ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

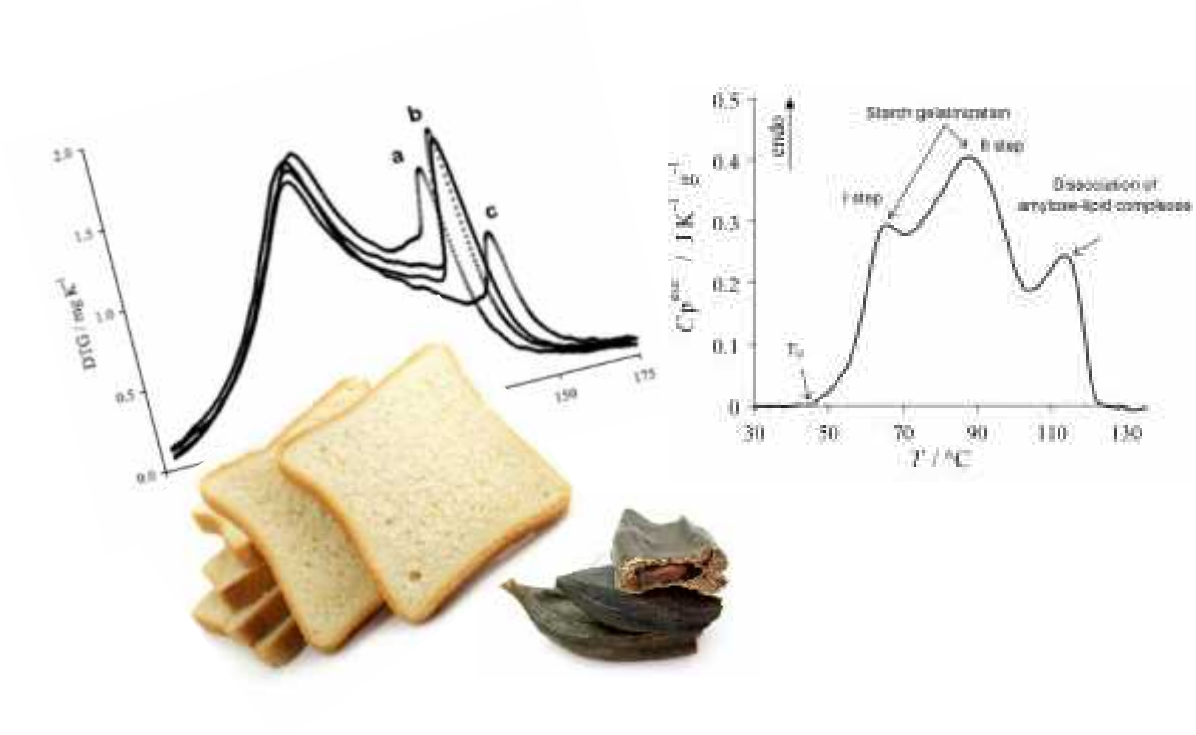
ΕΠΙΣΤΗΜΗ ΚΑΙ ΤΕΧΝΟΛΟΓΙΑ ΤΡΟΦΙΜΩΝ ΚΑΙ ΔΙΑΤΡΟΦΗ ΤΟΥ ΑΝΘΡΩΠΟΥ
Εργαστήριο Μηχανικής Τροφίμων, Επεξεργασίας & Συντήρησης Γεωργικών Προϊόντων

ΠΜΣ "ΕΠΙΣΤΗΜΗ ΚΑΙ ΤΕΧΝΟΛΟΓΙΑ ΤΡΟΦΙΜΩΝ ΚΑΙ ΔΙΑΤΡΟΦΗ ΤΟΥ ΑΝΘΡΩΠΟΥ"

ΜΕΤΑΠΤΥΧΙΑΚΗ ΔΙΑΤΡΙΒΗ ΜΕ ΤΙΤΛΟ:

