moieties are denaturated during gum solution preparation (90 ^{o}C, 90 min) and previous gum isolation process. Fenugreek seed proteins are mainly composed of S-like globulins and vicilins (Kruse Fæste et al., 2010). Tang (2008), who studied the denaturation of vicilin fractions by means of DSC (Differential Scanning Calorimetry), found that their denaturation temperature peak lays between 85-90 ^{o}C, but the role of thermal denaturation on emulsifying properties of proteins is considered ambiguous. Moderate heating for 5 min at 90 ^{o}C was beneficial regarding the emulsifying properties of soy whey isolate due to denaturation of 7S and 11S globulins (Palazolo et al., 2004; Mitidieri and Wagner, 2002). However, extended thermal treatment (95 ^{o}C - 15 min) decreased their emulsifying properties by shifting the average droplet size to higher values, assigned

to denaturation of both β-conglycinin and glycinin causing formation of aggregates adsorbing at the interface. As a consequence more protein was necessary to obtain stable emulsions (Keerati-u-rai and Corredig, 2009). Additonally, galactomannan gum derived proteins represent a small proportion (<0.03 % in the total emulsion formulation) in comparison to whey proteins contained in our samples, therefore their effect on droplet size could be considered negligeable.

Table 3.2 Particle size (D_{50}, μm) of emulsions prepared by HS and US method.

	HS method		US method	
	0.25 %wt	**0.5 %wt**	**0.25 %wt**	**0.5 %wt**
FGB	$4.06^{de} \pm 0.03$	$3.77^{d} \pm 0.11$	$1.38^{ab} \pm 0.06$	$1.38^{ab} \pm 0.13$
FGF	$4.25^{ef} \pm 0.04$	$4.01^{de} \pm 0.06$	$1.23^{a} \pm 0.15$	$1.29^{ab} \pm 0.08$
GG	$5.68^{g} \pm 0.08$	$4.42^{f} \pm 0.05$	$2.04^{c} \pm 0.09$	$1.46^{ab} \pm 0.08$
LBG	$4.53^{f} \pm 0.07$	$4.22^{e} \pm 0.05$	$2.15^{c} \pm 0.19$	$1.56^{b} \pm 0.05$
XG	$7.32^{h} \pm 0.31$	$9.76^{i} \pm 0.24$	$1.60^{b} \pm 0.13$	$2.18^{c} \pm 0.11$

[a-i] Samples with different superscripts differ significantly ($p<0.05$).

3.3.3 Effect of ultrasonication on the viscosity of gum solutions and emulsions

Ultrasonic irradiation and other high shear treatments such as high pressure homogenization have been reported to influence the flow behavior of hydrocolloid dispersions due to reduction of their molecular weight via "mechanochemical" depolymerization reactions (Bashari et al., 2014). When sonication is applied, the decrease in molecular weight reduces the number per chain of hydrogen bonding sites leading, at the same time, to a reduction of the intermolecular hydrogen bonding between polymer molecules, that is, the attractions between the gum chains (Cheng et al., 2002).

Apparent viscosities as a function of shear rate for galactomannan and XG dispersions are shown in Fig. 3.1. The flow curves of untreated samples exhibit shear thinning behavior, which is an important characteristic in oil-in-water emulsions, in that the droplets are prevented from gravitational separation but the emulsion still flows easily when poured from a container (Barnes, 1994; Taherian et al., 2007). Consistency values (k) of galactomannan gum dispersions ranged from 1.31 up to 5.37 Pa-s^n for FGB and GG respectively, while for XG it was appreciably higher, reaching 19.83 Pa-s^n (Table 3.3). The viscosity of the FGF dispersions was greater than FGB ones for untreated dispersions (Fig. 3.2), in line to their galactommanan content as seen in Table 3.1. Among galactomannan stabilizers investigated, GG exhibited the highest consistency values. No significant differences were observed regarding the consistency values of FGF and LBG, thereby the overall consistency values followed the trend FGB<LBG≤FGF<GG<<XG.

The application of ultrasonication caused a sharp decrease of the apparent viscosity (Fig. 3.1b) and consistency values of gum solutions, and increased the flow behavior index values (n) (Table 3.3). More specifically, consistency values reduction reached 97.6 % for XG while in the case of galactomannans it ranged between 98.7 up to 99.8 %. This reduction rate is similar to other findings reported (Tiwari et al., 2010) concerning the consistency index reduction of ultrasonically treated gum solutions (80 ml processing volume, 1 %wt solution, 10.1 W/cm^2, 5 min), which reached 89.7 and 98.7 % for XG and GG respectively. Overall, no significant differences in consistency values were observed among sonicated gums ($p>0.05$).

The shear thinning behavior of ultrasonicated XG is maintained, whereas all the other solutions demonstrated Newtonian behavior in the region of shear rate >1 s^{-1}, as

evidenced by the the flow curves (Fig. 3.1b) and corresponding n values in Table 3.3. More specifically, the flow behavior values of ultrasonically treated galactomannan dispersions ranged between 0.922 up to 1.047, while it was considerably lower for XG (0.571). These values are also in accordance to the values measured by Tiwari et al (2010) who report n values of ~ 0.6 and slightly below 1 for ultrasonicated XG and GG.

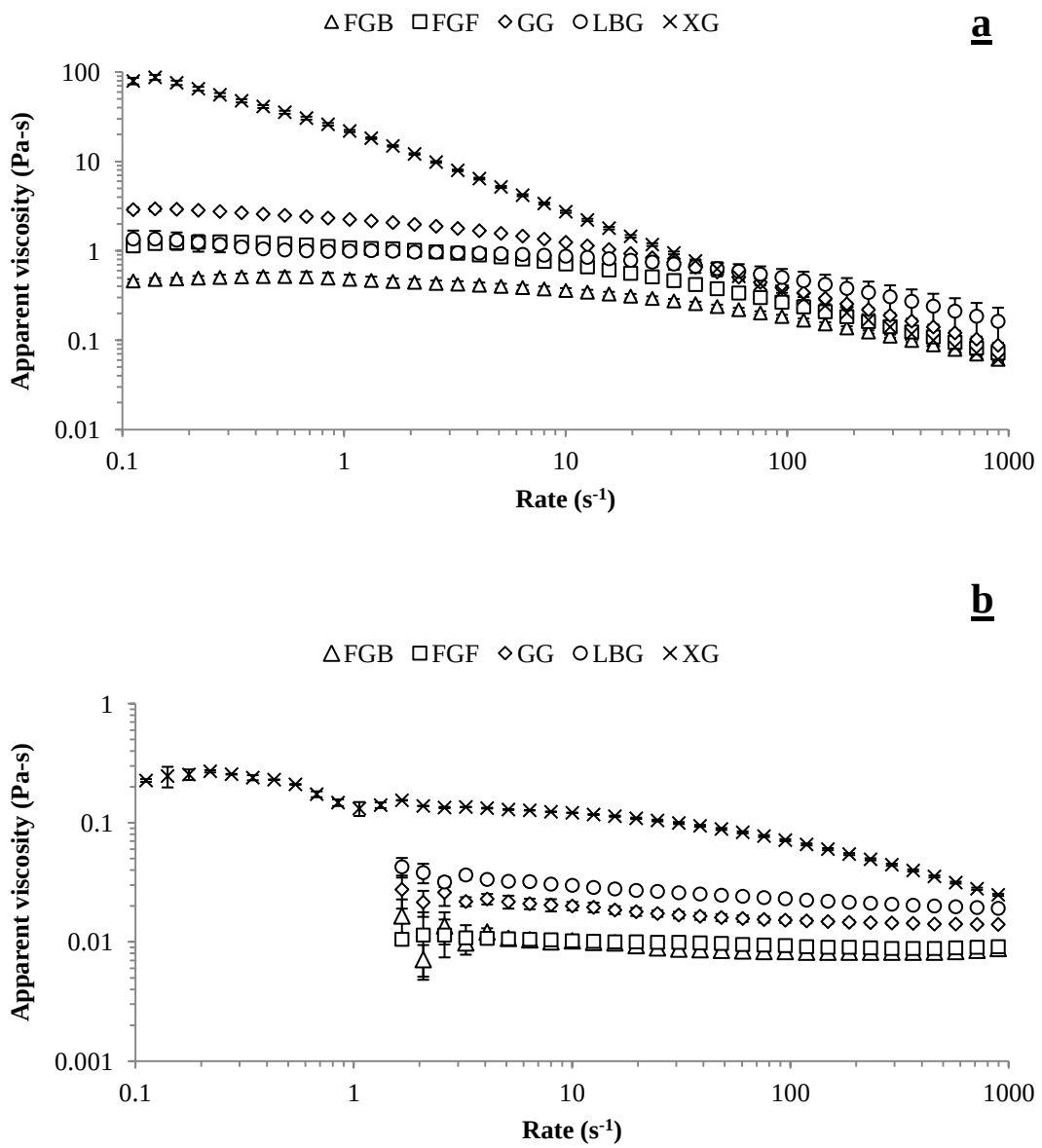


Figure 3.1 Apparent viscosity flow curves of a) untreated and b) ultrasonically treated 1 %wt gum solutions.

.

Table 3.3 Viscosity parameters of untreated and ultrasonically treated 1 %wt gum solutions.

	Untreated		US treated	
	k (Pa-s^n)	**n (-)**	**k (Pa-s^n)**	**n (-)**
FGB	1.314^{b} ±0.075	0.558^{d} ±0.009	0.006^{a} ±0.001	1.047^{g} ±0.011
FGF	3.208^{c} ±0.626	0.458^{c} ±0.014	0.008^{a} ±0.000	1.015^{g} ±0.002
GG	5.372^{d} ±0.062	0.404^{b} ±0.003	0.017^{a} ±0.002	0.968^{f} ±0.017
LBG	2.473^{c} ±1.205	0.556^{c} ±0.055	0.032^{a} ±0.002	0.922^{e} ±0.005
XG	19.833^{e} ±0.384	0.133^{a} ±0.008	0.478^{ab} ±0.007	0.571^{d} ±0.001

$^{a-g}$ Samples with different superscripts differ significantly ($p<0.05$).

R^2 values range between 0.99 -0.999.

Viscosity measurements were performed only for emulsions at 0.5 %wt concentration, which were more stable (no phase separation observed for at least 24 hours). Consistency values of HS galactomannan emulsions varied between 1.86 up to 4.73 Pa-s^n depending on gum type (Table 3.4). Overall, the viscosity of coarse galactomannan emulsions followed the trend FGB<LBG≤GG≤FGF, similarly to the trend established regarding the viscosity of pure gum solutions (FGB<LBG≤FGF<GG), indicating that the emulsion viscosity is not governed solely by the viscosity of the continuous phase, as differences can be also observed due to droplet size variations. FGF emulsions had slightly lower droplet size compared to GG ones, thus leading to increased droplet compaction, which enhances viscosity. Unexpectedely, XG emulsions presented the lowest consistency values, which could be due to observed increased droplet size. In addition, as explained by Tolstoguzov (1996), when proteins and polysaccharides interact to form a complex, the junction zones are less hydrophilic and the hydration capacity of the complex lowers, resulting in a decrease of the viscosity. Moreover, the electrostatic binding of the XG on the droplet surface leads to a decrease of the total concentration of the polysaccharide on the continuous phase and thus, to viscosity decrease. The decrease in pseudoplasticity and apparent viscosity upon mixing a protein with a polysaccharide, at acidic pHs has

also been reported by other authors (Delben and Stefancich, 1997; Sanchez et al., 1997; Launeville et al., 2000; Koupantsis and Kiosseoglou, 2009).

It can be denoted that ultrasonication caused a decrease in viscosity and pseudoplasticity of the emulsions due to polymer degradation (Table 3.4), with the exception of XG emulsions. Regarding galactomannan emulsions, after ultrasonication differences in k values among samples were not statistically significant ($p<0.05$). Even though droplet size reduction may lead to higher emulsion viscosity via increase in droplet packing and subsequent immobilization throughout the emulsion matrix, this was not observed in the case of galactommanan samples. The continuous phase viscosity was in all cases very low and any increase by droplet packing was not able to increase it considerably. Ultrasonically prepared XG emulsions though, exhibited higher consistency and lower n values compared to their HS counterparts. This could be due to the combined effect of sonication, towards decreasing the average droplet size and reducing the interaction strength between the protein and the polysaccharide (Hosseini et al., 2013), in favor of its hydration capacity.

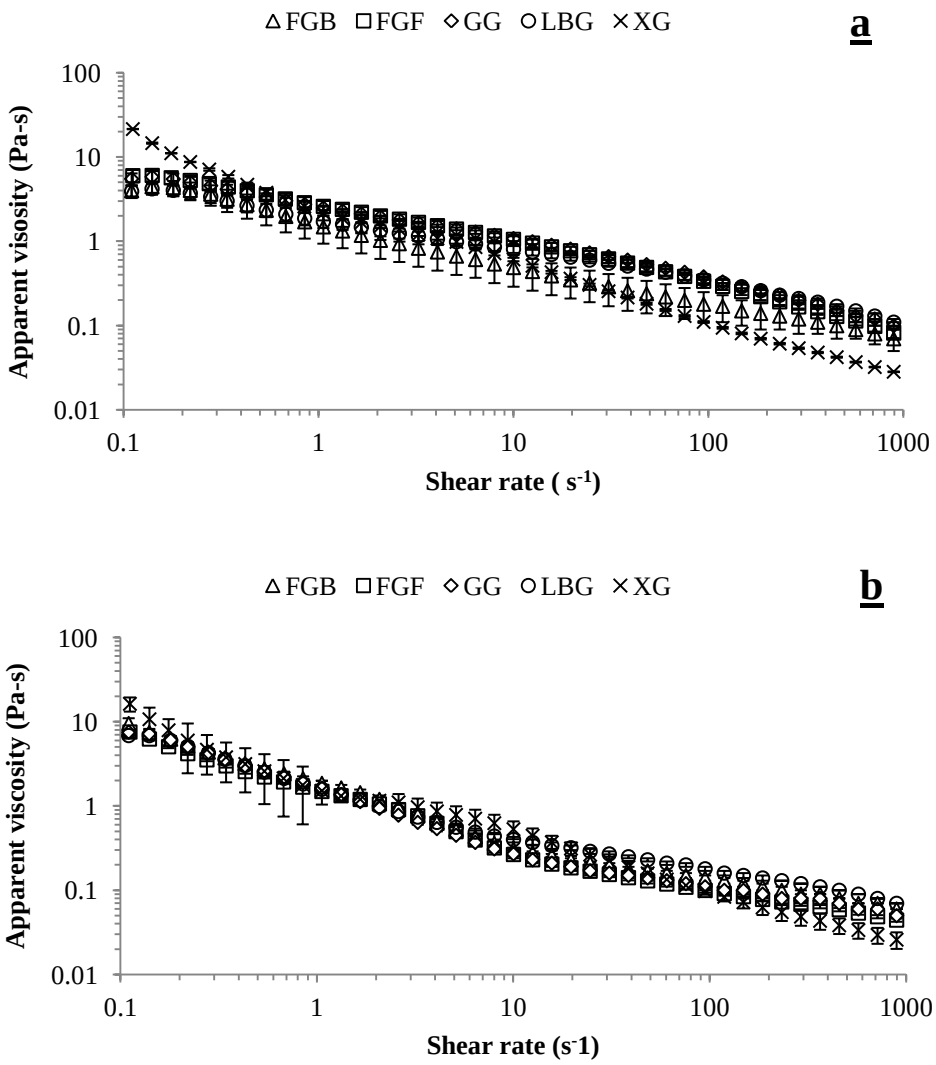


Figure 3.2 Apparent viscosity flow curves of emulsions containing 0.5 %wt galactomannans and XG prepared by a) HS and b) US method.

Table 3.4 Viscosity parameters of emulsions containing 0.5 %wt gums prepared by HS and US method.

	HS emulsions		US emulsions	
	k (Pa-s^n)	**n (-)**	**k (Pa-s^n)**	**n (-)**
FGB	1.856^{a} ±0.943	0.545^{e} ±0.064	0.720^{a} ±0.052	0.652^{f} ±0.013
FGF	4.729^{d} ±0.664	0.429^{b} ±0.022	0.609^{a} ±0.045	0.614^{e} ±0.005
GG	4.338^{cd} ±0.274	0.451^{c} ±0.021	0.571^{a} ±0.053	0.651^{f} ±0.005
LBG	3.902^{c} ±0.137	0.531^{d} ±0.008	1.063^{a} ±0.072	0.610^{e} ±0.010
XG	0.617^{a} ±0.119	0.545^{d} ±0.014	2.318^{b} ±0.281	0.340^{a} ±0.014

$^{a-d}$ Samples with different superscripts differ significantly ($p<0.05$).

R^2 values range between 0.992 to 0.999.

3.3.4 Stability of emulsions prepared by HS and US emulsification

In Fig. 3.5a,b the evolution of serum percentage values during storage of HS emulsions are depicted. Samples containing 0.25 %wt gum concentration became quickly very unstable and the serum percentage at day 10 of storage ranged between 36.3 to 40.1 %, without any signifant difference among samples ($p<0.05$). However, it should be noticed that the kinetics of the serum percentage for galactomannan emulsions was similar to the opposite order of the viscosity of the gums (FGB< LBG ≤ FGF <GG). Thus, emulsions containing FGB had the smallest droplet size (D_{50} = 4.06 μm) compared to all other emulsions, but they destabilized faster, assuming that creaming is majorly governed by the continuous phase viscosity. This phenomenon though was not observed in emulsions containing 0.25 %wt gums prepared by ultrasonication and all galactomannan emulsions exhibited similar kinetics (Fig. 3.5c). This observation did not apply for XG emulsions which clearly destabilized faster even than those containing FGB, despite the at least four-fold increased consistency values of XG solutions compared to galactomannan gums. This could be due to the combined effect of higher droplet mean diameters observed for XG emulsions and lower consistency values observed in these systems (Table 3.4).

Overall, the stability of emulsions containing 0.25 %wt gums was increased for samples by applying sonication treatment, since the % serum was reduced, ranging from 26.8 to 30.7 %, with the exception of FGB samples. The above improvement occurs due to the reduction of the average droplet size, taking into account the severe concurrent continuous phase viscosity reduction during sonication, although in the case of FGB it may not be sufficient to counterbalance the viscosity loss.

As expected, the stability of the emulsions was increased upon increase of gum concentration at 0.5 %wt (Fig. 6 d), as a result of the combined effect of droplet size reduction and the increased viscosity of the aqueous phase. High-molecular weight polysaccharides keep the droplets apart after formation and thus, protect them against creaming, flocculation and coalescence (Akthar and Dickinson, 2003).

GG presented the highest stability against creaming (3.5 %), accompanied by FGF and LBG that statistically performed the same ($p>0.05$), whereas an improvement to a lesser extent was assessed for FGB samples. Despite the fact that no significant differences were identified on day 10 of storage between samples containing 0.25 and 0.5 % wt stabilizer, it can be seen that the kinetics of serum formation at higher gum concentration (Fig. 3.5b) are distinguishably retarded.

In contrast to 0.25 %wt HS emulsions, a lag time period was observed up to 5 days for GG emulsions before they became phase separated. It is also interesting to note that the same trend of stability kinetics was maintained as in the case of 0.25 %wt HS galactomannan emulsions. In particular, the serum percentage of 0.5 %wt HS galactomannan emulsions followed the order FGB>LBG≥FGF≥GG, similarly to the opposite viscosity order of the gums. The effect of ultrasonic application on the stability against creaming of the 0.5 %wt emulsions was not beneficial regarding the majority of the samples. For US emulsions, the % serum ranged between 3.5 to 20.7 % for GG and FGB emulsions respectively, the later being the less stable of all, whereas no significant differences were identified among the rest samples including XG as well. Sonication treatment resulted in total reduction of the % serum by ~41, 43, 48.5, 47 and 36 % for FGB, FGF, GG, LBG and XG respectively, in comparison to their HS counterparts. Despite this, significant improvement was only detected in the case of FGB and XG samples (Fig. 3.6a). In the case of XG emulsions, higher viscosity values were also obtained in comparison to their HS counterparts and US galactomannan emulsions, which explains the overall beneficial impact of sonication in these samples and equal stability rates respectively.

As seen in Fig. 3.7, emulsions were highly aggregated regardless of gum type or method applied. For presentation reasons only emulsions at 0.5 %wt concentration are depicted, while aggregation was also present in all cases of 0.25 %wt gum concentration. Several studies have indicated that the presence of > 2% protein (sodium caseinate) in emulsions induces rapid creaming via depletion flocculation mechanism due to the presence of unabsorbed protein in the aqueous phase (Dickinson and Golding, 1997, Euston and Hirst, 1999 and Srinivasan et al., 2000). However in the case of emulsions containing the anionic XG, the underlying cause of extensive droplet aggregation would be different than that of the used galactomannans, since the later are non ionic (Funami et al., 2005; McClements, 2015). Whey proteins are known to interact electrostatically with a number of charge-carrying polysaccharides (pectin, xanthan, chitosan, carrageenan, carboxymethylcellulose e.tc.), both in solution and emulsion systems and extensive research has been conducted on this topic (Koupantsis and Kiosseoglou, 2009). In the case of anionic polysaccharides extensive interaction is expected to take place under low concentrations of total solids (<3-4 %wt) and low ionic strengths (<0.4 M) between the pI of the protein and the pK_a of the polysaccharide (=3.1 for XG) (Turgeon and Launeville, 2009; Oprea et al., 2013).

The extensive flocculation observed in emulsions containing negatively charged pectin and sodium caseinate at pH 3 or 4, could also be caused due to droplet charge neutralization by pectin adsorption, thereby minimizing the magnitude of electrostatic repulsion of droplets (Weibreck et al., 2004; Surh et al., 2006). Under these conditions, which cause droplet charge neutralization and extensive viscosity reduction, (explained previously in detail), the highest creaming rates have also been observed at pH ~3.8 in other related studies involving emulsions formed with milk proteins and anionic polysaccharides (Weibreck et al., 2004).

With regards to galactomannan emulsions, Huang et al. (2001) who compared the stability of emulsions containing different hydrocolloids, have shown that long term stability without phase separation could be achieved upon use of higher concentration of fenugreek, guar and locust gums (1-1.5 %wt), attributed to droplet movement restriction caused by continuous phase viscosity increase. More recently, different studies (Perrechil and Cunha, 2010; Farshchi et al., 2013; Chung et al., 2013) have shown that a concentration of 0.6-0.8 %wt locust gum was needed to stabilize

via viscosity enhancement mechanism emulsions more susceptible to creaming due to pH (close to the protein's isoelectric point) or low oil concentration.

However, such concentration levels would not favor the formation of submicron emulsions since it could inevitably cause an increase in the average droplet size in coarse or sonicated emulsions as a consequence of viscosity increase (Tzoumaki et al., 2011). Also, as seen in Table 3.2 only a slight decrease was noticed upon the increase of gum concentration from 0.25 to 0.5%. Hence, lowering the protein content could primarily be used in order to examine if it could lead to formation of more stable emulsions by reducing the amount of the unabsorbed protein in the aqueous phase near the monolayer coverage region (Dickinson, 1999).

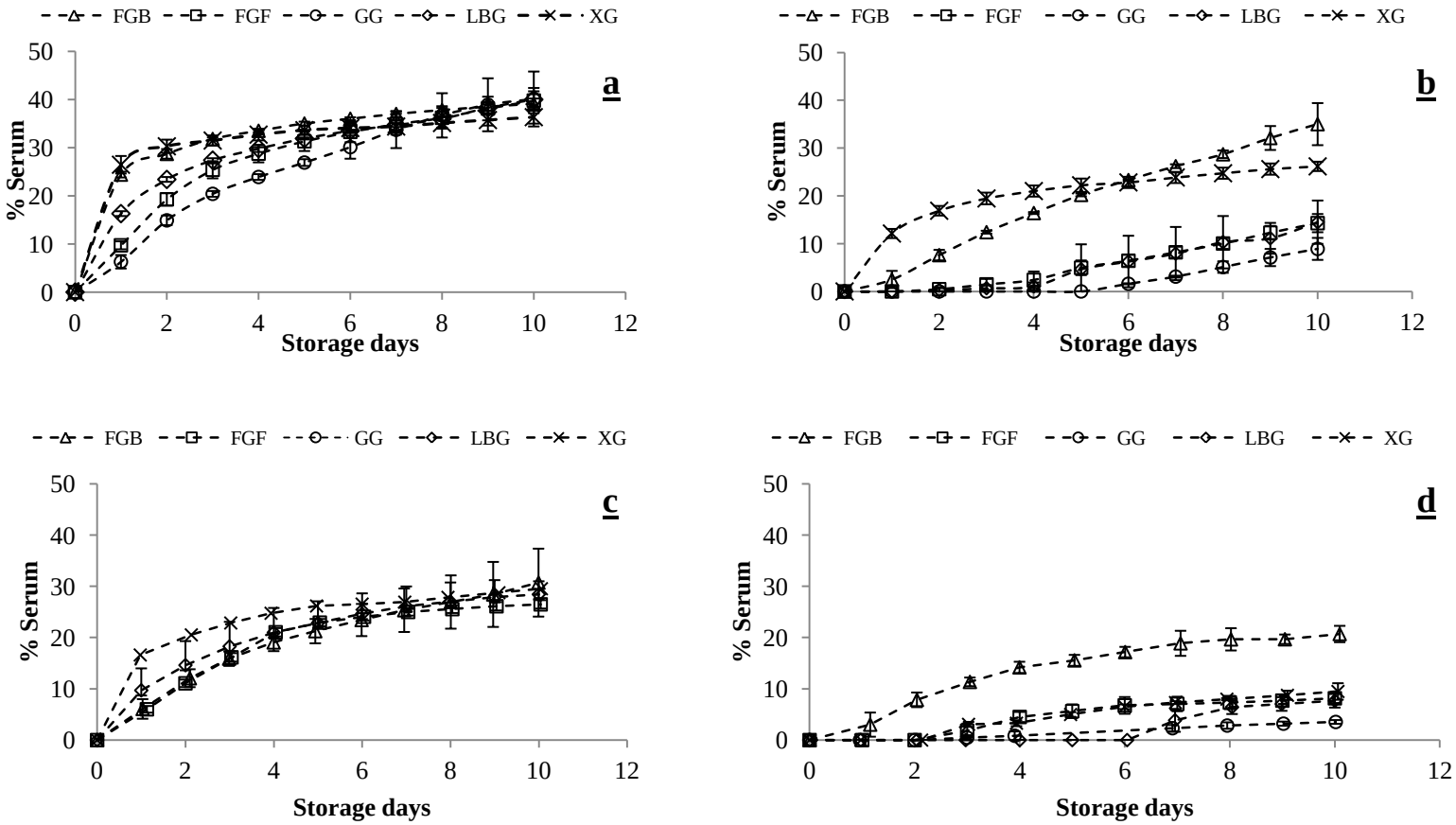


Figure 3.3 Stability of emulsions during storage prepared by HS method: a) 0.25 %wt and b) 0.5 %wt gum and US method: c) 0.25 %wt and d) 0.5 %wt gum concentration..

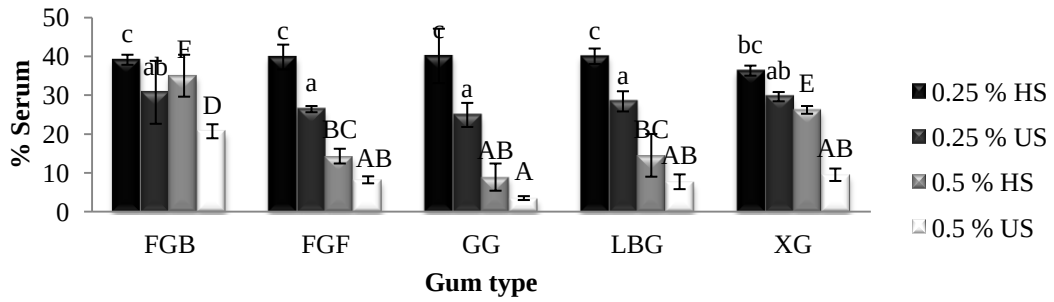


Figure 3.4 Stability of emulsions prepared by HS and US method.

[a-c, A-E] Samples with different superscripts differ significantly ($p<0.05$).

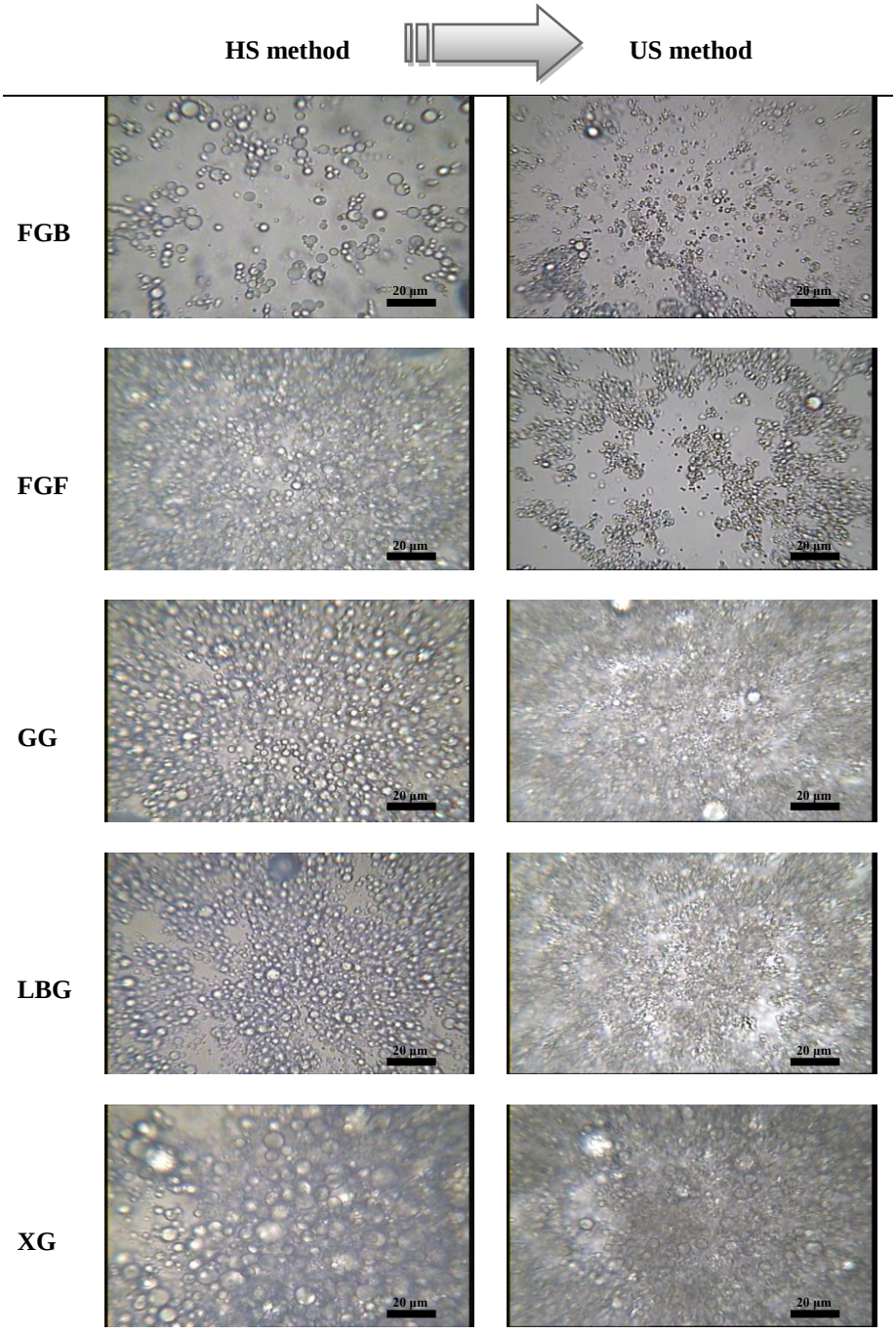


Figure 3.5 Light microscopy pictures of freshly prepared emulsions containing various gums at 0.5 %wt concentration, prepared by HS and US method. Bar corresponds to 20 µm.

3.3.5 Stability and properties of HS emulsions containing 1 %wt WPI and 0.5 %wt gums

In Table 3.5 the properties of emulsions emulsions containing 1 %wt WPI and 0.5 %wt gums (droplet size, viscosity and stability) are summarized. Among two different fenugreek gum types previously studied, FGF (Fenulife®) was chosen to be

used in this section since it demonstrated higher viscosity properties and performed better in terms of emulsion stability.

The size of the emulsion droplets was affected by gum type used. Emulsions containing 0.5 %wt XG presented higher droplet size (D_{50}= 10.34 μm) compared to the other stabilizers at the same concentration, while FGF the smallest ones (5.16 μm). In comparison to our previous findings, the reduction of WPI concentration from 2.7 to 1 %wt resulted in emulsions with higher droplet sizes, at a given gum concentration 0.5 %wt. XG emulsions exhibited higher viscosity in comparison to galactomannans, despite considerably higher D_{50} values at a given concentration (0.5 %wt). In the case of XG emulsions therefore, emulsion viscosity is mainly governed by the viscosity properties of the continuous phase. The type of galactomannan used affected the consistency values of emulsions, hence FGF and LBG samples exhibited relatively lower consistency values compared to GG. These findings are merely in accordance to those of Wu et al. (2009), who compared the emulsifying and stabilizing properties of the same type of galactomannans. It was shown that for emulsions formed solely by gums, a relation between their viscosity and emulsion stability was revealed. More specifically, the trend for viscosity followed the trend GG>FGF>LBG, in line with that observed for emulsion capacity and stability. In the presence of whey protein though, it seems that the viscosity of emulsions is influenced by their droplet size and gum properties (molecular weight or galactomannan content) may vary.

Table 3.5 Effect of gum concentration on the properties of HS emulsions containing 1 %wt WPI and 0.5 %wt gums.

Gum type	D_{50} (μm)	k (Pa-s^n)	n (-)	Serum (%)
FGF	$5.16^{a} \pm 0.03$	$0.857^{a} \pm 0.097$	0.659±0.020	$38.7^{b} \pm 1.1$
GG	$5.68^{bc} \pm 0.12$	$2.114^{c} \pm 0.147$	0.519±0.015	$33.0^{a} \pm 1.9$
LBG	$5.86^{c} \pm 0.05$	$1.461^{b} \pm 0.236$	0.554±0.018	$38.2^{b} \pm 1.0$
XG	$10.34^{f} \pm 0.41$	$5.415^{d} \pm 0.615$	$0.383^{a} \pm 0.021$	stable

$^{a-f}$ Samples with different superscripts per column differ significantly ($p<0.05$).

In Fig. 3.6 the evolution of serum indices during storage of emulsions are shown. The serum percentage of emulsions containing 0.5 %wt galactomannans varied between 32.97 to 38.2 %, while no phase separation was observed in emulsions

upon incorporation of XG under the specific storage conditions (5 ^{o}C). Recently it has been reported (Bouyer et al., 2013) that emulsions containing 30 % almond oil stabilized by 1 %wt b-lactoglobulin in the presence of 1 %wt xanthan gum (pH 4.2) were stable within a 6 month period attributed to the higher viscosity and shear-thinning behavior of xanthan compared to gum arabic emulsions which became phase separated within 3 days. As demonastrated in Fig. 3.6, emulsions appeared flocculated, despite decreased WPI amounts. Compared to emulsions containing higher protein amounts (2.7 %wt WPI) at 0.5 %wt gum concentration galactomannan emulsions containing 1 %wt WPI exhibited lower stability (Fig. 3.7). This could be due to lower viscosities (k) observed as a consequence of lower WPI amount used and/or higher droplet sizes formed. Therefore, it appears that WPI concentration has a major influence on emulsion viscosity leading to increase of emulsion stability via the formation of a viscous aggregated matrix at higher protein concentration, despite depletion flocculation observed.

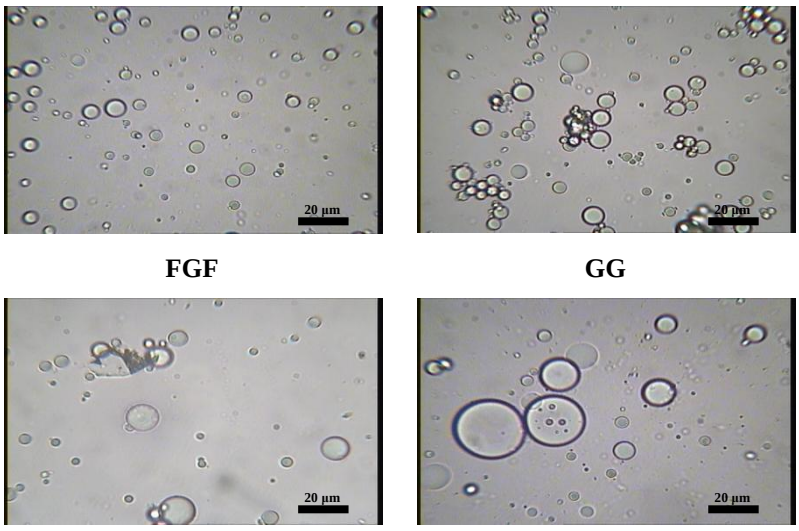


Figure 3.6 Light microscopy photos of freshly prepared HS emulsions containing 1 %wt WPI and 0.5 %wt of various gums. Bar corresponds to 20 µm.

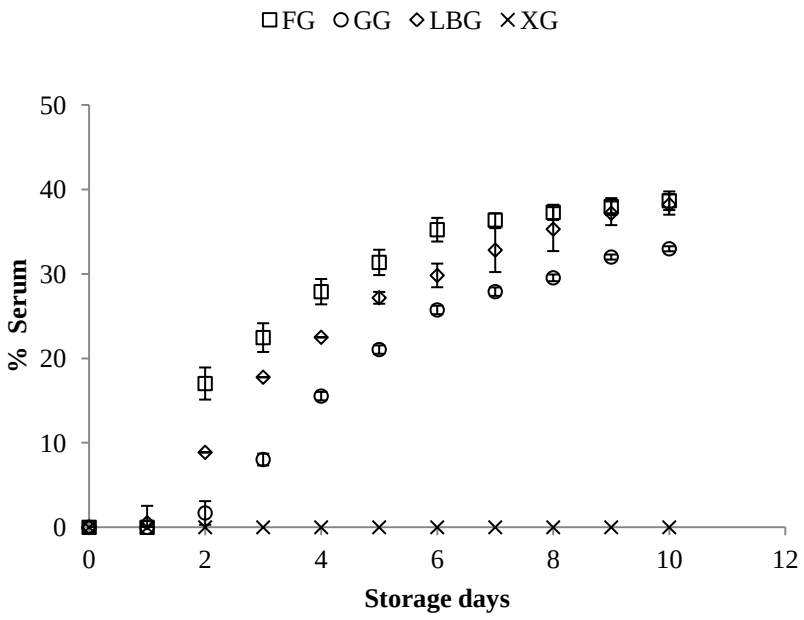


Figure 3.7 Stability of emulsions containing 1 %wt WPI and various types of gums at 0.5 %wt concentration.

3.4 Conclusions

The droplet size, viscosity and stability of emulsions were affected by galactomannan type and concentration as well as emulsification method used for preparation. The droplet size was reduced in respect with the protein amount of FGs, whereas stability depended on their viscosity. In total, under the experimental conditions used, unpurified FG, with proteins (FGB), was more efficient in reducing the droplet size compared to LBG or GG in most of the cases, whereas FGF type with higher galactomannan content and viscosity resulted in the formation of more stable emulsions. Increase in their concentration might have a positive contribution to droplet size reduction, due to further absorption on droplet surface, and to stability improvement, via viscosity increase. Overall, the application of ultrasonics did not remarkably increase emulsion stability, attributed to inferior thickening ability caused by extreme stabilizer degradation.

In contrast to emulsions previously prepared at ph 7 (Chapter 2), droplet size reduction within the submicron scale was not observed, which could be due to over-processing conditions. For this reasons a more detailed research considering ultrasonication parameters would be required and alternative methods of preparation

e.g. pre-emulsification of oil-emulsifier blend and dilution with stabilizer solution should be examined.

Reducing the amount of WPI to 1 %wt had a mixed effect on emulsion stability depending on stabilizer type used. Galactomannans emulsions were not more stable compared to those prepared with higher WPI amounts, as a consequence of droplet size increase and emulsion viscosity decrease observed. XG on the other hand was more efficient stabilizer, since emulsions formed at the same concentration were stable against phase-separation under conditions of cold storage, attributed to the formation of a network made by entrapped droplets through bridging flocculation mechanism.

CHAPTER 4

INFLUENCE OF ULTRASONICATION PARAMETERS ON PHYSICAL CHARACTERISTICS OF MODEL EMULSIONS CONTAINING XANTHAN.

4.1 Introduction

The use of ultrasonics in the food industry is gaining more interest. In-line implementation of ultrasonic processing is well established for the chemical and pharmaceutical industry and is being developed for food products such as fruit juices, mayonnaise and tomato ketchup and dairy systems (Wu et al., 2000; Zisu et al., 2010).

Concerning emulsion technology, ultrasonic emulsification (USE) can offer several improvements compared to conventional high shear methods or novel ones like micro-fluidization apart from power efficiency (Tang et al., 2013). This stands for production of oil droplets in the sub-micron/nano-scale (up to 100 nm), improved stability over time and need for small amounts of emulsifying agents (Chendke and Fogler, 1975; Li and Chiang, 2012). Although microfluidization has been found to be more efficient than ultrasound, it is considered less practicable with respect to production cost as well as equipment contamination (Karbstein and Schubert, 1995; Freitas et al. 2006).

Despite the referred advantages, a number of restrictions considering USE have arisen. These involve demulsification (Canselier et al., 2002), deterioration in quality of edible oils and off-flavoring due to oxidation (Chemat et al., 2004; Gharibzahedi et al., 2012) and polymer degradation. Although there are many reports on ultrasonic degradation of polysaccharides (Lorimer et al., 1995; Tayal and Khan, 2000; Wasikiewicz et al., 2005; Goodwin et al., 2011), these findings focus on rheological properties as affected by molecular weight reduction, thus information are provided in terms of gum fractionation and viscosity tailoring. Therefore, there are only limited data to our knowledge relating the effect of gum viscosity decrease on emulsion stability (Vinod and Sashidhar, 2009; Karaman et al., 2012; Ansari et al., 2012). On the other hand sonication conditions' choice seems quite difficult, when food is treated and viscosity influences process and further product quality.

Optimization of sonication conditions deals with a careful determination and further choice of the variables influencing cavitation. These variables are related to the reaction medium (e.g. viscosity and vapor pressure), to the reaction conditions (e.g. frequency, power but temperature control as well) and to the type of the sonic system (e.g. frequency range) including the choice of the chemical reactor (e.g. horn shape and dimensions) (Santos et al., 2009). In the present research some reactions conditions were changed (amplitude and sonication time) keeping sample formula

constant and using the same sonic system. Amplitude choice in a sonication process is critical, because it is proportional to the intensity of sonication and its increase is related to an increase in the sonochemical effects. A minimum amplitude is required to achieve the cavitation threshold, whereas high amounts can lead to rapid deterioration of the ultrasonic transducer resulting in liquid agitation instead of cavitation and in poor transmission of the ultrasound through the liquid media. However, high viscosity samples may require higher amplitude in order to obtain the necessary mechanical vibrations and to promote cavitation in the sample thereafter (Santos et al., 2009).

The type of cavitation is also related to the ultrasonic intensity and consequently to the amplitude applied. When intensity exceeds 10 W/cm^2 a transient cavitation occurs. In this range bubbles expand through a few acoustic cycles to a radius of at least twice their initial size before collapsing violently on compression (Santos et al., 2009). On the other hand cavitation bubbles' number and collapse are influenced by the temperature of the reaction medium. Temperature affects the vapor pressure, surface tension, and viscosity of the liquid medium (Muthukumaran et al., 2006). In a higher temperature cavitation bubbles form more easily, because of medium viscosity decrease, but their collapse may be cushioned or dampened by the high vapor pressure (Muthukumaran et al., 2006; Zhou and Shi, 2006). Thus, there is an optimum temperature at which viscosity is low enough to enhance cavitation bubbles formation, yet the temperature is low enough to avoid the dampering effect (Moncada Reyes, 2011).

In food systems, when ingredients degradation and structure change may occur by small changes in the ultrasonication process, a careful choice of process parameters helps to gain scientific and technological knowledge, but also to prevent undesirable quality effects. In this research different sonication parameters (time and amplitude) were applied on a whey protein emulsion containing olive oil and xanthan gum produced in a batch-type process. Extra virgin olive oil was selected for nutritional reasons and health benefits related to its composition in combination with the fact that presents great stability under ultrasonication. No oxidation or hydrolysis effects were observed when ultrasounds (20 kHz, 120 s) were applied during extraction of oil procedure from olive paste (Jiménez et al., 2007). Additionally, in comparison to other edible oils that are primarily used in salad dressings such as corn and sunflower

oil, olive oil presented the highest absence of off-flavoring after sonication which is related to the composition of the unsaturated fatty acids.

The formulation of these model o/w emulsions resembles that of a low fat and cholesterol-free mayonnaise or salad dressing. The main objective of this study was to establish an optimum of sonication conditions that lead to maximum stability emphasizing on gum viscosity characteristics and droplet size. For this reason, experimental findings relating gum viscosity, emulsion stability and droplet size with ultrasonic parameters are discussed.

4.2 Materials and methods

4.2.1 Materials

Whey protein isolate (WPI) Lacprodan DI-9224 was kindly provided by Arla (Arla Foods Ingredients, Amba-Denmark). The composition of the WPI was 92 ± 2 % protein, maximum amounts of fat 0.2 %, ash 4.5 % and lactose 0.2 % as stated by the manufacturer. Xanthan gum XG was obtained from Sigma (St. Louis, MO, USA). Virgin olive oil Altis (Elais Unilever, Greece) was purchased from a local store. Citric acid, phosphate and sodium azide were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland).

4.2.2 Emulsion preparation

Whey protein isolate stock solution 10 %wt in citric acid-phosphate buffer at pH 3.8 was prepared by agitation with a magnetic stirrer for 90 min. Sodium azide 0.02 %wt was added to the aqueous solutions as an antibacterial agent. Xanthan gum solution 1 %wt was prepared by hot stirring in a water bath at 90 ^{o}C for 90 min. Thereafter, solutions were kept overnight at 5 ^{o}C to ensure complete hydration. Coarse emulsions containing 2.7 %wt WPI, 20 %wt extra virgin olive oil and 0.25 %wt XG of total 50 g were prepared by mixing appropriate amounts of the aqueous stock solutions and oil with a high-shear device Ultraturrax T25 device (IKA Werke, Staufen, Germany) at 13.500 rpm for 2 min. Small adjustments on emulsion pH were made with a few drops of HCl 1 M. 40 ml of the coarse emulsion were placed in a glass beaker (38 mm internal diameter) covered by ice to prevent overheating (< 50 ^{o}C). Secondary emulsions were produced following a batch-type sonication approach. The ultrasonic tip of a 13 mm diameter cylindrical titanium probe (VS 70T) was immersed in the centre of the glass beaker at a repeatable depth of 1 cm and different

sonication treatments were applied generated from an ultrasonic device (Sonopuls 3200, Bandelin GmbhandCo, Berlin, Germany) operating at constant frequency of 20 kHz. The sonication device operates by controlling either the percentage of amplitude (100 % amplitude corresponding to 170 μm_{ss} for the specific probe used) or power (150 W nominal maximum power). It also has the ability to display or monitor the energy input (kJ) in the sample during sonication. Sonication treatments were applied by controlling the percentage of amplitude (40, 60, 80 and 100 %) for constant time (1 min) and time (1, 2, 3 and 4 min) at constant amplitude (70 %). The 70 % amplitude was identified as the maximum applicable percentage that could be used for elongated emulsification (up to 4 min) in order to avoid overheating and subsequent protein denaturation. The nominal total energy applied on the system was recorded from the device readings and a digital thermometer was used to measure the temperature of the emulsion at the end of the sonication.