ΦΥΣΙΚΟΧΗΜΙΚΕΣ ΙΔΙΟΤΗΤΕΣ ΔΙΑΦΟΡΩΝ ΑΛΕΥΡΩΝ - ΚΑΤΑΝΟΜΗ ΤΟΥ ΝΕΡΟΥ ΣΕ ΜΙΓΜΑΤΑ ΑΛΕΥΡΩΝ ΑΠΟ ΧΑΡΟΥΠΙ, ΣΙΤΑΡΙ ΚΑΙ ΡΥΖΙ: ΘΕΡΜΙΚΗ ΠΡΟΣΕΓΓΙΣΗ

ΑΝΘΟΥΛΑ Ι. ΑΠΟΣΤΟΛΙΔΟΥ



ΜΙΛΑΝΟ-ΑΘΗΝΑ 2012-2013

Επιβλέπων καθηγητής: κος Σ. Γιαννιώτης

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PHYSICOCHEMICAL PROPERTIES OF DIFFERENT FLOURS - WATER DISTRIBUTION IN CAROB, WHEAT AND RICE MIXED SYSTEMS: thermal analysis approach

ΑΝΘΟΥΛΑ Ι. ΑΠΟΣΤΟΛΙΔΟΥ

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Τριμελής εξεταστική επιτροπή: κος Σ. Γιαννιώτης, κα Ι. Μαντάλα, κα Β. Ευαγγελίου

Η ολοκλήρωση της εργασίας αυτής έγινε στο πλαίσιο της υλοποίησης του μεταπτυχιακού προγράμματος το οποίο συγχρηματοδοτήθηκε μέσω της Πράξης «Πρόγραμμα χορήγησης υποτροφιών Ι.Κ.Υ. με διαδικασία εξατομικευμένης αξιολόγησης ακαδημαϊκού έτους 2011-2012» από πόρους του Ε.Π. «Εκπαίδευση και Δια Βίου μάθηση» του Ευρωπαϊκού Κοινωνικού Ταμείου (ΕΚΤ) και του ΕΣΠΑ, του 2007-2013.

ΠΕΡΙΛΗΨΗ

Σκοπός της μελέτης είναι η διερεύνηση της συμπεριφοράς των κύριων συστατικών του χαρουπάλευρου (Κόμμι χαρουπιού και Πρωτεΐνη χαρουπιού) χωριστά και σε μίγματα με σιτάλευρο και ρυζάλευρο χρησιμοποιώντας θερμική ανάλυση, προκειμένου να διερευνηθεί ο ρόλος της πρωτεΐνης χαρουπιού ως ενισχυτικό της δομής μιας ζύμης. Ψωμιά από μικτά συστήματα αλεύρων παρήχθησαν επίσης για την επιβεβαίωση των αποτελεσμάτων.

Η υψηλή περιεκτικότητα σε πρωτεΐνη (≥ 50%) του σπέρματος του χαρουπιού, που περιέχει μεγάλη ποσότητα γλουταμινικού οξέος και αργινίνης και η απουσία προλαμίνης, το καθιστά κατάλληλο για τη διατροφή των αθλητών με σκοπό την αύξηση της επίδοσής τους και για τους πάσχοντες από κοιλιοκάκη.

Η θερμική ανάλυση βρίσκει εφαρμογή στον τομέα της επιστήμης και της τεχνολογίας τροφίμων, όπου οι θερμικές επεξεργασίες είναι συχνές. Η Διαφορική θερμιδομετρία σάρωσης (DSC) είναι ένα χρήσιμο διαγνωστικό εργαλείο για την ένδειξη της σταθερότητας κάθε φάσης που σχηματίζεται σε ένα σύστημα, όπως μια ζύμη. Η Θερμοσταθμική Ανάλυση (TGA) παρέχει πληροφορίες σχετικά με την κατανομή του νερού, που παγιδεύεται μέσα στις διάφορες φάσεις που δημιουργούνται στη ζύμη (π.χ. πλέγμα αμύλου, γλουτένης, άλλων πρωτεϊνών, κ.α.), λόγω του διαχωρισμού φάσεων που προκαλείται από τη θερμοδυναμική ασυμβατότητα.

Δεδομένα από τις 2 αυτές θερμικές μεθόδους (DSC και TGA) έδειξαν ότι η πρωτεΐνη χαρουπιού δε συμβάλλει στην ενίσχυση της δομής της ζύμης, αλλά ταυτόχρονα δεν οδηγεί στην καταστροφή της δομής. Επιπλέον, λόγω της θερμοδυναμικής της ασυμβατότητας με το άμυλο δεν επηρεάζει τη ζελατινοποίηση του αμύλου. Αντιθέτως, το κόμμι του χαρουπιού, λόγω της ικανότητάς του να δεσμεύει μεγάλη ποσότητα νερού, περιορίζει τη ζελατινοποίηση, ανεξάρτητα από τη φύση του αμύλου. Ως εκ τούτου, μια ζύμη που περιέχει κόμμι χαρουπιού θα πρέπει να είναι υπερενυδατωμένη, για να αναπτυχθεί σε ψωμί. Η επίδραση του κόμμεος στη γλουτένη είναι επίσης εμφανής, δεδομένου ότι, υπό την παρουσία του σχηματίζεται έναν ασθενέστερο δίκτυο γλουτένης. Σε μίγματα κόμμεος και πρωτεΐνης χαρουπιού, λόγω του διαχωρισμού των φάσεών τους, η πρωτεΐνη χαρουπιού ανταγωνίζεται το κόμμι παγιδεύοντας το νερό, σε όλες τις αναλογίες μιγμάτων που μελετήθηκαν. Το κόμμι οδηγεί σε συσσωμάτωση της πρωτεΐνης χαρουπιού σε συστήματα με χαμηλή περιεκτικότητα υγρασίας. Η συνολική υγρασία των μιγμάτων τροποποιεί τόσο το διαχωρισμό φάσεων όσο και τον τρόπο με τον οποίο απελευθερώνεται το νερό.

Τα αποτελέσματα από την παρασκευή ψωμιού, δείχνουν ότι τα ψωμιά από σιτάλευρο και 2% κόμμι παρουσιάζουν καλύτερη υφή και πορώδες, από εκείνα με προσθήκη 4% κόμμεος. Η προσθήκη 2% κόμμεος οδηγεί στη βελτίωση της εμφάνισης του ψωμιού σίτου. Σε συγκέντρωση πάνω από 4%, το κόμμι αρχίζει να έχει επίπτωση στη ζελατινοποίηση του αμύλου, επιβεβαιώνοντας τα αποτελέσματα της θερμικής ανάλυσης. Στα ψωμιά από ρυζάλευρο, η προσθήκη του κόμμεος οδηγεί σε ψωμιά με διαφορετικά χαρακτηριστικά. Τα ψωμιά από σιτάλευρο ή ρυζάλευρο με προσθήκη 5% πρωτεΐνης χαρουπιού παρουσιάζουν έντονη ομοιότητα με τα πρότυπα ψωμιά (παρασκευασμένα μόνο από σιτάλευρο ή ρυζάλευρο) με εξαίρεση τη μικρή κίτρινη απόχρωση που προσδίδει η πρωτεΐνη χαρουπιού στα ψωμιά από ρυζάλευρο.

Επιστημονική περιοχή: Μηχανική Τροφίμων
Λέξεις κλειδιά: DSC, TG, πρωτεΐνη χαρουπιού, κόμμι χαρουπιού, αλληλεπιδράσεις πολυμερών

ABSTRACT

Thermal analysis was used to check the role of the main components of carob flour (Locust Bean Gum-LBG and Carob Protein-CP) in blends with wheat and rice flour, in order to investigate the performance of carob as structural enhancer in formulated foods. Breads from mixed flour systems were also produced to confirm the results, from the technological point of view.

Carob germ's high protein content (≥50% CP), rich in glutamic acid and arginine aminoacids and poor in prolamin, makes it suitable for functional foods development for sportspeople and celiac sufferers.