4.2.3 Droplet size evaluation

Optical microscopy is arguably the most precise among the existing general methods for drop-size determination, but it is time consuming, so it is used mainly in scientific studies in which very accurate results are needed (Denkova et al., 2004). Oil droplet size measurements were performed by means of optical microphotography and image analysis software processing (Krstonošić et al., 2009; Tonon et al., 2011; Zúñiga et al., 2013). The final emulsions were diluted with the same buffer solution (pH 3.8) at a 20:1 ratio and several micrographs were recorded from an optical microscope (Kruss Optronik, Germany) with a 40x magnification connected with a camera (SONY, Topica TP-1002DS). The oil droplets' diameters were measured with an image analysis software (Image-Pro Plus 7.0, Media Cybernetics, Rockville USA) and conversion of micrographs' pixels to μm were made by using the micrometer ruler of the microscope. In total, a number of 1000-1200 droplets were measured and results are demonstrated as the arithmetic median (d_{50}), the volume-surface mean diameter or Sauter diameter ($d_{32} = \Sigma n_i\ d_i^3/\Sigma n_i\ d_i^2$) that represents the average size based on the specific surface per unit volume and better characterizes small and spherical particles (Zúñiga et al., 2013) and the equivalent volume mean diameter or De Brouckere diameter ($d_{43} = \Sigma n_i\ d_i^4/\Sigma n_i\ d_i^3$), where n_i is the number of droplets of diameter d_i (Galazka et al., 1996; Huang et al., 2001; Leroux et al., 2003; Tonon et al., 2011). d_{43} is more sensitive to the presence of large particles in an emulsion (Walstra,

2003), so it is more sensitive to fat droplet aggregation (Huck-Iriart et al., 2011; Relkin and Sourdet, 2005). Therefore d_{43} could give a good indication about droplet re-coalescence. An analytical mathematical explanation for the above attribute of the de Brouckere mean diameter could be found in Rawle (2002). The procedure was at least duplicated with differently prepared samples.

4.2.4 Emulsion stability

Emulsion stability was evaluated with a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France) during 10 days of storage at 5 ^{o}C. Emulsion samples of about 6 ml were put in a glass tube and scanned from bottom to 80 mm tube height on a daily basis in order to obtain back scattering and transmission profiles that allow the calculation of instability due to creaming, sedimentation, coalescence as well as other phenomena.

The BS profiles were used to monitor the creaming kinetics (clarification) at the bottom of the tubes (6-20 mm zone) during cold storage. This zone was not affected by other destabilization phenomena. The obtained data was expressed as a percentage of the average backscattering intensity (% BS) and plotted against storage time.

4.2.5 Viscosity measurements

Viscosity flow curves of 1 %wt XG aqueous solutions were obtained using a controlled stress SR-5 rheometer (Universal Stress Rheometer/Rheometrics Scientific, Inc., NJ) with plate-plate geometry and a 0.2 mm gap. The diameter of the upper plate was 20 mm. The temperature was maintained at 25 ± 0.2 ^{o}C by circulating water from a constant temperature circulator. Flow curves (steady shear measurements) were obtained under controlled stress in the range of 0.5-50 Pa. All measurements were performed in freshly prepared samples before serum phase separation was observed. The linear part of the viscosity (η) curves was fitted in a Power-law or Herschel-Buckley model according to the maximum R^2 values per sample achieved:

$$\eta = k\gamma^{n-1} \qquad \text{Eq. 4.1}$$

$$\eta = \frac{\tau_o}{\gamma} + k'\gamma^{n-1} \qquad \text{Eq. 4.2}$$

where, γ is the shear rate, τ_o the yield stress, n is flow behavior index and *k, k'* are consistency indices to obtain rheological (*n* and *k, k'*) and statistical (R^2) parameters. *K,k', τ_o* and *n* values derived from Eq. 4.1 and 4.2 are reported as the average of 2-3 separately prepared samples.

4.2.6 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and the F-test was applied in order to compare the mean values of emulsions and XG solutions properties at 95 % level of confidence.

4.3 Results and discussion

4.3.1 Sono-emulsification conditions

Energy input and final temperature of the samples derived from the different treatments are presented in Table 4.1. It should be noted that the initial temperature of the samples was ~10 ^oC. It is shown that by varying either the amplitude or the duration of sonication treatment, the energy released in samples follows a linear behavior (R^2 = 0.997 and 0.999), while the temperature of the samples at the end of the emulsification was exponentially influenced (R^2 = 0.997 and 0.999) according to amplitude and time change. From the slope of the energy curves it can be seen that the energy input rate on the emulsions is much faster by increasing the amplitude but the sonication time is restricted. As a consequence the emulsification is conducted in much higher temperatures leading to heating of the emulsions up to ~ 44 ^oC after 4 min at 70 % amplitude. On the contrary, in all cases of amplitude increments between 40 – 100 % and constant time the final temperature was kept below ~ 32 ^oC. The effect of energy input and emulsification temperature (even though it was not kept constant in this batch type method) on emulsion stability attributes is discussed in the following paragraphs.

Table 4.1 Nominal energy input applied, intensity and temperature rise during emulsification by various sonication treatments.

Sonication treatment	Energy (kJ)	Ultrasonic intensity (W/cm^2)	Equation	R^2	Temperature (^oC)	Equation	R^2
Amplitude percentage (%) (at constant time = 1min)							
40	3.48 ± 0.08	43.72	E = 0.0832xAmp. + 0.129*	0.997	16.00 ± 0.94	$T=1.142xAmp.^{0.717}$	0.997
60	5.00 ± 0.24	62.81			21.91 ± 1.87		
80	6.92 ± 0.11	86.94			26.02 ± 0.80		
100	8.39 ± 0.24	105.40			31.20 ± 0.36		
Time (min) (at constant amplitude percentage =70%)							
1	6.03 ± 0.09	75.75	E = 5.696xTime + 0.4148	0.999	25.10 ± 1.85	$T=25.182xTime^{0.407}$	0.999
2	11.86 ± 0.26	74.50			33.55 ± 1.76		
3	17.63 ± 0.16	73.83			39.60 ± 0.26		
4	23.09 ± 0.22	72.52			44.03 ± 0.47		

*E is energy (kJ) and Amp. the amplitude percentage (%).

4.3.2 Influence of sonication treatment on emulsions and XG solutions viscosity

Ultrasonication and other high shear treatments such as high pressure homogenization have been reported to influence the flow behavior of hydrocolloid dispersions due to reduction of their molecular weight (Price, 1990; Marcotte et al., 2001; Syed Gulrez et al., 2012). The degradation of the macromolecules is considered of "mechanochemical" nature (Striegel, 2003), since the depolymerization reactions that lead to chain cleavage can follow distinct mechanisms such as homolytic (formation of free macro-radicals), heterolytic (oppositively charged macro-ions) or intramolecular degradation (stable macro-fragments) (Stellbrink et al., 1998). Ultrasonic degradation of xanthan gum solutions leads to samples characterized by a low polydispersity and a narrow range of molecular weight that can be representative for the given sample (Milas et al., 1986). Apparent viscosities of XG solutions as affected by sonication treatments are demonstrated in Figure 4.1. Consistency (*k, k'*) and flow behavior (*n*) indices for all samples at each amplitude-time level are presented in Table 4.2. Flow curves and flow parameters indicate that all samples exhibit shear thinning behavior at all ultrasonic amplitudes and duration levels. The experimental results show that the viscosity of polymer solutions and final emulsions decreased with an increase in the ultrasonication time and amplitude and approached a limiting value after 4 min of sonication (Fig. 4.1, Table 4.2). Solutions of untreated 1 %wt XG solutions are characterized by high consistency (k' = 24.0 Pa-s^n) and low flow behavior index (n = 0.181), which dramatically changed even when the mildest ultrasonication treatment (40 % amplitude-1 min) was applied ($p<0.05$). Also, the yield stress, characteristic for xanthan solutions disappears at amplitude values greater than 40 % (Fig. 4.1). The sonication introduced a decreased shear thinning behavior, but still not Newtonian since maximum $n << 1$ for both solutions and emulsions. In particular, emulsions presented greater *n* values, indicative for a less shear-thinning behavior due to lower concentration of XG used in the formulation (0.25 %). This trend is in accordance with the findings of Tiwari et al. (2010), who studied the influence of high intensity ultrasound on the rheological characteristics of guar, xanthan and pectin dispersions. They have concluded that the reduction of *k v*alues in XG solutions depended strongly on intensity magnitude (W/cm^2) and followed a linear decrease regression with increasing intensity level. Similarly to our findings, as

discussed below, the consistency index was reduced by 89.7 % at the highest intensity input (10.1 W/cm^2) compared to control samples.

In our experiments it was found that ultrasonication time increase (at constant amplitude 70 %) had as result a more pronounced effect on polymer viscosity compared to that obtained by amplitude increments (Table 4.2). Thus, in solutions, a consistency reduction of 77 % was observed by amplitude increase from 40 to 100 % and 81 % by time increase from 1 min to 4 min, whereas in emulsion *k* reduction was 45 and 52 % respectively. Considering the flow index values the greatest changes were observed for XG solutions sonicated for elongated time (4 min), for which n = 0.499. Nevertheless, for emulsions treated with the maximum amplitude or time for which n = 0.490 and 0.552 respectively no significant difference was found ($p>0.05$).

In Figure 4.2 k values are presented as a function of energy applied on the system in order to better understand the influence of amplitude and sonication duration on samples consistency. Consistency values of gum solutions and emulsions followed a power-law decrease as a function of amplitude and time, as can be seen. It is also shown that the consistency change rate (slope) of xanthan solutions is much greater, almost double, by increasing the amplitude than by increasing the sonication time. The same trend is observed by comparing emulsions' consistency, although the slopes are similar. In the case of *n* values, although a clear trend was identified as discussed above, the correlation of models was poor to demonstrate in most cases ($R^2< 0.9$).

Regarding the role of sonication temperature on viscosity properties over the range of 40-60 ^oC it is considered negligible (Karaman et al., 2012). However, in another research related to the effect of ultrasonics on chitosan fragmentation it has been concluded that the chain scissions were strongly affected by sonication power as well as by the solution temperature (Kasaai et al., 2008). Nevertheless, the influence of temperature in the case of xanthan could be considered minor since it displays a structural stability over a wide range of temperature and shifts from an ordered to disordered structure at temperatures above 40 ^oC (Kang and Pettit, 1993; Casas et al., 2000).

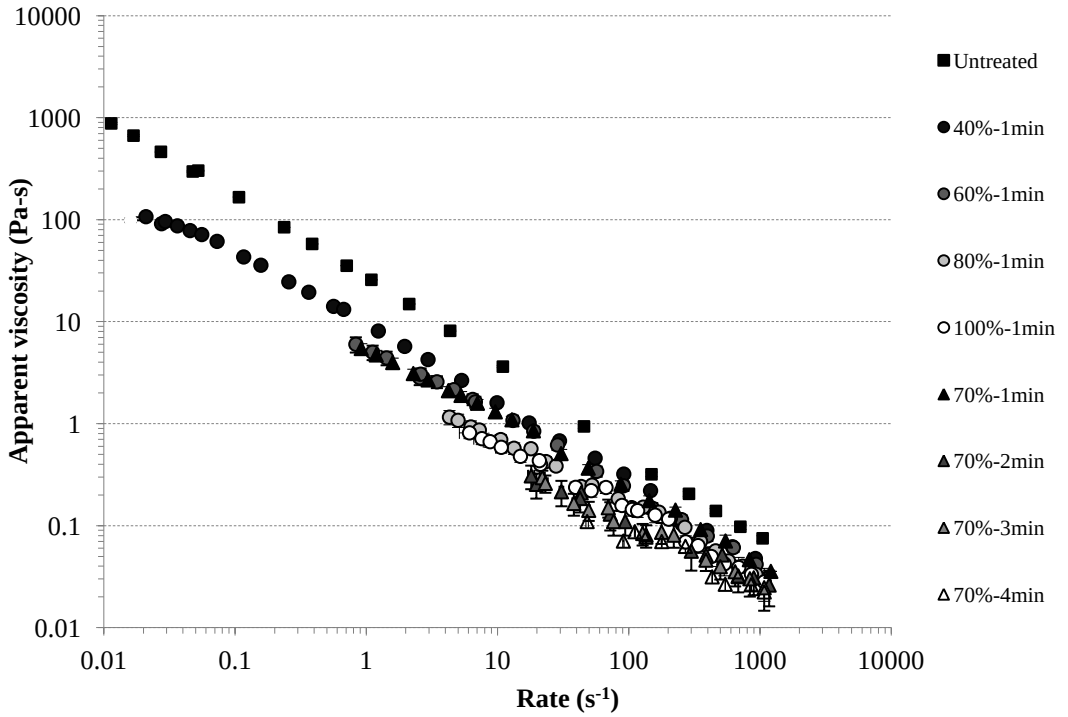


Figure 4.1 Influence of various sonication treatments on the flow behavior of XG 1 %wt solutions: ■ untreated sample, as affected by amplitude (● up to ○), or time (▲ up to Δ).

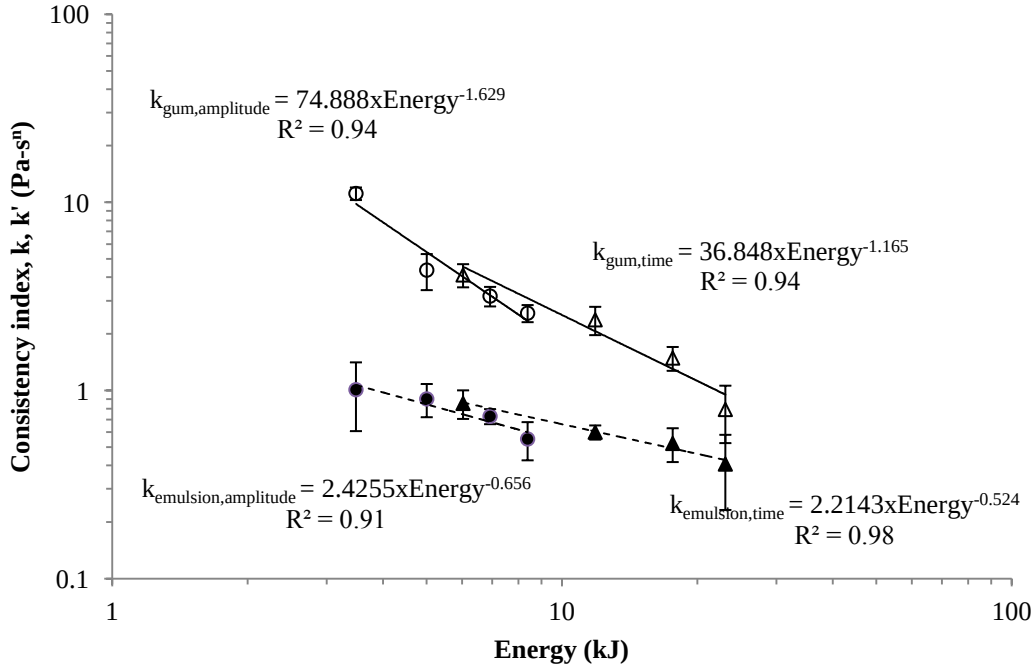


Figure 4.2 Consistency index (*k*) of XG 1 wt% solutions (Δ, ○) and emulsions (▲, ●) as a function of sonication energy applied. Circles represent amplitude (40-100 %) and triangles time increments (1-4 min).

Table 4.2. Flow behavior parameters of XG 1 %wt solutions and emulsions prepared with different sonication treatments.

	1 %wt XG solution			Emulsions	
	τ_o (Pa)	*k, k'* (Pa-s^n)*	*n* (-)*	*k, k'* (Pa-s^n)	*n* (-)*
Untreated	6.93 ± 0.188^{b}	24.00 ± 0.247^{f,**}	0.181 ± 0.008^{a,**}	-	-
Amplitude percentage (%), (at constant time = 1min)					
40	1.92 ^{a} ± 0.125	11.16 ^{e,**}±0.856	0.196 ^{a,**} ± 0.012	1.011 ^{c} ± 0.402	0.332 ^{a} ± 0.062
60	-	4.37 ^{d} ± 0.949	0.309 ^{b} ± 0.014	0.903 ^{cd} ± 0.181	0.461 ^{bc} ± 0.096
80	-	3.18 ^{cd} ± 0.378	0.331^{b} ± 0.024	0.730 ^{bcd} ± 0.067	0.477 ^{bc} ± 0.015
100	-	2.58^{c} ± 0.269	0.354 ^{bc} ± 0.011	0.553 ^{ab} ± 0.127	0.490 ^{bc} ± 0.028
Time (min), (at constant amplitude percentage = 70%)					
1	-	4.12 ^{d} ± 0.580	0.308 ^{b} ± 0.022	0.855 ^{bcd} ± 0.148	0.413 ^{ab} ± 0.052
2	-	2.38 ^{c} ± 0.408	0.359 ^{bc} ± 0.016	0.601 ^{abc} ± 0.052	0.514 ^{bc} ± 0.018
3	-	1.49 ^{b} ± 0.216	0.420 ^{c} ± 0.020	0.524 ^{a} ± 0.107	0.521 ^{c} ± 0.024
4	-	0.79 ^{a} ± 0.268	0.499 ^{d} ± 0.052	0.407 ^{a} ± 0.175	0.552 ^{c} ± 0.103

*Values followed by the same letters at the same column are not significantly different ($p>0.05$).

**Values estimated by Herschel-Buckley model.

R^2 ranging between 0.92-0.996

4.3.3 Influence of sonication treatment on droplet size

During the emulsification process two different phenomena (droplet disruption and re-coalescence) occur at the same time. The kinetics of each one individually affects the final droplet size of emulsions (Jafari et al., 2008). Thus, an optimum energy input should be established to avoid over-processing regarding droplet size increase (Kentish et al., 2008). The influence of sonication treatment on droplet size according to amplitude (%) and time increase is demonstrated in Figures 4.3-4.5.

As shown in Figure 4.3 the particle diameters of emulsions were decreased upon increase of amplitude and sonication duration. These differences were more evident in the case of d_{32} and d_{43}, whereas d_{50} values did not decrease remarkably and statistical differences were not noticed in most of the cases ($p>0.05$). According to Behrend et al. (2000), a logarithmic reduction of d_{32} occurs when increasing the energy density (energy per unit volume). In the present study size reduction was also reduced in a logarithmic way when increasing the energy input by means of amplitude and a limiting value was reached after ~7 kJ (80 % amplitude) (Fig. 4.3b). On the contrary when longer sonication was applied at 70 % amplitude the diameter decrease followed a linear trend. This suggests that ultrasonication, at these experimental conditions, is more efficient in decreasing the polydispersity of the emulsions rather than remarkably minimizing the size of the smaller droplets in this system. Furthermore, samples prepared at 40 % amplitude presented the highest values of d_{43} compared to samples prepared with all other treatments applied ($p<0.05$), meaning that over-processing in terms of re-coalescence was not observed even for samples treated with maximum amplitude or most elongated time. In another research it has been suggested that a lower amplitude percentage would be more appropriate in order for the protein to better adsorb at the interfacial area of droplet surface (Tang et al., 2012), thus decreasing more efficiently the final droplet size compared to higher amplitude. Apparently this has not been our case, assuming that the emulsification time at 40 % amplitude was restricted. An increase of amplitude from 40 to 100 % was able to decrease the median diameter of oil droplets from 1.432 to 0.943 μm. At the same time an increase in sonication time from 1 to 4 min led to a droplet size decrease from 1.141 to 0.891 μm assuming that the increase in amplitude is more effective in reducing the average droplet size of the samples. This is in agreement with previous findings about consistency and flow index change by changing the amplitude. An

explanation for the significant role of the amplitude change in droplet size is that as ultrasonication power is a measure of the pressure amplitude an increase in the applied pressure amplitude leads to more intensive cavitation. Then an increased number of bubbles are generated as a result of a more frequent liquid threads breaking and collapse. Additionally, the energy input in the system increases the temperature which facilitates the dispersion of both liquid phases into another by means of interfacial tension, viscosity and Laplace pressure decrease, thereby smaller droplets are produced; whereas at lower amplitude a wider droplet distribution is observed (Gaikwad and Pandit, 2008).

It can be noted that by increasing the amplitude or sonication time, differences between the d_{50}, d_{32} and d_{43} values decrease, thus similar values at 80 and 100 % amplitude and after 4 min of sonication are presented ($p>0.05$). Thus, upon increase of sonication duration the standard deviations of diameter values were noticeably decreased. This phenomenon is in accordance with the findings of other researches (Tang et al., 2013; Cucheval and Chow, 2008; Li and Fogler, 1978) and as mentioned indicative of a polydispersity reduction. On the other hand, this trend was not clearly identified when amplitude increased, therefore the repeatability of experiments is lower when emulsification duration is restricted at 1 min. It is obvious that increase in time and amplitude resulted in narrower droplet size distributions (Figures 4.4 and 4.5). For presentation reasons only data for 40 and 100 % amplitude and 1 and 4 min treatments by means of frequency histograms are demonstrated (Figure 4.4). Prolonged sonication induces sample mixing due to extended turbulent flow and consequently an effective disruption of most droplets is achieved. Therefore, the application of 4 min sonication resulted in finer emulsions with the minimum d_{43} value, although the decrease rate of droplet size was much greater by amplitude change rather than by sonication time increase. Coupled with the temperature rise that occurs at these conditions, which reduces the minimum thermodynamic energy necessary for emulsification, the production of smaller droplets is facilitated (Canselier et al., 2002; McClements, 2005).

Overall, no overprocessing thresholds could be detected among different treatments, when increasing sonication amplitude or when using extended sonication times, as evidenced by monomodal distributions and d_{43} values decrease.

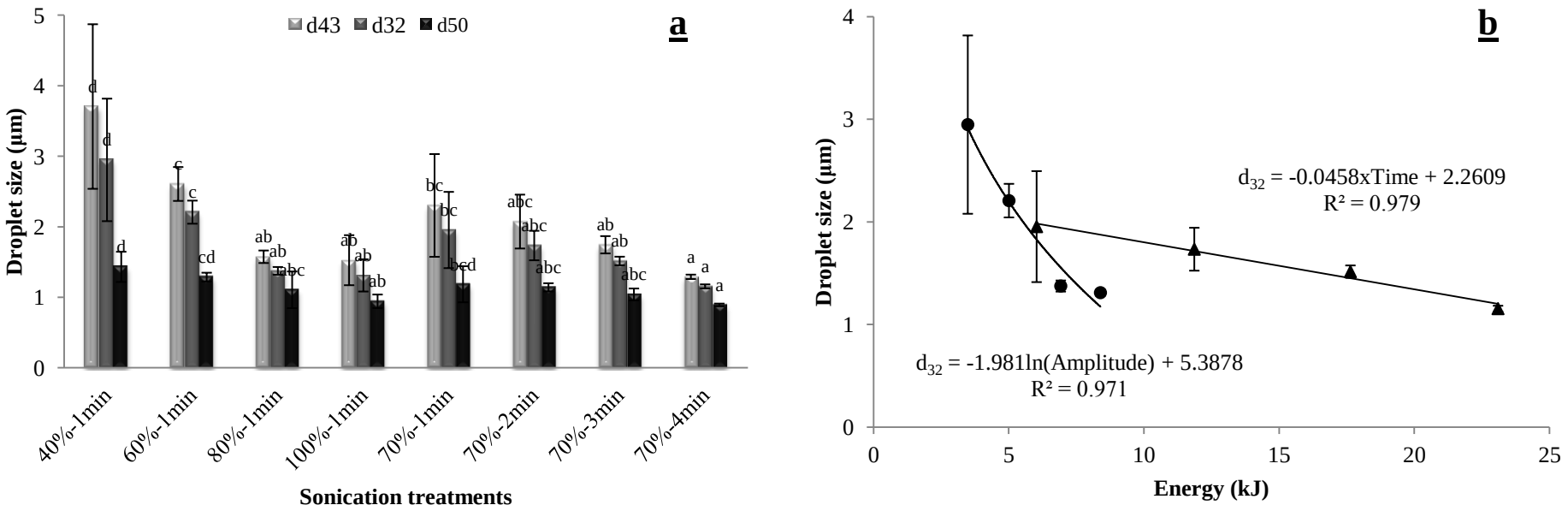


Figure 4.3 (a) Surface (d_{32}) and volume (d_{43}) weighted diameters and arithmetic median diameter (d_{50}) of freshly prepared emulsions as affected by various sonication treatments, (b) Sauter mean (d_{32}) diameters as a function of energy applied. Circles represent amplitude (40-100 %) and triangles time increments (1-4 min).

[a-d] Values followed by the same letters are not significantly different ($p>0.05$).

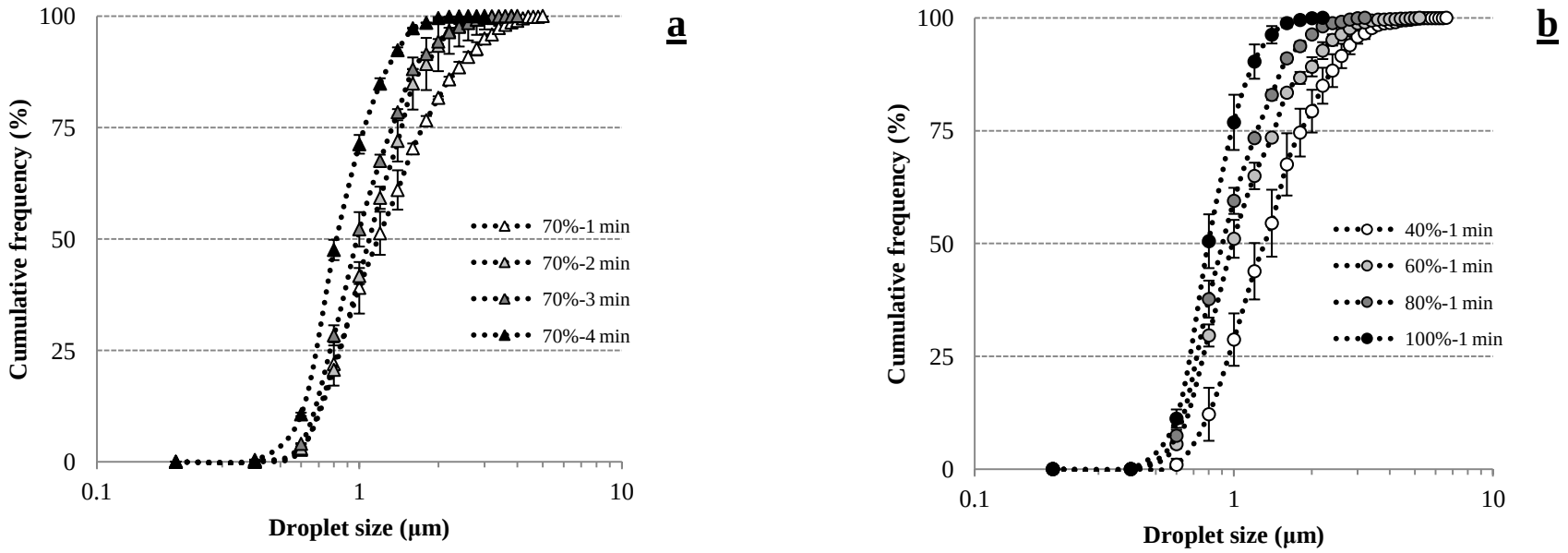


Figure 4.4 Droplet size cumulative distributions of emulsions as affected by sonication (a) amplitude at constant time 1 min and (b) time at constant amplitude 70 %.

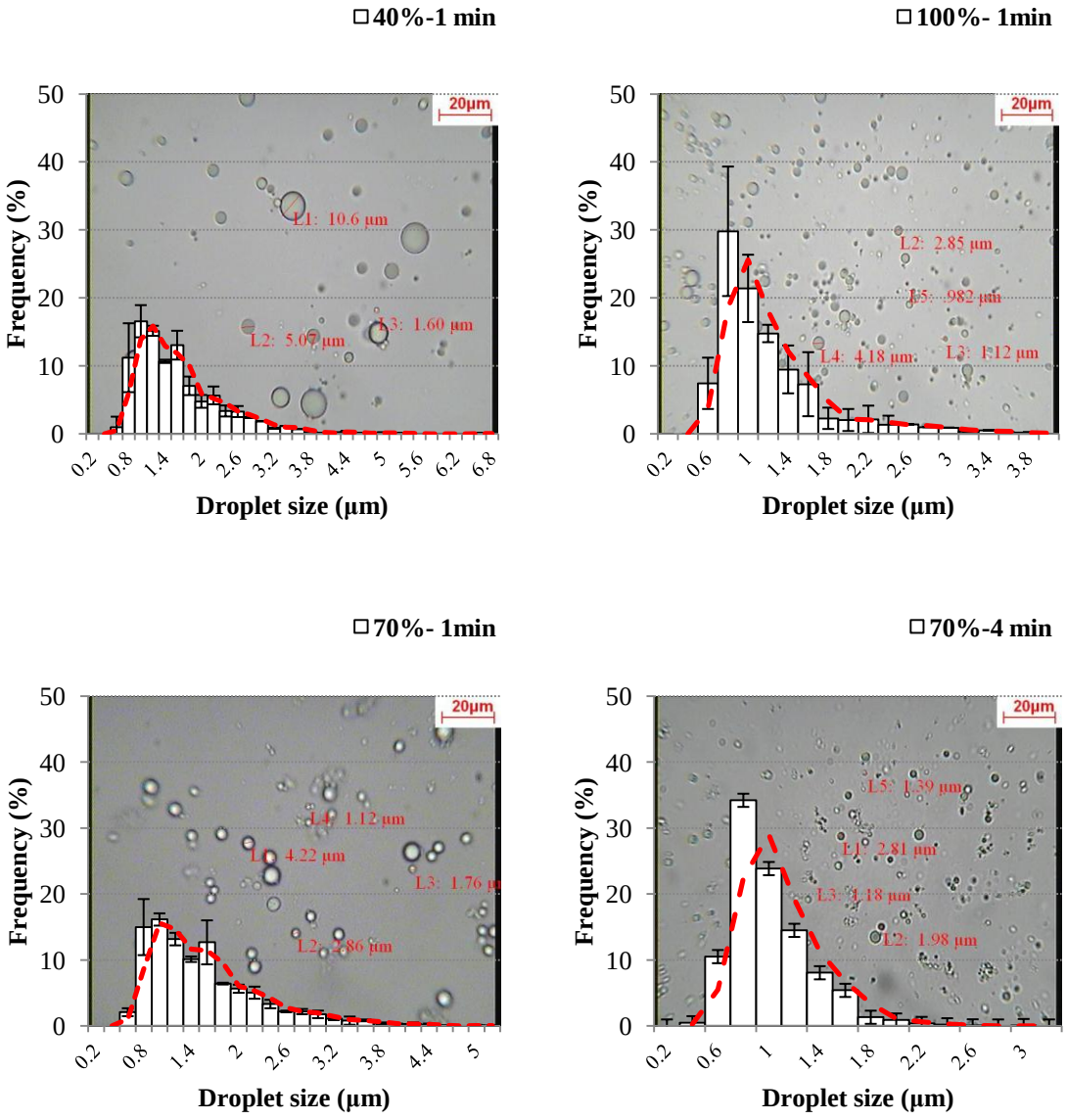


Figure 4.5 Distribution histograms of oil droplets of freshly prepared emulsions as affected by various sonication treatments. At the background: Micro-photographs processed by Image-Pro Plus 7.0. Scale bar at upper right side represents 20µm.

4.3.4 Influence of sonication treatment on emulsion stability

Turbiscan measurements gave the time evolution over a 10 day period of the back scattering intensity of emulsions along the tube height. The analysis of BS profiles as a function of time offers the advantage to allow a continue evaluation of the emulsion destabilization without previous dilution. The initial BS intensity (t=0) is a value related to the size and density of particles in emulsions; BS enhances as the increase of the number of particles, and decreases as a function of the size of

individual droplets and/or presence of flocs (Palazolo et al. 2004, 2005). Hence, creaming kinetics can be easily monitored by the decrease of BS intensity at the bottom of the tube due to decrease of the concentration of the particles in this part (clarification) (Fameau, 2012). It has also to be noted though that according to Mie's law, the BS intensity decreases when the droplet size increases only for particles with diameter larger than 0.5 μm (Mengual, 1999; Buron, 2009; Pizzino, 2009).

The creaming of emulsions was strongly affected by sonication duration and amplitude as seen in Figure 4.6. In the case of amplitude (Fig. 4.6a) there was an improvement of emulsion stability expressed by the increased BS at the bottom zone; from 49.4 % (40 % amplitude) to 64.2 % (100 % amplitude) after 10 days of storage. Emulsions prepared by varying sonication time (at constant 70 % amplitude) presented an even more pronounced stability (Fig. 6b). Hence, an increase in duration from 1 to 3 min led to an increase of the BS from 58.9 to 72.1 % accordingly. Extended sonication time (4 min, 70 %) seems to have a negligible influence over stability and this suggests a limiting boundary since the BS was only slightly increased to 72.7 % ($p > 0.05$). The marginal increase in BS occurring could be due to the decrease in viscosity of the continuous phase (Figure 4.1, Table 4.2), since the oil droplet size was further decreased, and re-coalescence (Jafari et al., 2006; Olson et al., 2004) was not observed. From Stokes' Law, $v_s = D^2 g(\rho_p - \rho_f)/18\mu$, where v_s the rising velocity of the droplet (m/s), g the gravitational constant (9.81 m/s^{-2}), D the particle diameter (m), ρ_p the density of settling particle and ρ_f the density of the continuous phase (kg/m^3) and μ the dynamic viscosity (Pa-s) provides a qualitative indication of the physical factors that influence the stability of an emulsion. The critical importance of particle size, occurring as a squared term, can be derived as well as why emulsions are more stable when density differences are small and when the viscosity of the continuous phase is high. However, in our case, even low viscosities values resulted in improved stability but there is a threshold value to assume that. This is because the relative flow of the particles under gravitational forces may break the emulsion, so stability is enhanced by small settling velocities. It should be noted that a small delay in creaming was observed when 3 and 4 min of sonication was applied, thus emulsions started to destabilize after 3 days of storage. Creaming delay was observed in emulsions, where different amplitude increments were applied, only at 100 % amplitude restricted at the first day of storage. In all the other cases of amplitude treatments, a sharp diminution of BS was observed even after 24 hours of storage,

corresponding to phase separation. Apart from decreasing the average droplet size it has been shown that sonication treatment (40 % amplitude, 30 s, 20 kHz) was used to improve the stability of emulsions containing casein against creaming by disrupting the flocs formed upon acidification of emulsions from pH 7 to pH 3 which enhanced electrostatic repulsion (Surh et al., 2006). Finally, among different treatments studied, the 100 % - 1 min sonication presented similar effects as the 70 %-2 min in terms of emulsion stability, XG solution and emulsion viscosity and droplet size, owing to similar values of energy input.

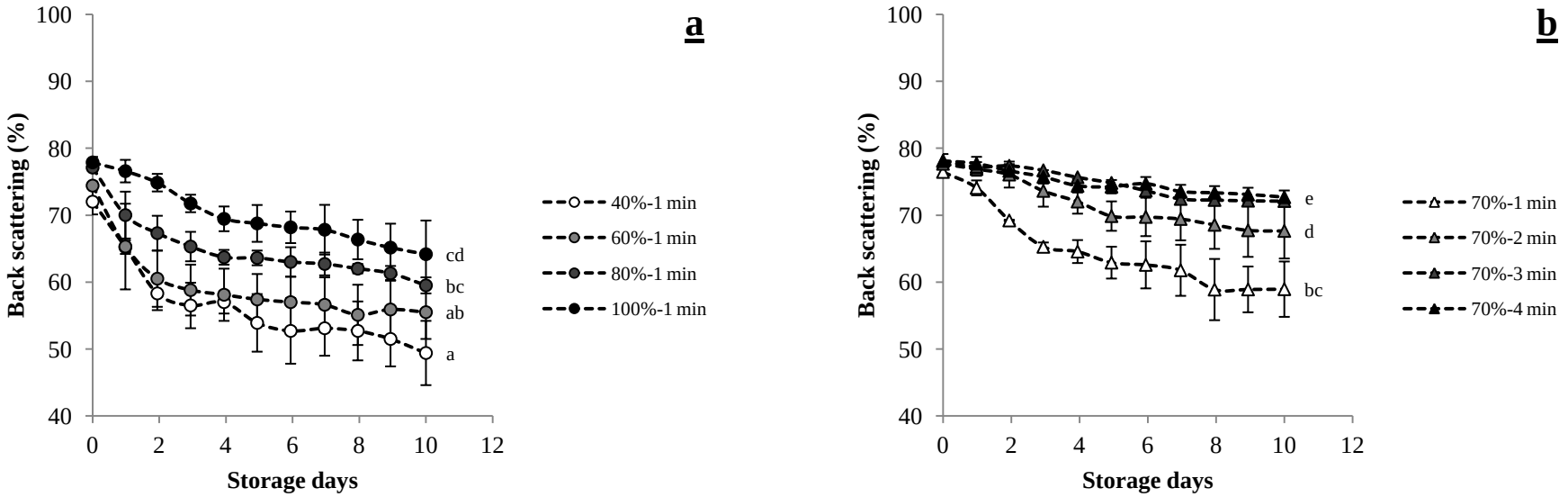


Figure 4.6 Creaming kinetics (clarification) of emulsions during cold storage (5 ^{o}C) as affected by sonication (a) amplitude at constant time 1 min and (b) time at constant amplitude 70 %.

[a-d] Values followed by the same letters are not significantly different ($p>0.05$).

4.4 Conclusions

An ultrasonication process application, in which the pressure amplitude (40-100 % for 1 min) and the sonication time (70 % amplitude, 1-4 min) varied, resulted in emulsions treated in different ways. The increase in either of amplitude or time improved sample stability and led to a significant reduction of droplet size and viscosity. The impact of the varied parameters on emulsion quality became less pronounced as variables values approached maximum limits and differences among samples produced at higher amplitudes applied (80 % and 100 %) or prolonged times (3 or 4 min) were not great ($p<0.05$) in most of the quality parameters measured. However, no overprocessing conditions in terms of recoalescence could be identified under application of amplitude or time extremes.

Optimum emulsification conditions were neither at highest amplitude values applied nor at the greatest sonication time used. Amplitude increase at maximum did not reduce remarkably droplet polydispersity, for which increase in time applied was required. Temperature increase was also noticed. Thus, a sonication time at 3 min is more appropriate at amplitude values of 70 %. Viscosity measurements showed that stability did not reduce at low viscosity values resulted after longer treatments. However, a very low viscosity is responsible for instability increase (70 %, 4 min) during storage. In terms of energy saving, the 100 % - 1 min treatment would be more preferable compared to 70 % - 2 min, since the time of sonication would be decreased by 50 % and energy required by 29 %.

CHAPTER 5

EFFECT OF SONICATION ON THE PROPERTIES OF EMULSIONS CONTAINING WHEY OR PEA PROTEIN.

5.1 Introduction

Within the last few years there has been an increasing interest for the replacement of commonly used emulsifiers in the food sector, such as egg protein, soy lecithin, or low molecular weight emulsifiers by proteins of plant origin, especially from pulses. This is derived by both consumer preferences as well their relatively lower cost compared to animal proteins (Stone et al., 2014). For example milk proteins are not suitable for use in a vegan diet or observers of the Kosher diet laws.

The ability of pea proteins to stabilize oil-in-water emulsions is related to the surface properties of their constitutive protein units: the storage globulins 7S (vicilin) and 11S (legumin) (Guéguen, 1989), which lead to the decrease of the interfacial tension between oil and water and the formation of a rigid membrane at the oil interface (Ducel, Richard, Popineau, & Boury, 2004a; Ducel, Richard, Saulnier, Popineau, Boury, 2004b).

The main draw-back of using plant proteins is their relatively poor functional properties related to emulsion formation (interfacial tension and elasticity, solubility, emulsifying activity and stability) in comparison to casein, bovine serum albumin and milk whey proteins (Popineau et al., 2002; Karaka et al., 2011; Arzeni et al., 2012; Amine et al., 2014).

Ultrasonication represents a well commercialized technique in the field of emulsified products such as mayonnaise or dressings due to several reasons including large scale equipment availability, high efficiency, low energy and maintenance cost and installation easiness (Patist and Bates, 2008).

Eventhough there are several studies on plant protein emulsions formed in the presence of polysaccharides (Makri et al., 2006; Martínez et al., 2007; Klassen and Nickerson, 2012; Qiu et al., 2015; Varankovich et al., 2015), there are no information regarding the influence of sonication on salad dressing model emulsions containing pea protein as emulsifier.

The objective of this research was to compare the stability properties of pea and whey protein isolates in salad dressing model emulsions containing xanthan prepared by conventional homogenization (high shear) and ultrasonication, as well as to examine the possibility of submicron droplets formation.

5.2 Materials and methods

5.2.1 Materials

Pea protein isolates Nutralys® S85F and F85M were a kind gift from Roquette Freres SA (Lestrem, France). Whey protein isolate (WPI) Lacprodan DI-9224 was kindly provided by Arla (Arla Foods Ingredients, Amba-Denmark). Xanthan gum (XG) was bought from Sigma (St. Louis, MO, USA). Virgin olive oil Altis (Elais Unilever, Greece) was purchased from a local store. Citric acid, phosphate and sodium azide were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland).

5.2.2 Protein content

Total nitrogen contents were determined by Dumas method using a flash EA 1112 NC analyzer (Thermo Fisher Scientific Inc., Waltham, Ma, USA) and 6.25 was used as protein conversion factor (Koocheki et al., 2009).

5.2.3 Solubility

For the estimation of isolates solubility 2 g of WPI and adjusted PPI amounts to equal amounts of protein were stirred for 30 min in deionized water (100 g total mass). Then the pH was adjusted to values from 2 up to 7 with NaOH or HCL 1 M and the mixture was allowed to stir for another 30 min (Liang and Tang, 2013). The mixture was centrifuged for 15 min at 3000 rpm, the supernatant was removed and the sediment was air dried (90 min/105 ^{o}C). Solubility was calculated as in Panaras et al (2011) according to Eqs. (5.1) and (5.2).

$$\text{Insolubility (\%)} = \frac{\text{weight of dried insoluble sample}}{\text{sample weight}} \text{x} 100 \qquad \text{Eq. 5.1}$$

$$\text{Solubility (\%)} = 100 - \text{Insolubility (\%)} \qquad \text{Eq. 5.2}$$

5.2.4 Surface tension

The surface tension of WPI solution 1 % wt in citric acid-phosphate buffer at pH 3.8 and PPI containing equal amount of protein was measured by using the Du Nouy ring method via a Kruss tensiometer (Hamburg, Germany) at 23 °C. A platinum ring was used, rinsed with MilliQ water and if necessary with ethanol and then passed through a Bunsen flame and left to cool before each measurement. The samples were

placed in the apparatus and left undisturbed for 30 min before the measurements were carried out.

5.2.5 Solution preparation

Pea and whey protein stock solutions 10 %wt in citric acid-phosphate buffer at pH 3.8 were prepared by agitation with a magnetic stirrer for 90 min. Xanthan gum solutions 1 %wt were prepared by hot stirring in a water bath at 90 ^{o}C for 90 min. Thereafter, solutions were kept overnight at 5 ^{o}C to ensure complete hydration. Sodium azide 0.02 %wt was added to the aqueous solutions as an antibacterial agent.

5.2.6 Preparation of emulsions

Coarse o/w emulsions (50 g) containing 5, 10 or 20 %wt olive oil and 0.5 %wt XG and appropriate protein isolate solutions were prepared by homogenizing the oil and aqueous phases using an Ultra Turrax (T25 basic, Janke & Kunkel IKA Labortechnik, Staufen, Germany) for 2 min (13.500 RPM) at room temperature. Emulsions contained 5, 10 and 20 %wt olive oil and 1 %wt WPI. For emulsions containing PPI, adjustments were made in order to contain the same amount of protein as the WPI emulsions.

Both WPI and PPI coarse emulsions were further subjected to ultrasonication in order to produce finer emulsions. In this section, a cooling system was used to control temperature rise during sonication, hence allowing longer processing duration compared to the previous experiments. 10 ml of the coarse premixes with an ultrasonic homogenizer model Sonopuls 3200 (Bandelin Electronic Gmbh & Co, Berlin) equipped with a 3 mm in diameter microtip (MS 73, 245 μm_{ss} peak-to peak amplitude). The tip was immersed 10 mm from the surface of the sample which was placed in a double jacketed flow-through vessel (model DG-3). The temperature was maintained at 30 (± 1) ^{o}C by circulating cold water with a pump. The amplitude was set at 40 %. Although the maximum allowed amplitude of use for the specific probe was 65 % it was noticed that higher amplitudes led to increased fluctuations in the vessel which caused spilling of the sample. The ultrasonication processing time was 1, 2, 4, and 8 min. All samples were prepared in triplicate.

5.2.7 Droplet size evaluation

Droplet-size distributions of the emulsions were determined by using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Emulsion droplets were characterized under high dilution conditions by dispersing the samples in acidified water (pH 3.8). The refractive indices of water and olive oil were taken as 1.330 and 1.467 (Paximada et al., 2014), respectively, and the Mie theory was used for the analysis. Average droplet sizes were characterized in terms of the volume mean diameter d_{50} and polydispersity (Span).

$$\text{Span} = (D_{90}-D_{10})/D_{50,} \qquad \text{Eq. 5.3}$$

Where, D_{10} and D_{90}, and D_{50} are the mean diameters at the 90, 10 and 50 % of the cumulative distribution volume. Measurements were made at ambient temperature on at least two separately prepared samples, and the results are expressed as the average value.

5.2.8 Emulsion stability

The storage stability (at 5 ^{o}C) dressing emulsions was determined by obtaining backscattering profiles with a Turbiscan 2000 device (Turbiscan 2000, Formulaction, Toulouse). For unstable emulsions the serum percentage (% Serum) was calculated as in Chapter 2. For stable samples (macro- and nanoemulsions), the average value of the backscattering (BS) at the middle zone of the cell (between 30 and 40 mm of sample height) has been plotted as a function of ageing time to monitor the occurring destabilization phenomena (coalescence and flocculation) and its respective variation (dBS) was estimated as in Chapter 2.

5.2.9 Emulsion rheology

Viscosity measurements were performed on coarse emulsions as well as selected emulsions which exhibited the highest stability. A hybrid rheometer DHR (TA Instruments) equipped with plate-plate geometry (upper plate diameter 50 mm, gap 1 mm) was used to obtain flow curves between 0.1 to 1000 s^{-1} at 25 ± 0.01 ^{o}C by circulating water from a peltier. The total measurement time was 900 s. All measurements were performed at least in triplicate and data reported as average and standard deviations. The Power law model was used to calculate the consistency (k)

and flow behavior index (n) of the most stable emulsions with TRIOS software (TA Instruments).

The viscoelastic behavior of selected emulsions which exhibited the highest stability was also investigated. The linear viscoelastic region (LVR) was assessed at 6.28 rad/s (= 1 Hz) by amplitude sweep experiments; for all samples a constant deformation of $\gamma = 0.1$ was used, which was within the linear viscoelastic region of all the samples. Small deformation oscillatory measurements for evaluation of the viscoelastic properties, G′ (storage modulus), G″ (loss modulus), and tanδ (G″/G′), were performed over the frequency range of 0.1–100 rad/s at 25 °C. Deviation from the linear viscoelastic region occurs when the sample starts to permanently deform, implying the destruction of the transient network structure.

5.2.10 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and F-test was applied in order to compare the mean values at 95% level of confidence.

5.3 Results and discussion

5.3.1 Surface activity and solubility of protein isolates

Whey protein isolate demonstrated superior emulsifying properties in comparison to plant derived PPI, since it exhibited improved surface activity (Fig. 5.1) and higher solubility in a range of pH (Fig. 5.2).

The solubility profile of PPI appeared “bell shaped” and pH-dependant with values ranging between 11.8 to 24 %, and solubility minimum in the pH region near 4 to 5 (Fernadez-Quintela et al., 1997; Liang and Tang, 2013). Our results are in accordance to those reported in several studies regarding the solubility of commercial PPIs (Shand et al., 2007; Taherian et al., 2011; Adebiyi and Aluko 2011). According to Adebiyi (2011) commercial PPIs consist of different protein fractions depending on their solubility in water and aqueous ethanol, NaCl or NaOH solutions. It was shown that among different protein fractions the water-soluble fraction was characterized by high solubility within the pH range (3-8), whereas the opposite was reported for the salt and alkaline soluble fractions. According to the same research, despite the fact that the water soluble fraction was highly soluble (>80%), it only represents a small

percentage of the total PPI (~ 7 %), while the dominant fraction is the alkaline one (~ 87 %), hence resulting in poor solubility of the commercial PPI.

WPI presented a solubility minimum of 93.6 % at pH 5, which is near the typical value of whey proteins' pI ~ 4.5 (Pelegrine and Gasparetto, 2005), but still well above the maximum observed solubility of the PPIs at pH 2 and 7. This is in accordance to other findings concerning pea and whey protein solubility (Jackman and Yada, 1989). In comparison to PPI it can be seen (Fig. 5.2) that WPI is more efficient in reducing the surface tension, as also pointed in relative comparative studies. More specifically, the capability of interfacial tension reduction order of β-lg (β-lactoglobulin) and pulse proteins was: β-lg >lupin >soy ≈pea (Benjamin et al., 2014). Additionally, Na-caseinate was able to reduce the interfacial tension more efficiently, since lower concentrations were required to reach a plateau at lower values in comparison to pea or potato proteins (Amine et al., 2014).

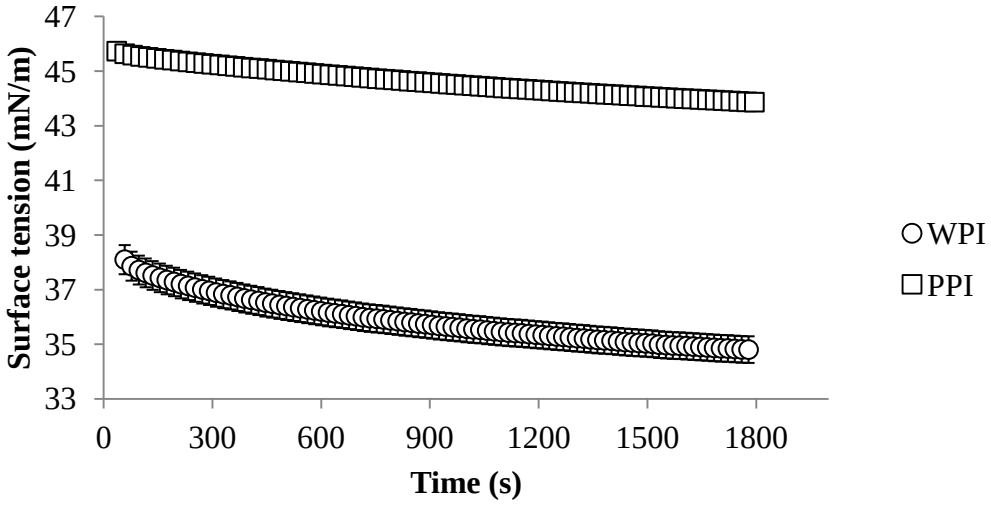


Figure 5.1 Surface tension of WPI and PPI solutions.

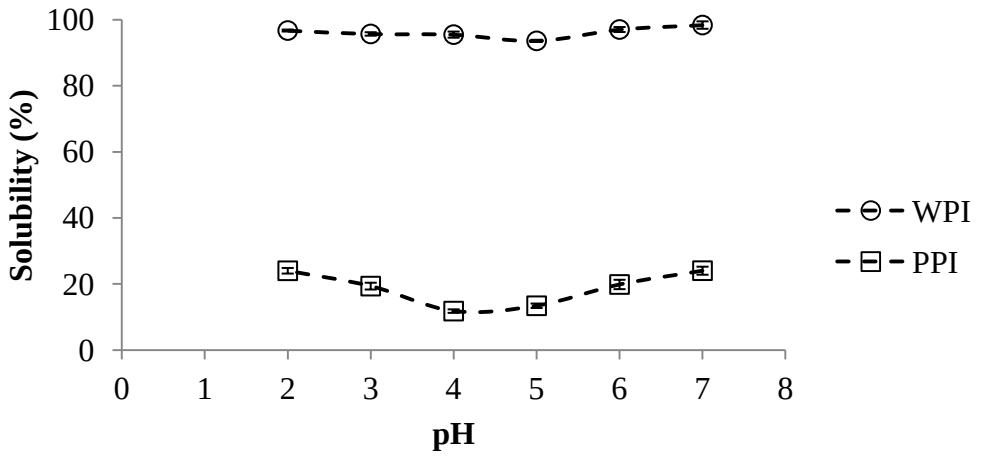


Figure 5.2 Solubility of WPI and PPI solutions as a function of pH.

5.3.2 Emulsion droplet size

In Table 5.1 the effect of homogenization method on WPI and PPI emulsions droplet size (D_{50}) and polydispersity (Span) is depicted. It can be noticed that coarse emulsions prepared with WPI resulted in emulsions characterized by smaller diameters ranging from 11.43 to 14.06 μm in comparison to PPI emulsions which ranged between 13.2 to 17.66 μm, depending on oil content. This is in accordance to other comparative studies concerning milk and plant proteins (Palazolo et al., 2004; Amine et al 2014) it was shown that finer particles and were obtained (at pH 7) for whey protein or caseinate emulsions in comparison to pea protein. This result in the case of caseinate was attributed to imrpoved properties such as lower interfacial tension, although data for whey protein are not demonstrated.

Additionally, as shown in Fig. 5.3 coarse emulsions prepared with PPI, exhibited higher viscosities in comparison to those with WPI. Significantly higher viscosity values of blends containing pea protein compared to whey protein (in the presence or absence of xanthan gum) have been reported elsewhere, resulting in a more structured and viscous mixtures (Tarrega et al., 2012). Therefore, the droplet disruption efficiency of the high shear device used for the preparation of PPI emulsions could also be hindered, resulting in larger particles (McClements, 2005; Håkansson, 2009).

The increase of oil concentration from 5 to 20 %wt led to an increase of the mean droplet diameter in both types of protein, although the greatest differences were observed between emulsions containing 5 and 20 %wt oil. The increase in mean

droplet diameter in o/w emulsions is due to two main reasons: unsufficient emulsifier to cover the oil droplets and increase of coalescence due to increased oil concentration (Sun and Gunasekaran, 2009).

Polydispersity (Span) is a property of high interest, as it can affect both emulsion creaming and rheology characteristics (Mirhosseini et al., 2008). More uniform emulsions of similar droplet size are characterized by higher viscosity, since in emulsions with uniform polydispersity the empty space between larger droplets can be used to enclose smaller ones, hence decreasing the friction of the droplets and consequently emulsions' apparent viscosity. Polydispersity was affected by both emulsifier type and oil concentration (Table 5.1). Coarse WPI emulsions presented higher Span values compared to PPI ones, while values increased from 2.18 to 3.96 and from 1.75 to 2.05 for whey and pea emulsions respectively by increasing the oil concentration. This is also in accordance to other reported studies concerning the effect of oil fraction on emulsion polidispersity (Mirhosseini et al., 2008; Dapčević Hadnađev et al., 2013).

The application of sonication on coarse emulsions resulted in a sharp, approximately 3-fold decrease of the mean diameters after 1 min of processing. However, no significant differences were found among all samples even after 8 min of homogenization, with the exception of WPI emulsion containing 10 % olive oil and PPI emulsion at 5 %wt oil concentration which presented the lowest (2.33 μm) and highest (3.37 μm) droplet size respectively (Table 5.1). Similarly, Span values were not significantly affected by the composition of emulsions within 2 min of sonication. The only effect of oil concentration on polydispersity was noticed between WPI emulsions containing 10 and 20 % wt oil processed for 4 min. Still, this effect was diminished after elongated sonication (8 min).

WPI emulsions containing 5 % oil sonicated for 8 min presented significantly lower polydispersity in comparison to their PPI counterparts. It is most likely that this is related to the different emulsifying properties that these protein posses. PPI are characterized by lower solubility, hence limited ability against recoalescence of the droplets. Their stability against coalescence should mainly depend on the viscous properties of the continuous phase. Thus, after elongated sonication which causes severe degradation of XG and considerable viscosity decrease (as extensively analyzed in previous sections), PPI emulsions are more susceptible to droplet coalescence.

Considering the effect of sonication on each emulsion type the following observations can be denoted. For PPI emulsions, no significant further decrease of D_{50} values occurred for homogenization longer than 1 min ($p>0.05$). Span values for PPI emulsions at 10 and 20 %wt oil were not diversified during US application, while a significant increase was found in the case of 5 %wt oil. For WPI emulsions, polydispersity was not affected by the application of ultrasounds and Span values of sonicated emulsions were not significantly different than those of coarse emulsions. In contradiction to PPI samples, WPI emulsions containing lower oil concentrations (5 and 10 %wt), D_{50} values were considerably decreased after 8 and 4 min of sonication respectively. However, for whey emulsions of higher oil content (20 %wt), the same observation as in the case of PPI emulsions applied.

Taking into account the above findings it could be concluded that sonication duration longer than 1 min does not contribute to further size reduction, or ameliorates emulsion homogeneity of PPI emulsions or WPI emulsions containing 20 %wt olive oil. Ultrasonic processing of 1 min could be considered sufficient to considerably decrease the average size of coarse emulsions, thus avoiding excessive power consumption.

As a final remark it should be noted that despite the fact that high energy input and elongated processing time was used which could facilitate the formation of finer particles by allowing more time for the protein to orientate on the surface, the production of submicron particles was not achieved even though sonication energy input was high (~ 15 kJ/ 10 ml). For instance, the formation of nanoemulsions with a minimum particle size of ~ 13 nm at similar energy input (~16.8 kJ, 100 g premix mass) was achieved, by using low molecular weight emulsifier, lower gum concentration and considerably lower oil amounts (1 %wt) (Hashtjin and Abbasi, 2015). Additionally, according to our previous findings (Chapter 2), the formation of fine particles (~ 0-6- 0.8 μm) was achieved for emulsions containing the same gum concentration with an energy input of 26 kJ/ 100 g premix (~33 ^{o}C). The amount of emulsifier might also be low to reduce further the droplet diameter, hence ultrasonic processing longer than 1 min seems to be pointless, since it does not reduce the droplet size nor it ameliorates emulsion polydispersity.

Table 5.1 Droplet size parameters of emulsions containing whey or pea protein and various oil concentrations (5, 10 and 20%wt) as a function of homogenization treatment.

	D_{50} (µm)						Span (-)					
	WPI			PPI			WPI			PPI		
	5 %	10 %	20 %	5 %	10 %	20 %	5 %	10 %	20 %	5 %	10 %	20 %
Coarse emulsions												
	$11.4^{a/C}$ ±0.18	$12.2^{b/D}$ ±0.45	$14.06^{bc/B}$ ±1.51	$13.20^{bc/C}$ ±0.18	$14.82^{cd/B}$ ±0.12	$17.66^{d/B}$ ±3.07	$2.18^{b/A}$ ±0.07	$2.98^{c/A}$ ±0.01	$3.96^{d/A}$ ±0.26	$1.75^{a/A}$ ±0.05	$1.75^{a/A}$ ±0.04	$2.05^{b/A}$ ±0.11
US emulsions												
1 min	$3.48^{a/B}$ ±0.03	$3.96^{a/C}$ ±0.26	$3.73^{a/A}$ ±0.83	$2.87^{a/AB}$ ±0.22	$3.58^{a/A}$ ±0.76	$3.25^{a/A}$ ±0.50	$4.40^{a/A}$ ±1.48	$3.67^{a/A}$ ±0.63	$5.92^{a/A}$ ±3.44	$5.91^{a/BC}$ ±0.73	$4.31^{a/B}$ ±0.60	$5.11^{a/B}$ ±0.20
2 min	$3.12^{a/AB}$ ±0.03	$2.97^{a/B}$ ±0.08	$3.28^{a/A}$ ±0.25	$2.69^{a/A}$ ±0.26	$3.09^{a/A}$ ±0.59	$2.85^{a/A}$ ±0.22	$4.85^{a/A}$ ±1.25	$5.44^{a/A}$ ±0.53	$8.13^{a/A}$ ±0.19	$5.89^{a/BC}$ ±0.97	$6.46^{a/B}$ ±0.87	$4.36^{a/B}$ ±0.51
4 min	2.87^{aAB} ±0.45	$2.34^{a/A}$ ±0.11	$2.95^{a/A}$ ±0.35	$3.13^{a/AB}$ ±0.23	$2.91^{a/A}$ ±0.51	$2.73^{a/A}$ ±0.16	$2.49^{a/A}$ ±1.34	$3.27^{a/A}$ ±0.84	$7.08^{b/A}$ ±0.30	$5.29^{ab/BC}$ ±1.30	$5.59^{ab/B}$ ±1.76	$3.99^{b/B}$ ±0.40
8 min	$2.48^{ab/A}$ ±0.37	$2.33^{a/A}$ ±0.06	$2.88^{ab/A}$ ±0.30	$3.37^{b/B}$ ±0.10	$2.75^{ab/A}$ ±0.76	$2.69^{ab/A}$ ±0.42	$2.44^{a/A}$ ±0.39	$3.99^{ab/A}$ ±2.19	$6.41^{ab/A}$ ±1.56	$8.33^{b/C}$ ±0.28	$5.11^{ab/B}$ ±2.28	$5.07^{ab/B}$ ±1.30

[a-d] Different superscripts per property per row indicate significant differences ($p<0.05$).

[A-C] Different superscripts per column indicate significant differences ($p<0.05$).

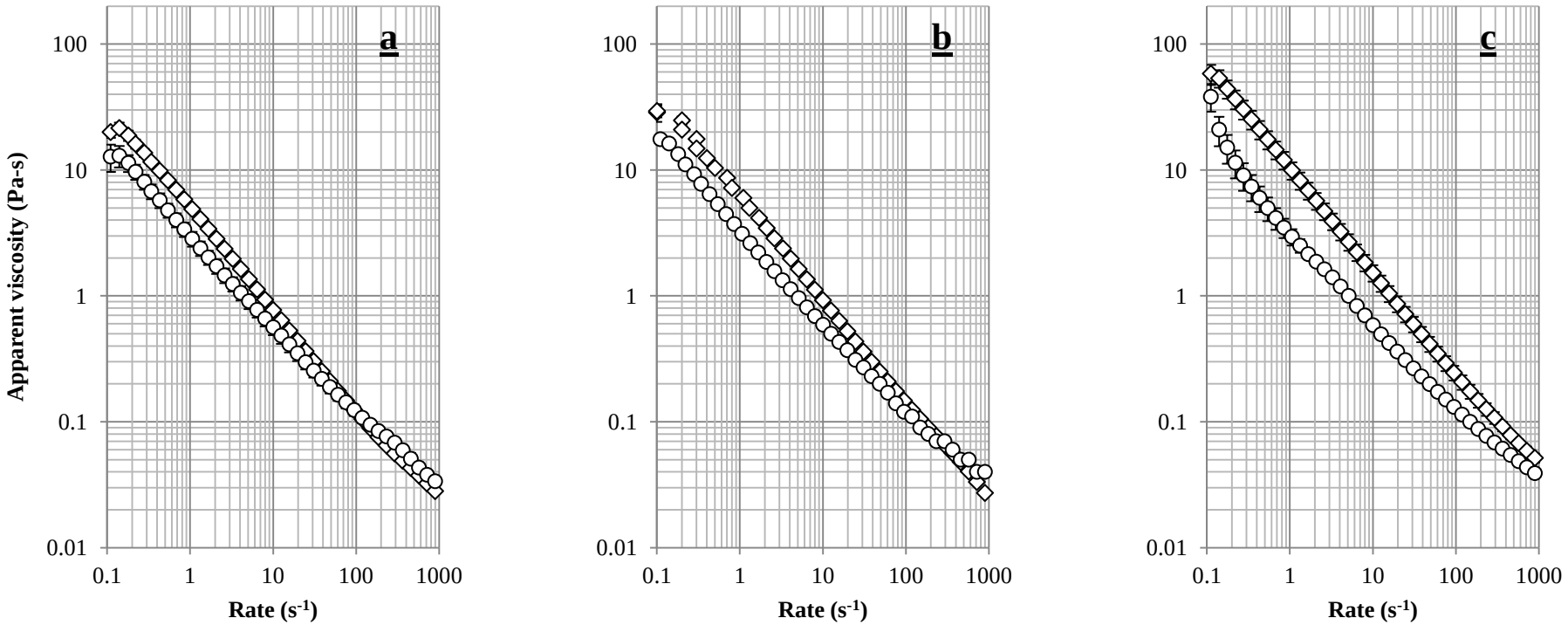


Figure 5.3 Apparent viscosity flow curves of coarse emulsions containing PPI (circles) and WPI (diamonds) at various oil contents: a) 5 %wt, b) 10 %wt and c) 20 %wt oil.

5.3.3 Emulsion stability

Emulsion stability of PPI and WPI emulsions was strongly affected by protein type, oil content and homogenization method used, as shown in Fig. 5.4 and Table 5.2. All coarse emulsions were stable against creaming regardless of oil content, and did not exhibit phase separation within the storage period examined. Therefore their destabilization mechanism involves flocculation or coalescence phenomena evolution, described by the variation of backscattering (dBS) (Protonotariou et al., 2013). Generally, emulsions containing PPI presented higher stability as evidenced by the lower dBS values compared to WPI samples. Additionally WPI samples, despite their smaller droplet size, they presented higher dBS values as seen in Table 5.2, inversely depending on oil content. WPI emulsions containing 5 %wt olive oil presented the highest dBS value (15.04 %), whereas for 10 and 20 % oil content the variation was 10.78 and 5.96 % respectively. The same phenomenon was observed for PPI coarse emulsions, for which dBS values ranged between 4.87 to 0.97 % upon increase of oil concentration. This suggests higher flocculation-coalescence rates for emulsions with low oil concentration, hence faster destabilization during storage. Higher oil concentrations result in higher apparent viscosity and gel or gel-like structures, thereby restricting droplet mobility throughout the emulsion matrix. As a consequence, droplet flocculation and intra-droplet collisions causing coalescence are reduced. Another factor that could be related with decreased flocculation-coalescence rates observed in PPI emulsions would be associated to their chemical composition. In contrast to whey proteins, pea proteins contain low amounts of sulfur-containing aminoacids (cysteine and metheionine) (Christou et al., 1992; Singh et al., 2014). This suggests that the overall ability for disulfide linkage formation between droplets -owed to partial protein unfolding on the interface- would be limited. Such bonds increase the viscoelasticity of the interfacial film, giving it enhanced stability against coalescence, but also promote flocculation (Kim et al., 2002; McClements et al., 2006), that is the case of WPI emulsions. Furthermore, the pea protein being mostly unsoluble in acidic pH may stabilize emulsions due to Pickering effect (Liang and Tang, 2014).

In general, the application of ultrasonication was more detrimental on emulsion stability of WPI emulsions, whereas PPI exhibited significant resilience against phase separation. WPI emulsions became phase separated even when the lowest processing

level (1 min) was applied, regardless of the oil content. The serum percentage of WPI emulsions decrease upon increase of the oil concentration, hence those containing 5 %wt oil became quickly unstable (within 1 day of storage) and serum percentage increased from 18.2 to 24.2 % (day 10) when sonication time increased from 1 to 8 min. A lag time period prior to destabilization was noticed for WPI emulsions containing 10 and 20 %wt oil which decreased from 6 to 2 days when homogenization time was increased up to 8 min. The respective serum percentage at the highest sonication input for samples prepared with 10 and 20 %wt oil concentration was 19.5 and 17 % accordingly, but no significant differences were observed ($p>0.05$). The sonication threshold for serum formation initiation in PPI emulsions depended on their oil content, hence for those containing 5 and 10 %wt oil, serum formation after 10 days of storage was observed after being homogenized for 1 and 2 min respectively (Table 5.3 and Fig. 5.4). Emulsions containing 20 % wt on the other hand were the most stable against creaming among all samples prepared. Ultrasonic treatment up to 4 min did not cause significant dBS increase, since values were not significantly different ($p>0.05$). Further input induced phase separation and 1.9 % Serum formation after 10 days of storage.

To conclude, the application of 1 min would be suggested for PPI formulations in order to achieve equal stability compared to their coarse counterparts.

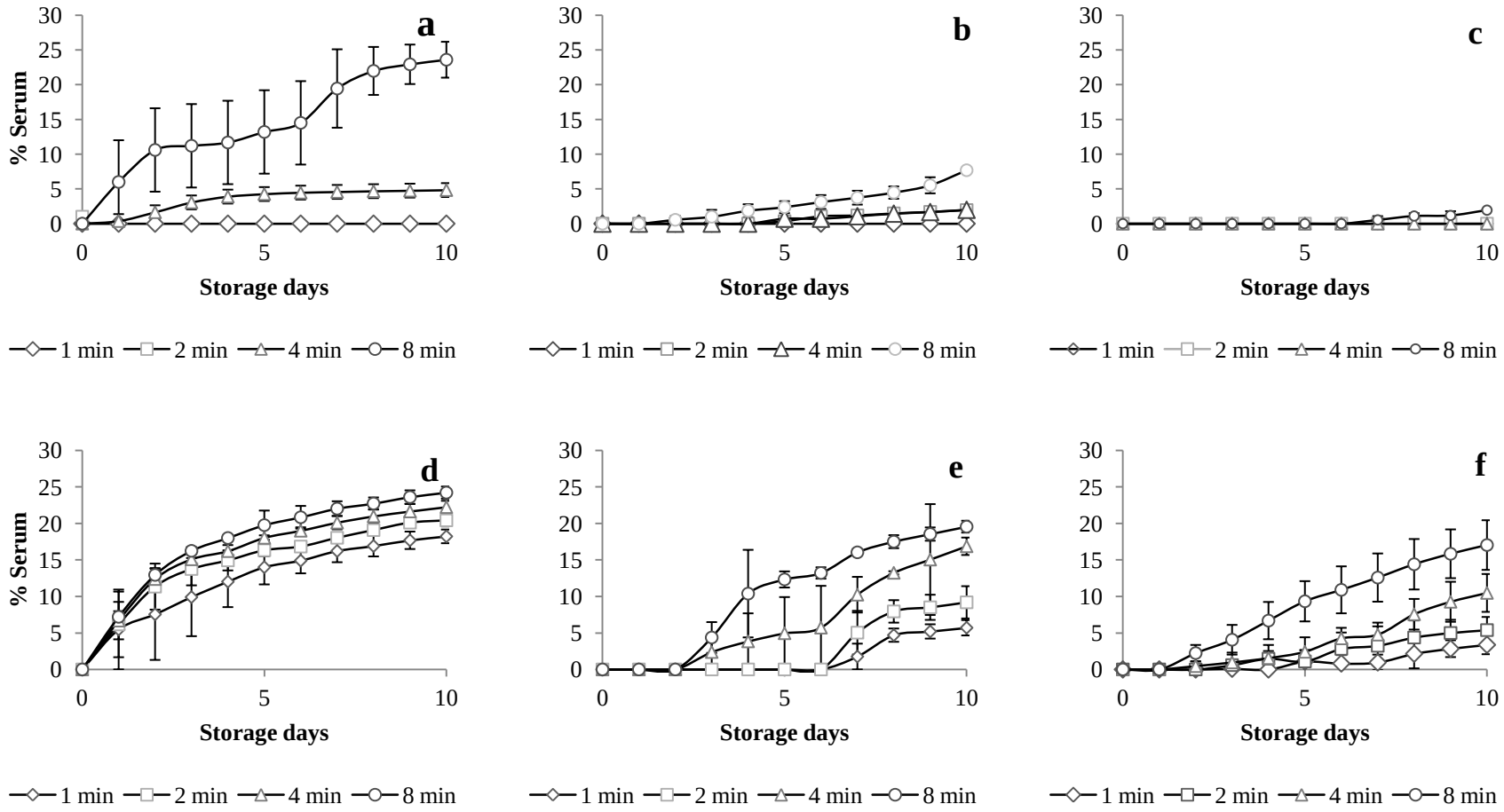


Figure 5.4. Effect of ultrasonic homogenization time on emulsion stability during storage. Upper row: PPI samples containing a) 5, b) 10 and c) 20 %wt oil. Lower row: WPI emulsions containing c) 5, d) 10 and e) 20 %wt oil.

Table 5.2 BS variation (dBS, %) of PPI and WPI emulsions as affected by oil concentration and preparation method.

	PPI			WPI		
	Oil content (% wt)					
	5	**10**	**20**	**5**	**10**	**20**
Coarse emulsions						
	$4.87^{c}\pm0.81$	$1.99^{ab}\pm0.86$	$0.97^{ab}\pm0.22$	$15.04^{e}\pm0.65$	$10.78^{d}\pm0.87$	$5.96^{c}\pm1.79$
US emulsions						
1 min	$1.43^{ab}\pm0.98$	$0.86^{a}\pm0.21$	$0.64^{a}\pm0.28$	ps	ps	ps
2 min	ps*	$2.39^{b}\pm1.30$	$0.62^{a}\pm0.40$	ps	ps	ps
4 min	ps	ps	$1.61^{ab}\pm0.88$	ps	ps	ps
8 min	ps	ps	ps	ps	ps	ps

*Where ps, phase separated emulsions.

$^{a-e}$ Different superscripts indicate significant differences ($p<0.05$).

Finally, to ensure long term stability of coarse and US emulsions processed for 1 min which would be prerequisite in the case of salad dressing or mayonnaise, samples were also kept in ambient storage for up to 6 months (180 days). Their BS profiles are depicted in Fig. 5.5 and it can be asserted that formulations were highly stable exhibiting only flocculation/coalescence phenomena (no serum formation was observed) as seen from BS decrease upon storage. To conclude, they could be considered suitable for the preparation of these types of emulsified products.

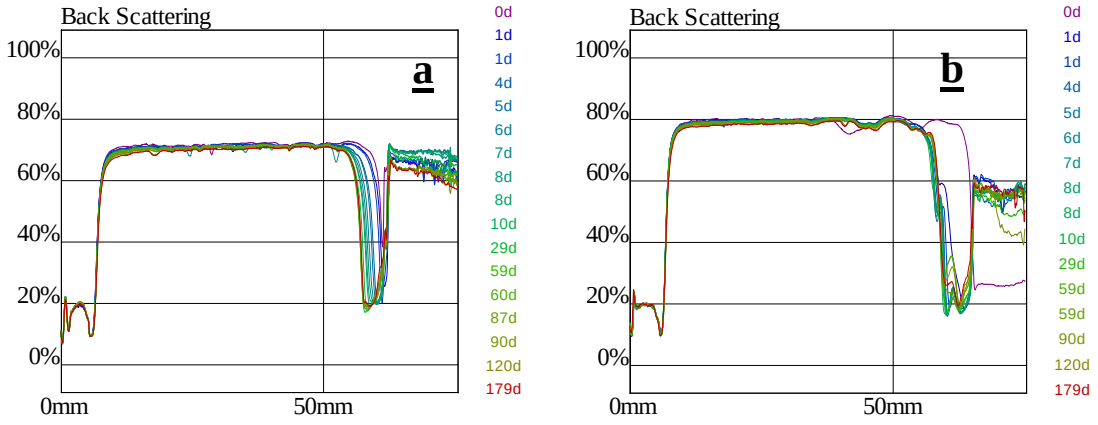


Figure 5.5 BS profiles of a) coarse and b) US treated (1 min), PPI emulsions containing 20 %wt olive oil during long term ambient storage.

5.3.4 Effect of sonication treatment on rheological properties of 20 %wt o/w PPI emulsions

Considering the previous screening of different coarse and ultrasonically homogenized formulations, 20 %wt olive oil PPI emulsions presented the highest stability, hence they are considered of increased interest for use in food products like salad dressings and mayonnaise. For this reason, a deeper analysis concerning the correlation between its rheological properties, mainly emulsion apparent viscosity and continuous phase viscosity to corresponding emulsion stability would provide an insight for the production of stable emulsions. Naturally, as extensively referred in previous sections, emulsion viscosity is dramatically reduced as a consequence of polymer degradation. In Fig. 5.6 the apparent viscosity flow curves of emulsions and 0.5 %wt XG solutions as a function of treatment is displayed. Table 5.3 summarizes the calculated values of Power law model (k, n). In Fig. 5.7 consistency and n indices are plotted as a function of sonication duration and best fit equations (maximum R^2) are displayed.

Emulsions consistency (k) values of coarse emulsions decreased from 8.87 to 2.29 Pa-s^n and flow behavior (n) values increased from 0.217 to 0.384 as a result of ultrasonication up to 8 min. Interestingly XG solutions experienced a more intensive degradation, as denoted by the decrease in k values from 4.24 to a limiting 0.038 Pa-s^n, while the pseudoplastic behavior was reduced considerably, approaching Newtonian region (n = 0.798).

Although the variation of k and n of XG solutions over time was best described by power law equations, this did not apply in the case of emulsions, for which an exponential decrease for consistency and linear increase of n as a function of sonication time suited better. It should also be noted that for emulsions, k and n reduction was less acute in comparison to solutions, assuming that their continuous phase was less susceptible to ultrasonic degradation. This is probably due to considerably higher viscosity of the emulsions, which hindered the propagation of ultrasound waves in the emulsion sample. Despite the stark sonication input applied on the samples which led to approximately 100-fold reduction of gum solution after 8 min of processing, the shear thinning behavior of XG was maintained, since the maximum n observed was considerably lower than that of Newtonian fluids (n = 1).

It should also be noted that the viscosities of emulsions (k values) do not necessarilly correlate to dBS values. Coarse emulsions exhibited the same dBS as ultrasonicated ones (up to 4 min) despite their considerable higher droplet size (17.7 μm). Moreover, even though significant differences in k values were detected between 2 and 4 min of sonication, emulsions performed the same in terms of coalescence/flocculation stability. This suggests the existence of a viscosity threshold in order for the emulsions to become considerably more unstable against these phenomena.

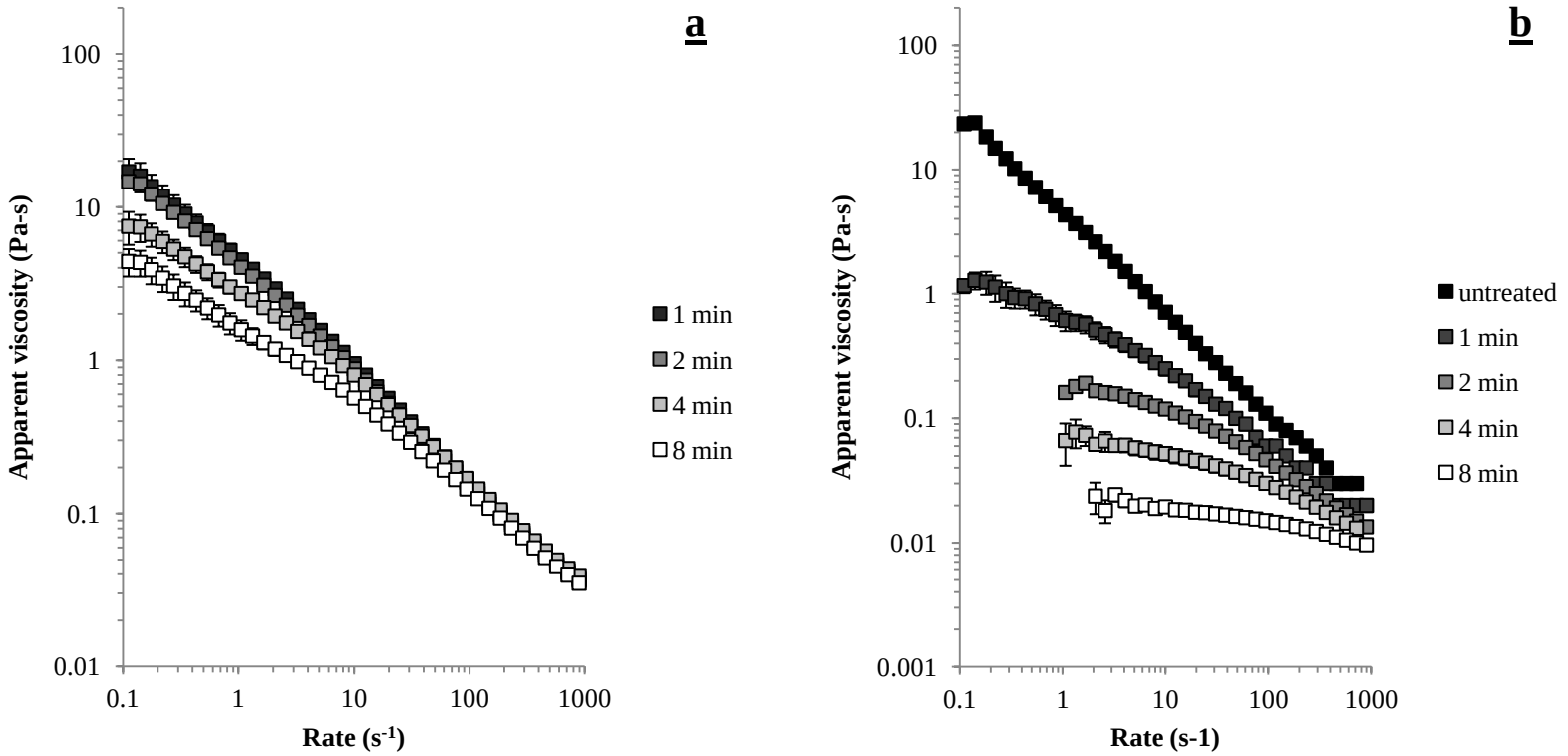


Figure 5.6 Viscosity flow curves of (a) sonicated PPI emulsions containing 20 %wt oil and (b) untreated or sonicated 0.5 %wt XG solutions.

Table 5.3 Viscosity paramenters of coarse and ultrasonicated PPI emulsions containing 20 %wt oil and untreated and ultrasonicated 0.5 %wt XG solutions.

	Emulsions		**XG solutions**	
	k (Pa-s^n)	**n (-)**	**k (Pa-s^n)**	**n (-)**
Coarse/Untreated	8.872^e ±0.621	0.217^a ±0.002	4.249^e ±0.063	0.215^a ±0.007
Ultrasonically treated				
1 min	4.579^c ± 0.364	0.282^b ±0.010	1.032^d ±0.121	0.381^b ±0.008
2 min	4.120^c ±0.111	0.293^b ±0.012	0.452^c ±0.019	0.485^c ±0.002
4 min	3.438^b ±0.135	0.337^c ±0.005	0.165^b ±0.025	0.618^d ±0.010
8 min	2.290^a ±0.102	0.384^d ±0.006	0.038^a ±0.001	0.798^e ±0.004

$^{a-e}$ Different superscripts per parameter indicate significant differences ($p<0.05$).

R^2 values range between 0.98 – 0.999.

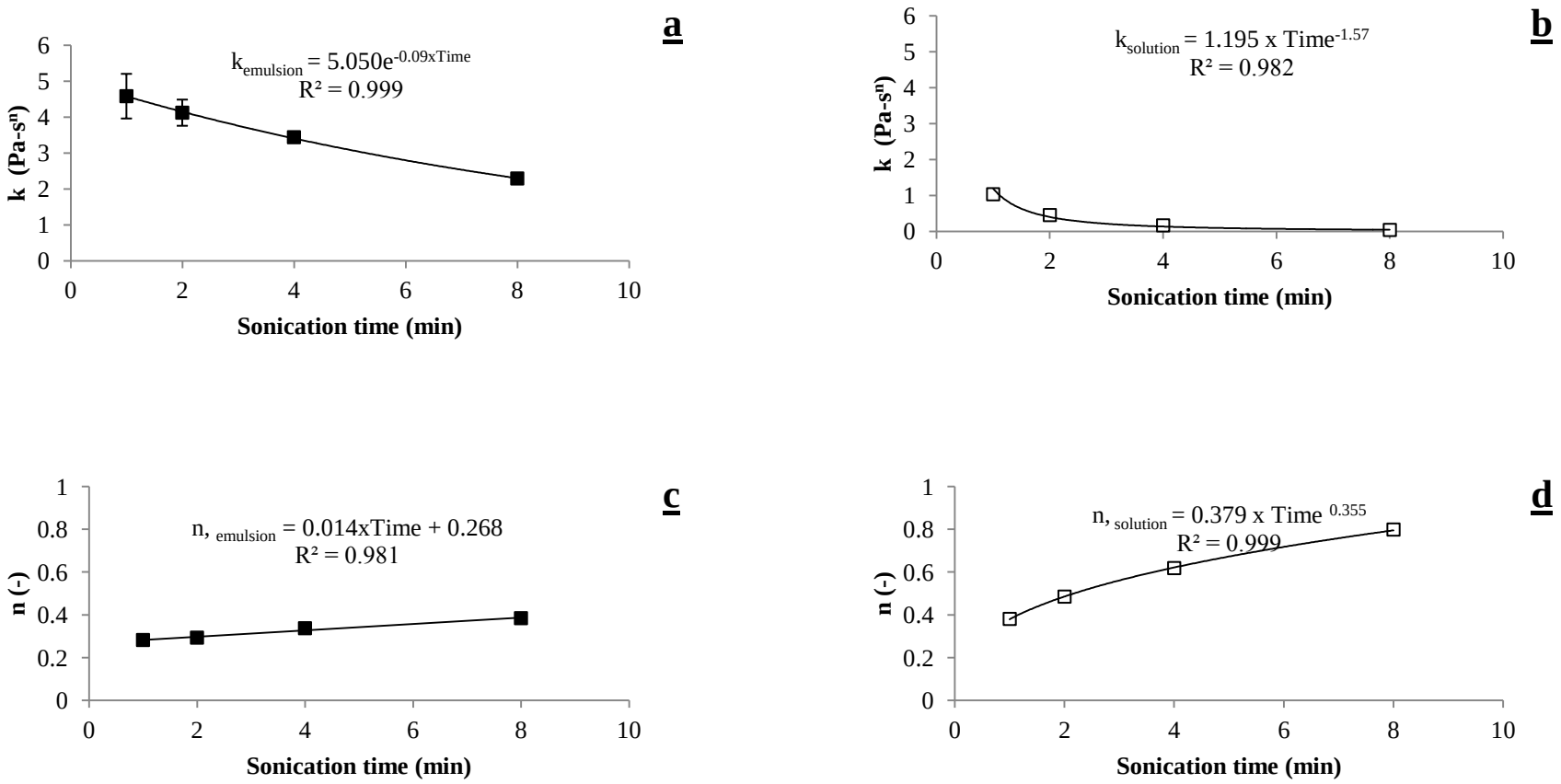


Figure 5.7 Consistency (k) and flow behaviour (n) index of PPI emulsions (a, c) and 0.5 %wt XG solutions (b, d) as a function of sonication time.

Coarse emulsions were characterized by the dominance of G' over the G'' modulus (Fig. 5.8) within the whole frequency range tested (0.1<tan delta < 0.5) (data not shown). Both moduli were slightly frequency dependant and loss tangent decreased by increasing frequency, indicative of higher droplet interactions. These findings suggest the existence of gel-like structure in the system (Diftis et al., 2005; Taherian et al., 2006). However the relatively high values of tan delta (>0.1) are typical for so called weak gel emulsions (Krstonošić et al 2015). Ultrasonication decreased both storage and loss moduli resulting in less thickened emulsions. A slight dominance of the G'' modulus was observed at low frequencies, which became more intensive as sonication time increased. After 8 min of sonication, tanδ values of emulsions were above unity within the majority of region of 0.1- 100 rad/s, hence the samples were mostly liquid-like.

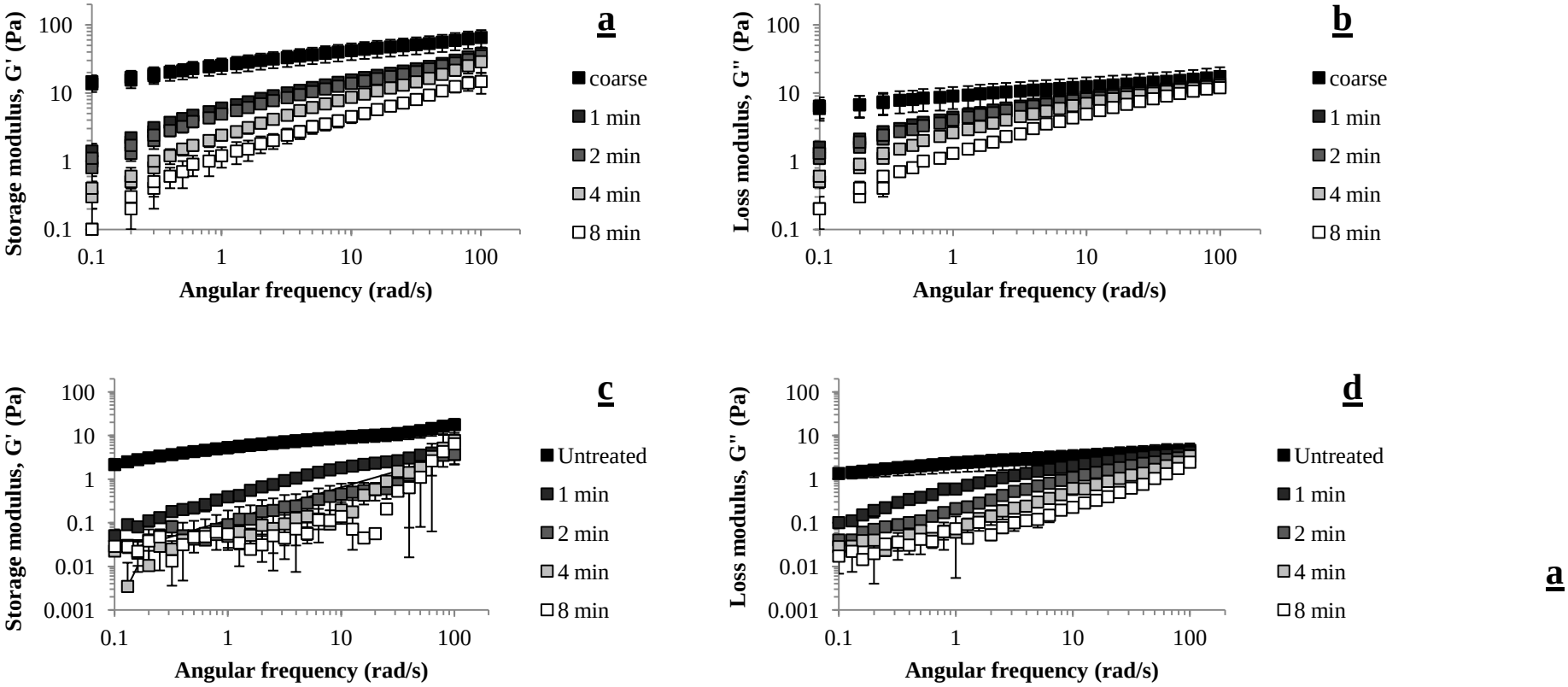


Figure 5.8 Storage (G') and loss (G") moduli of coarse and sonicated PPI emulsions containing 20 %wt oil (a,b), and untreated or sonicated 0.5 %wt XG solutions (c,d).

5.4 Conclusions

In this study the effect of different types of protein emulsifiers (milk or plant derived), oil concentration and ultrasonication time on emulsion physical characteristics was investigated.

Coarse emulsions prepared with WPI had smaller particle diameters than those stabilized by PPI, owned to both higher efficacy in reducing surface tension and lower viscosity in the presence of xanthan. However, WPI emulsions were characterized by lower viscosities and increased destabilization rate concerning flocculation/coalescence phenomena.

Even though extended sonication time was used to homogenize the samples, this did not result in formation of fine emulsion within the submicron range, probably due to the increased viscosity of XG emulsions, regardless of the oil content. WPI samples were highly unstable against sonication and became phase separated even after 1 min of sonication in all cases of oil content. PPI emulsions containing 20 %wt olive exhibited the highest stability as coarse or sonicated emulsions, and 8 min of sonication was required to induce phase separation. This is mostly related to enhanced viscoelastic properties of these emulsions which hinder the propagation of US, since the properties of XG solutions were affected in greater extent. 20 %wt o/w coarse and US (1 min) PPI emulsions would be of great interest for the preparation of salad dressings or mayonnaise since they exhibited the same stability and no phase separation after long term storage was noticed.

Finally, the processing of solely oil and protein aqueous phases, in the absence of stabilizers should be considered in order to minimize the droplet size.

CHAPTER 6

STABILITY AND PROPERTIES OF MACRO- AND NANO/SUBMICRON WHEY EMULSIONS CONTAINING FENUGREEK GUM.

6.1 Introduction

Nanoemulsions are acknowledged for numerous advantages, including inceased bioavailability of functional bioactive compounds, as well as higher physical stability against creaming, coalescence or flocculation phenomena. The later is considered significant towards the strategies and actions undertaken globally by several initiatives and governments. The World Health Organization suggestions over fat include consumption shift from saturated to unsaturated fats and limitation of energy intake from fat (WHO, 2015).

Eventhough the stability of properties of simple whey nanoemulsions formed by homogenization of the oil and protein aqueous phase has been the object of many studies, to our best of knowledge there are only a few available information regarding model formulations resembling mayonnaise or salad dressings. Commonly used polysaccharide stabilizers undergo severe degradation owed to extreme shearing generated during the homogenization processes such as ultrasonication or high pressure, hence their incorporation in the initial formulation is not suggested (Tiwari et al., 2010). Additionally, the presence of stabilizers may also hinder the efficacy of the homogenization process, due to increased viscosity of the formulation, which hinders the propagation of soundwaves, increases cavitation threshold to higher amplitudes and reduces shearing turbulence (Wooster et al., 2008).

Therefore, the objective of this study is to investigate the efficacy of nanoemulsion formation by controlling the oil fraction and ultrasonication processing time. In general low oil fractions (<10 %) favor the formation of nanoemulsions below 200 nm, while higher ones promote the formation of larger ones (Leong et al., 2009; Mohammadifar et al., 2015; Tabibiazar et al., 2015). Extended sonication time may also be helpful towards reducing the mean droplet diameter of nanoemulsions by allowing more time for the protein to orientate on the oil interface and by increasing the number of cavitation events (Jafari and Bhandari, 2007). The stability of the final formulations (secondary dressing model emulsions) produced by further dilution with fenugreek gum was assessed and comparisons were made between nanoemulsions and their macroemulsion counterparts produced by high-speed mixing.

6.2 Materials and methods

6.2.1 Materials

Whey protein isolate (WPI) Lacprodan DI-9224 was kindly provided by Arla (Arla Foods Ingredients, Amba-Denmark). Fenugreek gum powder Fenulife® was a kind gift from Frutarom Virgin olive oil Altis (Elais Unilever, Greece) was purchased from a local store. Sodium azide was purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland) and glacial acetic acid from Sigma–Aldrich.

6.2.2 Solution preparation

Whey protein stock solutions (10 g in deionized water in total weight 95, 90 or 80 g) were prepared by agitation with a magnetic stirrer for 90 min. Fenugreek gum (FGF) solutions 1, 1.5 and 2 %wt in deionized water were prepared by hot stirring in a water bath at 90 ^{o}C for 90 min. Thereafter, solutions were kept overnight at 5 ^{o}C to ensure complete hydration. Sodium azide 0.02 %wt was added to the aqueous solutions as an antimicrobial agent.

6.2.3 Coarse emulsion preparation and screening

Oil-in-water primary macro-emulsions were prepared by homogenizing 5, 10 or 20 g of oil phase with 95, 90 or 80 g of the WPI aqueous solutions accordingly. The initial step in preparing macro-emulsions involved production of a coarse premix emulsion by homogenizing the oil and aqueous phases using an Ultra Turrax (T25 basic, Janke & Kunkel IKA Labortechnik, Staufen, Germany) for 2 min (13.500 RPM) at room temperature. The coarse emulsions were further diluted with fenugreek gum solutions (2 %wt) in a ratio 1:1 by weight with a magnetic stirrer (for 1 min in order to avoid recoalescence of the droplets) to yield secondary coarse emulsions. The pH of the resulting macro- and nano-emulsions was adjusted to 3.8, which is typical for products such as mayonnaise and salad dressings, with a few drops of glacial acetic acid.

Nanoemulsions were also produced by further homogenizing 10 ml of the primary coarse premixes with an ultrasonic homogenizer model Sonopuls 3200 (Bandelin Electronic Gmbh & Co, Berlin) equipped with a 3 mm in diameter titanium probe (MS 73). The tip was immersed 10 mm from the surface of the sample which was placed in a double jacketed flow-through vessel (model DG-3). The temperature

was maintained at 30 (± 1) ^{o}C by circulating cold water with a pump. The amplitude was set at 40%. Although the maximum allowed amplitude for usage of the specific probe was 65% it was noticed that amplitudes higher than 40 % led to increased fluctuations of the samples in the vessel which caused sample spilling. The ultrasonication processing time ranged between 1 up to 12 min. The energy input applied on samples during sonications as a function of time is demonstrated in Fig. 6.1. The emulsification time which resulted in minimum droplet sizes or polydispersity was chosen for the incorporation of nano-emulsions in 2 %wt fenugreek solutions as mentioned above for macro-emulsions. All samples were prepared in triplicate.

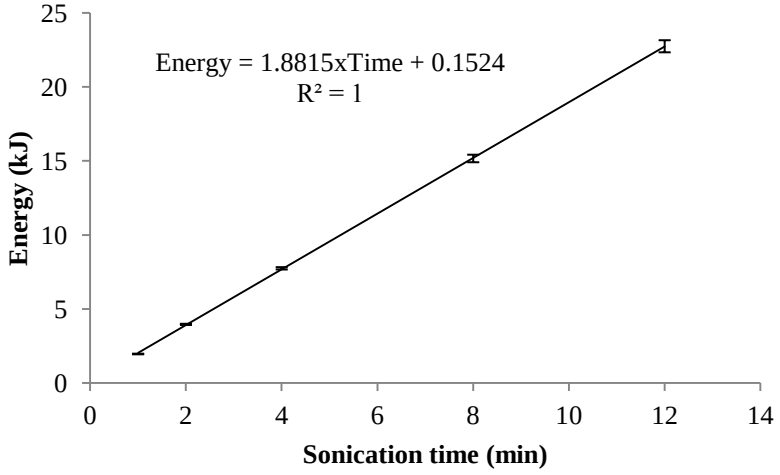


Figure 6.1 Nominal ultrasonic energy input (kJ) as a function of time.

6.2.4 Droplet size measurement

Droplet size distributions of the primary coarse emulsions were determined by using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Emulsion droplets were characterized under high dilution conditions by dispersing the samples in deionized water. The refractive indices of water and olive oil were taken as 1.330 and 1.467, respectively, and the Mie theory was used for the analysis (Paximada et al., 2014). Average droplet sizes were characterized in terms of the volume mean diameter D_{50} and polydispersity (Span). All measurements were made at ambient temperature on three separately prepared samples.

The mean droplet size (z-average diameter) and polydispersity index (PDI) of nanoemulsions were measured using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). Measurements were performed at

25 °C in triplicate. Prior to measurement, the nanoemulsions were diluted (1:500) with deionized water to avoid multiple scattering during measurements.

6.2.5 Emulsion stability

The storage stability (at 5 ^{o}C) of macro- and nanoemulsions was determined by obtaining backscattering profiles with a Turbiscan 2000 device (Turbiscan 2000, Formulaction, Toulouse). For unstable macro emulsions the serum percentage was calculated (Zinoviadou et al., 2012). For stable samples (macro- and nano/submicron emulsions), the average value of the backscattering (BS) measurement at the middle zone of the cell (between 30 and 40 mm of sample height) has been plotted as a function of ageing time and BS variation (dBS) during 10 days of storage was estimated as in Chapter 2.

6.2.6 Emulsion rheology

Viscosity measurements were performed with a hybrid rheometer DHR (TA Instruments) equipped with plate-plate geometry (upper plate diameter 50 mm, gap 1 mm) to obtain flow curves between 0.1 to 1000 s^{-1} at 25 ± 0.01 ^{o}C by circulating water from a peltier. The total measurement time was 900 s. All measurements were performed in triplicate and data reported as average and standard deviations.

The linear viscoelastic region (LVR) was assessed at 6.28 rad/s (= 1 Hz) by strain sweep experiments; for all samples a constant deformation of $\gamma = 0.1$ % was used, which was within the linear viscoelastic region of all the samples. Deviation from the linear viscoelastic region occurs when the sample starts to permanently deform, implying the destruction of the transient network structure. Small deformation oscillatory measurements were performed for the evaluation of the viscoelastic properties, G′ (storage modulus), G″ (loss modulus), and tanδ (G″'/G′) (Steffe, 1996; Metzger, 2006), over the frequency range of 0.1–100 rad/s at 25 °C.

6.3 Results and discussion

6.3.1 Droplet size

The droplet mean diameter (D_{50}) of primary coarse emulsions ranged between 9.48 and 13.76 μm (Table 6.1). Although emulsion diameter usually increases when increasing oil concentration (Christensen et al., 2001; Turchiuli et al., 2012), only a slight increase of the mean diameter was observed for samples containing 20 %wt

olive. Nevertheless, no statistically significant differences were observed compared to emulsions containing lower amounts of olive oil ($p>0.05$). No significant differences were observed either considering the polydispersity of samples, since Span values ranged between 1.46 up to 2.48 (Table 6.1). This allows us to assume that the emulsification time and protein isolate concentration used, were sufficient to equally minimize the droplet size in all formulations examined regardless of the oil content.

Primary coarse emulsions were subjected to sonication in order to achieve further droplet size reduction. As shown in Fig. 6.2a, a sharp decrease of the droplet size is achieved within 1 min of sonication, hence the z-average diameter ranged between 323 - 288 nm depending on the oil content. Thereafter, the droplet size was moderately reduced by increasing sonication time up to 12 min. In more detail, the z-average diameter of samples containing 5 and 10 %wt oil did not show any considerable droplet reduction after 1 or 2 min of sonication accordingly, while for those containing 20 %wt oil, longer time (8 min) was needed to reach droplet size plateau.

Regarding the polydispersity of sonicated emulsions, it is shown that the PDI decreased by increasing the sonication processing time (Fig. 6.2b). Although the PDI values for samples containing 5 and 10 %wt oil, was 0.434 and 0.51 respectively after 1 min of sonication the reduction was less dramatic for those containing higher oil amounts. More specifically, emulsions containing 20 %wt olive oil were characterized by very high PDI values (>0.9), indicative of the strong polidispersity of emulsions (Tiwari et al., 2015) and results should be treated with caution since DLS measurements can be considered reliable when values of PDI <0.5 are obtained. PDI values at low oil fractions 5 and 10 %, was practically unaffected after 4 or 8 min accordingly, whereas the lowest value was observed after 12 min of sonication when higher oil amounts were used.

Considering the above findings, a sonication time of 12 min was selected in order to prepare salad dressing models by incorporation of FGF gum, because it resulted in the lowest and acceptable PDI values for the total of samples produced. Nanoemulsions with an average size of 207 nm and of narrow polydispersity (~ 0.2) were prepared with the lowest oil fraction (5 %wt) (Fig. 6.3a,b). This size is slightly higher than that reported by Adjonu et al. (2014), who preparted WPI nanoemulsions of 160 nm, which could be attributed to slightly lower oil fraction used (4 %wt). Lower droplet sizes reaching 75 nm have been referred by using bovine serum

albumin (BSA) in the presence of polyethylene-glycol (PEG) (Tabibiazar et al., 2015). Increasing the oil fraction led to higher droplet sizes (~280 nm) and PDI values (~0.38), although no significant differences were observed after 12 min of sonication when 10 or 20 %wt olive oil was used (Table 6.1) ($p>0.05$).

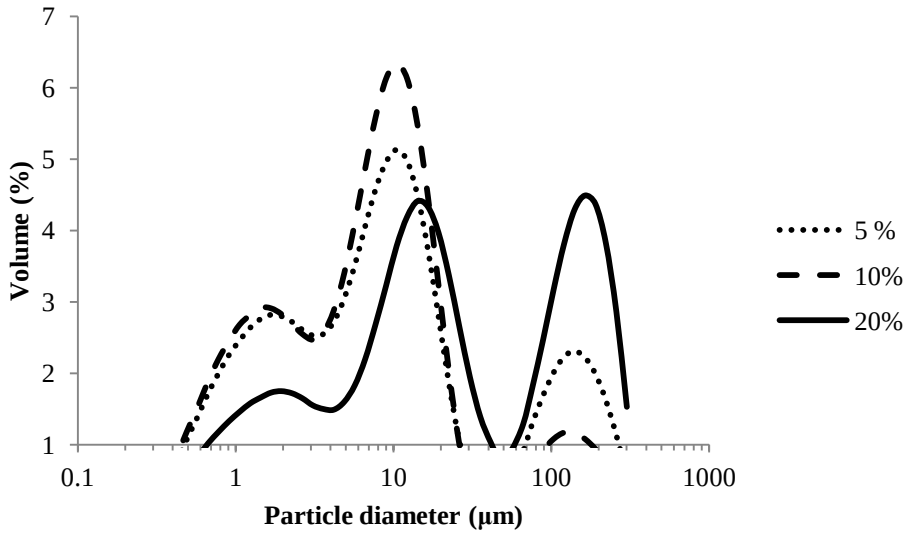


Figure 6.2 Volume weighted size distributions of primary emulsions as affected by oil concentration.

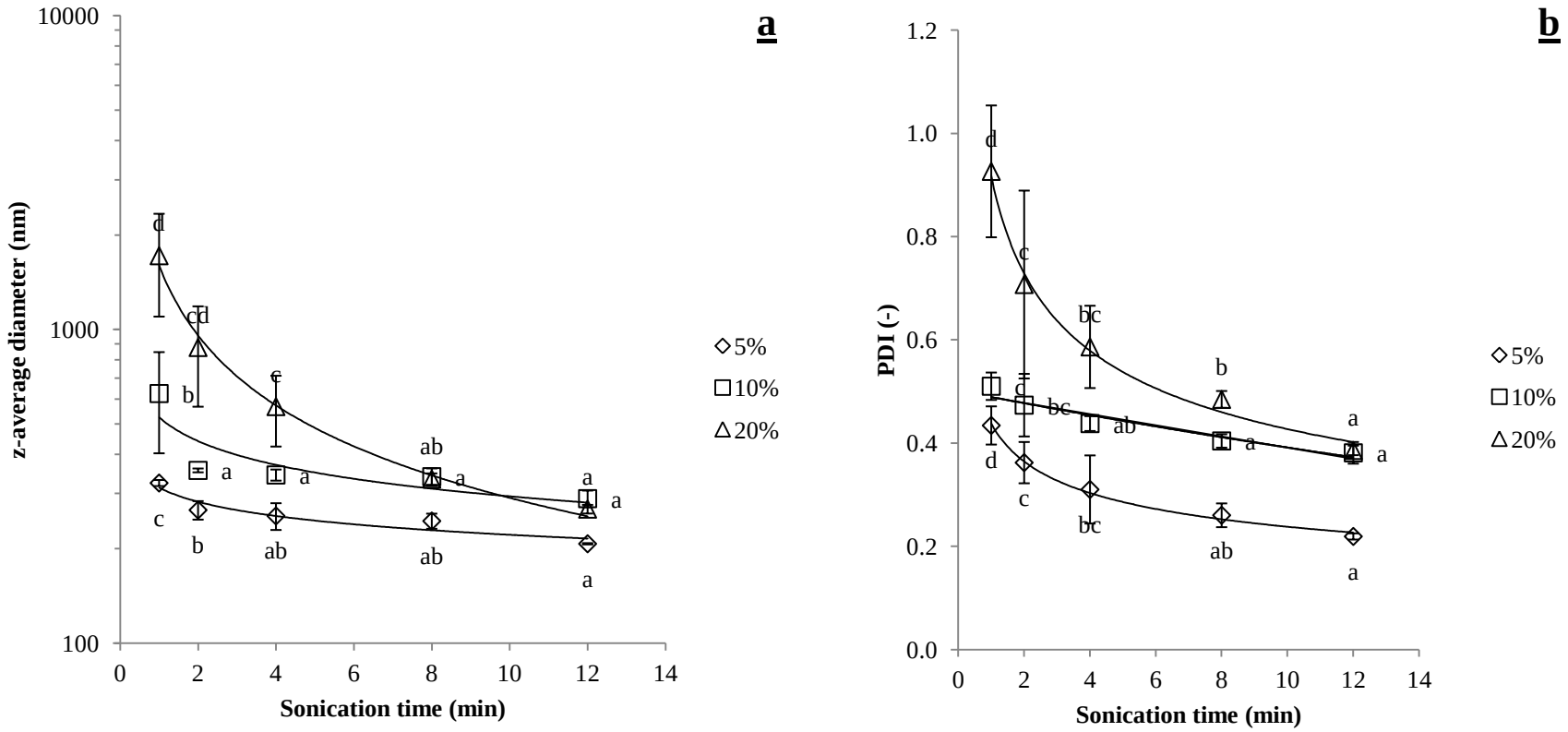


Figure 6.3 Droplet size (a) and polydispersity index (PDI) (b) of emulsions as a function of sonication time and oil concentration. Diamonds correspond to 5 %, squares 10 % and triangles to 20 % oil.
[a-d] Different superscripts per oil content sample indicate significant differences ($p<0.05$).

Table 6.1 Droplet size (volume median diameter (D_{50}) and z-average diameter) and polydispersity (Span and PDI) of primary emulsions as affected by olive oil concentration and emulsification method applied.

	Oil concentration (%wt)		
	5	**10**	**20**
Coarse emulsions			
D_{50} (μm)	$9.73^{a} \pm 3.3$	$9.48^{a} \pm 4.09$	$13.76^{ab} \pm 3.96$
Span (-)	$1.81^{a} \pm 0.23$	$2.68^{a} \pm 1.08$	$1.46^{a} \pm 0.54$
Nano- emulsions			
Z-average diameter (nm)	$207.3^{a} \pm 1.0$	$288.7^{b} \pm 17.6$	$267.3^{b} \pm 8.5$
PDI (-)	$0.219^{a} \pm 0.006$	$0.381^{b} \pm 0.021$	$0.386^{b} \pm 0.009$

[a-b] Different superscripts per parameter indicate significant differences among samples ($p<0.05$).

6.3.2 Screening of FGF concentration for secondary coarse emulsion preparation

Initially, a screening procedure was conducted in order to assess the FGF concentration required to obtain stable secondary coarse emulsions containing 2.5, 5 or 10 %wt olive oil in the final formulation. The curves of creaming (% Serum) versus storage days of coarse emulsions containing 0.5 up to 1 %wt FGF are shown in Fig. 6.4. The oil phase content significantly affected the serum percentage values ($p<0.05$). As can be seen the stability of emulsions improved by increasing the oil content from 2.5 to 10 %wt. For emulsions containing 0.5 %wt FGF serum percentage decreased from 71.3 to ~ 9.5 %, while for those containing 0.75 %wt FGF, a reduction from 42.2 to 3.2 % was noticed. Stability improvement by increasing the oil is associated to the increase of oil droplet packing, which leads to enhanced interdroplet interactions and more structured emulsions (Nikiforidis et al., 2012). Nevertheless, the incorporation of higher FGF concentrations and subsequent emulsion thickening was more beneficial towards stability compared to increased oil volume fractions. The addition of 1 %wt FGF resulted in formation of stable coarse emulsions which did not show phase separation during 10 days of storage. Therefore this concentration was used in subsequent formulations and comparisons were made between coarse and nanoemulsion formulations.

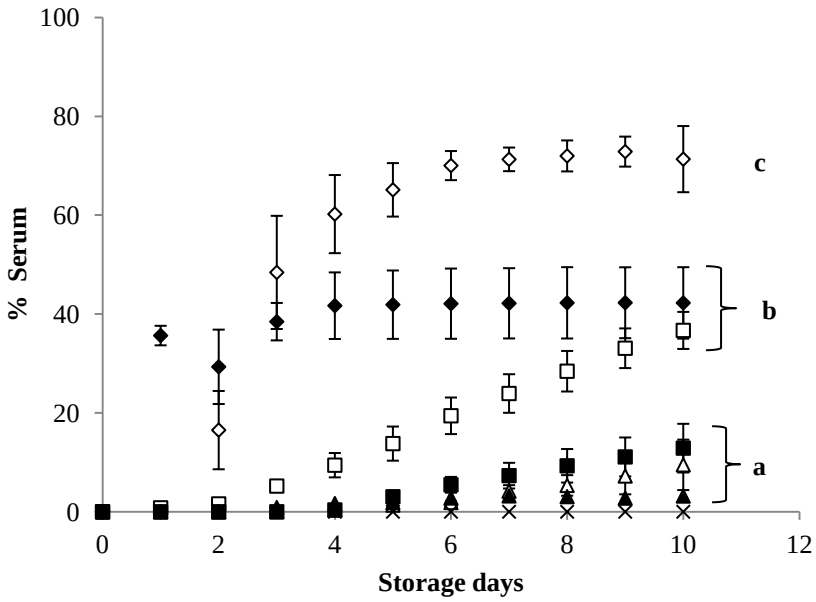


Figure 6.4 Stability of coarse emulsions containing as affected by oil (diamonds, 2.5 %wt, squares 5 %wt and triangles 10 %wt) and FGF concentration (open symbols: 0.5 %wt, closed symbols: 0.75 %wt and "x", all other emulsions containing 1 %wt).
[a-c] Different superscripts indicate significant differences among samples ($p<0.05$).

6.3.3 Emulsion rheological properties

The Power law model was adopted to describe the viscosity properties of coarse and nanoemulsion analogues. Although higher regression coefficients (R^2) were obtained when fitting the Herschel-Buckley model, it had no physical meaning, since negative values for yield stress were obtained. Hence, these results are omitted and data were fitted to the Power law model. Table 6.2 summarizes the estimated consistency (k) and flow behavior (n) values of prepared secondary coarse and nano-emulsions containing 1 %wt FGF and various amounts of olive oil. All model emulsions prepared were highly pseudoplastic with flow coefficient (n) values varying from 0.29 to 0.35.

Both consistency and flow behavior values were affected by the oil content and preparation method used. In the case of coarse emulsions, the increase of oil content from 2.5 to 10 %wt increased the consistency of the samples from ~7.4 to 11.7 Pa-s^n. The increase of oil fraction causes an upward shift of the flow curves to higher viscosities, attributed to the increased packing of the oil droplets. This leads to enhanced droplet interactions, thus more viscous emulsions (Sun and Gunasekaran 2009). However, significant differences were observed between all coarse emulsions regardless of the oil content ($p<0.05$). Nevertheless, this was also observed in nano-

emulsions, for which an increase in k values was observed from 8.59 to 15.69 Pa-s, and similar values were obtained among samples containing 2.5 and 5 %wt oil ($p>0.05$).

Increasing the oil fraction also led to an increase of the pseudoplastic character in both coarse and nano-emulsions, as evidenced by the decrease in flow behavior values (n). In the case of consistency index, nano-emulsions containing 2.5 and 5 %wt oil exhibited similar pseudoplasticity ($p>0.05$), whereas between macroemulsions increased values were obtained for emulsions with 5 %wt oil ($p<0.05$).

In comparison to their coarse counterparts, nanoemulsions were also more viscous and more pseudoplastic in all cases of oil concentration. The decrease of the droplet size within the submicron scale resulted in an increase of the k values by 16 up to 34 % depending on oil concentration. Flow behavior values ranged between 0.333 – 0.352 and 0.288 – 0.324 for coarse and nanoemulsions respectively, similarly to those reported for 1 %wt fenugreek solutions (Wu et al., 2009).

Table 6.2 Estimated consistency (k) and flow behavior (n) values of coarse and nano-emulsions containing various amounts of olive oil and 1 %wt FGF.

Oil content (%wt)	**k (Pa-s^n)**	**n (-)**
Coarse emulsions		
2.5	$7.40^{a} \pm 0.45$	$0.352^{d} \pm 0.006$
5	$8.31^{b} \pm 0.32$	$0.343^{d} \pm 0.011$
10	$11.72^{c} \pm 1.05$	$0.333^{c} \pm 0.003$
Nano-emulsions		
2.5	$8.59^{b} \pm 0.71$	$0.324^{bc} \pm 0.006$
5	$9.09^{b} \pm 0.35$	$0.320^{b} \pm 0.001$
10	$15.69^{d} \pm 0.24$	$0.288^{a} \pm 0.012$

$^{a-d}$ Different superscripts indicate significant differences among samples ($p<0.05$).

R^2 values range from 0.987 to 0.994.

Dynamic oscillatory shear test was used to characterize the viscoelastic properties of coarse and nano-emulsions containing 1 %wt FGF and the determined storage (G') and loss (G") moduli are shown in Fig. 6.5(a-c). Loss tangent (tanδ) was also used to assess the elastic or viscous character predominance in the samples (Fig. 6.6).

Overall, samples exhibited frequency-dependent storage and loss moduli values, which increased during frequency ramp from 0.1 to 100 rad/s.

In all cases of emulsions prepared, G" was dominant over G' in low frequency region, thereby showing a liquid like behavior. This implies that the most input energy cannot be stored at this low frequency region, thus it dissipates through viscous flow. At higher frequencies (approximately > 1 rad/s) a solid-like behavior is observed for all emulsion preparations (G'>G"). Additionally, the crossover point of the moduli is located at lower frequencies in the viscoelastic spectrum of nanoemulsions. The tanδ values were higher than 0.3 over the entire frequency range and decreased by increasing frequency (Fig. 6.6), which is indicative of the viscous character of the emulsions (Mandala et al., 2004). This finding can be explained based on the fact that galactomannans are capable of forming highly viscous solutions due to their high water binding capacity even at low concentrations. Unlike xanthan gum, galactomannans such as fenugreek and guar gum do not form gels on their own, whereas for locust bean gum subzero temperatures (Dea et al., 1977) or long storage is required to form gels at high concentration (Richarson et al., 1999). According to Wu (2009), fenugreek gum solutions with concentration ranging between 0.5 -2 %wt, presented a liquid like behavior at lower frequencies (G">G') and a solid like at higher ones. Moreover, in another study the mechanical spectra of 1 %wt fenugreek gum aqueous dispersions showed a variety of behavior depending on the molecular weight. Fenugreek gum fractions with higher molecular weight ($Mw \geq 18.3x10^5$ g/mol) showed a concentrated polymer solution pattern, exhibiting modulus crossover (G'>G'') at high angular frequencies, whereas those of lower Mw presented a dilute polymer solution pattern, as evidenced by moduli values (G''>G') and absence of crossover (Funami et al., 2008).

The increase of droplet packing caused by oil fraction increase and sonication resulted in emulsion thickening as evidenced by the enhancement of G' and G'' values (Sun and Gunasekaran, 2009). At the same time, a decrease of loss tangent occurred, revealing the increase of the solid like behavior. Minor differences on storage and loss moduli and tanδ were detected between formulations with low amounts of oil (2.5 and 5 %wt). Obviously, findings of dynamic properties are in-line with those obtained by examining the flow behavior of emulsions.

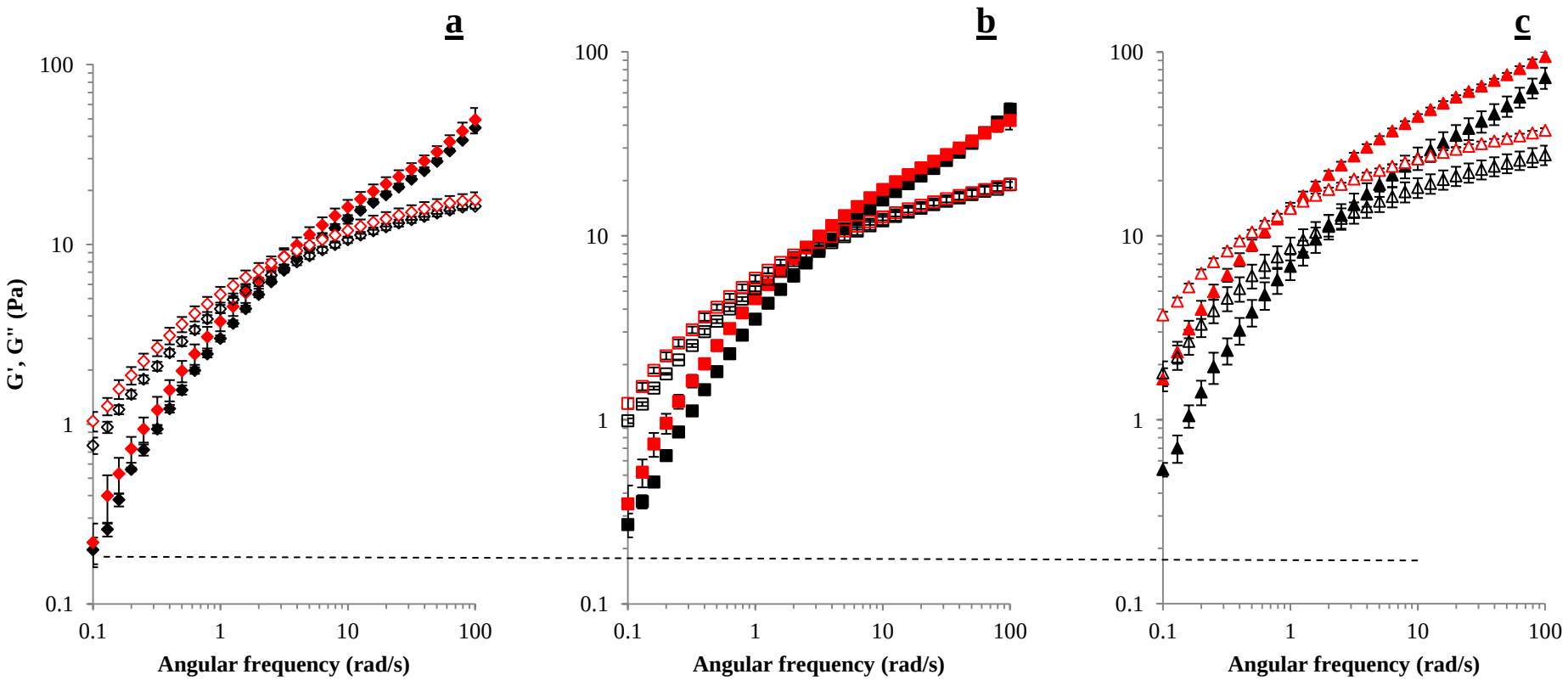


Figure 6.5 Effect of oil content on storage (G', closed symbols) and loss modulus (G", open symbols) of emulsions containing a) 2.5 %wt (diamonds), b) 5 %wt (squares) and c) 10 %wt (triangles) olive oil. Black symbols indicate coarse emulsions and red ones nanoemulsions.

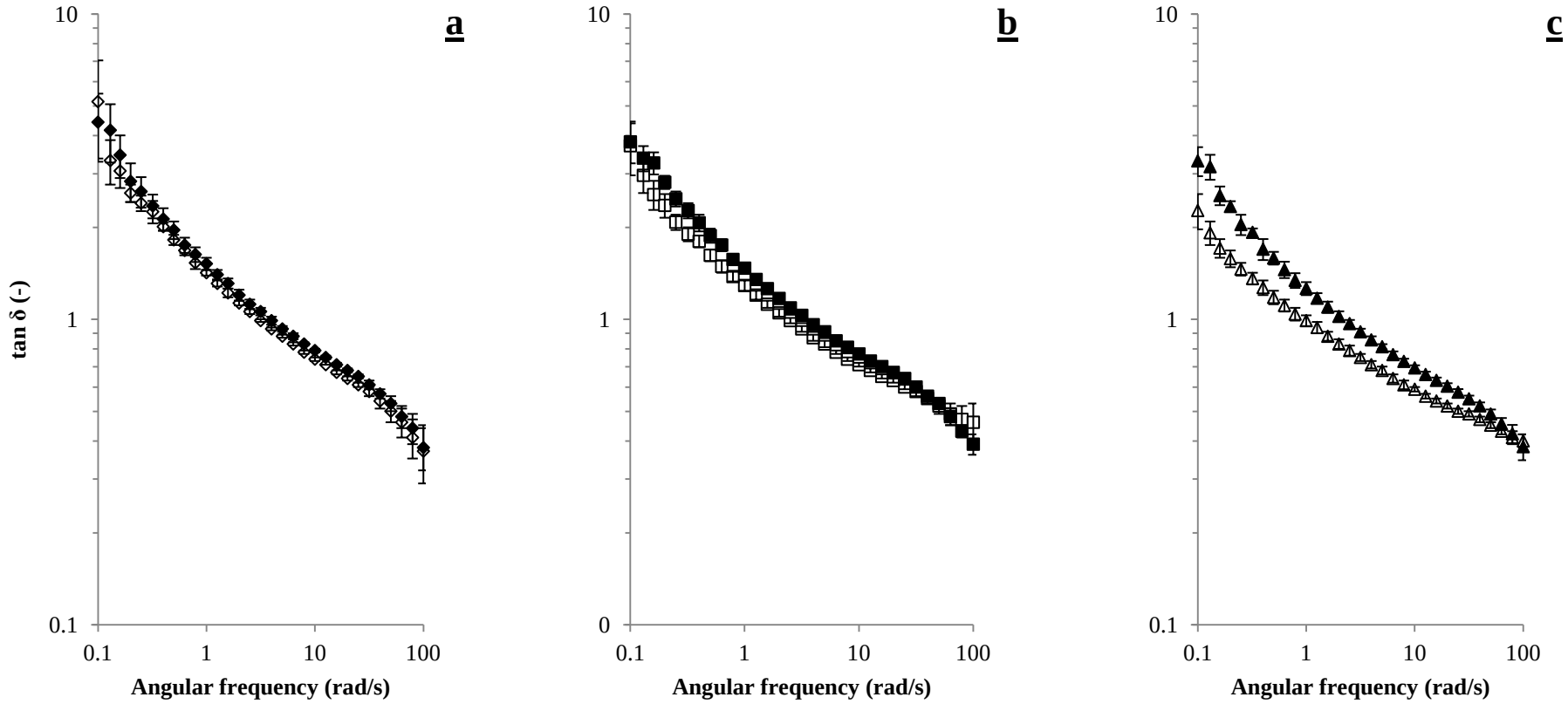


Figure 6.6 Effect of oil content on loss tangent (tanδ) of coarse (closed symbols) and nanoemulsions (open symbols) containing a) 2.5, b) 5 and c) 10 %wt olive oil.

6.3.4 Stability of coarse and nano- emulsions containing 1 %wt FGF

Coarse and nanoemulsion formulations at 1 %wt FGF did not exhibit phase separation within the period tested (10 days, 5^{o}C), hence their stability in terms of coalescence/flocculation phenomena was monitored by means of back-scattering variation as a function of storage time (Fig. 6.7). It can be noticed that the initial BS intensity values are affected by oil content and emulsification method. According to several studies BS intensity is a value affected by the size and density (population) of the oil particles in emulsions. It is enhanced by the increase of the oil droplet number and by decreasing their size or the size of flocs present (Palazolo et al., 2004, 2005). In our case, the increase of the oil fraction from 2.5 to 10 %wt increased the initial BS values in the case of coarse emulsions from 40.3 to 61.6 % and for nanoemulsions from 45.6 to 71.8 %. For coarse emulsions containing 5 and 10 %wt oil, a reduction of approximately ~4 % in BS (dBS) was noticed within 10 days of storage while for those containing 2.5 %wt dBS reached 11.4 % (Fig. 6.7c). For nanoemulsions the BS variation (dBS) ranged between ~ 1-1.9 %, hence an improvement in emulsion stability in terms of coalescence-flocculation was achieved by sonication in all cases of oil content attributed to droplet size reduction and thickening of the samples as discussed above. Normally, an increase in BS should be noticed with storage time in the case of nanoemulsions as a consequence of coalescence or flocculation phenomena occurring during storage as previously observed in other studies (Porras et al., 2008; Wulff-Pérez et al., 2009). According to Mie theory the back scattering intensity increases by decrease of particle size for particles with diameter > 500 nm, while the opposite applies for those of smaller size e.g. nanoemulsions. This could be explained by the fact that nanoemulsions may have undergone flocculation during the incorportation of FGF solution and subsequent pH adjustment from 6.5 to 3.8 passing through the isoelectric point of proteins. The net negative charge that the oil droplets posse at high pH values is suppressed by the decrease of pH values near the pI of the protein (4.8), thus a few droplets of an average diameter 200 - 300 nm could easily form flocs, the size of which could be detected by the Turbiscan device wave length. Considering long storage (5 months), as demonstrated in Fig. 6.8 and 6.9 coarse emulsions prepared with 2.5 %wt oil were the least stable, since phase separation occurred as evidenced by the stark BS decrease near zero values at the bottom of the tube (Fig. 6.8).

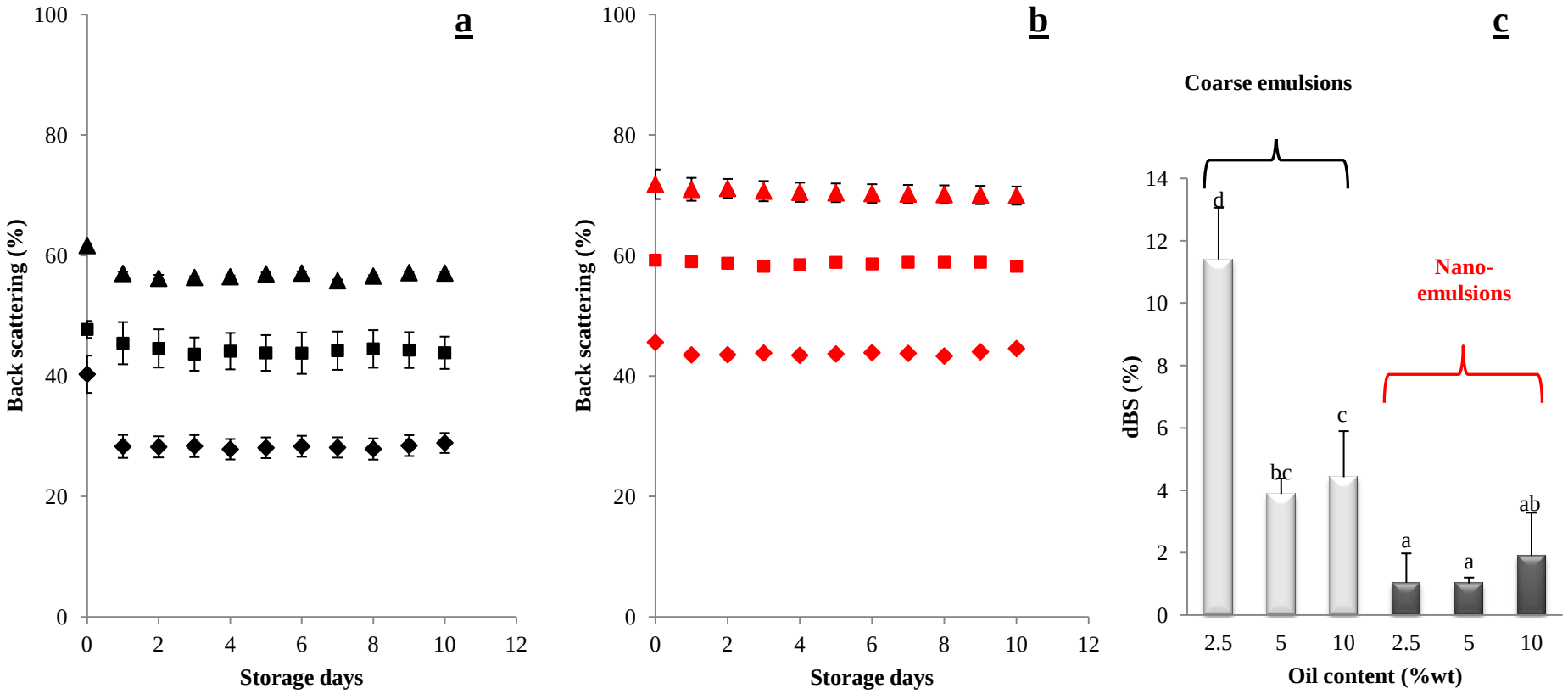


Figure 6.7 Stability of emulsions containing 2.5 (diamonds), 5 (squares) and 10 %wt oil (triangles): a) Coarse emulsions, b) Nanoemulsions and c) Back scattering variation (dBS) after 10 days of storage (5 ^{o}C).

[a-d]Different superscripts indicate significant differences among samples ($p<0.05$).

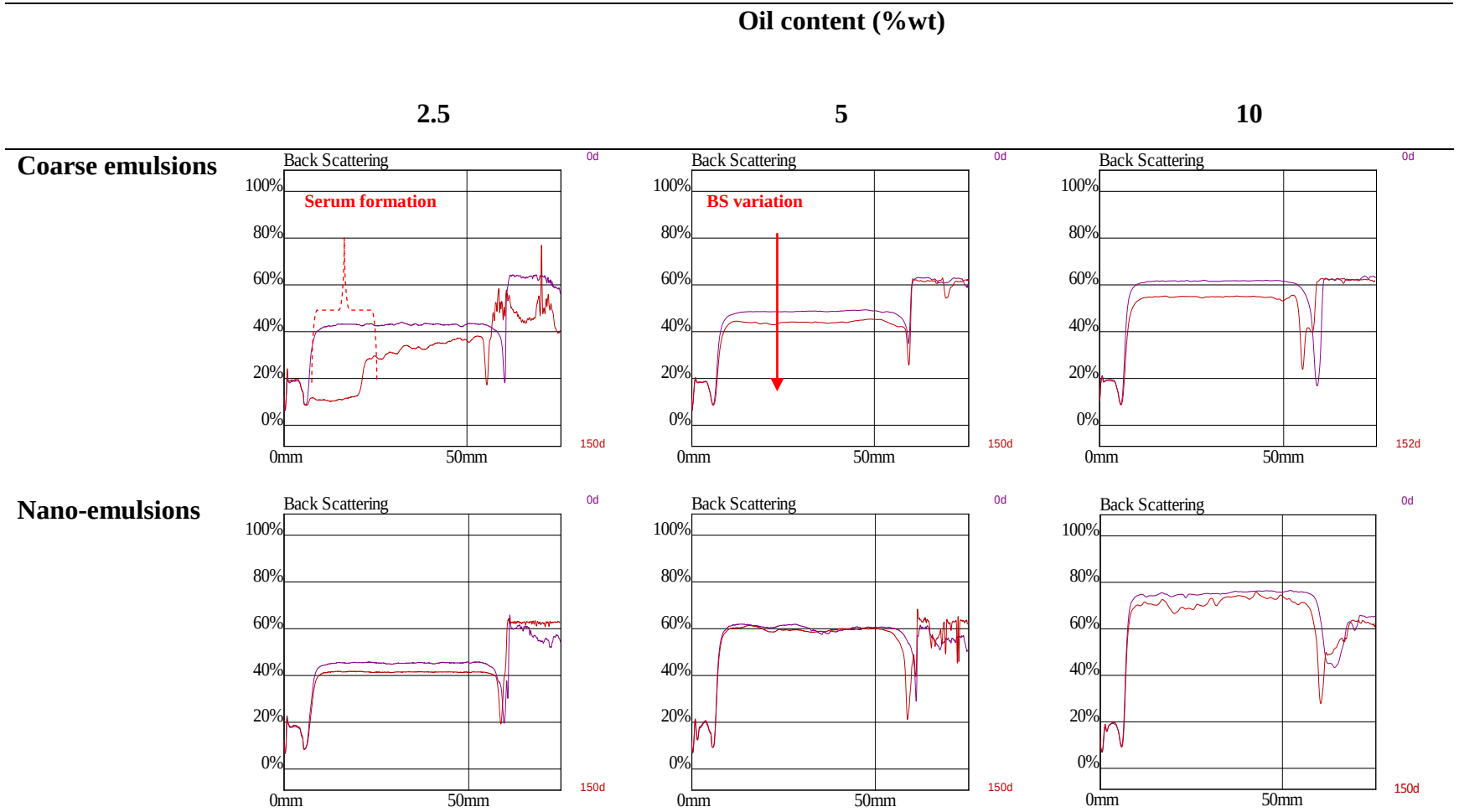


Figure 6.8 Back scattering profiles of coarse and nano- emulsions containing 1 %wt FGF during storage (5 ^{o}C).

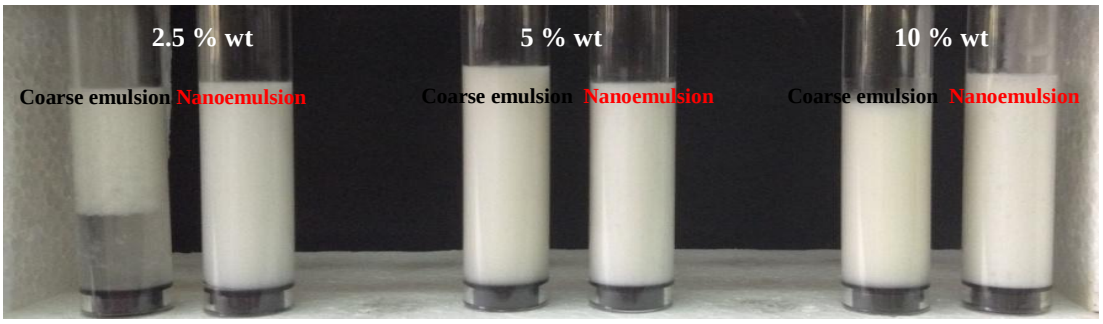


Figure 6.9 Stability of coarse and nano- emulsions containing 1 %wt FGF after 5 months of storage (5 ^{o}C).

6.4 Conclusions

Low oil content salad dressing model emulsions were developed and stabilized by FGF addition. Stable coarse formulations were achieved upon the addition of 1 wt % FGF (final concentration) in all cases of oil content (2.5, 5 or 10 %wt). Emulsion stability was further ameliorated by preparing nano/submicron emulsions by ultrasonic homogenization. Nanoemulsions with the smallest droplet diameter (~200 nm) and PDI (~0.2) were prepared at the lowest oil content after 12 min of sonication. Droplet size reduction in the nano/submicron scale affected the viscoelastic properties of emulsions, hence more structured and viscous emulsions were obtained. All nano/submicron emulsions exhibited higher stability regarding coalescence/flocculation phenomena during storage, attributed to droplet size reduction and enhanced viscoelastic properties. Considering long term storage, sonication was able to hinder phase separation in formulations containing 2.5 %wt oil.

CHAPTER 7

INCORPORATION OF SAFFRON AND POMEGRANATE JUICE POWDER IN EMULSION DRESSINGS: EFFECT ON AROMA PROFILE AND OXIDATION DURING STORAGE.

7.1 Introduction

Current food production trends focus on the protection of food components, as well as on the production of foodstuffs with health promoting properties through the incorporation of antioxidants (Maat et al., 2005).

Pomegranate, a fruit cultivated in the Mediterranean basin (Spain, Italy, Turkey, Greece, Israeli, etc.) and in Southern Asia, in India and in North and South America, has gained worldwide popularity as a functional food and nutraceutical source. The *in vitro* antioxidant activity of pomegranate has been attributed to its high concentration in polyphenols, especially punicalagins, punicalins, gallic acid, and ellagic acid (Seeram et al., 2005). The health effects of the whole fruit and its products (juices and extracts) have been studied in relation to a variety of chronic diseases. Promising results against cardiovascular disease, diabetes, and prostate cancer have been reported from human clinical trials (Melnyk et al., 2010).

Spices are also rich sources of bioactive compounds with potential protective effects against diseases (Embuscado, 2015). Saffron, world's most expensive spice, (*Crocus sativus* L.) exhibits numerous medicinal activities. The antihypertensive, anticonvulsant, antitussive, antigenotoxic and cytotoxic effects, anxiolytic aphrodisiac, antioxidant, antidepressant, anti-inflammatory and relaxant activity of saffron has been well documented in scientific literature (Patel et al., 2008; Bolhassani et al., 2014).

Despite related health benefits with saffron and pomegranate ingredients, only limited information is available to date regarding their antioxidant effect and properties in food systems (Licón et al., 2012a,b; Topuz et al., 2014; Chranioti et al., 2015). The oxidation of lipids is a deleterious chemical reaction that occurs in emulsified products, leading to development of rancid flavor and aroma/odor and harmful compounds. As a concequence, deteriorated foods become unpalatable and unacceptable (Decker, et al., 2010). Meanwhile, ultrasonic technology shows great potential for wider use in the food industry due to its implementation easiness.

Therefore, the aim of this study was to determine and incorporate the most favourable concentration of saffron and pomegranate juice powder in salad dressing model emulsions, prepared by high shear (HS) or ultrasonic (US) homogenization and examine their effect on aroma profile and oxidation during storage.

7.2 Materials and methods

7.2.1 Materials

Organic Greek Red Saffron (*Crocus sativus* L.) filaments were purchased from the Cooperative of Saffron (Kozani) and macerated in a lab scale mill (M 20 Universal mill, IKA Werke GmbH & Co. KG, Stauffen, Germany) in order to obtain a fine powder. Organic freeze-dried pomegranate juice powder from whole fruit (*Punica granatum* L.) was obtained from Navitas Naturals (Novato, California). The product contained no protein, fiber or fats, and ~26 %wt sugars, as stated by the manufacturer. Low fat mayonnaise containing 26 %wt oil (Hellman's Light, Unilever), was purchased from a local store. Glacial acetic acid, isooctane, p-anisidine, methanol and butanol were purchased from Sigma–Aldrich.

7.2.2 Characterization of organic red saffron and pomegranate juice powder

The quality of red saffron filaments was evaluated according to the ISO/TS 3632-2 standards (ISO, 2003). For the pomegranate juice powder Total Soluble Solids (TTS) were estimated in an aqueous solution of 25 %wt powder with a digital refractometer model N1 (Atago Co. Ltd., Tokyo, Japan) at 20°C. Total Phenolic Content (TPC) was estimated by the Folin-Ciocalteu method in 1 %w/v aqueous solution (Singleton and Rossi, 1965).

7.2.3 Consumer preference test

In order to estimate the optimum levels of added saffron and pomegranate powders, two independent sensory evaluations were conducted by using a commercial emulsified product (low fat mayonnaise) containing various amounts of saffron or pomegranate juice powder. Saffron powder was mixed with low fat mayonnaise in appropriate ratios in order to contain various saffron concentrations: 50, 100, 150, 200, 250 and 300 mg/kg in the final formulation taking into account the levels suggested by Selim et al. (2000). Freeze-dried pomegranate juice powder was added in different concentrations: 2.5, 5, 7.5 and 10 %wt. Consumer likeness tests were elaborated with 35-40 untrained individuals, all members of the staff and students of Food Science and Human Nutrition Department. Samples containing saffron powder were evaluated in terms of color, aroma, taste, bitterness, aftertaste and overall acceptance, on a nine-point hedonic scale, with 1 being "dislike extremely" and 9

being “like extremely”. The same applied for pomegranate juice powder samples with the exception that instead of bitterness, sweetness was evaluated due to the high concentration of sugars in the powder.

7.2.4 Color measurement

The color of prepared commercial mayonnaise containing different amounts of saffron or pomegranate juice powder used in consumer likeness test was measured with a Minolta CR-200 colorimeter and Hunter L, a, b parameters were recorded in duplicate per sample. The colorimeter was initially calibrated with a white ceramic plate (L = 96.98, a = -0.81 and b = 3.19).

7.2.5 Salad dressing model emulsion preparation

Salad dressing model emulsions containing 20 %wt olive, 1.06 w% PPI, and 0.5 %wt XG were prepared by high speed or ultrasonication method demonstrated in Chapter 5. Samples homogenized by HS and US treatment (40% amplitude-1min), presented the highest stability, and where therefore chosen for incorporating the optimum saffron and pomegranate juice powder amounts. In total, 6 salad dressing formulations were prepared: reference (REF, no added powders), samples with 100 mg/kg saffron powder (SP) and 5 %wt pomegranate juice powder (PJP).

7.2.6 Quantitative Descriptive Analysis (QDA)

Quantitative Descriptive Analysis (Stone and Sidel, 2004) was used to describe the key aromatic notes and odors of freshly prepared and stored (4 weeks, 25 ^{o}C) model pea protein salad dressing emulsions.

The QDA procedure used in the study was conducted in agreement with requirements of the International Standard (ISO/DIS 13299:1998). 15 experienced panelists from the staff of Laboratory of Food Engineering participated in the QDA evaluation of the model emulsions and were all trained according to the International Standards, including the detection and recognition of aroma, odors and training in the use of scales. The majority of the panelists had more than 2 years of experience in sensory evaluation. The panelists deliberated in order to select the aroma or odor attributes recognized in the samples from a given list for saffron (Maggi et al., 2010) and pomegranate (Koppel and Chaambers, 2010) and a common lexicon was developed. Odors such as oily, acid, rancid and metallic were also enlisted due to their

high importance in emulsified salad dressings. Samples were evaluated in two different sessions (fresh and stored samples) by using an unstructured scale (1 = no intensity, increasing intensity to 9 = strong intensity).

Table 7.1 Standards used during training to analyze the aroma profile of salad dressings.

Aroma/odor descriptor	Standard	Position
Fruity	Isoamyl acetate solutions	2 and 9 respectively
Grassy	Hexenal solutions	2 and 9 respectively
Floral	Phenyl-2-ethylacetate solution 10^{-3} (g/L)	2 and 9 respectively
Spicy	Thymol solution $5x10^{-4}$ (g/L)	5
Buttery	Diacetyl solutions	2 and 9 respectively
Saffron	Aqueous solutions of 30 and 300 mg/L	2 and 9 respectively
Oily	o/w emulsion containing 5 or 50 %wt oil	2 and 9 respectively
Acid	citric acid solution 0.1 %wt	5
Rancid	Unheated and heated oil (50 ^{o}C/1 hour)	1 and 5 respectively
Metallic	metallic coin	9

7.2.7 Emulsion oxidation

7.2.7.1 Measurement of conjugated diene hydroperoxides (CD)

The determination of conjugated diene hydroperoxides in model pea protein salad dressing emulsions was according to Dimakou et al. (2007). Emulsion samples (50 μL) were added to 4 mL of methanol/butanol (2:1 v/v) mixture and vortexed (30 sec). The sample mixture was then filtrated through Macherey–Nagel (Düren, Germany) filters (25 mm, pore 0.2 μm) to remove residual protein from the sample to diminish its spectrum interference near the measurement region. The absorbance of conjugated dienes was measured at 232 nm using a UV–VIS scanning spectrophotometer (Hitachi U-2000 Spectrophotometer) in order to monitor the formation of primary oxidation products.

7.2.7.1 Measurement of p-Anisidine Value (p-AV)

An adaptation of IUPAC Method No. 2.504 was used to analyse p-Anisidine values (pAv) (IUPAC, 1979). Adaptations were made to destabilise the emulsions: 2 ml ethanol were added, followed by 5 ml iso-octane. Ethanol was used to destabilise the emulsion so that the oil was then exposed, and free to disperse in the iso-octane. The tubes were capped, vortexed for ten seconds and centrifuged for 10 min, at 3000 rpm. The absorbance of the solution was measured, using a Hitachi U-2000 UV–Visible spectrophotometer. The p-Anisidine value was calculated by the formula: p-Av = [25 × (1.2 As − Ab)]/m, where: As = absorbance of the oil solution after reaction with the p-Anisidine reagent, Ab = absorbance of the solution of the oil, and m = mass (in g) of the sample.

7.3 Results and discussion

7.3.1 Quality characteristics of organic red saffron and pomegranate juice powder

Table 7.2 demonstrates the results obtained by application of ISO/TS 3632 test methods, as well as ISO requirements set for saffron filaments. The Red Organic Saffron filaments fulfilled the requirements for maximum moisture and volatile content and safranal limitations set. The absorbance values at 257 and 440 nm were 87.9 and 259.2, which allows the classification of the sample used in Category I.

The 1 % w/v aqueous solution of freeze dried pomegranate juice powder contained 65 mg GAE/L. Total polyphenols vary among different cultivars and contents in juice may range between 3.15–7.43 mg GAE/mL, while ^{o}Brix range from 13.97 to 16.30 (Li et al., 2015). The TSS of a 25 %wt pomegranate juice powder aqueous solution was found to be 10.8 ^{o}Brix.

Table 7.2 ISO norm for the quality of saffron and results for the classification of Red Organic Saffron filaments used.

Characteristic	ISO requirements	Sample results
Moisture and volatile matter, % (m/m), max	12	10.44
Bitterness, expressed as direct reading of the absorbance of picrocrocine at 257 nm ($E^{1\%}_{1cm,\ 257nm}$) , on dry basis, Minimum:		
Category I	70	87.9
Category II	55	
Category III	40	
Saffranal expressed as direct readning of the absorbance at 330 nm , ($E^{1\%}_{1cm,\ 330nm}$) on dry basis:		
Minimum	20	41.6
Maximum	50	
Coloring strength, expressed as direct readning of the absorbance of crocine at 440 nm ($E^{1\%}_{1cm,\ 440nm}$), dry basis, Minimum:		
Category I	190	259.2
Category II	150	
Category III	100	

7.3.2 Consumer preference test and color properties of commercial low fat mayonnaise containing saffron or pomegranate juice powder

Color plays a key role in food choice since it is the first attribute that consumers deal with when buying a food product. It is also worth noting that food color has a psychological impact on other food sensory properties such as flavor and taste and modify their perceived intensity (Pangborn, Berg and Hansen, 1963; Lavin and Lawless, 1998).

In Table 7.3 the effect of saffron and pomegranate addition on commercial mayonnaise L, a, and b color parameter is demonstrated. It can be noticed that the addition of increased powder concentration decreased the L values of all samples prepared. For those containing saffron, lightness decreased from 81.22 to 79.77 upon concentration increase from 50 to 300 mg/kg. For pomegranate samples a decrease from 84.72 to 77.95 was noticed by increasing the concentration of juice powder from 2.5 to 10 %wt. The decrease of L values is attributed to the addition of colorants, which cause a decrease in the light being reflected back to the detector (McClements, 2002). The color of saffron is primarily attributed to the presence of crocins (mono-, di- and tri-glycosyl esters of crocetin), while the color of pomegranates is due to the presence of anthocyanins. In contrast to L values, increased saffron powder

concentration decreased the a values (increased greenness) from -10.3 to -11.7, while pomegranate powder concentration increase led to increased positive a values (increased redness). Positive b values which represent the yellowness of the samples, were positively affected by increasing the concentrations of both powders. For saffron dressings, b ranged between 41.08 and 58.88, while for pomegranate samples minor changes from 13.55 to 14.41 were observed.

Table 7.3 Color parameters of commercial mayonnaise containing various amounts of saffron or pomegranate juice powder.

	L	**a**	**b**
Saffron powder (mg/kg)			
50	81.22 (±0.09)	-10.35 (±0.30)	41.08 (±0.21)
100	81.45 (±0.15)	-10.08 (±0.01)	41.57 (±0.04)
150	81.88 (±0.11)	-10.86 (±0.04)	47.86 (±0.13)
200	81.19 (±0.05)	-10.88 (±0.04)	52.41 (±0.05)
250	80.69 (±0.09)	-10.73 (±0.10)	56.52 (±0.31)
300	79.77 (±0.38)	-11.71 (±0.14)	58.88 (±0.25)
Pomegranate juice powder (%wt)			
2.5	84.72 (±0.14)	-0.60 (±0.04)	13.55 (±0.22)
5	82.77 (±0.24)	0.26 (±0.11)	13.53 (±0.13)
7.5	80.39 (±0.01)	1.84 (±0.10)	14.38 (±0.02)
10	77.95 (±0.23)	2.77 (±0.16)	14.41 (±0.02)

Table 7.4 summarizes the results of the consumer test sensory evaluation for the mayonnaise containing saffron powder. All samples scored equally or well above the moderate likeness (~5 - 6.5), with the exception of the sample that contained the lowest concentration (50 mg/kg). Considering color, an optimum saffron incorporation level was detected when 100 mg/kg were added in the mayonnaise formulation, resulting in the highest score (6.6) observed. Meanwhile, on of the least favorable samples was that containing the lowest saffron level (50 mg/kg). Increasing the saffron concentration above 100 mg/kg increased color vividness (increased a nd b values) while gradually decreased sample likeness up to 300 mg/kg, which were also the least favorable. In general the lowest saffron concentration received the lowest

scores in all examined attributes. Considering both aroma and taste, the addition of saffron powder ≥ 100 mg/kg did not cause any significant effect on samples ($p>0.05$). The optimum level for bitterness and aftertaste likeness was set at 100 mg/kg. At the same concentration the highest score for total acceptance was also recorded.

Table 7.5 shows the estimated sensory scores for the dressings containing pomegranate juice powder. Color likeness was negatively correlated with powder concentration, as it was considerably reduced from 6.9 to 5.7 when pomegranate juice powder increased from 2.5 to 10 %wt. In relation to color parameters, this could be attributed to the decrease of lightness as well as increased redness and yellowish hues represented by a and b values accordingly, leading to samples appearing more reddish-brown. Samples containing 5 %wt pomegranate powder scored also better in terms of aroma (7.3) and total acceptance (7.3) ($p<0.05$). Considering the attributes of taste and aftertaste, no significant differences were detected among the samples. A positive correlation of sweetness when increasing the concentration of powder was found, and samples containing the highest amounts were the best rated.

To conclude saffron powder incorporation at a level of 100 mg/kg and pomegranate juice powder at a level of 5 %wt would be suggested for incorporation in emulsified products, since these concentrations received the highest scores for the majority of the sensory attributes tested.

Table 7.4 Sensory attributes of commercial mayonnaise containing various saffron powder concentrations.

Saffron powder (mg/kg)	color	aroma	taste	bitterness	aftertaste	total acceptance
50	5.8^{a} (±2.1)	5.7^{a} (±2.2)	4.2^{a} (±2.3)	4.5^{a} (±2.6)	5.2^{a} (±2.2)	5.8^{a} (±2.1)
100	6.6^{b} (±1.7)	6.2^{b} (±1.9)	5.6^{b} (±2.2)	5.8^{b} (±2.7)	6.2^{b} (±2.1)	6.6^{b} (±1.7)
150	6.2^{ab} (±1.9)	5.8^{b} (±2.0)	5.9^{b} (±1.9)	5.4^{ab} (±2.6)	5.9^{ab} (±2.0)	6.2^{ab} (±1.9)
200	6.5^{ab} (±1.6)	6.2^{b} (±1.7)	6.2^{b} (±2.1)	5.5^{ab} (±2.6)	5.8^{ab} (±2.2)	6.5^{ab} (±1.6)
250	6.0^{ab} (±2.0)	5.9^{b} (±2.1)	5.9^{b} (±2.0)	5.1^{ab} (±2.6)	5.6^{ab} (±2.4)	6.0^{ab} (±2.0)
300	5.8^{a} (±2.2)	5.7^{b} (±1.9)	6.1^{b} (±2.2)	5.0^{ab} (±2.6)	5.7^{ab} (±2.3)	5.8^{a} (±2.2)

$^{a-b}$ Different superscripts indicate significant differences among samples ($p<0.05$).

Table 7.5 Sensory attributes of commercial mayonnaise containing various pomegranate juice powder concentrations.

Pomegranate juice powder (%wt)	color	aroma	taste	sweetness	aftertaste	total acceptance
2.5	6.9^{b} (±1.1)	6.3^{a} (±1.0)	6.6^{a} (±1.2)	5.9^{a} (±1.2)	6.0^{a} (±1.4)	6.8^{ab} (±1.3)
5	6.8^{ab}(±1.2)	7.3^{b} (±0.8)	6.7^{a} (±1.8)	6.4^{ab} (±1.0)	6.5^{a} (±1.3)	7.3^{b} (±0.6)
7.5	6.8^{ab} (±1.8)	6.4^{a} (±1.6)	6.8^{ab} (±0.9)	6.2^{ab} (±1.3)	6.7^{a} (±1.2)	6.7^{ab} (±0.9)
10	5.7^{a} (±2.1)	6.3^{a} (±1.8)	7.6^{ab} (±0.6)	6.9^{b} (±1.2)	6.9^{a} (±1.7)	6.1^{a} (±1.1)

$^{a-b}$ Different superscripts indicate significant differences among samples ($p<0.05$).

7.3.3 Effect of pomegranate and saffron addition on salad dressing model emulsion oxidation during storage

The pea protein dressing emulsions were allowed to oxidize for 28 days at 25 ^{o}C. The progress of oxidation according to the primary lipid oxidation marker (CD) is shown in Fig. 7.1. The major factors governing the rate of lipid oxidation include interfacial characteristics (emulsifier membrane thickness, surface charge), droplet characteristics (size and concentration), ingredient interactions and the use of chelating agents and antioxidants (McClements and Decker, 2006). The lowest CD levels were observed in the presence of pomegranate powder and the highest in the presence of saffron powder. The homogenization method also affected the oxidation of emulsions. Those prepared by US homogenization presented considerably higher absorbance values compared to their HS counterparts, indicating that ultrasonication promotes oxidation. The influence of ultrasonication on emulsion oxidation is considered relatively contradictive. For instance, several studies report that sonication may result in increased oxidation of nanoemulsions or milk (Juliano et al., 2014; Tabibiazar et al., 2015). This phenomenon is due to the reduced droplet size of the US treated dressings which increases the total surface area available for oxidation, as well as the number of transient radicals generated during sonication (OH^-, H^+) (Gülseren et al., 2007). US emulsions are also characterized by lower viscosities compared to HS ones (as seen in Chapter 5) due to XG gum degradation which can lead to increased diffusive mobility of reactants and reaction products, hence increased oxidation rates. On the contrary the CD of flaxseed oil/mik emulsion was unaffected after 8 min of sonication (Shanmugam and Ashokkumar, 2014). Considering the positive effect of pomegranate juice powder incorporation against lipid oxidation, our findings are in accordance to those recently reported by Topuz et al. (2014), who showed that the incorporation of pomegranate juice in olive oil emulsified sauce at 25 up to 50 %wt level was able to retard lipid oxidation when added in fish marinades, attributed to increased polyphenol content compared to controlled samples. Although a numerous studies have been conducted regarding the antioxidant and health promoting properties of saffron as a spice, there has been limited information to our knowledge concerning the antioxidant potential of saffron in food matrices (Martínez-Tomé, 2001). As shown by Martínez-Tomé (2001), saffron exhibits lipid peroxidation inhibitory action when added in oil, but the studied concentration was considerably

higher (5 %wt), while saffron ethanolic extracts (20-100 mg/ml) showed no antioxidant activity *in vitro* by DPPH method. Significant radical scavenging avtivity was identified for two of the main bioactive constituents of saffron, crocin and safranal at levels higher than 500 ppm (mg/L) in ethanolic solutions (Assimopoulou et al., 2005). No radical scavenging activity was detected in methanolic extracts (500 or 5000 mg/L) was also detected according to Ordoudi et al. (2008), who stated that their results indicate that saffron extracts could not be considered as sources of strong food antioxidants. These concentrations are significantly higher than those incorporated for the preparation of dressing model emulsions in our case (100 mg/kg), which could justify the absence of antioxidant activity. The negative effect of saffron addition could be associated with interactions with proteins present on the emulsion matrix. The binding of saffron apocarotenoids (crocin, crocetin and dimethylcrocetin) with lysine-rich proteins has been identified by means of spectrofluorometry (Ashafi et al., 2005). The secondary metabolites of saffron are also known to bind with DNA molecules or human serum albumin (Kanakis et al., 2007a, b). In general proteins can prevent or retard emulsion oxidation by two distinctive mechanisms. They can form thick viscoelastic membranes around the droplet interface which restricts the diffusion of oxidative initiators inside it. Secondly, when present in the aqueous phase they act as free radical scavengers, being preferably self-oxidized (Coupland and McClements, 1996). A similar mechanism for the inhibition of the antioxidant properties of unabsorbed whey proteins by interacting with high concentrations of xanthan gum (0.5 %wt) has been also proposed (Sun et al., 2007). Although, polyphenols can also bind with proteins (Genovese et al., 2015), which could promote oxidation in dressings containing pomegranate juice powder as well, this was not observed. In this case, the amount of phenolics present in pomegranate juice could be high enough so that excessive phenols are present in the food matrix. Moreover, other ingredients contained in the pomegranate juice powder like sugars, which represent its major ingredient may have a protective effect against emulsion oxidation (Ponginebbi et al., 1999; Faraji and Lindsay, 2004). Eventhough a more detailed research is required to fully elucidate the underlying mechanisms involved in lipid oxidation after the addition of saffron or pomegranate juice powder, at concentration investigated pomegranate juice seems to be more efficient against primary oxidation.

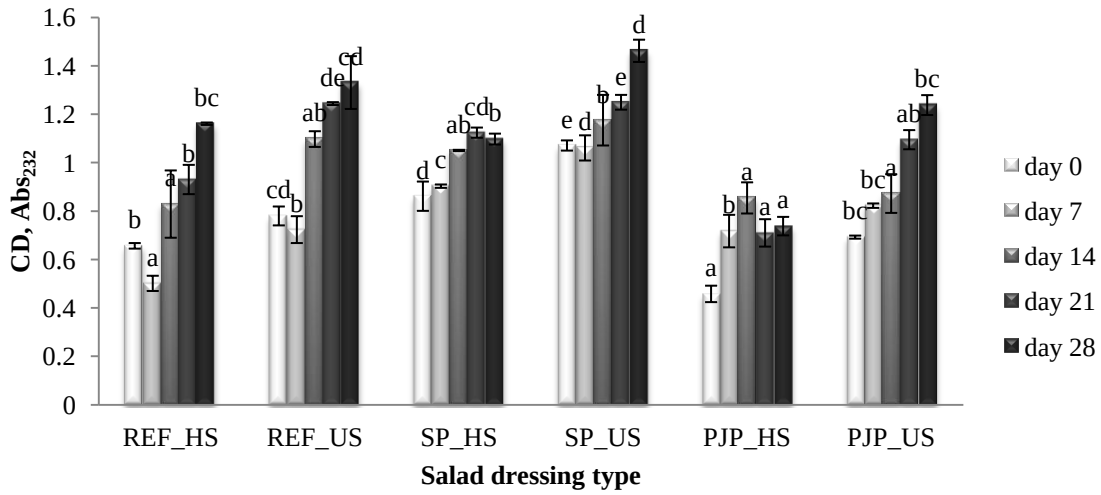


Figure 7.1 Evolution of CD concentration measured as change in absorbance (Abs_{232}) in dressing model emulsions prepared by HS and US methods as a function of storage time (28 days, 5 ^{o}C).

$^{a-e}$ Different superscripts per storage day indicate significant differences ($p<0.05$).

The p-Av is a reliable measurement for the evaluation and monitoring of the secondary oxidation products. During lipid oxidation the primary reaction products decompose to secondary products (aliphatic aldehydes, ketones, alcohols, acids and hydrocarbons), responsible for off-flavors and off-odors of edible oils (Poiana, 2012).

As shown in Fig. 7.2 fresh samples (day 0) containing pomegranate powder prepared by HS method exhibited the lowest p-Av, while no significant differences were obtained between the reference and that containing saffron powder. Considering the emulsification method applied no significant differences were observed among HS and US samples for the majority of the measurements, with the exception US ones containing pomegranate powder on days 14 and 21 which unexpectedly exhibited higher values compared to their HS counterparts. Storage had a detrimental effect over the chemical stability of dressing model emulsions, resulting in increased p-AV values ranging between 0.48-0.53 and 0.44-0.63 for HS and US respectively. However, no significant differences could be detected among samples regarding their formulation or homogenization method used. Obviously, results from the measurement of secondary products do not correlate with those obtained by measuring the CD values, which clearly revealed higher oxidation rates for US samples and lowest oxidation in the presence of pomegranate powder. One possible explanation for this observation would be that, the value of the CD absorbance is

affected by the presence of other conjugated dienes (not hydroperoxides) i.e. carotenenoids (i.e. crocin and crocetin) present in saffron (Akoh and Min, 2008). In contradiction to the suggestions and findings of some studies and reviews (Shahidi and Zhong, 2005) that the CD values correlate well with those of the peroxide value – the latter being a more sensitive and specific index of the primary formed hydroxides-, this may not apply in the case of olive oil. Oils such as olive oil or high-oleic sunflower oil, contain low amounts of polyunsaturated fatty acids, hence the appropriatness of the method for monitoring the formation of primary oxidation products would be limited in this case (Marmesat et al., 2009; Frankel, 2012).

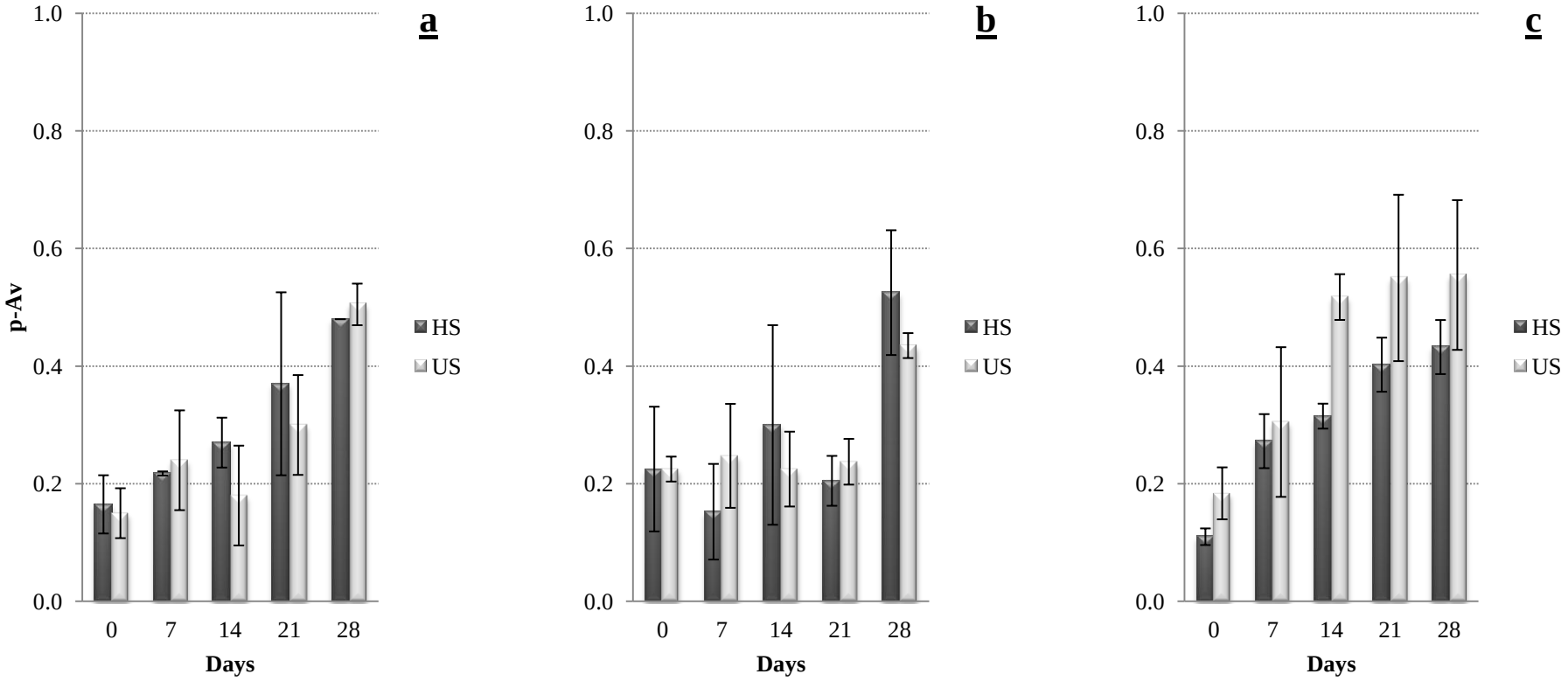


Figure 7.2 p-Anisidine values (p-Av) of dressing model emulsions prepared by HS and US methods as a function of storage: a) REF, b) SP and c) PJP samples.

7.3.4 Influence of pomegranate and saffron addition on aroma profile of salad dressing model emulsions during storage

Mean intensity ratings of descriptive aroma and odor attributes and the analysis of varience of the pea protein salad dressings are documented in Table 7.6. Results from sensory QDA indicated that with the exception of the oily odor all other sensory attributes were not affected by homogenization method applied -which modifies emulsion droplet size and viscosity- or storage. The viscosity of the emulsions is an important parameter that influences the diffusion of volatile molecules since the Stokes–Einstein equation predicts that the diffusion is dependent on the square root of the viscosity (Karaiskou et al., 2008).

Considering droplet size, emulsions characterized with lower droplet diameters may retard the volatization of the compounds due to reduced interfacial surface area (Karaiskou et al., 2008). It could be therefore assumed that despite polysaccharide degradation the viscosity of ultrasonically treated pea protein emulsions (as seen in Chapter 5) was efficient to prevent a rapid diffusion of aromatic volatile compounds and subsequent release at the air space. The addition of pomegranate powder resulted in salad dressings with increased scores in fruity aroma compared to reference and saffron containing dressings. Moreover, these dressings (PJP) were also characterized by increased values of grassy note, especially those prepared with HS method (day 0). The addition of pomegranate resulted in increased flower aroma intensity but only in the case of fresh samples prepared by HS homogenization. No significant differences were detected among the samples in terms of saffron, spicy and butter notes and rancid or metallic odors. Metallic odors can be caused by metal ions migration from the sonication probe immersed in the processing samples (Riener et al., 2009). Considering rancidity, results obtained from QDA evaluation are in accordance with those obtained by p-Av method, which suggest no significant effect of saffron or pomegranate juice powder addition or emulsification method on the formation of secondary oxidation derived off-flavors.

Table 7.6 Aroma and odor descriptor scores of dressing emulsions as affected by storage.

		fruity	grassy	flower	spicy	buttery	saffron	oily	acid	rancid	metallic
REF HS	*day 0*	$1.2^{a} \pm 0.6$	$1.8^{ab} \pm 0.8$	$2.2^{ab} \pm 1.5$	$3.8^{a} \pm 2.6$	$1.7^{a} \pm 0.8$	-	$5.7^{ab} \pm 2.0$	$3.9^{ab} \pm 2.0$	$2.4^{a} \pm 1.4$	$2.6^{a} \pm 1.5$
	day 28	$1.8^{a} \pm 0.2$	$2.3^{ab} \pm 1.9$	$1.5^{a} \pm 0.5$	$3.0^{a} \pm 1.9$	$1.2^{a} \pm 0.4$	-	$6.4^{b} \pm 2.9$	$5.8^{b} \pm 1.8$	$3.7^{a} \pm 2.5$	$3.5^{a} \pm 2.5$
REF US	*day 0*	$1.3^{a} \pm 0.5$	$1.7^{ab} \pm 0.8$	$1.5^{a} \pm 0.5$	$4.0^{a} \pm 2.7$	$1.7^{a} \pm 0.5$	-	$6.6^{b} \pm 2.6$	$3.9^{ab} \pm 2.5$	$3.3^{a} \pm 2.1$	$3.5^{a} \pm 2.6$
	day 28	$1.5^{a} \pm 0.4$	$1.3^{a} \pm 0.5$	$2.0^{ab} \pm 0.5$	$2.6^{a} \pm 1.9$	$1.5^{a} \pm 0.8$	-	$4.9^{ab} \pm 2.8$	$4.7^{ab} \pm 2.3$	$1.7^{a} \pm 0.5$	$2.3^{a} \pm 1.9$
SP HS	*day 0*	$1.2^{a} \pm 0.8$	$1.7^{ab} \pm 0.8$	$2.0^{ab} \pm 1.3$	$3.6^{a} \pm 2.1$	$1.3^{a} \pm 0.5$	$6.8^{a} \pm 1.2$	$6.4^{b} \pm 1.7$	$4.4^{ab} \pm 2.7$	$3.3^{a} \pm 2.1$	$3.2^{a} \pm 2.6$
	day 28	$1.5^{a} \pm 0.7$	$2.5^{ab} \pm 1.8$	$2.7^{ab} \pm 0.9$	$4.3^{ab} \pm 2.1$	$2.2^{a} \pm 1.2$	$5.0^{a} \pm 2.4$	$3.9^{a} \pm 1.8$	$2.8^{a} \pm 2.8$	$3.1^{a} \pm 2.0$	$2.9^{a} \pm 2.7$
SP US	*day 0*	$1.5^{a} \pm 0.6$	$2.2^{ab} \pm 1.9$	$2.7^{ab} \pm 2.3$	$4.0^{a} \pm 2.4$	$2.0^{a} \pm 1.5$	$6.0^{a} \pm 2.7$	$6.7^{b} \pm 2.1$	$4.7^{ab} \pm 2.6$	$3.7^{a} \pm 2.8$	$3.8^{a} \pm 3.1$
	day 28	$1.3^{a} \pm 0.5$	$2.5^{ab} \pm 1.7$	$2.3^{ab} \pm 2.0$	$4.7^{ab} \pm 3.0$	$1.3^{a} \pm 0.5$	$4.3^{a} \pm 2.9$	$3.7^{a} \pm 2.2$	$3.5^{ab} \pm 2.3$	$3.5^{a} \pm 2.5$	$3.2^{a} \pm 2.2$
PJP HS	*day 0*	$2.8^{b} \pm 0.5$	$4.8^{c} \pm 2.9$	$3.7^{b} \pm 2.4$	$4.6^{ab} \pm 2.5$	$2.0^{a} \pm 1.8$	-	$5.3^{ab} \pm 3.3$	$4.0^{ab} \pm 2.9$	$3.4^{a} \pm 2.5$	$3.4^{a} \pm 2.4$
	day 28	$3.0^{b} \pm 0.7$	$3.3^{bc} \pm 2.6$	$2.0^{ab} \pm 1.3$	$3.9^{a} \pm 2.0$	$1.8^{a} \pm 1.2$	-	$6.1^{ab} \pm 2.5$	$4.9^{ab} \pm 2.0$	$3.7^{a} \pm 3.1$	$4.5^{ab} \pm 2.8$
PJP US	*day 0*	$2.7^{b} \pm 0.5$	$3.8^{bc} \pm 3.4$	$3.0^{ab} \pm 2.3$	$4.4^{ab} \pm 2.1$	$2.5^{a} \pm 1.3$	-	$4.7^{ab} \pm 2.7$	$3.8^{ab} \pm 2.5$	$3.3^{a} \pm 2.4$	$3.3^{a} \pm 2.4$
	day 28	$3.3^{b} \pm 0.6$	$4.0^{bc} \pm 1.8$	$3.5^{b} \pm 3.3$	$5.4^{ab} \pm 1.9$	$2.2^{a} \pm 1.2$	-	$4.0^{a} \pm 2.5$	$2.8^{a} \pm 1.5$	$3.0^{a} \pm 2.4$	$3.3^{a} \pm 2.3$

[a-b] Different superscripts indicate significant differences among samples ($p<0.05$).

7.4 Conclusions

The addition of saffron and pomegranate juice powder altered the color properties and modified the preference of commercial emulsified products. With regards to added saffron an optimum concentration was set at 100 mg/kg which resulted in maximum response in all sensory attributes tested, in addition to being the lowest concentration.

Commercial samples containing pomegranate juice were mostly preferred at 5 %wt addition level, which scored the best with respect to aroma and total acceptance. Saffron addition resulted in slightly more oxidized model dressing emulsions compared to control and pomegranate juice samples, the later being the least oxidized, as evidenced by CD values. Ultrasonicated model emulsions experienced increased CD levels, due to decrease of droplet size.

Results from CD evaluation were not in line with those from p-Av measurements, probably due to the reduced specificity and sensitivity of the conjugated dienes' method. The emulsification method did not influence the formation of secondary oxidation metabolites in any sample.

The addition of saffron and pomegranate affected the intensity of aroma and odor descriptors in various ways. However, the droplet size and viscosity decrease in US samples did not affect the aroma profile for most of the dressings. Storage also had no remarkable effect on aroma profile for the vast majority of the aroma descriptors for the time period examined.

CHAPTER 8

PROPERTIES OS SALAD DRESSINGS PREPARED BY HIGH-SHEAR AND ULTRASONICATION METHOD, CONTAINING SAFFRON (*Crocus sativus* L.) AND POMEGRANATE JUICE POWDER DURING LONG TERM STORAGE.

8.1 Introduction

Emulsified sauces such as mayonnaise and salad dressings represent a very significant category for the food industry and their nutritive and economic importance continues to grow within the last two decades (Sikora et al., 2008). Mayonnaise is the most widely consumed sauce today and traditionally is an o/w emulsion containing high amounts of fat (70-80 %) and egg yolk (Worrasinchai et al., 2006), while for dressings the fat content varies between 30-60 %. Regarding their formulation, low cost vegetable oils (sunflower oil, corn oil, soy bean oil) are mainly incorporated, whereas olive oil would suggest a healthier alternative (Di Mattia et al., 2015).

Due to the high amount of fat, its origin and the type of common emulsifiers/stabilizers used, a great deal of interest has been developed by the consumers towards reformulation of such products, which could fulfill consumer demands as well as strategy requirements addressing health concerns, such as obesity (Buttriss et al., 2013; Laurian, 2013; Malik et al., 2013).

However the most common problem of low fat emulsion formulations is their instability problems during storage attributed to creaming, flocculation and coalescence phenomena. For this reason, polysaccharides are often incorporated in emulsion formulations, because of their thickening effect (Tarrega and Costell, 2006). Additionally, such thickeners have been mentioned as possible additives to contribute in fat replacement (Frøst et al., 2001). Xanthan gum has a high efficacy in stabilizing such products, due to its superior pseudoplastic character and gelling properties compared to other commonly used hydrocolloid stabilizers (locust bean gum, guar gum) (Kennedy et al., 2015). More recently, resistant starch (RS) has received much attention for both its potential health benefits (Sajilata et al., 2006) and functional properties (viscosity, gel formation) (Fuentes-Zaragoza et al., 2010; Protonotariou et al., 2013). Pea protein is suitable for human consumption, and especially for the development of novel protein foods, which could be used for meat replacement, whey replacement in protein beverages, or as alternative emulsifiers (Sandberg et al., 2011; Karaca et al., 2011).

The antioxidant potential of pomegranate juice and the health benefits of saffron have been extensively studied (Fernández, 2006; Kanakis et al., 2009; Melnyk et al., 2010; Mena et al., 2013; Vegara et al., 2013; Aboonabi et al., 2014; Bhandari, 2015; Emami et al., 2015). However, despite their health benefits, only a few studies have

been conducted regarding the use of saffron in final products and their quality characteristics (Carpino et al., 2008; Licón et al., 2012a, b). Although pomegranate juice has been extensively studied in terms of chemical composition, quality characteristics, influence of processing and storage, still only little information is available considering its use in food products such as sauces, emulsified salad dressings and mayonnaise (Gokoglu et al., 2009; Topuz et al., 2014).

8.2 Materials and methods

8.2.1 Materials

Pea protein isolate (PPI) Nutralys® S85F was a kind gift from Roquette Freres S.A. (Lestrem, France). Resistant starch (RS), (Hi-Maize 260, National Starch, USA) was from Ingredion Gmbh (Hamburg, Germany). Xanthan gum (XG) was bought from Sigma (St. Louis, MO, USA). Virgin olive oil (Altis, Elais Unilever, Greece) was purchased from a local store. Organic red saffron was purchased from the Cooperative of Kozani Saffron and was grinded to a fine powder with a lab mill. Freeze-dried pomegranate juice powder was purchased from Navitas Naturals (California). Citric acid and phosphate were purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland). White pepper, garlic powder and salt were purchased from a local store.

8.2.2 Solution preparation

Xanthan gum solution 1% wt in citric acid-phosphate buffer at pH 3.8 was prepared by hot stirring for 90 min at 90 ^{o}C. Pea protein isolate solution 10.6 %wt was also prepared by stirring for 90 min in ambient temperature. The solutions were kept at 5 ^{o}C overnight to ensure complete hydration. Sodium azide (0.02 %wt) was added as an antibacterial agent.

8.2.3 Preparation of salad dressings

HS salad dressings (50 g in total mass) were prepared by homogenizing the olive oil, aqueous phases of XG and PPI and other ingredients listed in Table 8.1 (formulation given as g/100 g dressing) with an Ultra Turrax (T25 basic, Janke & Kunkel IKA Labortechnik, Staufen, Germany) for 2 min (13.500 RPM) at room temperature. The amounts of added saffron and pomegranate juice powder were the optimum ones determined by sensory evaluation in Chapter 7. For the preparation of

US samples, the coarse HS formulations were further subjected to processing by an ultrasonic homogenizer model Sonopuls 3200 (Bandelin Electronic Gmbh & Co, Berlin) equipped with a 3 mm in diameter titanium probe (MS 73). The tip was immersed 10 mm from the surface of the sample which was placed in a double jacketed flow-through vessel (model DG-3). The temperature was maintained at 30 ±1 ^{o}C by circulating cold water with a pump. The amplitude was set at 40% and ultrasonication processing time was 1 min. In total, 6 salad dressings were prepared by HS and US homogenization: reference (REF, no added powders), with saffron powder (SP) and dressings with added pomegranate juice powder (PJP). All samples were prepared and analyzed in triplicate.

Table 8.1 Composition (g/100g dressing) of salad dressings' formulae.

Ingredients (g/100 g dressing)	**REF**	**SP**	**PJP**
Olive oil	20	20	20
PPI solution 10.6 %wt	10	10	10
XG solution 1% wt	50	50	50
Resistant starch	2	2	2
Saffron powder	-	0.01	-
Pomegranate juice powder	-	-	5
White pepper	0.25	0.25	0.25
Garlic powder	0.25	0.25	0.25
Salt	1	1	1
Buffer pH 3.8	16	15.99	11.5

8.2.4 Microstructure

Emulsion structure was observed in freshly prepared and stored samples (day 180) with a conventional optical microscope (Kruss Optronik, Germany) and a 40x magnification. The micrographs were recorded using a camera (SONY, Hyper HAD, CCD-Iris) connected to a computer.

8.2.5 Rheological properties of salad dressings

A hybrid rheometer DHR (TA Instruments) equipped with plate-plate geometry (upper plate diameter 50 mm, gap 1 mm) was used to obtain flow curves between 0.1

to 1000 s^{-1} at 25 ± 0.01 ^{o}C by circulating water from a peltier. The total measurement time was 900 s and stress vs rate data were fitted to the Power law model:

$$\tau = K \gamma^{n} \quad \text{Eq. 8.1}$$

Where, τ is shear stress (Pa), γ shear rate (s^{-1}), K consistency index (Pa-s^{n}) and n behavior index (dimensionless).

The linear viscoelastic region (LVR) was assessed at 6.28 rad/s (= 1 Hz) by strain sweep experiments; for all samples a constant deformation of γ = 0.1 % was used, which was within the linear viscoelastic region of all samples. Small deformation oscillatory measurements for evaluation of the viscoelastic properties, G′ (storage modulus), G″ (loss modulus), and tanδ (G″/G′), were performed over the frequency range of 0.1–100 rad/s at 25 °C.

In order to establish a quantitative analysis of the frequency dependence of G′ modulus, the following power law model was used (Wang et al., 2009):

$$G' = K' \omega^{n'} \quad \text{Eq. 8.2}$$

Where, K′ is constant and n′ is referred to as the frequency exponent, and ω is the angular frequency.

8.2.6 Storage stability

Storage stability of the salad dressings was determined by obtaining backscattering profiles with a Turbiscan 2000 device (Turbiscan 2000, Formulaction, Toulouse). The average value of the backscattering (BS) measurement over the whole height of the sample (approximately zone 10-55 mm) was plotted as a function of ageing time (180 days). BS variation (dBS) between days 0 and 180 was calculated as in Chapter 2.

8.2.7 Color properties

Color measurement of salad dressings was performed with a Minolta colorimeter (CR-200, Minolta Company, Ramsey, NJ, USA) after being standardized using Hunter lab colour standards. The parameters recorded were: L = lightness (black/white), a = chroma (green/red) and b = hue (blue/yellow). These parameters were used for the determination of color properties. Total Color Difference (TCD) was calculated from Eq. (8.3) (Khazaei et al., 2014).

$$TCD = [(L-L_0)^2 + (a_0-a)^2+(b_0-b)^2]^{½} \quad \text{Eq. 8.3}$$

Where, L_0, a_0 and b_0 color values of freshly prepared samples, L, a, b, color values on day 180 of storage.

8.2.8 Sensory evaluation

Sensory evaluation was performed by a small panel consisting of 15 experienced assesors from Food Science and Human Nutrition Department of Agricultural University (Athens) the vast majority of whom had more than 3 years experience in sensory evaluation. Their age ranged between 22 and 44, 6 males and 9 females participated in the sensory evaluation of each product. The pots filled with 10 g dressing samples were codified with three digit numbers and presented to the panelists in randomized order. Panelists were asked to rate the likeness on color, taste, aftertaste, viscosity, flowability, aroma and total acceptance of the samples by using a 9-point structured hedonic scale (9 = like extremely, 1 = dislike extremely). The tasters were asked to eat an unsalted biscuit and drink water between the samples to avoid an aftertaste and cleanse the palate.

8.2.9 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and F-test was applied in order to compare the mean values at 95 % level of confidence.

8.3 Results and discussion

8.3.1 Salad dressing rheological properties

Salad dressing emulsions are pseudoplastic materials with yield stress, since their shearing stress, and hence viscosity, depend on the applied shear rate and they begin to flow provided their yield stress value is exceeded. Typically, this behavior is explained in terms of the continuous break down of emulsion structure during the course of shear application and flow (McClements, 1999).

To identify the flow characteristics of dressings, data from flow measurements were fitted to the Power law model, which is commonly used for the rheological analysis of such systems. The associated parameters, consistency (K) and flow behavior index (n) values are summarized in Table 8.2. Overall, the reference and

saffron dressings prepared by HS method demonstrated fairly similar rheological behavior. On the contrary, pomegranate powder addition resulted in thicker samples, as evidenced by the consistency values. K values for HS reference and saffron samples were 9.66 and 10.03 (Pa-s^n), but significantly higher values (11.74 Pa-s^n) were observed for pomegranate dressings ($p<0.05$), because of its considerably higher concentration compared to saffron.

All systems were highly pseudoplastic with flow behavior (n) values around 0.21 without significant differences among them. Sonication resulted in less viscous and less pseudoplastic samples characterized by lower K and increased n values ranging from 7.83 to 9.32 Pa-s^n and 0.238 to 0.259 respectively, as a consequence of xanthan mechanochemical degradation. Again the pseudoplasticity of dressings did not depend on composition ($p>0.05$).

The consistency and flow behavior index of salad dressing emulsions may differ to an appreciable extent, depending on oil volume fraction or the presence in the emulsion of thickening agents. In our case, the estimated viscosity parameter values of HS and US emulsion dressings are significantly different from those reported for 20% o/w emulsions containing 0.5% wt XG gum in the presence of 2 %wt WPI, for which lower K and higher n values have been reported (3.328 Pa-s^n and 0.3 respectively) (Sun and Gunasekaran, 2009). This could be the result of the thickening effect of resistant starch incorporated in the samples. The role of RS as a solid particle stabilizer has been reported by Protonotariou et al. (2013). It was shown that a three-fold viscosity increase was the result of 2 %wt RS addition in low fat olive oil emulsions containing 0.3 %wt XG. However, direct comparisons cannot be made since, preparation methods and the resulting colloidal attributes of the emulsion matrices (droplet size, flocculation) may vary considerably.

Storage had a major influence on dressing consistency index, which is a measure of viscosity. It is worth noticing that within the first two months of storage, the consistency of the HS dressings remained the same, regardless of the composition, whereas for the US samples a significant reduction was observed. Additionally, for the HS samples, a gradual reduction of K values was noticed up to 4 months of storage, after which no significant reduction occurred. For the US samples on the other hand, no decrease of the consistency index was observed during elongated storage (>2 months). The decrease of emulsion viscosity is related to the existence of droplet coalescence in the system (Tadros et al., 2004) (as discussed below in the

segment for stability). After six months of storage, a reduction of about 25% in the consistency values was noticed regardless of the treatment or emulsion composition. Regarding the flow behavior, values were practically unaffected by long term storage, with the exception of HS samples containing pomegranate powder. In this case, n values increased gradually with storage from 0.221 up to 0.245, even though no significant increase was observed between 2 and 6 months.

Dynamic oscillatory shear test was used to characterize the viscoelastic properties of salad dressings. The G' and G" moduli calculated are shown in Fig. 8.1 (a-d). Both viscoelastic moduli values showed angular frequency dependence. Moreover, there is a dominance of G' over the G'' and absence of crossover point in all samples regardless of preparation method and storage time. Ideally, for a true gel G' value should be frequency independent and G'>>G" ($\tan\delta = G''/G' < 0.1$), whereas such frequency dependence is typical for weak gel behavior in emulsions (Ross-Murphy et al., 1995; Ikeda and Nishinari, 2001; Tzoumaki et al., 2011).

In general, freshly prepared reference and saffron HS samples presented similar values of G' and G'' values within the examined frequency range. This indicates that no further interactions take place among the emulsions' ingredients when saffron is added. Slightly higher values were detected for both moduli when pomegranate powder was added, due to concentration effects. Dominant elastic properties have been referred by Lee et al. (2012) for mixtures of RS with 0.5 % xanthan and pectin gum, indicating the continuous phase impact on the elastic character of the emulsion. The US treated dressings presented lower G' values than the HS ones as a consequence of polymer degradation. Thus, structural modifications occurring during ultrasonication, influence the rheological properties of the emulsions.

Long term ambient storage greatly affected the viscoelastic behavior of emulsions by decreasing the values of both storage and loss moduli, indicative of less thick emulsions. In particular, a slight higher dependence at high frequencies can be observed on the 120 days of storage samples, with the exception of the HS dressings containing pomegranate (Fig. 8.1,c), which exhibited a similar behavior at the end of storage. These results indicate structural changes of the samples upon storage for four months and are in accordance to the changes observed in viscosity values for the same storage period.

In Table 8.3, the effect of storage is presented according to the calculated parameters of the model described by Eq. 8.2. Similar results can be reached by using

the parameters of the model for G'', but for simplicity reasons, only K' and n' values are presented for all samples. K' represents the solid character strength and n' is the elastic exponent according to some recent reference data (Saxena et al., 2011; Bagliotti Meneguin et al., 2014). In this sense, the solid character will be enhanced as the K' values become higher, while lower n' values indicate that the network stability increases (lower dependency on frequency). In our samples, K' values decrease, whereas n' increase during storage indicating a less solid behavior with storage. After 180 days storage, K' values decreased by 25 % in all HS samples. A similar rate of decrease also found with viscosity experiments. The US treated samples presented a slightly higher decrease at in K' values, for the same storage period, reaching a 30 % reduction. Moreover, the frequency dependence, as n' values indicate, was similar upon storage for the HS samples upon a 120 days storage, whereas for the US samples, n' values on day 120 were significantly increased compared to those on days 0 and 60 of storage ($p<0.05$).

Table 8.2 Consistency (K) and flow behavior (n) indices according to Power law model of HS and US salad dressings during long term ambient storage (180 days).

	K (Pa-s^n)	n (-)	K (Pa-s^n)	n (-)
	REF HS		**REF US**	
day 0	9.66^{bc} ±0.48	$0.207^{a/*}$ ±0.001	7.83^{a} ±0.42	$0.238^{bc/*}$ ±0.020
day 60	9.39^{ab} ±0.94	0.22^{a} ±0.004	$7.26^{a/*}$ ±0.25	0.257^{cd} ±0.008
day 120	$8.03^{abc/*}$ ±0.33	0.215^{a} ±0.014	6.65^{a} ±0.62	0.253^{c} ±0.006
day 180	7.17^{bc} ±0.69	0.229^{a} ±0.008	6.71^{ab} ±0.37	0.263^{c} ±0.005
	SP HS		**SP US**	
day 0	10.03^{c} ±0.05	$0.222^{ab/*}$ ±0.006	8.72^{ab} ±0.25	$0.249^{c/*}$ ±0.006
day 60	9.10^{bc} ±0.77	0.223^{a} ±0.002	$7.12^{a/*}$ ±0.37	0.254^{c} ±0.005
day 120	$8.38^{bc/*}$ ±0.28	0.224^{ab} ±0.007	7.13^{ab} ±0.45	0.258^{c} ±0.004
day 180	7.78^{c} ±0.49	0.225^{a} ±0.004	6.23^{a} ±0.46	0.259^{c} ±0.02
	PJP HS		**PJP US**	
day 0	11.74^{d} ±0.41	0.221^{ab} ±0.001	9.32^{bc} ±1.20	$0.259^{c/*}$ ±0.015
day 60	10.53^{c} ±0.40	$0.238^{b/*}$ ±0.003	$7.61^{ab/*}$ ±0.17	0.268^{d} ±0.004
day 120	$9.81^{c/*}$ ±1.06	0.245^{b} ±0.003	7.80^{ab} ±1.21	0.256^{c} ±0.007
day 180	8.39^{d} ±0.20	0.240^{b} ±0.002	7.30^{bc} ±0.31	0.264^{c} ±0.005

Regression coefficients (R^2) range between 0.975-0.986.
[a-c] Different superscripts for each parameter per storage day indicate significant differences ($p<0.05$).
*Denotes no significant differences from that point on during storage ($p>0.05$).

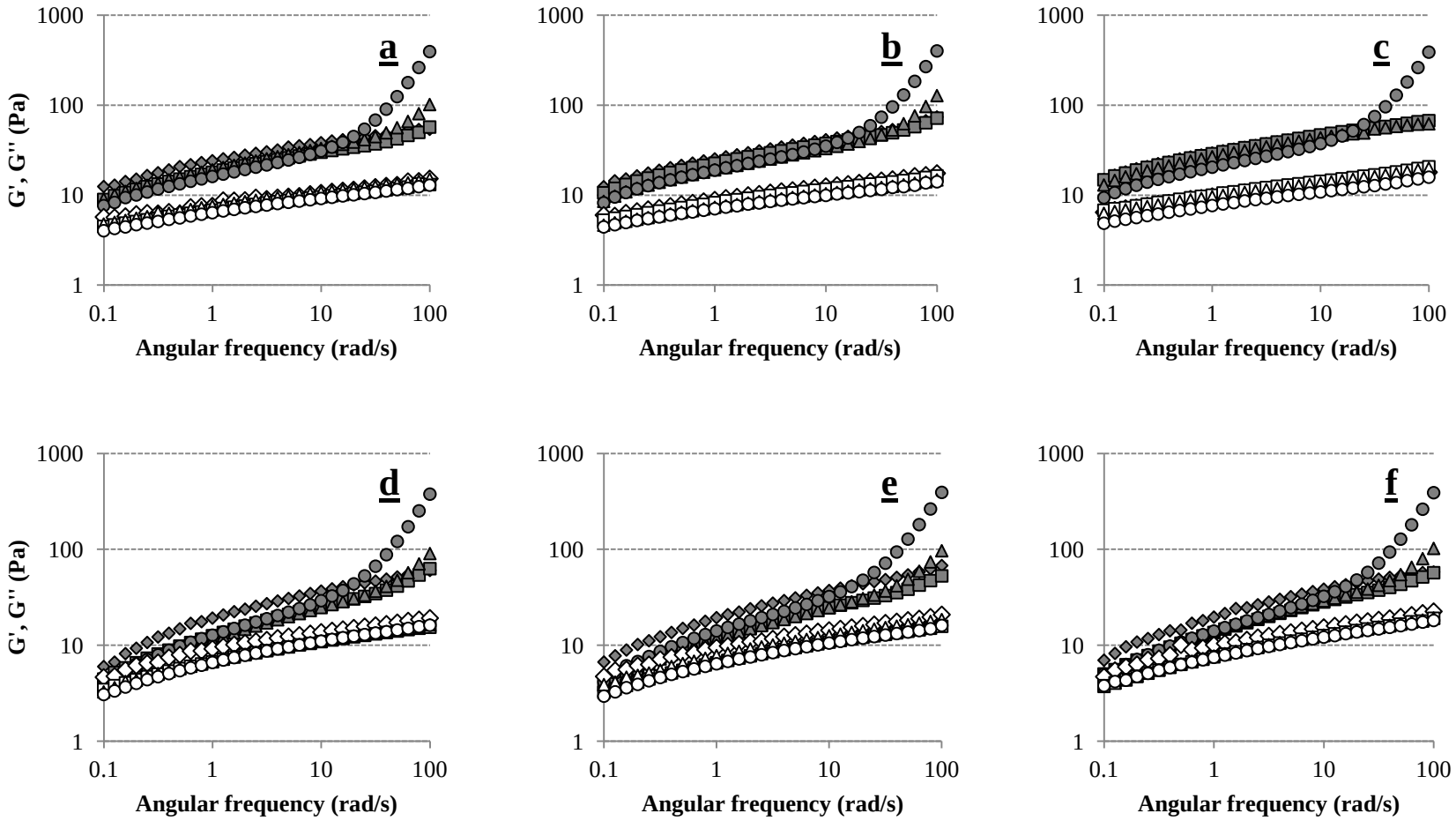


Figure 8.1 Storage and loss moduli mean values of salad dressings prepared by: HS method (closed symbols) a) REF, b) SP, c) PMJ, and by US method (open symbols) d) REF, e) SP and f) PMJ as a function of long term ambient storage.

Table 8.3 Evolution of viscoelastic parameters (K' and n') according to Power law model for HS and US salad dressings, during long term (6 months) ambient storage.

	K'	n'	K'	n'
	REF HS		**REF US**	
day 0	22.50 $^{abc/B}$ ±1.50	0.209 $^{ab/A}$ ±0.009	16.54 $^{a/B}$ ±3.45	0.309 $^{c/A}$ ±0.016
day 60	19.82 $^{b/AB}$ ±0.70	0.237 $^{a/A}$ ±0.004	11.22 $^{a/A}$ ±0.39	0.339 $^{b/A}$ ±0.005
day 120	18.26 $^{bc/A}$ ±1.03	0.253 $^{a/A}$ ±0.028	10.79 $^{a/A}$ ±0.50	0.398 $^{b/B}$ ±0.035
day 180	17.01 $^{b/A}$ ±1.27	0.442 $^{a/B}$ ±0.016	11.63 $^{a/A}$ ±0.55	0.536 $^{b/C}$ ±0.009
	SP HS		**SP US**	
day 0	24.18 $^{bc/C}$ ±0.09	0.230 $^{b/A}$ ±0.009	17.53 $^{a/B}$ ±3.05	0.308 $^{c/A}$ ±0.006
day 60	20.08 $^{b/B}$ ±0.95	0.244 $^{a/A}$ ±0.012	10.91 $^{a/A}$ ±0.82	0.341 $^{b/A}$ ±0.009
day 120	18.38 $^{c/A}$ ±0.50	0.297 $^{a/B}$ ±0.011	10.63 $^{a/A}$ ±0.86	0.475 $^{b/B}$ ±0.061
day 180	17.90 $^{bc/A}$ ±0.02	0.436 $^{a/C}$ ±0.007	12.66 $^{a/A}$ ±1.07	0.532 $^{b/B}$ ±0.016
	PJP HS		**PJP US**	
day 0	27.66 $^{d/B}$ ±0.91	0.200 $^{a/A}$ ±0.008	20.65 $^{ab/B}$ ±1.89	0.296 $^{c/A}$ ±0.009
day 60	27.58 $^{c/B}$ ±2.55	0.212 $^{a/A}$ ±0.013	12.64 $^{a/A}$ ±0.31	0.332 $^{b/A}$ ±0.023
day 120	21.56 $^{d/A}$ ±1.36	0.218 $^{a/A}$ ±0.007	12.86 $^{ab/A}$ ±1.09	0.420 $^{b/B}$ ±0.037
day 180	20.61 $^{c/A}$ ±1.18	0.419 $^{a/B}$ ±0.006	13.88 $^{a/A}$ ±1.92	0.521 $^{b/C}$ ±0.009

Regression coefficients (R^2) range between 0.88-0.993.
$^{a-d}$ Different superscripts for each parameter per storage day indicate significant differences ($p<0.05$).
$^{A-C}$ Different superscripts for each parameter per sample indicate significant differences during storage ($p<0.05$).

8.3.2 Salad dressing stability

Emulsified products such as salad dressings or sauces often exhibit stability problems during prolonged storage, either caused by emulsion instability or by changing polymer interactions (Mandala et al., 2004).

By monitoring the evolution of the backscattering profiles for salad dressings prepared with different samples, the information regarding particle migration in the emulsion such as creaming, coalescence and flocculation can be obtained. The initial mean values of BS along the entire tube (day 0) for the reference, and the dressings containing saffron and pomegranate powder prepared by the HS method were 69.88, 69.22 and 66.34 %, respectively, while for those prepared by the US method were

75.23, 75.44 and 71.9 % (Fig. 8.2a). The HS or the US salad dressings did not present any significant differences regarding their initial back scattering values ($p<0.05$). It was noticed that the dressings prepared with freeze-dried pomegranate juice powder had slightly lower initial BS values, suggesting that their relatively more compact network structure may have led to a decrease in the total surface area and thus decreased BS values. Physical stability is an important property of emulsion products. Stability of emulsions is usually attained by preventing droplet coalescence, flocculation, creaming and sedimentation. The stability of salad dressings is influenced, among other things, by their interfacial composition, emulsion droplet size, and/or continuous phase rheology (Zhang et al., 2008). In general, all the salad dressings were quite stable during 6-month storage and no phase separation was detected (Figure 8.2c). The variation of BS reflects their stability in terms of coalescence and/or flocculation phenomena, although it does not differentiate between them. A sharp decrease in BS occurred within the first two months of storage (60 days), after which an equilibrium was reached for all formulations applied and no significant decrease was observed thereafter ($p>0.05$) (Figure 8.2a). The variation of BS (dBS) after long term storage (180 days) is depicted in Figure 8.2b. It can be observed that the application of sonication did not improve the stability of the formulations since no differences were found in calculated dBS in all US samples ($p>0.05$). Pomegranate samples prepared by HS are considered highly stable emulsions despite their slightly larger droplets as evidenced by lower initial BS values and light microscopy photos (Fig. 8.3). They exhibited the highest stability as the BS decreased only by 1.04 % (Fig. 8.2b) as a result of the thickening effect of the pomegranate juice powder. It could be assumed that ultrasonication was not able to reduce the droplet size of the salad dressings to a point where a counterbalance of coalescence/flocculation rate would be achieved. The rheological properties have also shown that US influence was significant to changes observed upon storage. It should be noted that rheological properties and stability ones are not directly related, since in rheological properties great changes were observed after 120 days of storage, whereas changes in BS% values were observed at the beginning of storage (within 60 days).

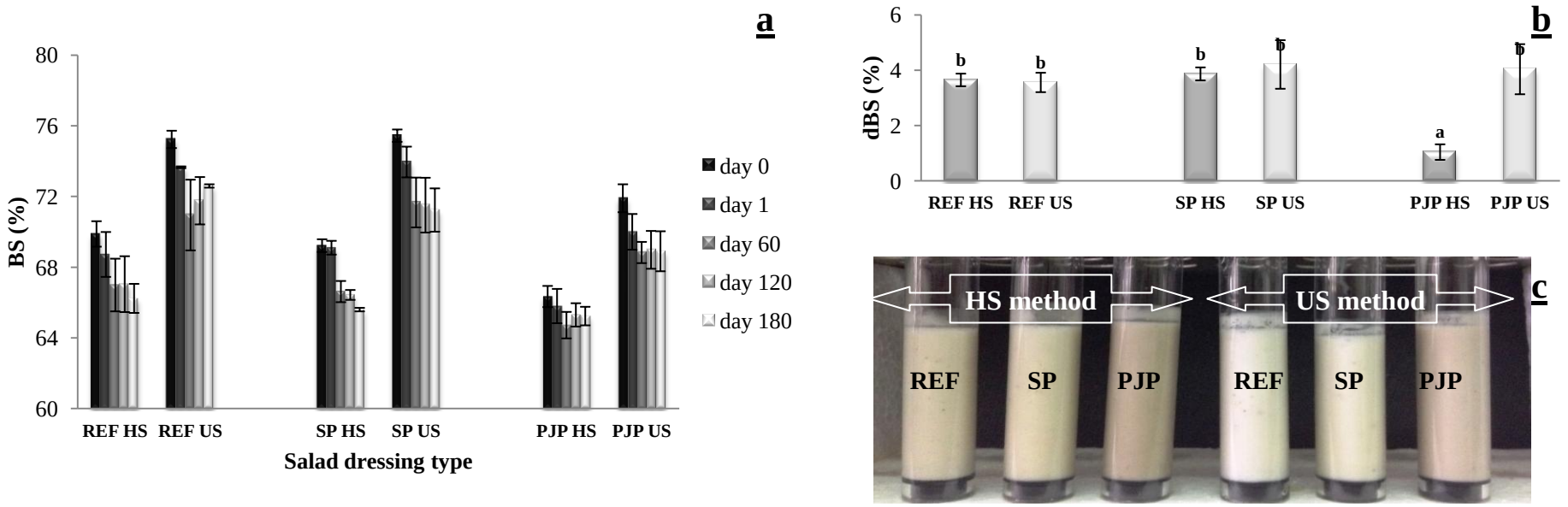


Figure 8.2 Evolution of backscattering values of prepared salad dressings (a), backscattering variation (dBS) (b) and appearance after 180 days of ambient storage (c).

[a-b] Different superscripts in samples indicate significant differences ($p<0.05$).

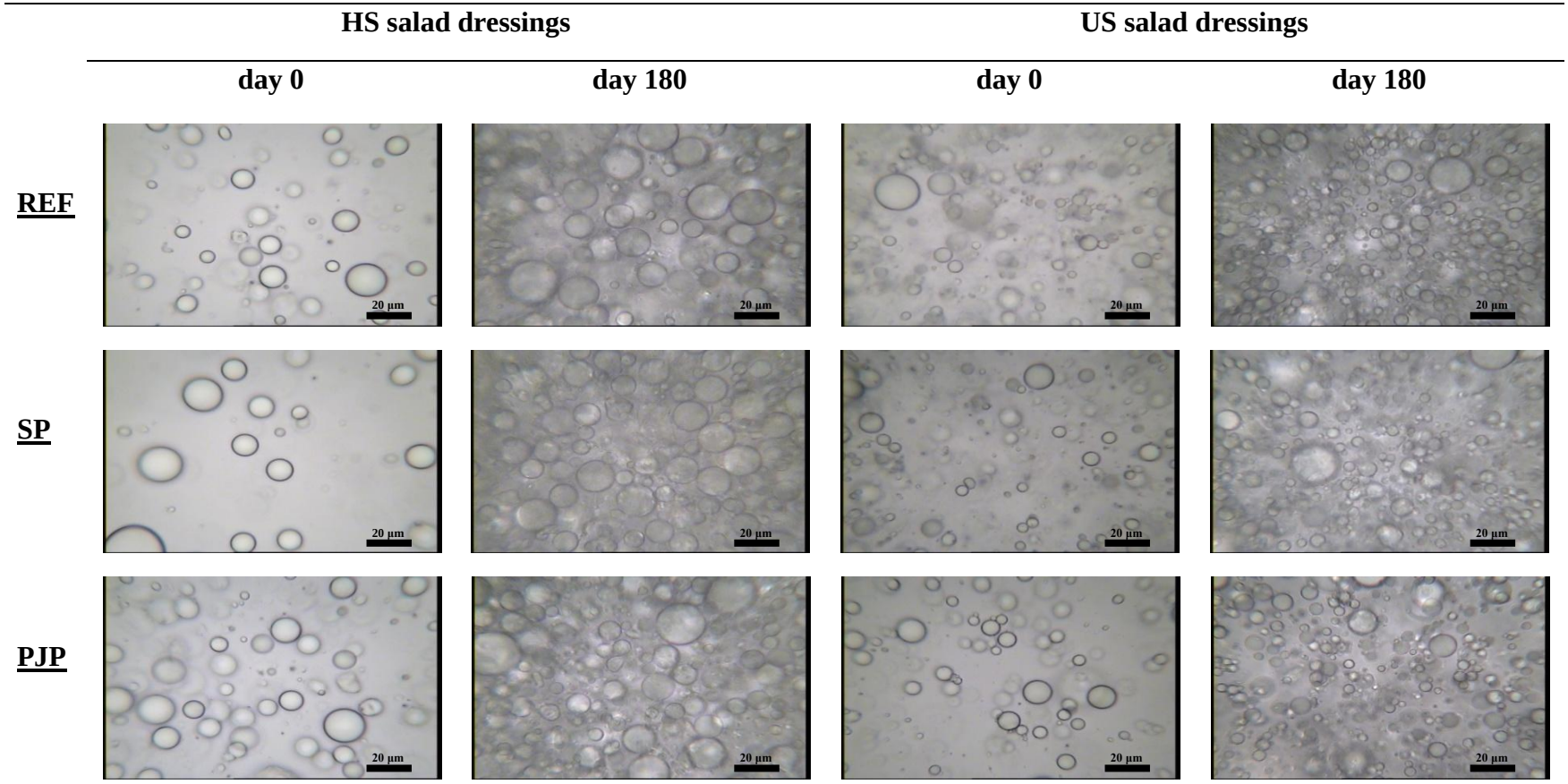


Figure 8.3 Microstructure of fresh and stored (180 days) salad dressings prepared by HS and US homogenization methods.

8.3.3 Color parameters

Color is an important attribute because it is usually the first property consumers interact with. In Table 8.4 and Figure 8.4, the evolution of estimated chromatic parameters (L, a, b and TCD) and appearance of the different salad dressings prepared over long term storage is demonstrated.

In emulsions with no added colorants, their color is determined by the colloidal characteristics of the system such as particle size, oil concentration, refractive index, as well as aggregation (Chantrapornchai et al., 1999; McClements, 2002). Apart from coalescence which increases the size of the particles over time, other types of destabilization such as Ostwald ripening have been associated with color changes (Weiss and McClements, 2001).

Color parameters were affected by saffron and pomegranate powder addition as well as emulsification method applied. The color of saffron is mainly owed to the presence of crocins (glycosylated esters of crocetins). The color of pomegranate varies from light pink to dark red or violet derived from the various anthocyanins. The concentration of anthocyanins in pomegranate juice varies between 10 and 700 mg/L depending on the pomegranate cultivar (Maskan, 2006). The 3-glucosides and 3, 5-diglucosides of delphinidin, cyanidin, and pelargonidin have been identified as the most important anthocyanins (Du et al., 1975). Delphinidin derivatives are responsible for blue and violet hues, while pelargonidin is related to red–orange colors (Brouillard et al., 1997).

Freshly prepared dressings containing pomegranate powder presented lower lightness values compared to saffron and reference samples, a phenomenon that was maintained throughout the whole storage period. The addition of colorants in emulsions resulted in decreased lightness as a consequence of less light being reflected back to the detector (Chantrapornchai et al., 1999).

As far as the factor of emulsification method is concerned, US application led to the formation of dressings with increased L values ranging from 75.1 to 85.5, while for HS samples lower values were obtained (71.5 to 82). This is attributed to the combined effect of increased droplet concentration (population/number) and decreased droplet radius. According to McClements (2002), emulsion lightness increases sharply with oil content (up to a few percent of oil), followed by a moderate increase afterwards. In the same study, it was also demonstrated that emulsion

lightness decreases as the particle size increases, for emulsions with droplets higher than 100 nm, while the opposite occurs for droplets with smaller diameters.

Powder addition and homogenization method affected a, b values of fresh samples in various ways. Reference and saffron samples exhibited a values near -3.0 and -11, while no significant differences were observed between the HS or the US samples ($p>0.05$). On the contrary, the pomegranate samples were characterized by slightly positive a values (1.6-1.1) indicative of redness, which decreased when US was applied. Concerning yellowness, saffron dressings showed considerably higher b values ranging between 40.2 and 45.3, whereas no significant differences were detected between the reference and the pomegranate samples ($p>0.05$). Unlike a values, b values of the fresh HS samples were higher than those reported for dressings prepared by US in all cases ($p<0.05$).

Even though ultrasound-assisted extraction is considered more effective over conventional methods for pigment extraction and is commonly used for the extraction of saffron's color and volatile constituents (Kanakis et al., 2007; Kamel et al., 2009; Xu and Pan, 2013; Yolmeh et al., 2014), ultrasonically homogenized salad dressing appeared less yellowish than those containing saffron and less reddish than those with pomegranate. It can be therefore assumed that the particle characteristics of the emulsions affect the color of the samples more than a possible extraction of pigments.

Long-term ambient storage affected color values by slightly decreasing *L* in all cases with the exception of the saffron samples for which it was practically unaffected (Table 8.4). The main reduction in *L* occurred during the first 60 days of storage and then remained practically constant up to the end of storage period. The initial decrease in *L* values occurring within 60 days of storage correlates well with the sharp decrease of BS values referred previously. For longer storage times, BS as well as lightness were not affected. In the case of the saffron samples, similar *L* values with that of the reference ones were found ($p>0.05$). The value of a in reference and saffron samples increased (lower negative values) throughout storage resulting in less green hues ($p<0.05$). In pomegranate dressings, changes of a value were slight and no trend was detected upon storage. For *b* values, changes during storage were more pronounced in both saffron and pomegranate samples in all treatments used, whereas in reference samples, *b* was unaffected during storage. In particular, a major reduction of *b* to about one third of its initial values was observed in the case of HS and US saffron dressings. This is most probably due to the degradation of saffron's color constituents.

Selim et al. (2000) investigated the degradation of encapsulated saffron apocarotenoids in amorphous polymer matrices under different a_w conditions. A first order kinetic of pigment degradation reaction was established from plots of the absorbance (ln $E^{1\%}_{440}$) as a function of time. Similar kinetic responses have been reported for factors such as pH and temperature (Tsimidou and Tsatsaroni, 1993; Tsimidou and Biliaderis, 1997). The color of saffron can be degraded either by a photochemical process, which promotes the *trans to cis* isomerization, or by a thermal process, which detaches the glycosyl moieties (Vickackaite et al., 2004). Cis isomerization causes color loss attributed to the hypsochromic shift (band shift) and hypochromic effect (intensity decrease), denoted by the appearance of a *cis* peak within or near the ultraviolet region (~320 nm), whereas *trans*-isomers absorb in the visible region (400-500 nm) (Rodriguez-Amaya, 2001).

Storage affected the *b* values of pomegranate samples, but in lesser extent compared to saffron ones. Findings on the evolution of *b* values of pomegranate juice are contradictory. It has been reported that *b* values of HHP (high hydrostatic pressurization) pomegranate juices increased from slightly negative (approximately -1.2) to near zero positive values, while for high temperature-short time treated juices, a slight decrease was initially observed followed by an increase to the initial levels (Chen et al., 2013). However, *b* values were still negative (~-2.4) indicative of the presence of blue pigments. On the contrary a reduction in *b* values during storage at 20 and 37 ^{o}C, at values well above or near +25 have been reported, in which delphinidin (violet-blue hue) and pelargonidin (red hue) were almost lost after 210 days of storage at elevated temperatures (Alighourchi and Barzegar, 2009). An increase in *b* values in our experiments is in accordance with the above references. However, the positive values obtained, depended on overall emulsion scattering characteristics as well.

Finally, regarding TCD, the dressings containing saffron powder exhibited the highest color change after 180 days of storage caused by the severe decrease in *b* values, regardless of the treatment used. The reference and the pomegranate dressings presented significantly lower TCD values without any statistical difference between them ($p>0.05$).

Table 8.4 L, a, b color parameters and TCD of salad dressings during storage.

	Lightness				a				b				TCD
	day 0	**day 60**	**day 120**	**day 180**	**day 0**	**day 60**	**day 120**	**day 180**	**day 0**	**day 60**	**day 120**	**day 180**	**day 180**
REF HS	82.0^{d} ±0.4	79.2^{c} ±0.1	78.6^{c} ±0.6	78.6^{c} ±0.6	-3.0^{b} ±0.2	-2.2^{c} ±0.1	-1.8^{b} ±0.0	-1.8^{b} ±0.1	11.3^{b} ±0.2	12.2^{b} ±0.5	11.7^{b} ±0.3	12.4^{b} ±0.1	3.89^{ab} ±0.8
REF US	85.5^{e} ±0.6	83.0^{d} ±1.2	84.0^{d} ±0.5	83.5^{d} ±0.4	-2.9^{b} ±0.1	-2.1^{c} ±0.1	-2.0^{a} ±0.1	-1.6^{b} ±0.1	9.7^{a} ±0.3	9.6^{a} ±1.0	9.6^{a} ±0.2	10.1^{a} ±0.1	1.85^{a} ±0.81
SP HS	79.8^{c} ±0.8	77.6^{c} ±0.3	77.8^{c} ±0.2	78.2^{c} ±0.4	-10.8^{a} ±0.3	-4.5^{a} ±0.5	-2.9^{bc} ±0.4	-2.6^{a} ±0.6	45.3^{d} ±0.8	18.9^{d} ±1.0	15.3^{d} ±0.6	15.8^{c} ±0.1	30.63^{c} ±2.69
SP US	83.3^{d} ±1.1	82.8^{d} ±0.7	83.3^{d} ±0.5	83.3^{d} ±0.2	-11.0^{a} ±0.2	-3.5^{b} ±0.3	-2.4^{c} ±0.1	-1.8^{b} ±0.3	40.2^{c} ±1.0	14.9^{c} ±2.1	11.4^{b} ±0.6	11.4^{ab} ±0.1	30.27^{c} ±0.58
PJP HS	71.5^{a} ±0.4	67.4^{a} ±0.3	67.6^{a} ±0.6	67.3^{a} ±1.2	1.6^{d} ±0.2	1.4^{d} ±0.2	1.4^{d} ±0.1	1.3^{c} ±0.1	11.2^{b} ±0.3	13.2^{bc} ±0.2	14.5^{c} ±0.0	14.5^{c} ±0.1	5.39^{b} ±0.91
PJP US	75.1^{b} ±0.6	70.0^{b} ±1.2	70.8^{b} ±0.2	71.1^{b} ±1.5	1.1^{c} ±0.1	1.4^{d} ±0.2	1.4^{d} ±0.1	1.1^{c} ±0.3	9.9^{a} ±0.3	11.6^{ab} ±0.4	12.8^{d} ±0.0	12.5^{b} ±0.1	4.80^{b} ±1.08

$^{a-d}$ Different superscripts for each parameter per storage day indicate significant differences ($p<0.05$).

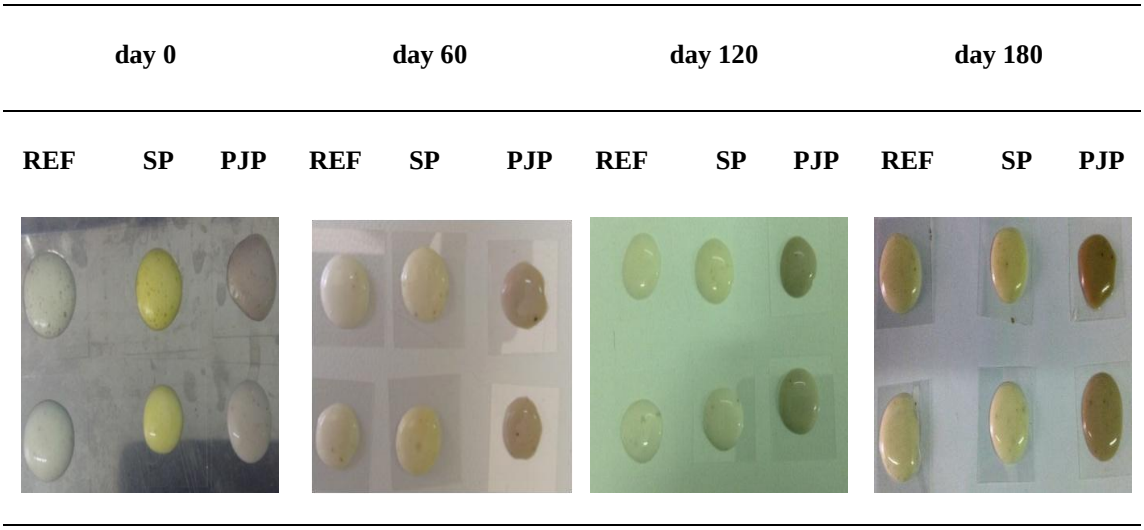


Figure 8.4 Color change of HS (upper samples) and US (lower samples) salad dressings during long term ambient storage (180 days).

8.3.4 Sensory evaluation

The effect of saffron and pomegranate juice powder on the sensory characteristics of the different salad dressings prepared is demonstrated on Table 8.5. Sensory evaluation scores for color did not reveal any significant difference in appearance between the samples, with the exception of pomegranate juice dressing prepared by HS, which was the least favorable (3.9). In all other cases color scores ranged from 5.9 to 6.9 ($p>0.05$). The application of sonication though was able to ameliorate the color of pomegranate juice powder dressings by decreasing the size of the droplets, however no differences were observed for reference and saffron samples.

In relation to instrumental color values this might be due to the darker (brownish) color evidenced by color values reported previously. Dressings in the presence of saffron powder received the highest taste score (6.9), for HS and US samples as well. For pomegranate juice powder a slight preference in comparison to the reference ones was observed although not statistically different ($p>0.05$). Homogenization method did not affect the taste between dressing analogs either.

No significant difference was observed in aroma, aftertaste, viscosity, and flowability at any level ($p>0.05$). All the products were equally acceptable as evidenced by the overall acceptability scores.

As also stated by Vingerhoeds et al. (2008) the perception of 10% fat-containing emulsion with added guar gum could resemble the fattiness of a 40% fat-containing emulsion. Emulsion thickness is often correlated to its creaminess Akhtar et al (2005),

which results in increased satiating expectations (Morell et al., 2014). In our case though, dressings prepared with HS method were not more preferable than their US analogs, despite significantly increased viscosity properties described above (apparent viscosity, k).

Table 8.5 Average scores obtained by sensory evaluation of freshly prepared salad dressings.

Salad dressing type	color	aroma	taste	aftertaste	viscosity	flowability	total acceptance
REF HS	$6.9^{c} \pm 1.0$	$5.9^{a} \pm 1.0$	$5.1^{a} \pm 1.2$	$5.8^{a} \pm 1.8$	$5.5^{a} \pm 1.0$	$5.5^{a} \pm 0.9$	$6.1^{a} \pm 1.1$
REF US	$6.4^{bc} \pm 1.3$	$6.1^{a} \pm 1.2$	$5.5^{a} \pm 1.5$	$5.9^{a} \pm 1.8$	$5.0^{a} \pm 1.2$	$5.4^{a} \pm 1.3$	$6.1^{a} \pm 1.3$
SP HS	$5.8^{b} \pm 1.9$	$6.1^{a} \pm 1.5$	$6.9^{b} \pm 1.4$	$5.7^{a} \pm 2.2$	$6.0^{a} \pm 1.7$	$6.0^{a} \pm 1.6$	$6.1^{a} \pm 1.7$
SP US	$6.0^{bc} \pm 1.8$	$6.0^{a} \pm 1.4$	$6.9^{b} \pm 1.2$	$5.5^{a} \pm 1.8$	$5.4^{a} \pm 1.3$	$5.3^{a} \pm 1.2$	$5.9^{a} \pm 1.3$
PJP HS	$3.9^{a} \pm 1.1$	$5.7^{a} \pm 1.6$	$5.9^{ab} \pm 1.8$	$5.5^{a} \pm 2.1$	$5.9^{a} \pm 1.6$	$6.1^{a} \pm 1.4$	$6.4^{a} \pm 1.6$
PJP US	$5.9^{bc} \pm 0.9$	$6.1^{a} \pm 1.5$	$6.1^{ab} \pm 1.9$	$5.8^{a} \pm 2.0$	$6.0^{a} \pm 1.4$	$5.8^{a} \pm 1.5$	$6.1^{a} \pm 1.3$

$^{a\text{-}c}$ Different superscripts for each parameter indicate significant differences ($p<0.05$).

8.4 Conclusions

In this study, the effect of saffron and pomegranate powder on the properties of salad dressings prepared with high shear and ultrasonic homogenization was studied.

The addition of saffron powder did not affect the stability or the viscoelastic properties of dressings in comparison to reference samples, whereas pomegranate juice powder resulted in viscosity and solid character increase in HS dressings, as its amount in the emulsion was much higher, which was also beneficial concerning emulsion stability during storage.

Ultrasonication resulted in less viscous, less structured and less stable emulsions due to degradation of the structure. US treatment did not produce any further improvement, since a significant droplet reduction was not achieved due to the large amount of oil used along with highly viscous samples. However, more pronounced differences concerning the viscoelastic behavior were noticed after four months of storage, whereas changes in BS values were noticed earlier. Ambient storage was detrimental for color parameters since a severe decrease of *b* value in the case of saffron salad dressings and an increase of *b* for pomegranate ones was observed as a consequence of degradation of the main color constituents and BS reduction. As a result, after 6 months of storage saffron salad dressings appearance was similar to that of reference samples, while pomegranate dressings became darker in color and appeared brownish. Cold storage would be more suitable for preserving the pigments from deterioration and thickening of the continuous phase during storage should be taken into account, in order to increase stability.

Pomegranate juice powder dressings prepared by HS were the least favorable in terms of color, whereas their US counterparts were more acceptable. The addition of saffron powder was beneficial towards taste in comparison to reference samples.

SUGGESTIONS FOR FURTHER WORK

This thesis examined the potential of nano-emulsion formation by using sonication techniques and formulations similar to those used in emulsified products, such as salad dressings or low fat mayonnaise. However, this was not achieved in highly viscous formulation systems. Hence, the formation of nanoemulsions was only feasible in the absence of stabilizers, and subsequent hydrocolloid incorporation is suggested. Fenugreek gum is a promising new type galactomannan, and can successfully replace commercially available approved galactomannans used by the food industry as thickeners.

However, fenugreek gum has not yet been approved for human consumption in EU and therefore a sensory evaluation in terms of taste could not be conducted in the context of this thesis. For this reason, once fenugreek gum becomes approved, a further study would be usefull in order to evaluate the effect of its addition in emulsified products and levels of preference, especially in the case of unbittered fractions which impart unfavourable sensory attributes (taste and aroma).

In this thesis it was shown that nanoemulsions can be usefull in terms of creating more stable emulsions with extremely low fat amounts. Moreover, with respect to nanoemulsions, there is still a great lack of knowledge regarding their toxicity issues and more studies are essential in this field to ensure their safety for human consumption, as well as from an environmental point of view.

Considering the antioxidant effects of saffron and pomegranate juice powder a deeper insight is required to fully elucidate their role in emulsified products, since foods are complex systems and interactions with different components may alter their effectiveness as antioxidants. As previously explained, not much work has been conducted in the field of the antioxidant activity of these components in food matrices. Additionally, it was shown that despite enhancing the taste of final products, some saffron constituents may undergo severe degradation leading to discoloration of the final product during storage. To address this negative phenomenon the following alternatives can be proposed:

- Storage condition (refrigeration, dark storage) or packaging optimization.
- Incorporation of other antioxidant compounds acting as protectants.

REFERENCES

A

AACC International. (2004). Approved Methods of Analysis. AACC Methods 44-16. St. Paul, MN: AACC International.

Abismaıl, B., Canselier, J. P., Wilhelm, A. M., Delmas, H., and Gourdon, C. (1999). Emulsification by ultrasound:drop size distribution and stability. *Ultrasonics Sonochemistry*, 6, 75–83.

Aboonabi, A., Rahmat, A., and Othman, F. (2014). Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. *Toxicology Reports*, 1, 915-922.

Acevedo-Fani, A., Laura Salvia-Trujillo, L., Rojas-Graü, M. A., and Martín-Belloso, O. (2015). Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties. *Food Hydrocolloids*, 47, 168–177.

Acharya, S.N., Thomas, J. E., and Basu, S. K. (2008). Fenugreek, an alternative crop for semiarid regions of North America. *Crop Science*, 48, 841-853.

Adebiyi, A. P., and Aluko, R. E. (2011). Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum* L.) seed protein isolate. *Food Chemistry*, 128(4), 1902-908.

Adjonu, R., Doran, G., Torley, P., and Agboola, S. Formation of whey protein isolate hydrolysate stabilised nanoemulsion. *Food Hydrocolloids, 41*, 169-177.

Akoh, C. C., and Min, D.B. (2008). Food lipids:Chemistry, Nutrition and Biotechnology. Boca Raton, CRC Press.

Akhtar, M., Stenzel, J., Murray, B. S., and Dickinson, E. (2005). Factors affecting the perception of creaminess of oil-in-water emulsions. *Food Hydrocolloids*, 19, 521–526.

Akhtar, S., Ismail, T., Fraternale, D., and Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, 174(1), 417-425.

Akthar, M., and Dickinson, E. (2003). Emulsifying properties of whey protein-dextran conjugates at low pH and different salt concentrations. *Colloids and Surfaces B: Biointerfaces*, 31, 125–132.

Alftrén, J., Peñarrieta, J. M., Bergenståhl, B., and Nilsson, L. (2012). Comparison of molecular and emulsifying properties of gum arabic and mesquite gum using asymmetrical flow field-flow fractionation. *Food Hydrocolloids*, 26(1), 54-62.

Alighourchi, H., and Barzegar. M. (2009). Some physicochemical characteristics and degradation kinetic of anthocyanin of reconstituted pomegranate juice during storage. *Journal of Food Engineering, 90(2), 179-185.*

Amin, R., Abdul-Ghani, A.S., and Suleiman, M. S. (1988). Effect of fenugreek and lupin seeds on the development of experimental diabetes in rats. *Planta Medica*, 54, 286–290.

Amine, C., Jing, D., Helgason, T., and Tadros, T. (2014). Investigation of emulsifying properties and emulsion stability of plant and milk proteins using interfacial tension and interfacial elasticity. *Food Hydrocolloids*, 39, 180–186.

Anon., (2012). Guar Gum Up Nearly 20×: Another Sticky Commodity Situation, Available from: http://www.spendmatters.com/index.cfm/2012/4/9/Guar-Gum-

Ansari, S. A., Matricardi, P., Di Meo, C., Alhaique, F., and Coviello, T. (2012). Evaluation of Rheological Properties and Swelling Behaviour of Sonicated Scleroglucan Samples. *Molecules*, 17, 2283-2297.

Ansari, S. A., Matricardi, P., Cencetti, C., Di Meo, C., Carafa, M., Mazzuca, C., Palleschi, A., Capitani, D., Alhaique, F., and T. Coviello. (2013). Sonication-Based Improvement of the Physicochemical Properties of Guar Gum as a Potential Substrate for Modified Drug Delivery Systems. *BioMed Research International*, 11 pages.

Anton, N., and Vandamme, T. F. (2009). The universality of low-energy nano-emulsification. *International Journal of Pharmaceutics*, 377(1–2), 142–147.

Anton, N. and Vandamme, T. F. (2011). Nano-emulsions and micro-emulsions: Clarifications of the critical differences. *Pharmaceutical Research*, 28, 978–985.

Arzeni, C., Martínez, K., Zema, P., Arias, A., Pérez, O. E., and Pilosof, A. M. R. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering*, 108(3), 463–472.

Ashrafi, M., Bathaie,, S. Z., Taghikhani, M., and Moosavi-Movahedi, A. A. (2005). The effect of carotenoids obtained from saffron on histone H1structure and H1–DNA interaction. *International Journal of Biological Macromolecules*, 36, 246–252.

Assimopoulou, A. N., Sinakos, Z., and Papageorgiou, V. P. (2005). Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. *Phytotherapy Research*, 19(11), 997-1000.

B

Bagliotti Meneguin, A., Stringhetti Ferreira Cury, B., and Evangelista R. C. (2014). Films from resistant starch-pectin dispersions intended for colonic drug delivery. *Carbohydrate Polymers*, 99(2) 140-149.

Bahamdan, A., and Daly, W. H. (2006). Poly(oxyalkylene) grafts to guar gum with applications in hydraulic fracturing fluids. *Polymers Advanced Technology*, 17, 679–681.

Bai, Y., Zhang, Y., Zhang, J., Mu, Q., Zhang, W., Butch, E. R., Snyder, S. E., and Yan, B. (2010). Repeated administrations of carbon nanotubes in male mice cause reversible testis damage without affecting fertility. *Nature Nanotechnology*, 5(9), 683-689.

Barak, S., and Mudgil, D. (2014). Locust bean gum: processing, properties and food applications--a review. *International Journal of Biological Macromolecules*, 66, 74-80.

Barati, R., Johnson, S. J., McCool, S., Green, D. W., Willhite, G. P., Liang, J.-T. (2011). Fracturing fluid cleanup by controlled release of enzymes from polyelectrolyte complex nanoparticles. *Journal of Applied Polymer Science*, 121, 1292–1298.

Barnes, H. A. (1994). Rheology of emulsions – A review. *Colloids and Surfaces A:Physicochemical and Engineering Aspects*, 91, 89–95.

Bartley, G. B., Hiltry, M. D., Andreson, B. D., Clairemont, A. C., and Maschke, S. P. (1981). "Maple-syrup" urine odor due to fenugreek ingestion. *The New England Journal of Medicine*, 305, 467.

Bashari, M., Abdelhai, M. H., Abbas, S., Eibaid, A., Xu, X., and Jin, Z. (2014). Effect of ultrasound and high hydrostatic pressure (US/HHP) on the degradation of dextran catalyzed by dextranase. *Ultrasonics Sonochemistry*, 21(1), 76–83.

Batista, A. P., Raymundo, A., Sousa, I. and Empis, J. (2006). Rheological characterization of coloured oil-in-water food emulsions with lutein and phycocyanin added to the oil and aqueous phases. *Food Hydrocolloids*, 20, 44–52.

Bazargani-Gilani, B., Aliakbarlu, J., and Tajik, H. (2015). Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. *Innovative Food Science and Emerging Technologies*, 29, 280-287.

Behrend, O., and Schubert, H. (2001). Influence of hydrostatic pressure and gas content on continuous ultrasound emulsification. *Ultrasonics Sonochemistry*, 8, 271–276.

Behrend, O., Ax, K. and Schubert, H. (2000). Influence of continuous phase viscosity on emulsification by ultrasound. *Ultrasonics Sonochemistry*, 7, 77–85.

Benjamin, O., Silcock, P., Beauchamp, J., Buettner, A., and Everett, D. W. (2014). Emulsifying properties of legume proteins compared to β-lactoglobulin and Tween 20 and the volatile release from oil-in-water emulsions. *Journal of Food Science*, 79(10), 2014-2022.

Berg van den, L., Rosenberg, Y., van Boekel, M. A. J. S., Rosenberg, M., and van den Velde, F. (2009). Microstructural features of composite whey protein/polysaccharide gels characterized at different length scales. *Food Hydrocolloids*, 23, 1288-1298.

Bhandari, P. R. (2015). *Crocus sativus* L. (saffron) for cancer chemoprevention: A mini review. *Journal of Traditional and Complementary Medicine*, 5(2), 81-87.

Bhargava, K., Conti, D. S., da Rocha, S. R., and Zhang, Y. (2015). Application of an oregano oil nanoemulsion to the control of foodborne bacteria on fresh lettuce. *Food Microbiology*, 47, 69-73.

Biliaderis C. G., and Izydorczyk M. S. (2007). Functional Food Carbohydrates. Boca Raton: Taylor and Francis.

Bleeker, E. a J., de Jong, W. H., Geertsma, R. E., Groenewold, M., Heugens, E. H. W., KoersJacquemijns, M., and Oomen, A. G. (2013). Considerations on the EU definition of a nanomaterial: science to support policy making. *Regulatory Toxicology and Pharmacology: RTP*, 65(1), 119– 125.

Bolhassani, A., Khavari, A., and Bathaie, S. Z. (2014). Saffron and natural carotenoids: Biochemical activities and anti-tumor effects. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 1845 (1), 20-30.

Bortnowska, G., and Makiewicz, A. (2006). Thechnological utility of guargum and xanthan for the production of low-fat inulin-enriched mayonnaise. *Acta Scientiarum Polonorum. Technologia Alimentaria*, 5(2), 135-146.

Brouillard, R, Figueiredo, P., Elhabiri, M., and Dangles, O. (1997). Molecular interactions of phenolic compounds in relation to the colour of fruit and vegetables. In F. A. Tomás-Barberán FA and R. J. Robins (Eds.). *Phytochemistry of fruit and vegetables* (pp. 29–49). Oxford, UK: Clarendon Press.

Brummer, R. (2006). Rheology Essentials of Cosmetic and Food Emulsions. Berlin, Heidelberg, New York: Springer.

Brummer, Y., Cui, W., and Wang, Q. (2003). Extraction, purification and physicochemical characterization of fenugreek gum. *Food Hydrocolloids*, 17, 229–236.

Bruschi, M. L. (2015). Strategies to modify the drug release from pharmaceutical systems. Cambridge: Woodhead Publishing Limited.

Buron, H., Mengual, O., Meunier, G., Cayre, I., and Snabre, P. (2004). Optical characterizationof concentrated dispersions: applications to laboratory analyses and on-line process monitoring and control. *Polymer International*, 53, 1205–1209.

Butt, M. S., Shahzadi, N., Sharif, M. K., and Nasir, M. (2007). Guar gum: A miracle therapy for hypercholesterolemia, hyperglycemia and obesity. *Critical Reviews in Food Science and Nutrition*, 47, 389–396.

Buttriss, J. L. (2013). Food reformulation: The challenges to the food industry. *Proceedings of the Nutrition Society*, 72 61–69.

Byrne, J. (2012). Eyes tara and locust bean gums as guar replacers. Available at:Food navigator.com. Accessed 26 March 2012. (http://www.foodnavigator.com/Market-Trends/Industry-eyes-tara-and-locust-bean-gums-as-guar-replacers).

C

Can Karaca, A., Low, N., and Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44(9), 2742-2750.

Canselier, J. R., Delmas, H., Wilhelm, A. M., and Abismail, B. (2002). Ultrasound emulsification-An overview. *Journal of Dispersion Science and Technology*, 23, 333–349.

Capron, I., Brigand, G., and Muller, G. (1998).Thermal denaturation and renaturation of a fermentation broth of xanthan: rheological consequences. *International Journal of Biological Macromolecules*, 23(3), 215–225.

Carpino, S., Rapisarda, T., Belvedere, G., and Licitra, G. (2008). Volatile fingerprint of Piacentinu cheese produced with different tools and type of saffron. *Small Ruminant Research*, 79(1), 16-21.

Casas, J. A., Mohedano, A. F., and Garcia-Ochoa, F. (2000). Viscosity of guar gum and xanthan/guar gum mixture solutions. *Journal of the Science of Food and Agriculture*, 80, 1722–1727.

Chalothorn, K, and Warisnoicharoen, W. (2012) Ultrasonic Emulsification of Whey Protein Isolate-Stabilized Nanoemulsions Containing Omega-3 Oil from Plant Seed. *American Journal of Food Technology*, 7, 532-541.

Champagne, C. P. and Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology*, 18, 184–190.

Chanamai, R., Herrmann, N., and McClements, D. J. (1998). Ultrasonic spectroscopy study of flocculation and shear-induced flock disruption in oil-in-water emulsions. *Journal of Colloid and Interface Science*, 204(2), 268-276.

Chandrapala, J., Zisu, B., Palmer, M., Kentish, S., and Ashokkumar M. (2011). Effects of ultrasound on the thermal and structural characteristics of proteins in reconstituted whey protein concentrate. *Ultrasonics Sonochemistry*,18(5), 951-957.

Chang, Y., McLandsborough, L., and McClements, D. J. (2012). Physical Properties and Antimicrobial Efficacy of Thyme Oil Nanoemulsions: Influence of Ripening Inhibitors. *Journal of Agricultural and Food Chemistry*, 60(48), 12056–12063.

Chantrapornchai, W. Clydesdale F.M., and McClements D. J. (2001). Influence of relative refractive index on optical properties of emulsions. *Food Research International*, 34 (9), 827-835.

Chantrapornchai, W., Clydesdale, F. M., and McClements. D. J. (1999). Theoretical and experimental study of spectra reflectance and color of concentrated oil-in-water emulsions. *Journal of Colloid and Interface Science*, 218, 324–330.

Charalampopoulos, D., Wang, R., Pandiella, S. S., and Webb, C. (2002). Application of cereals and cereal components in functional foods: A review. *International Journal of Food Microbiology*, 79, 131–141.

Charoen, R., Jangchud, A., Jangchud, K., Harnsilawat, T., Naivikul, O., and McClements, D. J. (2011). Influence of Biopolymer Emulsifier Type on Formation and Stability of Rice Bran Oil-in-Water Emulsions: Whey Protein, Gum Arabic, and Modified Starch. *Journal of Food Science*, 76(1), 165-172.

Chau, C. F., and Huang, Y. L. (2003). Comparison of the chemical composition and physicochemical properties of different fibres prepared from the peel of *Citrus sinensis* L. Cv. Liucheng. *Journal of Agricultural Food Chemistry*, 51, 2615–2618.

Chau, C. F., Cheung, P. C. K., and Wong, Y. S. (1997). Functional properties of protein concentrates from three Chinese indigenous legume seeds. *Journal of Agricultural and Food Chemistry*, 45(7), 2500–2503.

Chemat, F., Grondin, I., Costes, P., Moutoussamy, L., Shum Cheong Sing, A., and Smadja, J. (2004). High power ultrasound effects on lipid oxidation of refined sunflower oil. *Ultrasonics Sonochemistry*, 11(5), 281-285.

Chemat, F., Huma, Z., and Kamran Khan, M. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, 18, 813–835.

Chen, D., Xi, H., Guo, X., Qin, Z., Pang, X., Hu, X., Liao, X., and Wu, J. (2013). Comparative study of quality of cloudy pomegranate juice treated by high hydrostatic pressure and high temperature short time. *Innovative Food Science & Emerging Technologies*, 19, 85-94.

Chendke, P. K., and Fogler, H. S. (1975). Macrosonics in industry: 4 Ultrasonic innovations in the food industry: From the laboratory to commercial production. Chemical processing. *Ultrasonics*, 13, 31–37.

Cheng, Y., Brown, K. M., and Prud'homme, R. K. (2002). Characterization and intermolecular interactions of hydroxypropyl guar solutions. *Biomacromolecules*, 3, 456–461.

Cheng, Y., Brown, K. M., and Prud'homme, R. K. (2002). Characterization and intermolecular interactions of hydroxypropyl guar solutions. *Biomacromolecules*, 3(3), 456–461.

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M.T., Cerretani, L., and Bendini, A. (2008). Differential scanning calorimeter application to the detection of refined hazelnut oil in extra virgin olive oil. *Food Chemistry*, 110(1), 248-256.

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M.T., Cerretani, L. Bonoli, M., Bendini, A., and Lercker, G. (2007). Monovarietal extra virgin olive oils: Correlation between thermal properties and chemical composition. *Journal of Agricultural and Food Chemistry*, 55(26), 10779–10786.

Chranioti, C., Nikoloudaki, A., and Tzia, C. (2015). Saffron and beetroot extracts encapsulated in maltodextrin, gum Arabic, modified starch and chitosan: Incorporation in a chewing gum system. *Carbohydrate Polymers*, 127, 252-263.

Christou, P. (1992). Genetic Engineering and In Vitro Culture of Crop Legumes. Lancaster: Technomic Publishing.

Chung, C., Degner, B., and McClements, D .J. (2013). Designing reduced-fat food emulsions: Locust bean gum–fat droplet interactions. *Food Hydrocolloids*, 32(2), 263–270.

Chung, H.-J., Donner, E., and Liu, Q. (2011). Resistant starches in foods. In M. Moo-Young (Ed.). *Comprehensive biotechnology* (pp. 527–534.). Burlington: Academic Press.

Chung, K. M., Moon, T. W., Kim, H., and Chun, J. K. (2002). Physicochemical properties of sonicatedmung bean, potato, and rice starches. *Cereal Chemistry*, 79, 631–633.

Coles, D., and Frewer, L. J. (2013). Nanotechnology applied to European food production – A review of ethical and regulatory issues. *Trends in Food Science & Technology*, 34(1), 32-43.

Cortés-Muñoz, M., Chevalier-Lucia, D., and Dumay, E. (2009). Characteristics of submicron emulsions prepared by ultra-high pressure homogenisation: Effect of chilled or frozen storage. *Food Hydrocolloids*, 23(3), 640-654.

Coupland, J. N., McClements, D. J. (1996). Lipid oxidation in food emulsions. *Trends in Food Science and Technology*, 7(3), 83-91.

Croy, R. R., Gatehouse, J. A., Tyler, M., and Boulter, D. (1980).The purification and characterization of a third storage protein (convicilin) from the seeds of pea (Pisum sativum L.). *Biochemistry Journal*, 191(2), 509–516.

Cucheval, A., and Chow, R. C. Y. (2008). A study on the emulsification of oil by power ultrasound. *Ultrasonics Sonochemistry*, 15, 916–920.

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., and Cummins, E. (2012). Nanotechnologies in the food industry – Recent developments, risks and regulation. *Trends in Food Science & Technology*, 24(1), 30–46.

D

Dakia, P. A., Blecker, Ch., Robert, Ch., Wathelet, B., and Paquot, M. (2008). Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. *Food Hydrocolloids*, 22, 807-818.

Damodaran, S. (1996). Amino acids, peptide and proteins. In O. R. Fennema (Ed.). *Food Chemistry*, (pp. 321-429). New York, NY: Marcel Dekker.

Dapčević Hadnađev, T., Dokić, P. Krstonošić, V. and Hadnađev, M. (2013). Influence of oil phase concentration on droplet size distribution and stability of oil-in-water emulsions. *European Journal of Lipid Science and Technology*, 115(3), 313–321.

Das, K. P., and Kinsella, J. E. (1989). pH dependent emulsifying properties of b-lactoglobulin. *Journal of Dispersion Science and technology*, 10(1). 77-102.

de Lima, R., Oliveira Feitosa, L. Rodrigues Maruyama, C., Abreu Barga, M. Yamawaki, P. C., Vieira, I. J., Teixeira, Eliangela, M., Corrêa, A. C., Caparelli Mattoso, L. H. and Fernandes Fraceto, L. (2012). Evaluation of the genotoxicity of cellulose nanofibers. *International Journal of Nanomedicine*, 7, 3555–3565.

Dea, I.C.M., Morris, E. R., Rees, D. A., Welsh, E.J., Barnes, H. A., and Price J. (1977). Associations of like and unlike polysaccharides: Mechanisms and specificity in galactomannans, interacting bacterial polysaccharides, and related systems. *Carbohydrate Research,* 57, 249–272.

Decker, E. A., Elias, R. J., and McClements, D. J. (2010). Oxidation in foods and beverages and antioxidant applications. Oxford, UK: Woodhead Publishing.

Delben, F., and Stefancich, S. (1997). Interaction of food proteins with polysaccharides. I. Properties upon mixing. *Journal of Food Engineering*, 31, 325–346.

Delmas, T., Piraux, H., Couffin, A. C., Texier, I., Vinet, F., Poulin, P., Cates, M., and Bibette, J. (2011). How to prepare and stabilize very small nanoemulsions. *Langmuir,* 27(5), 1683-1692.

Denkova, P. S., Tcholakova, S., Denkov, N. D., Danov, K. D., Campbell, B., Shawl, C. and Kim, D. (2004). Evaluation of the precision of drop-size determination in oil/water emulsions by low-resolution NMR Spectroscopy. *Langmuir,* 20, 11402-11413.

Depree, J. A., and Savage, G. P. (2001). Physical and flavour stability of mayonnaise. *Trends in Food Science & Technology,* 12, 157-163.

Di Mattia, C., Balestra, F., Sacchetti, G., Neri, L., Mastrocola, D., and Pittia. P. (2015). Physical and structural properties of extra-virgin olive oil based mayonnaise. *LWT - Food Science and Technology*, 62(1), 764-770.

Dickinson, E. (1992). An introduction to food colloids. Oxford, UK: Oxford Univ. Press.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids,* 23(6), 1473-1482.

Dickinson, E. (2010). Flocculation of protein-stabilized oil-in-water emulsions. *Colloids and Surfaces B: Biointerfaces,* 81(1), 130-140.

Dickinson, E. (2011). Double emulsions stabilized by food biopolymers. *Food Biophysics*, 6, 1–11.

Dickinson, E., and Golding, M. (1998). Influence of Alcohol on Stability of Oil-in-Water Emulsions Containing Sodium Caseinate. *Journal of Colloid and Interface Science*, 197(1), 133-41.

Dickinson, E., and Stainsby, G. (1988). Emulsion stability. In E. Dickinson , and G. Stainsby (Eds). *Advances in Food Emulsions and Foams* (pp. 1-44). London,UK: Elsevier Applied Science.

Dickinson, E., Golding, M. and Povey, M.J.W. (1997). Creaming and Flocculation of Oil-In-Water Emulsions Containing Sodium Caseinate. *Journal of Colloid and Interface Science*, 185, 515-529.

Dickinson, E., and McClements, D. J. (1995). Advances in Food Colloids. Glasgow: Blackie Academic and Professional.

Diftis, N. G., Biliaderis, C. G., and Kiosseoglou, V. D. (2005). Rheological properties and stability of model salad dressing emulsions prepared with a dry-heated soybean protein isolate–dextran mixture. *Food Hydrocolloids,* 19(6), 1025-1031.

Dimakou, C. Kiokias, S. Tsaprouni, I. and Oreopoulou V. (2007). Effect of processing and storage parameters on the oxidative deterioration of oil-in-water emulsions. *Food Biophysics,* 2, 38–45.

Dolz, M., Hernández, M.J., Delegido, J., Alfaro, M. C., and Muñoz, J. (2007). Influence of xanthan gum and locust bean gum upon flow and thixotropic behaviour of food emulsions containing modified starch. *Journal of Food Engineering,* 81(1), 179–186.

Donsì, F., Cuomo, A., Marchese, E., and Ferrari, G. (2014). Infusion of essential oils for food stabilization: unraveling the role of nanoemulsion-based delivery systems on mass transfer and antimicrobial activity. *Innovative Food Science & Emerging Technologies,* 22, 212–220.

Donsì, F., Sessa, M., and Ferrari, M. (2012). Effect of Emulsifier Type and Disruption Chamber Geometry on the Fabrication of Food Nanoemulsions by High Pressure Homogenization. *Industrial and Engineering Chemistry Research*, 51(22), 7606–7618.

Du, C. T, Wang, P. L, and Francis, F.J. (1975). Anthocyanins of pomegranate, punica granatum. *Journal of Food Sci.ence,* 40(2), 417–418.

Ducel, V., Richard, J., Saulnier, P., Popineau, Y., and Boury, F. (2004). Evidence and characterization of complex coacervates containing plant proteins: Application to the microencapsulation of oil droplets. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 232(2–3), 239–247.

Ducel, V., Saulnier, P., Richard, J., and Boury, F. (2005). Plant protein–polysaccharide interactions in solutions: application of soft particle analysis and light scattering measurements. *Colloids and Surfaces B: Biointerfaces,* 41(2-3), 95-102.

Dumay, E, Chevalier-Lucia, D., Picart-Palmade, L., Benzaria, A., Gràcia-Julià, A., and Blayo, C. (2013). Technological aspects and potential applications of (ultra) high-pressure homogenisation. *Trends in Food Science & Technology,* 31(1), 13-26.

E

Emadzadeh, B., Razavi, S. M. A., and Hashemi, M. (2011). Viscous flow behavior of low-calorie pistachio butter: A Response Surface Methodology. *International Journal of Nuts and Related Sciences*, 2(1), 37-37.

Emami, A., Fathi Nasri, M. H., Ganjkhanlou, M., Zali, A., and Rashidi, L. (2015). Effects of dietary pomegranate seed pulp on oxidative stability of kid meat. *Meat Science*, 104, 14-19.

Embuscado, M. E. (2015). Spices and herbs: Natural sources of antioxidants – a mini review. *Journal of Functional Foods*. In Press, Available online 1 April 2015.

Englyst, H. N., Kingman, S. M., and Cummings, J.H (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46(2), 30–50.

Erçelebi, E. A., and Ibanoğlu, E. (2009). Rheological properties of whey protein isolate stabilized emulsions with pectin and guar gum. *European Food Research and Technology*, 229(2), 281-286.

EU, 2011. Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). O. J. L 275: 38-40.

F

Fameau, A. L., Ventureira, J., Novales, B., and Douliez, J. P. (2012). Foaming and emulsifying properties of fatty acids neutralized by tetrabutylammonium hydroxide. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 403(5), 87–95.

Faraji, H., and Lindsay R. C. (2004). Characterization of the Antioxidant Activity of Sugars and Polyhydric Alcohols in Fish Oil Emulsions. *Journal of Agricultural and Food Chemistry*, 5(23), 7164–7171.

Farshchi, A., Ettelaie, R., and Holmes, M. (2013). Influence of pH value and locust bean gum concentration on the stability of sodium caseinate-stabilized emulsions. *Food Hydrocolloids*, 32(2), 402–411.

Feng, L. Wang Z.-W., Zhang, J. Wang Z.-N., Liu F. (2009). Study on food-grade vitamin E microemulsions based on nonionic emulsifiers. *Colloids and Surfaces A*, 339 (1), 1–6.

Fernández, J.-A. (2006). Anticancer properties of saffron, *Crocus sativus* Linn. *Advances in Phytomedicine*, 2, 313-330.

Fernández-Quintela, A., Macarulla, M. T., Del Barrio, A. S., Martínez, J. A. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition*, 51(4), 331-342.

Ferreira, A. J., Cemlyn-Jones, J., and Robalo Cordeiro, C. (2013). Nanoparticles, nanotechnology and pulmonary nanotoxicology. *Revista Portuguesa de Pneumologia (English Edition)*, 19(1), 28-37.

Feys, D., Verhoeven, R., and de Schutter, G. (2007). Evaluation of time independent rheological applicable to fresh self-compactingconcrete. *Applied Rheology*, 17(5), 56244.

Finke J. H., Niemann, S. Richter, C., Gothsch, T., Kwade, A., Büttgenbach, S. and Müller-Goymann, C. C. (2014). Multiple orifices in customized microsystem high-pressure emulsification: The impact of design and counter pressure on homogenization efficiency. *Chemical Engineering Journal*, 248, 107-121.

Fischer, U. A., Carle, R., Kammere, D. R. (2011). Identification and quantification of phenolic compound from pomegranate (Punica granatum L.) peel, mesocarp, arils and differently produced juices by HPLC-DAD_ESI/MSn. *Food Chemistry*, 127, 807–21.

Foegeding, E. A., Davis, J. P., Doucet, D., McGuffey, M. K. (2002). Advances in modifying and understanding whey protein functionality. *Trends in Food Science and Technology*, 13, 151–159.

Foegeding, E. A., Luck, P., and Vardhanabhuti, B. (2011). Whey protein products. In J. Fuquay, P. Fox, and P. McSweeney (Eds.). *The Encyclopedia of Dairy Sciences* (pp. 873–878). Oxford, UK: Academic Press.

Ford, L. D., Borwankar, R. P., Pechak, D., and Schimmer, B. (1997). Dressing and sauces. In S.E. Friberg, and K. Larsson, (Eds.). *Food Emulsions* (pp. 361–412). New York, NY: Marcel Dekker.

Frankel, E. N. (2012). Lipid Oxidation. Cambridge, UK: Woodhead Publishing.

Fredrick , E., Walstra, P., and Dewettinck, K. (2010). Factors governing partial coalescence in oil-in-water emulsions. *Advances in Colloid and Interface Science*, 153(1–2), 30–42.

Freitas, S., Hielscher, G., Merkle, H. P., and Gander, B. (2006). Continuous contact and contamination free ultrasonic emulsification. *Ultrasonics Sonochemistry*, 13(1), 76-85.

Frøst, M. B., Dijksterhuis, G., and Martens, M. (2001). Sensory perception of fat in milk. *Food Quality and Preference*, 12(5–7), 327–336.

Fuentes-Zaragoza, E.. Riquelme-Navarrete, M. J., Sánchez-Zapata, E., and Pérez-Álvarez, J. A. (2010) Resistant starch as functional ingredient: A review. *Food Research International*, 43 (4), 931–942.

Funami, T., Kataoka, Y., Noda, S., Hiroe, M., Ishihara, S., Asai, I., Takahashi, R., and Nishinari, K. (2008). Functions of fenugreek gum with various molecular weights on the gelatinization and retrogradation behaviors of corn starch—1: Characterizations of fenugreek gum and investigations of corn starch/fenugreek gum composite system at a relatively high starch concentration; 15 w/v%. *Food Hydrocolloids*, 22(5), 763-776.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., and Nishinari, K. (2005). Effects of non-ionic polysaccharides on the gelatinization and retrogradation behavior of wheat starch. *Food Hydrocolloids*, 19(1), 1–13.

G

Gaikwad, S. G., and Pandit, A. B. (2008). Ultrasound emulsification: Effect of ultrasonic and physicochemical properties on dispersed phase volume and droplet size. *Ultrasonics Sonochemistry*, 15(4), 554–563.

Gaikwad, S. G., and Pandit, A. B. (2008). Ultrasound emulsification: Effect of ultrasonic and physicochemical properties on dispersed phase volume and droplet size. *Ultrasonics Sonochemistry*, 15(4), 554–563.

Galazka, V. B., Dickinson, E., and Ledward, D. A. (1996). Effect of high pressure on the emulsifying behaviour of b-lactoglobulin. *Food Hydrocolloids*, 10, 213–219.

Galla, N. R., and Dubasi, G. R. (2010). Chemical and functional characterization of gum karaya (Sterculia urens L.) seed meal. *Food Hydrocolloids*, 24, 479–485.

Gaonkar, A. G. (1991). Surface and interfacial activities and emulsion characteristics of some food hydrocolloids. *Food Hydrocolloids*, 5, 329–337.

García, M. C., Alfaro, M. C., Calero, N., and Muñoz, J. (2014). Influence of polysaccharides on the rheology and stabilization of α-pinene emulsions. *Carbohydrate Polymers*, 105, 177–183.

Genovese, A., Caporaso, N., De Luca, L., Paduano, A., and Sacchi, R. (2015). Influence of olive oil phenolic compounds on headspace aroma release by interaction with whey proteins. *Journal of Agricultural and Food Chemistry*, 63(15), 3838-3850.

Gharibzahedi, S. M. T., Mousavi, S. M., Hamedi, M, Khodaiyan, F., and Razavi, S. H. (2012). Development of an optimal formulation for oxidative stability of walnut-beverage emulsions based on gum arabic and xanthan gum using response surface methodology. *Carbohydrate Polymers*, 87(2), 1611-1619.

Gharibzahedi, S. M. T., Razavi, S. H., and Mousavi, S. M. (2013). Psyllium husk gum: An attractive carbohydrate biopolymer for the production of stable canthaxanthin emulsions. *Carbohydrate Polymers*, 92(2), 2002-2011.

Goddeeris , C., Cuppo, F., Reynaers, H., Bouwman, W. G., and Van den Mooter G. (2006). Light scattering measurements on microemulsions: Estimation of droplet sizes. *International Journal of Pharmaceutics*, 312, 187–195.

Gogate, P. R., Wilhelm, A. M., and Pandit, A. B. (2003). Some aspects of the design of sonochemical reactors. *Ultrasonics Sonochemistry*, 10(6), 325–330.

Goh, K. T., Sarkar, A., and Singh, H. (2009). Milk protein-polysaccharide interactions. In A. Thompson, M. Boland, and H. Singh (Eds.). *Milk Proteins: From Expression to Food* (pp.347-351). Elsevier.

Gokoglu, N., Topuz, O. K., and Yerlikaya, P. (2009). Effects of pomegranate sauce on quality of marinated anchovy during refrigerated storage. *LWT - Food Science and Technology*, 4(1), 113-118.

Goodwin, D. J., Picout, D. R., Ross-Murphy, S. B., Holland, S. J., Martini, L. G., and Lawrence, M. J. (2011). Ultrasonic degradation for molecular weight reduction of pharmaceutical cellulose ethers. *Carbohydrate Polymers*, 83(2), 843-851.

Goodwin, D. J., Picout, D. R., Ross-Murphy, S. B., Holland, S. J., Martini, L. G. and Lawrence, M. J. (2011). Ultrasonic degradation for molecular weight reduction of pharmaceutical cellulose ethers. *Carbohydrate Polymers*, 83(2), 843-851.

Gordon, L., and Pilosof, A. M. R. (2010). Application of high intensity ultrasounds to control the size of whey proteins particles. *Food Biophysics*, 5(3), 203-210.

Gorji, E. G., and Mohammadifar, M. A. (2014). Characterisation of gum tragacanth (Astragalus gossypinus)/ sodium caseinate complex coacervation as a function of pH in an aqueous medium. *Food Hydrocolloids*, 34, 161-168.

Goudappel, G.J.W., van Duynhoven, J.P.M., and Mooren M.M.W. (2001). Measurement of oil droplet size distribution in food oil/water emulsions by time domain pulsed field gradient NMR. *Journal of Colloid and Interface Science*, 239, 535-542.

Griffin, W. C. (1949). Classification of Surface Active Agents by HLB. Journal of the Society of Cosmetic Chemists, 1, 311-326.

Gu, Y. S., Decker, A. E., McClements, D. J. (2005). Influence of pH and carrageenan type on properties of β-lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids*, 19(1), 83-91.

Guéguen, J. (1989). Relation between conformation and surface hydrophobicity of pea (*Pisum sativum* L.) globulins. *Journal of Agricultural and Food Chemistry*, 37(5), 1236–1241.

Gülseren, I., Güzey, D. G., Bruce, B. D., and Weiss, J. (2007). Structural and functional changes in ultrasonicated bovine serum albumin solutions. *Ultrasonics Sonochemistry*, 14(2), 173–183.

Gunasekaran, S., and Mehmet, A. K. (2000). Dynamic oscillatory shear testing of foods – selected applications. *Trends in Food Science and Technology*, 11, 115–127.

Guttoff, M., Saberi, A. H., and McClements, D. J. (2015). Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability. *Food Chemistry*, 171, 117–122.

Guzey, D. and McClements, D. J. (2007). Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by β-lactoglobulin-pectin complexes. *Journal of Agricultural and Food Chemistry*, 55, 475-485.

H

Håkansson, A., Fuchs, L., Innings, F., Revstedt, J., Bergenståhl, B., and Trägårdh, C. (2010). Visual observations and acoustic measurements of cavitation in an experimental model of a high-pressure homogenize. *Journal of Food Engineering*, 100(3), 504–513.

Håkansson, A., Trägårdh, C., and Bergenståhl, B. (2009). Dynamic simulation of emulsion formation in a high pressure homogenizer. *Chemical. Engineering Science*, 64, 2915– 2925.

Hamden, K., Jaouadi, B., Salami, T., Carreau, S., Bejar, S., and Elfeki, A. (2010). Modulatory effect of fenugreek saponins on the activities of intestinal and hepatic disaccharidase and glycogen and liver function of diabetic rats. *Biotechnology and Bioprocess Engineering*, 15(5), 745-753.

Hannan, J. M. A., Ali L., Rokeya, B., Khaleque, J., Akhter, M., Flatt, P. R., et al. (2007). Soluble dietary fibre fraction of Trigonella foenum-graecum (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. *British Journal of Nutrition*, 97, 514–521.

Haq, M. A., Alam, M. J., and Hasnain, A. (2013). Gum *Cordia*: A novel edible coating to increase the shelf life of Chilgoza (*Pinus gerardiana*). *LWT - Food Science and Technology*, 50(1), 306-31.

Hashtjin, A. M., and Abbasi, S. (2015). Nano-emulsification of orange peel essential oil using sonication and native gums. *Food Hydrocolloids*, 44, 40-48.

He, W., Tan, Y., Tian, Z., Chen, L., Hu, F., and Wu, W. (2011). Food protein-stabilized nanoemulsions as potential delivery systems for poorly water-soluble drugs: preparation, in vitro characterization, and pharmacokinetics in rats. International Journal of Nanomedicine, 6, 521–533.

Henry, J. V. L., Fryer, P. J., Frith, W. J., and Norton, I. T. (2009). Emulsification mechanism and storage instabilities of hydrocarbon-in-water sub-micron emulsions stabilised with Tweens (20 and 80), Brij 96v and sucrose monoesters. *Journal of Colloid and Interface Science*, 338(1), 201-206.

Higiro, J., Herald, T. J., and Alavi, S. (2006). Rheological study of xanthan and locust bean gum interaction in dilute solution. *Food Research International*, 39(2), 165–175.

Hooda, S., and Jood, S. (2005). Organoleptic and nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour. *Food Chemistry*, 90(3), 427–435.

Hosseini, S. M. H., Emam-Djomeh, Z., Razavi, S. H., Moosavi-Movahedi, A. A., Saboury, A. A., Mohammadifar, M. A. *et al.* (2013). Complex coacervation of β-lactoglobulin – κ-Carrageenan aqueous mixtures as affected by polysaccharide sonication. *Food Chemistry*, 141, 215–222.

Huang, X., Kakuda, Y., and Cui, W. (2001). Hydrocolloids in emulsions: particle size distribution and interfacial activity. *Food Hydrocolloids*, 15(4-6), 533-542.

Huck-Iriart, C., Álvarez-Cerimedo, M. S., Candal, R. J., and Herrera, M. L. (2011). Structures and stability of lipid emulsions formulated with sodium caseinate. *Current Opinion in Colloid and Interface Science*, 16(5), 412–420.

I

Ikeda, S., and Nishinari, K. (2001). "Weak gel"-type rheological properties of aqueous dispersions of nonaggregated kappa-carrageenan helices. *Journal of Agricultural and Food Chemistry*, 49(9), 4436–4441.

Im, K. K. and Maliakel, B. (2008). Fenugreek dietary fibre a novel class of functional food ingredient. Agrofood industry hi-tech, 19, 18-21.

Işıklı, N. D., and Karababa, E. (2005). Rheological characterization of fenugreek paste (çemen). *Journal of Food Engineering*, 69, 185–190.

ISO 3632-1 Technical Specification. (2011). *Saffron* (*Crocus sativus L.*). *Part 1*(*specification*). Geneva, Switzerland: International Organization for Standardization.

ISO 3632-2 Technical Specification. (2010). *Saffron* (*Crocus sativus L.*). *Part 2* (*test methods*). Geneva, Switzerland: International Organization for Standardization.

ISO Technical specification. (2003). Saffron (*Crocus sativus* L.) Part 1. (Specification). Part 2. (Test methods). *International Organization of Standarization*, Geneva.

ISO/DIS 13299:1998. Sensory analysis – Methodology – General guidance for establishing a sensory profile. International Organization for Standardization, Geneva.

IUPAC. (1979). Standard methods for the analysis of oils, fats and derivatives. In International Union of Pure and Applied Chemistry (6th ed). Oxford: Pergamon Press Ltd.

J

Jackman, R. L., and Yada, R. Y. (1989). Functional Properties of Whey-Pea Protein Composite Blends in a Model System. *Journal of Food Science*, 54(5), 1287–1292.

Jafari, S. M., He, Y. and Bhandari B. (2006). Nano-emulsion production by sonication and Microfluidization – a comparison. *International Journal of Food Properties*, 9(3), 475–485.

Jafari, S. M., He, Y., and Bhandari, B. (2007). Production of sub-micron emulsions by ultrasound and microfluidization techniques. *Journal of Food Engineering*, 82, 478–488.

Jafari, S. M., Assadpoor, E. He, Y., and Bhandari, B. (2008). Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids*, 22(7), 1191–1202.

Jahnke, S. (1998). The theory of high-pressure homogenization. In R. H. Muller, S. Benita, and B. H. Bohm (Eds.). *Emulsions and nanosuspensions for the formulation of poorly soluble drugs* (pp. 177–200). Stuttgart: Medpharm Scientific Publishers.

Jambrak, A. R., Herceg, Z., Subaric, D., Babic, J., Brncic, M., Brncic, S. R., Bosiljkov, T., Cvek, D., Tripalo, B., and Gelo, J. (2010). Ultrasound effect on physical properties of corn starch *Carbohydrate Polymers*, 79, 91–100.

Jiménez, A., Beltrán, G., and Uceda, M. (2007). High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. *Ultrasonics Sonochemistry*, 14(6), 725-731.

Jiménez-Colmenero, F. (2013). Potential applications of multiple emulsions in the development of healthy and functional foods. *Food Research International*, 52(1), 64–74.

Jindal, M., Rana, V., Kumar V., Singh, R. S., Kennedy, J. F., and Tiwary, A. K. (2013). Sulfation of Aegle marmelos gum: Synthesis, physico-chemical and functional characterization. *Carbohydrate Polymers*, 92(2), 1660-1668.

Johns, M. L. (2009). NMR studies of emulsions. *Current Opinion in Colloid and Interface Science*, 14(3), 178–183.

Jourdain, L., Leser, M. E., Schmitt, C., Michel, M., and Dickinson, E. (2008). Stability of emulsions containing sodium caseinate and dextran sulfate: Relationship to complexation in solution. *Food Hydrocolloids*, 22(4), 647-659.

Juliano, P., Torkamani, A. E., Leong, T., Kolb, V., Watkins, P., Ajlouni, S., and Singh, T. K. (2014). Lipid oxidation volatiles absent in milk after selected ultrasound processing. *Ultrasonics Sonochemistry*, 21(6), 2165-2175.

K

Kafi, M. (2006). Saffron ecophysiology. In M. Kafi, A. Koocheki, M.H. Rashed, and M. Nassiri (Eds.). *Saffron (Crocus sativus) Production and Processing* (pp. 39-57). Enfield: Science Publishers.

Kaltsa, O, Michon, C, Yanniotis, S, and Mandala, I. (2013). Ultrasonic energy input influence on the production of sub-micron o/w emulsions containing whey protein and common stabilizers. *Ultrasonics Sonochemistry*, 20(3), 881-891.

Kaltsa, O., Gatsi, I., Yanniotis, S., and Mandala, I. (2014). Influence of Ultrasonication Parameters on Physical Characteristics of Olive Oil Model Emulsions Containing Xanthan. *Food and Bioprocess Technology*, 7(7), 2038-2049.

Kamel, M. M., Helmy, H. M., and El Hawary, N. S. 2009. Some Studies on Dyeing Properties of Cotton Fabrics with Crocus sativus (Saffron flowers) Using an Ultrasonic Method. *Journal of Natural Fibers*, - Taylor & Francis

Kamogawa, K., Okudaira, G., Matsumoto, M., Sakai, T., Sakai, H., and Abe M. (2004). Preparation of oleic acid/water emulsions in surfactant-free condition by sequential processing using midsonic–megasonic waves. *Langmuir*, 20(6), 2043–2047.

Kanakis, C. D, Tarantilis, P. A., Tajmir-Riahi H. A., and Polissiou, M. G. (2007). DNA Interaction with Saffron's Secondary Metabolites Safranal, Crocetin, and Dimethylcrocetin. *DNA and Cell Biology*, 26(1), 63-70.

Kanakis, C. D., Tarantilis, P. A., Pappas, C., Bariyanga, J., Tajmir-Riahi, H. A., and Polissiou, M. G. (2009). An overview of structural features of DNA and RNA complexes with saffron compounds: Models and antioxidant activity. *Journal of Photochemistry and Photobiology B: Biology*, 95(3), 204-212.

Kang, K. S., and Pettitt, D. J. (1993). Xanthan, gellan, welan, rhamsam. In R.L.Whistler and J.N. BeMiller (Eds.) *Industrial gums: polysaccharides and their derivatives* (pp. 341–371). New York, NY: Academic Press.

Karaca, A. C., Low, N., and Nickerson, M. (2011). Emulsifying properties of canola and flaxseed protein isolates produced by isoelectric precipitation and salt extraction. *Food Research International*, 44, 2991–2998.

Karaiskou, S., Blekas, G., and Paraskevopoulou, A. (2008). Aroma release from gum arabic or egg yolk/xanthan-stabilized oil-in-water emulsions. *Food Research International*, 41(6), 637–645.

Karaman, S., Tahsin Yilmaz, M., Ertugay, M. F., Baslar, M., and Kayacier, A. (2012). Effect of ultrasound treatment on steady and dynamic shear properties of glucomannan based salep dispersions: Optimization of amplitude level, sonication time and temperature using response surface methodology. *Ultrasonics Sonochemistry*, 19(4), 928-938.

Karbstein, H., and Schubert, H. (1995). Developments in the continuous mechanical production of oil-in-water macro-emulsions. *Chemical Engineering and Processing: Process Intensification*, 34(3), 205-211.

Kasaai, M. R., Arul, J. and Charlet, G. (2008). Fragmentation of chitosan by ultrasonic irradiation. *Ultrasonics Sonochemistry*, 15(6), 1001-1008.

Keerati-u-rai, M., and Corredig, M. (2009). Heat-induced changes in oil-in-water emulsions stabilized with soy protein isolate. *Food Hydrocolloids*, 23(8), 2141–2148.

Kennedy J. R., Kent, K. E., and Brown, J. R. (2015). Rheology of dispersions of xanthan gum, locust bean gum and mixed biopolymer gel with silicon dioxide nanoparticles. *Materials Science and Engineering C:Materials for Biological Applications*, 48, 347-53.

Kentish, S., Wooster, T. J., Ashokkumar, M., Balachandran, S., Mawson, R., and Simons, L. (2008). The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science and Emerging Technologies*, 9, 170-175.

Khazaei, K. M., Jafari, S. M., Ghorbani, M., and Kakhki, A. H. (2014). Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. *Carbohydrate Polymers*, 105, 57-62.

Kim , H-J. . Decker E. A, and McClements D. J. (2002). Impact of Protein Surface Denaturation on Droplet Flocculation in Hexadecane Oil-in-Water Emulsions Stabilized by β-Lactoglobulin. *Journal of Agricutural and Food Chemistry*, 50(24), 7131–7137.

Kimura, A., Fukuda, T., Zhang, M., Motoyama, S., Maruyama, N., and Utsumi, S. (2008). Comparison of Physicochemical Properties of 7S and 11S Globulins from Pea, Fava Bean, Cowpea, and French Bean with Those of Soybean French Bean 7S Globulin Exhibits Excellent Properties. *Journal of Agricultural and Food Chemistry*, 56, 10273–10279.

Kimura, T., Sakamoto, T., Leveque, J-M., Sohmiya, H., Fujita, M., Ikeda, S., and Ando, T. (1996). Standardization of ultrasonic power for sonochemical reaction. *Ultrasonics Sonochemistry*, 3(3), 157-161.

Kinsella, J. E. (1979). Functional properties of soy protein. *Journal of American Oil Chemists Society*, 56, 242–249.

Kiosseoglou, V., and Doxastakis, G. (1988). The emulsification properties of soybean protein in the presence of polysaccharides. *LWT - Food Science and Technology*, 21, 33–35.

Klassen, D. R., and Nickerson, M. T. (2012). Effect of pH on the formation of electrostatic complexes within admixtures of partially purified pea proteins (legumin and vicilin) and gum Arabic polysaccharides. *Food Research International*, 46(1), 167-176.

Koda, S., Kimura, T., Kondo, T., and Mitome, H. (2003). A standard method to calibrate sonochemical efficiency of an individual reaction system. *Ultrasonics Sonochemistry*, 10, 149–156.

Koda, S., Taguchi, K., and Futamura, K. (2011). Effects of frequency and a radical scavenger on ultrasonic degradation of water-soluble polymers. *Ultrasonics Sonochemistry*, 18(1), 276–281.

Kokias, S., Reiffers-Magnani, C. K. and Bot, A. (2004). Stability of whey-protein-stabilized oilin-water emulsions during chilled storage and temperature cycling. *Journal of Agricultural and Food Chemistry*, 52, 3823-3830.

Komaiko, J., and McClements, D. J. (2015). Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. *Journal of Food Engineering*, 146, 122-128.

Kong, B., Seog, J. H., Graham, L. M., and Lee, S. B. (2011). Experimental considerations on the cytotoxicity of nanoparticles. *Nanomedicine*, 6(5), 929–941.

Koocheki, A., and Kadkhodaee, R. (2011). Effect of Alyssum homolocarpum seed gum, Tween 80 and NaCl on droplets characteristics, flow properties and physical stability of ultrasonically prepared corn oil-in-water emulsions. *Food Hydrocolloids*, 25(5), 1149–1157.

Koocheki, A., Kadkhodaee, R., Mortazavi, S. A., Shahidi, F., and Taherian, A. R. (2009). Influence of Alyssum homolocarpum seed gum on the stability and flow properties of O/W emulsion prepared by high intensity ultrasound. *Food Hydrocolloids*, 23(8), 2416–2424.

Koppel, K., and Chambers, I.E. (2010). Development and application of a lexicon to describe the flavor of pomegranate juice. *Journal of Sensory Studies*, 25(6), 819–837.

Korman, S. H., Cohen, E., and Preminger, A. (2001). Pseudo-maple syrup urine disease due to maternal prenatal ingestion of fenugreek. *Journal of Paediatrics and Child Health*, 37, 403–404.

Koupantsis, T., and Kiosseoglou, V. (2009). Whey protein–carboxymethylcellulose interaction in solution and in oil-in-water emulsion systems. Effect on emulsion stability. *Food Hydrocolloids*, 23(4), 1156–1163.

Kovacs, T., Naish, V., O'Connor, B., Blaise, C., Gagné, F., Hall, L., Trudeau, V., and Martel, P. (2010) An ecotoxicological characterization of nanocrystalline cellulose (NCC). *Nanotoxicology*, 4(3), 255-270.

Koyoro, H., and Powers, J.R. (1987). Functional properties of pea globulin fractions. *Cereal Chemistry*, 64, 97–101.

Krešic, G., Lelas, V., Režek Jambrak, A. Herceg, Z., and Rimac Brnčic S. (2008). Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins. Journal of Food Engineering, 87(1), 64-73.

Krstonošić, V., Dokić, L., Dokić, P. and Dapčević, T. (2009). Effects of xanthan gum on physicochemical properties and stability of corn oil-in-water emulsions stabilized by polyoxyethylene (20) sorbitan monooleate. *Food Hydrocolloids*, 23(8), 2212-2218.

Krstonošić, V., Dokić, L., Nikolić, I., and Milanović, M. *(2015).* Influence of xanthan gum on oil-in-water emulsion characteristics stabilized by OSA starch. *Food Hydrocolloids*, 45, 9-17.

Kruse Fæstea, C., Christians, U., Egaas, E., and Jonscher, K. R. (2010). Characterization of potential allergens in fenugreek (Trigonella foenum-graecum) using patient sera and MS-based proteomic analysis. *Journal of Proteomics*, 73(7), 1321–1333.

Kunieda, H., and Friberg, S. E. (1981).Critical Phenomena in a surfactant/water/oil system. Basic study on the correlation between solubilization, microemulsion, and ultralow interfacial tensions. *Bulletin of the Chemical Society of Japan*, 54, 1010-1014.

L

Laneuville, S. I., Paquin, P., and Turgeon, S. L. (2000). Effect of preparation conditions on the characteristics of whey protein—xanthan gum complexes. *Food Hydrocolloids*, 14(4), 305–314.

Laurian, J. U. (2013). Food and health: Can economics contribute to improved outcomes? *American Journal of Agricultural Economics*, 95(2), 220–227.

Lavin, J., and Lawless, H. T. (1998). Effects of colour and odor on judgments of sweetness among children and adults. *Food Quality and Preference*, 9, 283–289.

Lawless, H.T., and Heymann, H. (2010). Sensory Evaluation of Food: Principles and Practices. New York, NY: Springer.

Lazos, E. S. (1992). Certain functional properties of defatted pumpkin seed flour. *Plant Foods for Human Nutrition*, 42, 257–273.

Leal-Calderon, F., Schmitt, V., and Bibette, J. (2007). Emulsion science: basic principles. New York, NY: Springer.

Lee, K. Y., Yao, H., Bae I. Y., Lee, S. and Hyeon H. G. (2012). Effect of hydrocolloids on the pasting and rheological characteristics of resistant starch (type 4). *Food Science and Biotechnology*, 21(3), 769-774.

Lee, L., and Norton, I. T. (2013). Comparing droplet breakup for a high-pressure valve homogeniser and a Microfluidizer for the potential production of food-grade nanoemulsions. *Journal of Food Engineering*, 114(2), 158–163.

Lee, L., Hancocks, R., Noble, I. and Norton, I. T. (2014). Production of water-in-oil nanoemulsions using high pressure homogenisation: A study on droplet break-up. *Journal of Food Engineering*, 131, 33–37.

Lehnert, S., Tarabishi, H., and Leuenberger, H. (1994). Investigation of thermal phase inversion in emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 91, 227-235.

Leman, J. (1999). Emulsification properties of alpha-lactalbumin. *Natural Sciences*, 3, 35–43.

Leong, T. S. H., Wooster, T. J., Kentish, S. E., and Ashokkumar, M. (2009). Minimising oil droplet size using ultrasonic emulsification. *Ultrasonics Sonochemistry*, 16, 721–727.

Leroux, J., Langendorff, V., Schick, G., Vaishnav, V. and Mazoyer, J. (2003). Emulsion stabilizing properties of pectin. *Food Hydrocolloids*, 17, 455–462.

Li, M. K. and Fogler, H. S. (1978). Acoustic emulsification. Part 1: The instability of the oil–water interface to form the initial droplet. *Journal of Fluid Mechanics*, 88, 499–512.

Li, P. H. and Chiang, B. H. (2012). Process optimization and stability of D-limonene-in-water nanoemulsions prepared by ultrasonic emulsification using response surface methodology. *Ultrasonics Sonochemistry*, 19(1), 192-197.

Li, R., and Feke, D. L. (2015). Rheological and kinetic study of the ultrasonic degradation of xanthan gum in aqueous solutions. *Food Chemistry*, 172, 808–813.

Li, X., Wasila, H., Liu, L., Yuan, T., Gao, Z., Zhao, B., and Ahmad, I. (2015). Physicochemical characteristics, polyphenol compositions and antioxidant potential of pomegranate juices from 10 Chinese cultivars and the environmental factors analysis. *Food Chemistry*, 175, 575-584.

Li, X., Wasila, H., Liu, L., Yuan, T., Gao, Z., Zhao, B., and Ahmad, I. (2015). Physicochemical characteristics, polyphenol compositions and antioxidant potential of pomegranate juices from 10 Chinese cultivars and the environmental factors analysis. *Food Chemistry*, 175, 575–584.

Liang, N.-H., and Tang, C. (2013). pH-dependent emulsifying properties of pea [Pisum sativum (L.)] proteins. *Food Hydrocolloids*, 33(2), 309-319.

Liang, N.-H., and Tang, C. (2014). Pea protein exhibits a novel Pickering stabilization for oil-in-water emulsions at pH 3.0. *LWT - Food Science and Technology*, 58(2), 463–469.

Licón, C. C., Carmona, M., Molina, A., and Berruga, M. I. (2012a). Chemical, microbiological, textural, color, and sensory characteristics of pressed ewe milk cheeses with saffron (*Crocus sativus* L.) during ripening. *Journal of Dairy Science*, 95(8), 4263-4274.

Licón, C. C., Carmona, M., Rubio, R., Molina, A., and Berruga, M. I. (2012b). Preliminary study of saffron (*Crocus sativus* L. stigmas) color extraction in a dairy matrix. *Dyes and Pigments*, 92(3), 1355-1360.

Lii, C., Chen, C. H., Yeh, A. I., and Lai, V. M.-F. (1999). Preliminary study on the degradation kinetics of agarose and carrageenans by ultrasound. *Food Hydrocolloids*, 13(6), 477-481.

López-Franco, Y. L., Cervantes-Montaño, C. I., Martínez-Robinson, K. G., Lizardi-Mendoza, J., and Robles-Ozuna, L. E. (2013). Physicochemical characterization and functional properties of galactomannans from mesquite seeds (*Prosopis* spp.). *Food Hydrocolloids*, 30(2), 656-660.

Lorimer, J. P., Mason, T. J., Cuthbert, T. C., and Brookfield, E. A. (1995). Effect of ultrasound on the degradation of aqueous native dextran. *Ultrasonics Sonochemistry*, 2, 55-57.

M

Maat, J., Rossi, D., Babuchowsk, A.. Beekmans, I, F. Castenmiller, J., Fenwick, R.. et al. (2005). European technology platform on food for life. The vision for 2020 and beyond. http:platformazywnosci.pl/pliki/BAT_Brochure_ETP.pdf on 23.01.13.

Madar, Z., Abel, R., Samish, S., and Arad, J. (1988). Glucose-lowering effect of fenugreek in non-insulin dependent diabetes. *European Journal of Clinical Nutrition*, 42, 51–54.

Maggi, L., Carmona, M., Zalacain, A. Kanakis, C. D., Anastasaki, E., Tarantilis, P. A., Polissiou, M. G., and Alonso, G. L. 2010. Changes in saffron volatile profile according to its storage time. *Food Research International*, 43(5), 1329-1334.

Maggi, L., Carmona, M., Zalacain, A., Kanakis, C. D., Anastasaki, E., Tarantilis, P. A., Polissiou, M. G., and Alonso, G. L. (2010). Changes in saffron volatile profile according to its storage time. *Food Research International*, 43(5), 1329–1334.

Maier, M., Anderson, M., Karl, C., and Magnuson, K. (1993). Guar, locust bean, tara, and fenugreek gums. In Whistly, L.R., and BeMiller, J.N. (Eds). Industrial gums: Polysaccharides and Their Derivatives. 3rd ed. Academic Press. New York.

Makri, E. A., and Doxastakis, G. I. (2006). Study of emulsions stabilized with *Phaseolus vulgaris* or *Phaseolus coccineus* with the addition of Arabic gum, locust bean gum and xanthan gum. *Food Hydrocolloids*, 20(8), 1141-1152.

Makri, E., and Doxastakis, G. (2006). Study of emulsions and foams stabilized with *Phaseolus vulgaris* or *Phaseolus coccineus* with the addition of xanthan gum or NaCl. *Journal of the Science Food and Agriculture*, 86(12), 1863-1870.

Malik, V. S., Willett, W. C., and Hu, F. B. (2013). Global obesity: Trends, risk factors and policy implications. *Nature Reviews Endocrinology*, 9, 13–27.

Mandala, I. G , Savvas, T. P, and Kostaropoulos, A. E. (2004). Xanthan and locust bean gum influence on the rheology and structure of a white model-sauce. *Journal of Food Engineering*, 64(3), 335-342.

Marchesini, G., Balzan, S., Montemurro, F., Fasolato, L., Andrighetto, I., Segato, S., and Novelli, E. (2012). Effect of ultrasound alone or ultrasound coupled with CO2 on the chemical composition, cheese-making properties and sensory traits of raw milk. *Innovative Food Science and Emerging Technologies*, 16, 391–397.

Marcotte, M., Taherian, A. R. and Ramaswamy, H. S. (2001). Evaluation of rheological properties of selected salt enriched food hydrocolloids. *Journal of Food Engineering*, 48(2), 157–167.

Mark, G., Tauber, A., Laupert, R., Schuchmann, H. P., Schultz, D., Mues, A., and von Sonntag, C. (1998). OH-radical formation by ultrasound in aqueous solution – part II: terephthalate and Fricke dosimetry and the influence of various conditions on the sonolytic yield. *Ultrasonics Sonochemistry*, 5, 41–52.

Marmesat, S. Morales, A., Velasco, J., Ruiz-Méndez, M. V., and Dobarganes, M. C. (2009). Relationship between changes in peroxide value and conjugated dienes during oxidation of sunflower oils with different degree of unsaturation. *Grasas y Aceites*, 60(2), 155-160.

Martínez, I., Riscardo, M. A., and Franco, J. M. (2007). Effect of salt content on the rheological properties of salad dressing-type emulsions stabilized by emulsifier blends. *Journal of Food Engineering*, 80(4), 1272-1281.

Martínez-Tomé, M., Jiménez, A. M., Ruggieri, S., Frega, N., Strabbioli, R., and Murcia, M. A. (2001). Antioxidant properties of Mediterranean spices compared with common food additives. Journal of Food Protection, 64(9), 1412-1419.

Marucci, G. (1969). A theory of coalescence. *Chemical Engineering Science*, 24, 975–985.

Maskan, M. (2006). Production of pomegranate (*Punica granatum L.) juice* concentrate by various heating methods: colour degradation and kinetics. *Journal of Food Engineering*, 72 (3), 218–224.

Mason, T. J. (1997). Ultrasound in Synthetic Organic Chemistry. *Chemical Society Reviews*, 26, 443-451.

Mason, T. J., Paniwnyk, L., and Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*, 3 (3), 253-260.

Mason, T., Wilking, J., Meleson, K., Chang, C., and Graves, S. (2006). Nanoemulsions: formation, structure, and physical properties. *Journal of Physics: Condensed Matter*, 18, 635.

Mazza, G., Di Tommaso, D., and Foti, S. (2002). Volatile constituents of sicilian fenugreek (*Trigonella foenum-graceum*L.) seeds. *Sciences des Aliments*, 22(3), 249–264.

McClements, D. J. (1999). Emulsion stability. In D.J. McClements (Ed.), Food emulsions, principles, practice and techniques (pp. 185-234). Boca Raton: CRC Press.

McClements, D. J. (2000). Comments on viscosity enhancement and depletion flocculation by polysaccharides. *Food Hydrocolloids*, 14, 173–177.

McClements, D. J. (2002). Colloidal basis of emulsion color. *Current Opinion in Colloid & Interface Science*, 7(5–6), 451–455.

McClements, D. J. (2005). Food Emulsions: Principles, Practices, and Techniques. Boca Raton: CRC Press.

McClements, D. J. (2008). Whey protein-stabilized emulsions. In C. Onwwulata, and P. Huth (Eds.). *Whey processing, functionality and health benefits* (pp. 63-98). Ames: Blackwell Publishing.

McClements, D. J. (2012). Nanoemulsions versus microemulsions: Terminology, differences, and similarities. *Soft Matter*, 8, 1719–1729.

McClements, D. J. (2013). Edible lipid nanoparticles: Digestion, absorption, and potential toxicity. *Progress in Lipid Research*, 52(4), 409–423.

McClements, D. J. (2015). Nanoparticle- and Microparticle-Based Delivery Systems. Encapsulation protection and Release of Active Compounds. Taylor and Francis, Boca Raton.

McClements, D. J., and Decker, E.A. (2000). Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *Journal of Food Science*, 65(8), 1270–1282.

McClements, D. J., Decker, E. A., and Weiss, J. (2007). Emulsion-based delivery systems for lipophilic bioactive components. *Journal of Food Science*, 72, 109–124.

McClements, D. J., Klinkesorn, U., Sophanodora, P., and Chinachoti, P. (2004). Stability and rheology of corn oil-in-water emulsions containing maltodextrin. *Food Research International*, 37(9), 851-859.

McClements, D. J., Monahan, F. J., and Kinsella, J. E. (2006). Disulfide bond formation affects stability of whey protein isolate emulsions. *Journal of Food Science*, 58(5), 1036-1039.

Meilgaard, M., Civille, G. V. and Carr, B. T. (1991). Sensory Evaluation Techniques, 2nd ed., CRC Press, Inc. Boca Raton, FL.

Meilgaard, M., Civille, G. V., and Carr, B. T. (1999). Controls for tests room, product, and panel; Attribute difference tests: How does attribute X differ between samples; Affective tests: consumer tests and in-house panel acceptance tests; Basic statistical methods; Guidelines for choice of technique. In M. Meilgaard, G.V. Civille, and B.T. Carr. *Sensory evaluation techniques* (pp. 23 36, 99 122, 231 304, 337 344). London: CRC Press.

Mela, D. J., Langley, K. R., and Martin, A. (1994). Sensory assessment of fat content: Effect of emulsion and subject characteristics. *Appetite*, 22(1), 67–81.

Melnyk, J. P., Wang, S., and Marcone M. F. (2010). Chemical and biological properties of the world's most expensive spice: Saffron. *Food Research International*, 43(8), 1981-1989.

Mena, P., Vegara, S., Martí, N., García-Viguera, C., Saura, D., and Valero, M. (2013). Changes on indigenous microbiota, colour, bioactive compounds and antioxidant activity of pasteurised pomegranate juice. *Food Chemistry*, 141(3), 2122-2129.

Mengual, O., Meunier, G., Cayre, I., Puech, K., and Snabre, P. (1999a). Characterisation of instability of concentrated dispersions by a new optical analyser: the TURBISCAN MA 1000. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 152(1), 111-123.

Mengual, O., Meunier, G., Cayré, I., Puech, K, and Snabre, P. (1999b). TURBISCAN MA 2000: multiple light scattering measurement for concentrated emulsion and suspension instability analysis. *Talanta*, 50(2), 445-56.

Merkus, H. G. (2009). Particle size measurements: Fundamentals, practice, Quality. Springer.

Milas, M., Rinaudo, M., and Tinland, B. (1986). Comparative depolymerization of xanthan gum by ultrasonic and enzymic treatments. Rheological and Structural properties, *Carbohydrate Polymers*, 6, 95-107.

Milas, M., Rinaudo, M., and Tinlands, B. (1986). Comparative depolymerization of xanthan gum by ultrasonic and enzymic treatments. Rheological and structural properties. *Carbohydrate Polymers*, 6, 95-107.

Mine, Y., Shimizu, M., and Nakashima, T. (1996). Preparation and stabilization of simple and multiple emulsions using a microporous glass membrane. *Colloids and Surfaces B: Biointerfaces*, 6 (4–5), 261–268.

Mirhosseini, H. and Amid, B. T. (2012). Influence of Chemical Extraction Conditions on the Physicochemical and Functional Properties of Polysaccharide Gum from Durian (Durio zibethinus) Seed. *Molecules*, 17(6), 6465-6480.

Mirhosseini, H., Tan, C. P., Hamid, N. S. A., and Yusof, S. (2008). Optimization of the contents of Arabic gum, xanthan gum and orange oil affecting turbidity, average particle size,polydispersity index and density in orange beverage emulsion. *Food Hydrocolloids*, 22 (7), 1212-1223.

Mitidieri, F. E., and Wagner, J. R. (2002). Coalescence of o/w emulsions stabilized by whey and isolate soybean proteins. Influence of thermal denaturation, salt addition and competitive interfacial adsorption. *Food Research International*, 35(6), 547–557.

Moncada Reyes, M. L. (2011). Influence of low sonication intensities at different temperatures on acid tolerance, bile tolerance, protease ativity, and growth of yogurt culture bacteria *Lactobacillus delbrueckii spp. Bulgaricus* and *Streptococcus salivarius ssp. Thermophilus*. Master Thesis. School of Animal Science, Louisiana State University, Louisiana, USA.

Morales, D., Gutierrez, J. M., Garcia-Celma, M. J., and Solans, C. (2003). A Study of the Relation between Bicontinuous Microemulsions and Oil / Water Nano-emulsion Formation. *Langmuir*, 19, 7196-7200.

Morell, P., Fiszman, S. M, Varela, P., and Hernando, I. (2014). Hydrocolloids for enhancing satiety: Relating oral digestion to rheology, structure and sensory perception. *Food Hydrocolloids*, 41, 343-353.

Mudgil, D., Barak, S. and Khatkar, B. S. (2012). X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. *International Journal of Biological Macromolecules*, 50, 1035–1039.

Murray, J. M., Delahunty, C. M., and Baxter, I. A. (2001). Descriptive sensory analysis: past, present and future. *Food Research International*, 34(6), 461–471.

Muthukumaran, S., Kentish, S. E., Stevens, G. W. and Ashokkumar, M. (2006). Application of Ultrasound in Membrane Separation processes: A Review. *Reviews in Chemical Engineering*, 22, 155-194.

N

Nakajima, H., Tomomossa, S., and Okabe, M. (1993). First Emulsion Conference, Paris, France.

Neirynck, N., Van Lent, K., Dewettinck, P., and Van der Meeren, P. (2007). Influence of pH and biopolymer ratio on sodium caseinate-guar gum interactions in aqueous solutions and in O/W emulsions. *Food Hydrocolloids*, 21, 862–869.

Nerina, A., Camino, A., and Pilosof, M. R. (2010). Hydroxypropylmethylcellulose at the oil/water interface. Part II. Submicron-emulsions as affected by pH. *Food Hydrocolloids*, 25, 1051-1062.

Nikiforidis, C. V.Biliaderis, C. G. Kiosseoglou, V. (2012). Rheological characteristics and physicochemical stability of dressing-type emulsions made of oil bodies–egg yolk blends. *Food Chemistry*, 134(1), 64–73.

Niknafs, N., Spyropoulos, F., and Norton, I. T. (2011). Development of a new reflectance technique to investigate the mechanism of emulsification. *Journal of Food Engineering*, 104, 603–611.

Nuncio-Jáuregui, N., Calín-Sánchez, Á., Vázquez-Araújo, L., Pérez-López, A. J., Frutos-Fernández, M. J., and Carbonell-Barrachina Á. A. (2015). *Processing Pomegranates for Juice and Impact on Bioactive Components* (pp. 629-636). In V. R. Preedy (Ed.). Processing and Impact on Active Components in Food. Waltham: Elsevier Academic Press.

O

Olson, D. W., White, C. H., and Richter, R. L. (2004). Effect of pressure and fat content on particle sizes in microfluidized milk. *Journal of Dairy Science*, 87(10), 3217–3223.

Onwulata, C. I., and Huth, P. J. (2009). Whey Processing, Functionality and Health Benefits. Oxford, UK: Wiley-Blackwell.

Oprea, A.-M., Nistor, M.-T., Profire, L., Popa, M. I., Lupusoru, C. E., and Vasile, C. (2013). Evaluation of the Controlled Release Ability of Theophylline from Xanthan/Chondroitin Sulfate Hydrogels. *Journal of Biomaterials and Nanobiotechnology*, 4, 123-131.

Ordoudi, S.A., Nenadis, N., and Tsimidou, M.Z. (2008). Comparative Study on the Radical Scavenging Properties of Methanol Extracts of *Crocus sativus* L., *Curcuma longa* L. and *Origanum hirtum* spp. 6th Euro Fed Lipid Congress, "Oils, Fats and Lipids in the 3rd Millennium", 7-10 September 2008, Athens, Greece.

Otoni, C.G. de Moura, M. R. Aouada, F.A. Camilloto, G.P. Cruz, R.S. Lorevice, M.V. *et al.* (2014). Antimicrobial and physical-mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids*, 41, 188–194.

P

Palazolo, G. G., Sorgentini, D. A, and Wagner, J. R. (2004). Emulsifying properties and surface behavior of native and denatured whey soy proteins in comparison with other proteins. Creaming stability of oil-in-water emulsions. *Journal of the American Oil Chemists' Society*, 81(7), 625-632.

Palazolo, G. G., Sorgentini, D. A., and Wagner, J. R. (2005). Coalescence and flocculation in o/w emulsions of native and denatured whey soy proteins in comparison with soy protein isolates. *Food Hydrocolloids*, 19(3), 595-604.

Panaras, G., Moatsou, G., Yanniotis, S., and Mandala, I. (2011). The influence of functional properties of different whey protein concentrates on the rheological and emulsification capacity of blends with xanthan gum. *Carbohydrate Polymers*, 86(2), 433-440.

Pangborn, R. M., Berg, H. W., and Hansen, B. (1963). The influence of color on discrimination of sweetness. *American Journal of Psychology*, 76, 492-495.

Paquin, P. (1999).Technological properties of high pressure homogenizers: the effect of fat globules, milk proteins, and polysaccharides. *International Dairy Journal*, 9(3–6), 329–335.

Patel, C.,Dadhaniya, P.,Hingorani, L., and Soni, M. G. (2008). Safety assessment of pomegranate fruit extract: Acute and subchronic toxicity studies. *Food and Chemical Toxicology*, 46(8), 2728–2735.

Pathak, M. (2012). Numerical Simulation of Droplet Dynamics in Membrane Emulsification Systems, In M. Andriychuk (Ed.). *Numerical Simulation - From Theory to Industry*, (pp. 415-438). INTECH Publisher.

Patist, A., and Bates, D. (2008). Ultrasonic innovations in the food industry: From the laboratory to commercial production. *Innovative Food Science & Emerging Technologies*, 9(2), 147-154.

Paximada, P., Tsouko, E., Kopsahelis, N., Koutinas, A. A., and Mandala, I. (2014). Bacterial cellulose as stabilizer of o/w emulsions. *Food Hydrocolloids*, In Press, Corrected Proof, Available online 10 December.

Pelegrine, D. H. G, and Gasparetto, C. A. (2005). Whey proteins solubility as function of temperature and pH. *LWT - Food Science and Technology*, 38(1), 77–80.

Pérez-Alvarez, J. A. (2008). Overview of meat products as functional foods. In J. Fernández-López, and J.A. Pérez-Alvarez (Eds.). *Technological strategies for functional meat products development*, (pp. 1–17). Kerala, India: Transworld Research Network.

Perrechil, F. A., and Cunha, R. L. (2010). Oil-in-water emulsions stabilized by sodium caseinate: influence of pH, high-pressure homogenization and locust bean gum addition. *Journal of Food Engineering*, 97, 441–448.

Phipps, L. W. (1985). The High Pressure Dairy Homogenizer. Reading, England: The National Institute for Research in Dairying.

Pizzino, A., Catte´, M., Van Hecke, E., Salager, J. L., and Aubry, J. M. (2009). On-line backscattering tracking of the transitional phase inversion of emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 338, 148–154.

Poiana, M. A. (2012). Enhancing Oxidative Stability of Sunflower Oil during Convective and Microwave Heating Using Grape Seed Extract. *International Journal of Molecular Sciences*, 13, 9240-9259.

Ponginebbi, L., Nawar, W. W., and Chinachoti, P. (1999). Oxidation of linoleic acid in emulsions: Effect of substrate, emulsifier, and sugar concentration. *Journal of the American Oil Chemists' Society*, 76(1), 131-138.

Popineau, Y., Huchet, B., Larre, C., and Berot, S. (2002). Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science*, 35, 327–335.

Porras, M., Solans, C., González, C., and Gutiérrez, J. M. (2008). Properties of water-in-oil (W/O) nano-emulsions prepared by a low-energy emulsification method. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 324(1–3), 181-188.

Povey, M. J. W., and Mason, T. J. (1998). Ultrasound in Food Processing. New York: Blackie Academic and Professional.

Prajapat, A. L., and Gogate, P. R. (2015). Depolymerization of guar gum solution using different approaches based on ultrasound and microwave irradiations. *Chemical Engineering and Processing: Process Intensification*, 88, 1-9.

Prajapati, V. D., Jani, G. K., Moradiya, N. G., Randeria, N. P., Nagar, B. J., Naikwadi, N. N., and Variya, B. C. (2013). Galactomannan: A versatile biodegradable seed polysaccharide. *International Journal of Biological Macromolecules*, 60, 83-92.

Price, G. (1990). The use of ultrasound for the controlled degradation of polymer solutions. In T. J. Mason (Ed.). *Advances in sonochemistry* (pp. 231–287). London, UK: JAI Press.

Protonotariou, S., Evageliou, V., Yanniotis, S., and Mandala, I. (2013). The influence of different stabilizers and salt addition on the stability of model emulsions containing olive or sesame oil. *Journal of Food Engineering*, 117(1), 124-132.

Q

Qian, C., and McClements, D. J. (2011). Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids*, 25(5), 1000–1008.

Qian, C., Decker, E. A., Xiao, H., and McClements, D. J. (2012). Inhibition of β-carotene degradation in oil-in-water nanoemulsions: Influence of oil-soluble and water-soluble antioxidants. *Food Chemistry*, 135(3), 1036–1043.

Qian, H. F., Cui, S. W., Wang, Q., Wang, C., and Zhou, H. M. (2011). Fractionation and physicochemical characterization of peach gum polysaccharides. *Food Hydrocolloids*, 25(5), 1285-1290.

Qiu, C., Zhao, M., and McClements, D., J. (2015). Improving the stability of wheat protein-stabilized emulsions: Effect of pectin and xanthan gum addition. *Food Hydrocolloids*, 43, 377-387.

R

Raghuram, T. C., Sharma, R. D., and Sivakumar, B. (1994). Effect of fenugreek seeds on intravenous glucose disposition in non-insulin dependent diabetic patients. *Phytotherapy Research*, 8, 83–86.

Rakhmanberdyeva, R. K., Rakhimov, D. A., Vakhabov, A. A., Khushbaktova, Z. A., and Syrov, V. N. (2005). *Chemistry of Natural Compounds*, 41, 11–13.

Rao, J., and McClements, D. J. (2011). Food grade microemulsions, nanoemulsions and emulsions: fabrication from sucrose nanopalmitate and lemon oil. *Food Hydrocolloids*, 25, 1413-1423.

Rao, J., and McClements, D. J. (2012). Lemon oil solubilization in mixed surfactant solutions:Rationalizing microemulsion & nanoemulsion formation. *Food Hydrocolloids*, 26(1), 268–276.

Rao, M. A. (2007). Rheology of Fluid and Semisolid Foods. Principles and Applications. Springer Press, New York, USA.

Rao, P. U., Sesikeran, B., Rao, P. S., Naidu, A.N., Rao, V. V., and. Ramacjandran, E. P. (1996). Short-term nutritional and safety evaluation of fenugreek. *Nutrition Research*, 16, 1495–1505.

Raso, J., Mañas, P., Pagán, R., and Sala, F. J. (1999). Influence of different factors on the output power transferred into medium by ultrasound. *Ultrasonics Sonochemistry*, 5(4), 157-62.

Rawle, A. (2002). The importance of particle sizing to the coatings industry. *Advances in Colour Science and Technology*, 5(1), 1-12.

Relkin, P. and Sourdet, S. (2005). Factors affecting fat droplet aggregation in whipped frozen protein-stabilized emulsions. *Food Hydrocolloids*, 19, 503–51.

Ribeiro, H. S., Guerrero, J. M. M., Briviba, K., Rechkemmer, G., Schuchmann, H. P., and Schubert H. (2006). Cellular uptake of carotenoid-loaded oil-in-water emulsions in colon carcinoma cells in vitro. *Journal of Agricultural and Food Chemistry*, 54(25), 9366–9369.

Ribes, G., Sauvaire, Y., Da Costa, C., Baccou, J. C., and Loubatieres-Mariani, M. M. (1986). Antidiabetic effects of subfractions from fenugreek seeds in diabetic dogs. *Proceedings of the Society for Experimental Biology and Medicine*, 182, 159–166.

Richardson, P. H., Clark, A. H., Russell, A. L., Aymard, P., and Norton, I. T. (1999). Galactomannan gelation: A thermal and rheological investigation analysed using the cascade model. *Macromolecules*, 32, 1519–1527.

Richardson, R. K., and Ross-Murphy, S. B. (1987). Non-linear viscoelasticity of polysaccharide solutions. 2: xanthan polysaccharide solutions. *International Journal of Biological Macromolecules*, 9(5), 250–256.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., Lyng, J.G. (2009). Characterisation of volatile compounds generated in milk by high intensity ultrasound. *International Dairy Journal*, 19, 269-272.

Rivera Calo, J., Crandall, P. G., O'Bryan, C. A., and Ricke, S. C. (2015). Essential oils as antimicrobials in food systems – A review. *Food Control*, 54, 111–119.

Roberts, K. T., Cui, S. W., Chang, Y. H., Ng, P. K. W., and Graham, T. (2012). The influence of fenugreek gum and extrusion modified fenugreek gum on bread. *Food Hydrocolloids*, 26(2), 350-358.

Robins, M. M. (1991). Effect of Polysaccharide on Flocculation and Creaming in Oil-In-Water Emulsion Flocculation and Creaming. ACS. Symp. Ser., 448, 230-246.

Robins, M. M. (2000). Emulsions-creaming phenomena. *Current Opinion in Colloid Interface Science*, 5, 265-272.

Rocha, C., Teixeira, J. A., Hilliou, L., Sampaio, P., and Gonçalves, M. P. (2009). Rheological and structural characterization of gels from whey protein hydrolysates/locust bean gum mixed systems. *Food Hydrocolloids*, 23(7), 1734-1745.

Rodriguez-Amaya, D. B. (2001). General procedure and sources of errors in carotenoid analysis. In: A guide to carotenoid analysis in foods. Intl. Life Sciences Inst. Washington, D.C.: ILSI Press. 23 p.

Roohinejad, S., Oey, I., Wen, J., Lee, S. J., Everett, D. W., and Burritt, D. J. (2015). Formulation of oil-in-water β-carotene microemulsions: Effect of oil type and fatty acid chain length. *Food Chemistry*, 174, 270-278.

Roosen, J., Bieberstein, A., Blanchemanche, S., Goddard, E., Marette, S., Vandermoere, F., Siegrist, M., Cousin, M., Kastenholz, H., and Wiek, A. (2007). Public acceptance of nanotechnology foods and food packaging: the influence of affect and trust. *Appetite*, 49, 459–466.

Ross-Murphy, S. B. (1995). Structure–property relationships in food biopolymer gels and solutions. *Journal of Rheology*, 39, 1451.

S

Sagalowicz, L., and Leser, M. E. (2010). Delivery systems for liquid food products. *Current Opinion in Colloid & Interface Science*, 15(1–2), 61–72.

Sajilata, M. G., Singhal, R. S., and Kulkarni, P. R. (2006). Resistant starch - A review. *Comprehensive Reviews in Food Science and Food Safety*, 5, 1–17.

Sajjadi, S. (2006). Nanoemulsion formation by phase inversion emulsification: On the nature of inversion. *Langmuir*, 22, 5597-5603.

Sajjadi, S., Jahanzad, F., and Yianneskis, M. (2004). Catastrophic phase inversion of abnormal emulsions in the vicinity of the locus of transitional inversion. *Colloids and Surfaces A Physicochemical and Engineering Aspects*, 240, 149-155.

Salager, J. L., Morgan, J. C, Schechter, R. S., Wade, W. H., and Vasquez, E. (1979). Optimum formulation of surfactant/water/oil systems for minimum interfacial tension or phase behavior. *Society of Petroleum Engineers Journal*, 19, 107–115.

Salager, J.L., Márquez, L., Peña, A., Rondón, M., Silva, F., and Tyrode, E. (2000). Current Phenomenological Know-How and Modeling of Emulsion Inversion. *Industrial and Engineering Chemistry Research*, 39(8), 2665-2676.

Salager, J. L., Forgiarini, A., Marquez, L., Peña, A., Pizzino, A., Rodriguez, M. P., and Rondón-González, M. (2004). Using emulsion inversion in industrial processes. *Advances in Colloid and Interface Science*, 108-109, 259-272.

Salvia-Trujillo, L., Rojas-Graü, M. A., Soliva-Fortuny, R., and Martín-Belloso, O. (2013). Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. *Food Hydrocolloids*, 30(1), 401-407.

Sanchez, C., Schmitt, C., Babak, V. G., and Hardy, J. (1997). Rheology of whey protein isolate–xanthan mixed solutions and gels: effect of pH and xanthan concentration. *Nahrung*, 41(6), 336–343.

Sandberg, A.-S. (2011). Developing functional ingredients: a case study of pea protein. In M. Saarela (Ed.). Functional Foods: Concept to Product (pp.358-382). Cambridge, UK: Woodhead Publishing Limited.

Sandolo, C., Bulone, D., Mangione, M. R., Margheritelli, S., Di Meo, C., Alhaique, F., Matricardi, P., and Coviello, T. (2010). Synergistic interaction of Locust Bean Gum and Xanthan investigated by rheology and light scattering. *Carbohydrate Polymers*, 82(3), 733–741.

Santos, H. M., Lodeiro, C., and Capelo-Martinez, J.-L. (2009). The Power of Ultrasound 1.1Introduction Ultrasound. In Capelo-Martinez (Ed.). *Chemistry: Analytical Applications* (pp. 1-16). Weinheim: WILEY-VCH.

Saxena, A., Kaloti, M., and Bohidar, H. B. (2011). Rheological properties of binary and ternary protein–polysaccharide co-hydrogels and comparative release kinetics of salbutamol sulphate from their matrices. *International Journal of Biological Macromolecules*, 48, 263–270.

Schärtl, W. (2007). Light Scattering from Polymer Solutions and Nanoparticle Dispersions. Springer, Berlin.

Schneider, A., and Lacampagne, J. P. (2000). Peas: a European production of protein-rich materials for feed and food. *Industrial Proteins*, 8, 3–6.

Seekkuarachchi, I. N., Tanaka, K., and Kumazawa, H. (2006). Formation and charaterization of submicrometer oil-in-water (O/W) emulsions using high-energy emulsification. *Industrial and Engineering Chemical Research*, 45, 372–390.

Seeram, N. P., Adams, L. S., Henning, S. M., Niu, Y., Zhang, Y., Nair, M. G., and Heber, D. (2005). In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *The Journal of Nutritional Biochemistry*, 16(6), 360-367.

Selim, K., Tsimidou, M., and Biliaderis C. G. (2000). Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices. *Food Chemistry*, 71(2), 199-206.

Sessa, M., Balestrieri, M. L., Ferrari, G., Servillo, L., Castaldo, D., D'Onofrio, N., Donsì, F., and Tsao, R. (2014). Bioavailability of encapsulated resveratrol into nanoemulsion-based delivery systems. *Food Chemistry*, 147, 42–50.

Sewell, A. C., Mosandl, A., and Bohles, H. (1999). False diagnosis of maple syrup urine disease owing to ingestion of herbal tea. *Massachusetts Medical Society*, 341, 769.

Shahidi, F., and Zhong, Y. (2005). Lipid Oxidation: Measurement Methods. In F. Shahidi (Ed.). *Bailey's Industrial Oil & Fat Products* (p. 357). New Jersey: Wiley-Interscience.

Shand, P.J., Ya, H., Pietrasik, Z., and Wanasundara, P. K. J. P. D. (2007). Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chemistry*, 102(4), 1119-1130.

Shankaracharya, N. B., Anandaraman, S., and Natarajan, C. P. (1973). Chemical composition of raw and roasted fenugreek seeds. *Journal of Food Science and Technology*, 10, 179–18.

Shanmugam, A., and Ashokkumar, M. (2014).Functional properties of ultrasonically generated flaxseed oil-dairy emulsions. *Ultrasonics Sonochemistry*, 21(5), 1649–1657.

Sharma, R., and Raghuram, T. C. (1990). Hypoglycemic effect of fenugreek seeds in non-insulin dependent diabetic subjects. *Nutrition Research*, 10, 731–739.

Shinoda, K., and Saito, H. (1968). The effect of temperature on the phase equilibria and the types of dispersions of the ternary system composed of water, cyclohexane, and nonionic surfactant. *Journal of Colloid and Interface Science*, 26(1), 70-74.

Siegrist, M., Cousin, M., Kastenholz, H., and Wiek, K. (2007). Public acceptance of nanotechnology foods and food packaging: the influence of affect and trust. *Appetite*, 49(2), 459-466.

Sikora, M., Badrie, N., Deisingh, A. K., and Kowalski, S. (2008). Sauces and dressings: a review of properties and applications. *Critical Reviews in Food Science and Nutrition*, 48(1), 50–77.

Singh, H., Boland, M., and Thompson, A. (2014). Milk proteins:From Expression to Food. London,UK: Academic Press.

Singleton, V., and Rossi, J. Jr. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.

Sivakumar, M., Tang, S. Y., Tan, K. W. (2014). Cavitation technology – A greener processing technique for the generation of pharmaceutical nanoemulsions. *Ultrasonics Sonochemistry*, 21(6), 2069-2083.

Slavin, J. L., and Greenberg, N. A. (2003). Partially hydrolysed guar gum: Clinical nutrition uses. *Nutrition*, 19, 549–552.

Smolkova, B., El Yamani, N., Collins, A. R., Gutleb, A. C., and Dusinska, M. (2015). Nanoparticles in food. Epigenetic changes induced by nanomaterials and possible impact on health. *Food and Chemical Toxicology*, 77, 64-73.

Solans, C. and Aramaki, K. (2008). Emulsions and Microemulsions. *Current Opinion in Colloid and Interface Science*, 14, 195–197.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N., and Garcia-Celma, M. J. (2005). Nano-emulsions. *Current Opinion in Colloid & Interface Science*, 10(3–4), 102–110.

Soleimanpour, M., Koocheki, and A., Kadkhodaee, R. (2013). Effect of Lepidium perfoliatum seed gum addition on whey protein concentrate stanilized emulsions stored at cold and ambient temperature. *Food Hydrocolloids*, 30(1), 292-301.

Srichamroen, A., Thomson, A. B. R., Field, C. J., and Basu, T. K. (2009). In vitro intestinal glucose uptake is inhibited by galactomannan from Canadian fenugreek seed (Trigonella foenum graecum L.) in genetically lean and obese rats. *Nutrition Research*, 29(1), 49–54.

Srinivasan, K. 2006. Fenugreek (*Trigonella foenum-graccum*): a review of health beneficial physiological effects. *Food Reviews International*, 22, 203–224.

Stang, M., Schuchmann, H., and Schubert, H. (2001). Emulsification in high-pressure homogenizers. *Engineering in Life Sciences*, 1, 151–157.

Stellbrink, J., Abbas, B., Allgaier, J., Monkenbusch, M., Richter, D., Likos, C. N., Löwen, H. and Watzlawek, M. (1998). Structure and dynamics of star polymers. *Progress Colloid Polymer Science*, 28, 110–125.

Stone, A. K., Karalash, A., Tyler, R. T., Warkentin, T. D., and Nickerson, M. T. (2014). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Research International, In Press, Corrected Proof, Available online 18 November 2014.*

Stone, H. and Sidel, J. L. (1995). Strategic applications for sensory evaluation in a global market. *Food Technology*, 49(2), 85-89.

Stone, H., and Sidel, J. L. (1992). Sensory evaluation practices. San Diego: Academic Press.

Stone, H., and Sidel, J. L. (2004). Sensory evaluation practices. Amsterdam: Elsevier Academic Press.

Striegel, A. M. (2003). Influence of chain architecture on the mechanochemical degradation of macromolecules. *Journal of Biochemical and Biophysical Methods*, 56, 117–139.

Sun, C., and Gunasekaran, S. (2009). Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. *Food Hydrocolloids*, 23(1), 165-174.

Sun, S., Gunasekaran, C., and Richards, M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocolloids*, 21(4), 555-564.

Surh, J., Decker, E. A., and McClements, D. J. (2006). Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food Hydrocolloids*, 20(50), 607–618.

Suslick, K. S. (1998). In Kirk-Othmer (Ed.). *Encyclopedia of Chemical Technology* (vol. 26, pp. 517-541). New York, NY: J. Wiley & Sons.

Syed Gulrez, K. H., Al-Assaf, S., Fang, Y., Phillips, G. O. and Gunning, A. P. (2012). Revisiting the conformation of xanthan and the effect of industrially relevant treatments. *Carbohydrate Polymers*, 90(3), 1235–1243.

T

Tabibiazar, M., Davaran, S., Hashemi, M., Homayonirad, A., Rasoulzadeh, F., Hamishehkar, H., and Mohammadifar, M. A. (2015). Design and fabrication of a food-grade albumin-stabilized nanoemulsion. *Food Hydrocolloids*, 44, 220-228.

Tadros, F. T. (2009). Emulsion science and technology. Weinheim: Wiley-VCH.

Tadros, F. T., Izquierdo, P., Esquena, J., and Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 108–109, 303–318.

Tadros, T. F. (1994). Fundamental principles of emulsion rheology and their applications. *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, 91, 39-55.

Tadros, T., Izquierdo, P., Esquena, J., Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 108, 303–318.

Taherian, A. R., Fustier, P., and Ramaswamy, H. S. (2006). Effect of added oil and modified starch on rheological properties, droplet size distribution, opacity and stability of beverage cloud emulsions. *Journal of Food Engineering*, 77, 687–696.

Taherian, A. R., Fustier, P., and Ramaswamy, H. S. (2007). Effects of added weighting agent and xanthan gum on stability and rheological properties of beverage cloud emulsions formulated using modified starch. *Journal of Food Process Engineering*, 30, 204–224.

Tan, C.P., and Che Man, Y.B. (2000). Differential scanning calorimetric analysis of edible oils: Comparison of thermal properties and chemical composition. *Journal of American Oil Chemists Society*, 77, 142–155.

Tang, C. T. (2008). Thermal denaturation and gelation of vicilin rich protein isolates from Phaseolus legumes: A comparative study. *LWT - Food Science and Technology*, 41, 1380-1388.

Tang, S. Y., Shridharan, P., and Sivakumar, M. (2013). Impact of process parameters in the generation of novel aspirin nanoemulsions –Comparative studies between ultrasound cavitation and microfluidizer. *Ultrasonics Sonochemistry*, 20, 485–497.

Tang, S.Y., Manickam, S., Wei, T. K., and Nashiru, B. (2012). Formulation development and optimization of a novel Cremophore EL-based nanoemulsion using ultrasound cavitation. *Ultrasonic Sonochemistry*, 19, 330-345.

Tarantilis, P.A., Beljebar, A., Manfait, M., and Polissiou, M. (1998). FT-IR, FT-Raman spectroscopic study of carotenoids from saffron (*Crocus sativus* L.) and some derivatives. *Spectrochimica Acta Part A*, 54 (44), 651–657.

Tarrega, A., and Costell, E. (2006). Effect of composition on the rheological behaviour and sensory properties of semisolid dairy dessert. *Food Hydrocolloids*, 20(6), 914-922.

Tarrega, A., Ramírez-Sucre, M. O., Vélez-Ruiz, J. F., and Costell, E. (2012). Effect of whey and pea protein blends on the rheological and sensory properties of protein-based systems flavoured with cocoa. *Journal of Food Engineering*, 109(3), 467–474.

Tayal, A., and Khan, S. A. (2000). Degradation of a Water-Soluble Polymer: Molecular Weight Changes and Chain Scission Characteristics. *Macromolecules*, 33, 9488-9493.

Tehranifar, A., Zarei, M., Nemati, Z., Esfandiyari, B., and Vazifeshenasb, M. R. (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, 126(2), 180–185.

Tiwari, A., Hihara, L., and Rawlins, J. (2014). Intelligent coatings for corrosion control. Butterworth Heinemann, Waltham.

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P. and Cullen, P. J. (2010). Rheological Properties of Sonicated Guar, Xanthan and Pectin Dispersions. *International Journal of Food Properties*, 13(2), 223-233.

Tolstoguzov, V.B. Structure–property relationships in foods,in: N. Parris, A. Kato, L. Creamer, J. Pearce (Eds.), Macromolecular interactions in foods technology, ACS Symposium Series 650, American Chemical Society, Washington, DC (1996), pp. 2–14.

Tonon, R. V., Grosso, C. R. F., Míriam, D. and Hubinger, M. (2011). Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food Research International*, 44, 282–289.

Topuz, O. K., Yerlikaya, P., Ucak, I., Gumus, B., and Büyükbenli, H. A. (2014). Effects of olive oil and olive oil–pomegranate juice sauces on chemical, oxidative and sensorial quality of marinated anchovy. *Food Chemistry*, 154, 63-70.

Tosi, A., Canna, L., Lucero, H., and Re, E. (2007). Foaming properties of sweet whey solutions modified by thermal treatment. *Journal of Food Chemistry*, 100, 794-799.

Tsaliki, E., Pegiadou, S., and Doxastakis, G. (2004). Evaluation of the emulsifying properties of cottonseed protein isolates. *Food Hydrocolloids*, 18, 631–637.

Tsimidou, M. and Biliaderis, C. (1997). Kinetic studies of saffron (*Crocus sativus* L.) quality deterioration. *Journal of Agricultural and Food Chemistry*, 45, 2890–2898.

Tsimidou, M., and Tsatsaroni, E. (1993). Stability of saffron pigments in aqueous extracts. *Journal of Food Science*, 58, 1073–1075.

Turfan, O., Türkyilmaz, M., Yemi, O., and Özkan, M. (2011). Anthocyanin and colour changes during processing of pomegranate (Punica granatum L.; cv. Hicaznar) juice from sacs and whole fruit. *Food Chemistry*, 129(4), 1644–1651.

Turgeon, S. L., and Laneuville, S. I. (2009). Protein þ polysaccharide coacervates and complexes: from scientific background to their application as functional ingredients in food products. In S. Kasapis, I. T. Norton, and J. B. Ubbink (Eds.). Modern biopolymer science (pp. 327-363). New York: Elsevier.

Tzitzikas, E. N., Vincken, J.-P., Groot, J., de Gruppen, H., and Visser, R. G. F. (2006). Genetic variation in pea seed globulin composition. *Journal of Agricultural and Food Chemistry*, 54, 425–433.

Tzoumaki, M. V., Moschakis, T., Kiosseoglou, V., and Biliaderis, C. G. (2011). Oil-in-water emulsions stabilized by chitin nanocrystal particles. *Food Hydrocolloids*, 25(6), 1521-1529.

U

Urban, K., Wagner, G., Schaffner, D., Röglin, D. and Ulrich, J. (2006). Rotor-Stator and Disc Systems for Emulsification Processes. *Chemical Engineering & Technology*, 29(1), 24–31.

USDA. (2009). Soy stats A reference guide to important soybean facts and figures. (http//:www.Soystats.com)

V

Van Duynhoven, J. P. M., Goudappel, G. J. W., van Dalen, G., van Bruggen, P. C., Blonk, J. C. G., and Eukelenboom, A.P.A.M. (2002). Scope of droplet size measurements in food emulsions by pulsed field gradient NMR at low field. *Magnetic Resonance in Chemistry*, 40, 51–S59.

Varankovich, N. V., Khan, N. H., Nickerson, M. T., Kalmokoff, M., and Korber, D. R. (2015). Evaluation of pea protein–polysaccharide matrices for encapsulation of acid-sensitive bacteria. *Food Research International*, 70, 118-124.

Vartiainen, J.,Pöhler, T.,Sirola, K., Pylkkänen, L., Alenius, H., Hokkinen, J., Tapper, U., Lahtinen, P., Kapanen, A., Putkisto, K., Hiekkataipale, P., Eronen, P., Ruokolainen, J., and Laukkanen, A. (2011). Health and environmental safety aspects of friction grinding and spray drying of microfibrillated cellulose. *Cellulose*, 18(3), 775-786.

Vanapalli, S.A., Palanuwech, J., and Coupland, J.N. (2002). Influence of fat crystallization on the stability of flocculated emulsions. *Journal of Agricultural and Food Chemistry*, 50(18), 5224-5228.

Van Bookel, M. A., and Walstra, P. (1981). Stability of oil-in-water emulsions with crystals in the disperse phase. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 3, 109–118.

Vaughn, S. F., Kenar, J. A., Felker, F. C., Berhow, M. A., Cermak, S. C., Evangelista, R. L, Fanta, G. F., Behle, R. W., and Lee E. (2013). Evaluation of alternatives to guar gum as tackifiers for hydromulch and as clumping agents for biodegradable cat litter. *Industrial Crops and Products*, 43, 798-801.

Vegara, S., Mena, P., Martí, N., Saura, D., and Valero, M. (2013). Approaches to understanding the contribution of anthocyanins to the antioxidant capacity of pasteurized pomegranate juices. *Food Chemistry*, 141(3), 1630–1636.

Vickackaite, V. Romani, A. Pannacci, D., and Favaro, G. (2004). Photochemical and thermal degradation of a naturally occurring dye used in artistic painting. A chromatographic, spectrophotometric and fluorimetric study on saffron. *International Journal of Photoenergy*, 6(4), 175-183.

Vilkhu, K., Mawson, R., Simons, L., and Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry-A review. *Food Science and Emerging Technologies*, 9 (2), 161-169.

Vingerhoeds, M. H. de Wijk, R. A. Zoet, F. D., Nixdorf, R. R., and van Aken, G. A. (2008). How emulsion composition and structure affect sensory perception of low-viscosity model emulsions. *Food Hydrocolloids*, 22(4), 631-646.

Vinod, V. T. P., and Sashidhar, R. B. (2009). Solution and conformational properties of gum kondagogu (Cochlospermum gossypium) – A natural product with immense potential as a food additive. *Food Chemistry*, 116(3), 686–692.

Vitale, S. A., and Katz, J. L. (2003). Liquid droplet dispersions formed by homogeneous liquid-liquid nucleation:"The ouzo effect". *Langmuir*, 19(10), 4105-4110.

W

Walstra, P. (2003). Physical chemistry of foods. New York, NY: Marcel Decker.

Walstra, P. and Smoulders P.E.A. (1998). In B.P. Binks (Ed.). Modern Aspects of Emulsion Science. (pp. 57-99).Cambridge, UK: Royal Society of Chemistry.

Walstra, P. Formation of emulsions. (1983). In P. Becher (Ed.). *Encyclopedia of Emulsion Technology*, (vol. 1, pp. 57–127). New York: Marcel Dekker.

Walstra, P., and Jenness, R. (1984). Dairy Chemistry and Physics. New York, NY:John Wiley & Sons.

Wang, B., Wang, L- J., Li , D., Özkan, N., Li, S-J., and Mao, Z-H. (2009). Rheological properties of waxy maize starch and xanthan gum mixtures in the presence of sucrose. *Carbohydrate Polymers*, 77(3), 472–481.

Wang, X. Q., Kluge, J. A., Leisk, G. G., and Kaplan, D. L. (2008). Improvement of physicochemical stabilities of emulsions containing oil droplets coated by non-globular protein–beet pectin complex membranes. *Biomaterials*, 29, 1054-1064.

Wasikiewicz, J. M., Yoshii, F., Nagasawa, N., Wach, R. A. and Mitomo, H. (2005). Degradation of chitosan and sodium alginate by gamma radiation, sonochemical and ultraviolet methods. *Radiation Physics and Chemistry*, 73, 287–295.

Weiss, J., and McClements, D. J. (2001). Color changes in hydrocarbon oil-in-water emulsions caused by Ostwald ripening. *Journal of Agricultural and Food Chemistry*, 49(9), 4372-7.

WHO. (2015). Global Strategy on Diet, Physical Activity and Health, (http://www.who.int/dietphysicalactivity/diet/en/).

Wientjes, R.H.W., Duits, M.H.G., Jongschaap, R.J.J., and Mellema, J. (2000). Linear rheology of guar gum solutions. *Macromolecules*, 33(26), 9594–9605.

Wood, R. W., and Loomis, A. L. (1927). The Physical and Biological Effects of High Frequency Sound Waves of Great Intensity. *Philosophical Magazine*, 4, 414.

Wooster, T. J,, Golding, M., and Sanguansri, P. (2008). Impact of oil type on nanoemulsion formation and Ostwald ripening stability. *Langmuir*, 24(22), 12758-12765.

Wörle-Knirsch, J. M., Pulskamp, K., and Krug, H. F. (2006). Oops They Did It Again! Carbon Nanotubes Hoax Scientists in Viability Assays. *Nano Lett*ers, 6(6), 1261–1268.

Worrasinchai, S., Suphantharika, M., Pinjai, and S., Jamnong, P. (2006). β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. *Food Hydrocolloids*, 20, 68–78.

Wu, H., Hulbert, G.J. and Mount, J. R. (2000). Effects of ultrasound on milk homogenization and fermentation with yogurt starter. *Innovative Food Science and Emerging Technologies*, 1, 211−218.

Wu, Y., Cui, W., Eskin, N. A. M., and Goff, H. D. (2009). An investigation of four commercial galactomannans on their emulsion and rheological properties. *Food Research International*, 42(8), 1141-1146.

Wulff-Pérez, M., Torcello-Gómez, A. Gálvez-Ruíz, M.J Martín-Rodríguez A. (2009). Stability of emulsions for parenteral feeding: Preparation and characterization of o/w nanoemulsions with natural oils and Pluronic f68 as surfactant. *Food Hydrocolloids*, 2(4), 1096-1102.

X

Xu, K., and Yao, P. (2009). Stable oil-in-water emulsions prepared from soy protein-dextran conjugates. *Langmuir*, 25(17), 9714-9720.

Xu, Y., and Pan, S. (2013). Effects of various factors of ultrasonic treatment on the extraction yield of all-trans-lycopene from red grapefruit (Citrus paradise Macf.). *Ultrasonics Sonochemistry*, 20(4), 1026-1032.

Xu, R. (*2015).* Light scattering: A review of particle characterization applications. *Particuology*, 18, 11-21.

Y

Ye, A., and Singh, H. (2006). Heat stability of oil-in-water emulsions formed with intact or hydrolysed whey proteins: influence of polysaccharides. *Food Hydrocolloids*, 20(2-3), 269-276.

Ye, A., Hemar, Y., and Singh, H. (2004). Enhancement of coalescence by xanthan addition to oil-in-water emulsions formed with extensively hydrolysed whey proteins. *Food Hydrocolloids*, 18(5), 737-746.

Yolmeh, M., Habibi Najafi M. B., and Farhoosh, R. (2014). Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Food Chemistry*, 155, 319-324.

Youssef, M. K., Wang, Q., Cui, S. W., and Barbut, S. (2009). Purification and partial physicochemical characteristics of protein free fenugreek gums. *Food Hydrocolloids*, 23, 2049–2053.

Yu, H., and Huang, Q. (2013). Investigation of the cytotoxicity of food-grade nanoemulsions in Caco-2 cell monolayers and HepG2 cells. *Food Chemistry*, 141 (1), 29-33.

Z

Zeeb, B., Gibis, M., Fischer, L., and Weiss, J. (2012). Influence of interfacial properties on Ostwald ripening in crosslinked multilayered oil-in-water emulsions. *Journal of Colloid and Interface Science*, 387(1), 65-73.

Zeliger, H. I. (2011). Human toxicology of chemical mixtures. London, UK: Elsevier.

Zhang, C., Quek, S., Lam, G., and Easteal, A. (2008). The rheological behaviour of low fat soy-based salad dressing. *International Journal of Food Science and Technology*, 43(12), 2204–2212.

Zhang, H., Tao, Y., Guo, J., Hu, Y., and Su, Z. (2011). Hypolipidemic effects of chitosan nanoparticles in hyperlipidemia rats induced by high fat diet. *International Immunopharmacology*, 11 (4), 457–461.

Zhou, Y., and Shi, J. (2006). Effects of extracellular calcium on cell membrane resealing in sonoporation. *Journal of Controlled Release*, 126, 34–43.

Zinoviadou, K. G., Scholten, E., Moschakis, T., and Biliaderis, C. G. (2012). Properties of emulsions stabilised by sodium caseinate–chitosan complexes. *International Dairy Journal*, 26(1), 94-101.

Zisu, B., Bhaskaracharya, R., Kentish, S., and Ashokkumar, M. (2010). Ultrasonic processing of dairy systems in large scale reactors. *Ultrasonics Sonochemistry*, 17(6), 1075-1081.

Zúñiga, R. N., Skurtys, O., Osorio, F., José, M., Aguilera, J. M., and Pedreschi, F. (2013). Optical properties of emulsion-based hydroxypropyl methylcellulose (HPMC) films: effect of their microstructure. InsideFood Symposium, 9-12 April 2013, Leuven, Belgium.

APPENDIX

List of publications in peer revied journals

1. **Kaltsa, O.,** Michon, C., Yanniotis, S., and Mandala I. (2013). Ultrasonic energy input influence on the production of sub-micron o/w emulsions containing whey protein and common stabilizers. *Ultrasonics Sonochemistry*, 20(3), 881-891.
2. **Kaltsa, O.,** Gatsi, I., Yanniotis, S., and Mandala I. (2014a). Influence of Ultrasonication Parameters on Physical Characteristics of Olive Oil Model Emulsions Containing Xanthan. *Food and Bioprocess Technology*, 7(2), 2038-2049.
3. **Kaltsa, O.,** Paximada, P., Mandala, I., and Scholten, E. (2014b). Physical characteristics of submicron emulsions upon partial displacement of whey protein by a small molecular weight surfactant and pectin addition. *Food Research International*, 66, 401-408.
4. **Kaltsa, O.,** Yanniotis, S., and Mandala, I. (2016). Stability properties of different fenugreek galactomannans in emulsions prepared by high-shear and ultrasonic method. *Food Hydrocolloids*, 52, 487-496.
5. **Kaltsa, O.,** Yanniotis, S., Polissiou, M., and Mandala, I. (2015). Properties of salad dressings prepared by High-Shear and Ultrasonication method, containing saffron (Crocus sativus L.) and pomegranate juice powder during long term storage. *Journal of Food Engineering*, (Submitted Manuscript).

List of presentations in International Conferences

1. **Kaltsa, O.,** Michon, C., Yanniotis, S., and Mandala I. (2011). The effect of different stabilizers on the production of sub-micron o/w emulsions by using ultrasound techniques. *11th International Congress on Engineering and Food: Food Process Engineering in a Changing World, ICEF11*, May 22-26, Athens, Greece (oral presentation).
2. **Kaltsa, O., C.,** Yanniotis, S., and Mandala I. (2012). Comparing different commercial fenugreek galactomannans for the production of emulsions with high intensity sonication. Effect on physical stability and rheological properties. *6th International Symposium on Food Rheology and Structure - ISFRS 2012*, 10-12 April, Zurich, Switzerland (poster).

3. **Kaltsa, O.,** Gatsi, I., Yanniotis, S., and Mandala I. (2012). Influence of ultrasonication parameters and NaCl on the stability of olive oil model emulsions containing xanthan. *6th International Symposium on Food Rheology and Structure – ISFRS, 2012*, 10-12 April, Zurich, Switzerland (poster).
4. **Kaltsa, O.,** Paximada, P., Mandala, I., and Scholten, E. (2012). O/W sub-micron emulsions prepared with whey protein-Tween 20 combinations and layer-by-layer pectin addition. *International COST Conference,* 15-16 October, Lunteren, The Netherlands (oral presentation).
5. **Kaltsa, O.,** Spiliopoulou, N., Yanniotis, S., and Mandala, I. (2014). The effect of olive oil and fenugreek gum content on the stability and oxidation of o/w macro- and submicron-nano emulsions. *3rd International ISEKI Food Conference,* May 21-23 Athens, Greece (poster).