Thermal techniques find application in food science and technology, where heat treatments are very frequent. Differential Scanning Calorimetry (DSC) may provide a useful diagnostic tool for indicating the stability of each phase formed in a mixed system, like a dough. Thermogravimetric Analysis (TGA) can provide information about the gross partition of water between the dough phases, especially for what concerns the water trapped within the different phases that occur, because of phase separation caused by thermodynamic incompatibility. The behaviour of a dough is directly related to the role played by the macromolecules, which induce phase separation and govern the water partition.

Data from DSC and TG analysis have shown that CP, due to its poor structure-related quality, does not contribute to dough structure enhancement, as it acts like a non gluten-like protein, but it does not lead to structure damage either. Furthermore, CP, because of its thermodynamic incompatibility with starch does not influence starch gelatinization. Conversely, LBG, due to its capacity to bind a large amount of water, restricts starch gelatinization regardless of starch nature. Therefore, a dough containing LBG should be hyper hydrated. The impact of LBG to wheat gluten's strength is also evident, since in the presence of LBG a weaker gluten network is formed, indicated by the earlier water evaporation from the gluten phase. In CP and LBG mixtures, due to their phase separation, CP demonstrates its high ability to trap water, competing LBG in all the mixture ratios studied. LBG leads to aggregation of CP in low mixture's water content. The overall moisture of the mixtures can modify both water partition between phases and the way water is released.

Results from bread making, show that wheat breads with 2% LBG present better characteristics, namely texture and porosity, than those with 4% LBG. It seems that addition of 2% LBG is the optimum LBG concentration for the enhancement of wheat bread. From concentrations up to 4%, LBG starts to show its impact to starch gelatinization, confirming the thermal analysis results. Rice breads were split into 2 groups; one group form rice bread and rice bread with 5% carob protein and the other group rice bread with 2 and 4%LBG. It was shown that the addition of LBG gives rice breads different characteristics. Breads with 5% CP present a strong resemblance to control breads either for rice or wheat flour, with exception of the slight yellow tint in rice breads.

Scientific area: Food Engineering

Keywords: DSC, TG, carob protein, Locust Bean Gum, food polymer interactions

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I want to thank my family for their patience and encouragement during the period of writing this work.

Finally, I would like to thank the State Scholarships Foundation for helping me to complete this work offering me a precious financial help throughout my master degree.

Σαν το σιτάρι σπέρνεται στον κόσμο η αλήθεια

Κι απ' τον καθάριο σπόρο της φυτρώνουν παραμύθια

Καλότυχος όποιος μπορεί τα στάχυα να θερίσει

Και το σιτάρι απ' τα' άχυρο καλά να ξεχωρίσει.

Για τον μικρό τον κόπο του μεγάλο κέρδος μένει:

όλη η αλήθεια που θα βρει στα ψέματα κρυμμένη.

(Γεώργιος Δροσίνης, Παιδικά παραμύθια, Εκδότης Ι. Ν. Σιδέρης)

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Abbreviations

LBG: Locust Bean Gum
CP: Carob Protein
CF: Carob Flour
W: Wheat Flour
R: Rice Flour
P: Protein
DSC: Differential Scanning Calorimetry
TGA: ThermoGravimetric Analysis and TG: Thermogravimetry
MC: Moisture Content
DTG: Derivative of Thermogravimetry
MC: Moisture Content

A. Prologue

Mixed flour systems present a particular interest due their nutritional and technological aspects. Wheat flour is the most common flour used in the field of bread making since hundreds of years. Rice flour is not so commonly used, but its natural absence of gluten, the low levels of sodium, protein, fat and fiber and a high amount of easily digested carbohydrates, make it appropriate for certain special diets, i.e. for celiac people. More recently, carob flour and its components, mainly carob germ has gained more attention, because of its high protein content (≥ 50%), its well-balanced amino acid composition and its prolamin absence that make it suitable ingredient for functional foods, which can be included in the nutrition of sportspeople and celiac people, being a low cost competitor to other food proteins like dairy or soy proteins. LBG that derives also from carob seed's processing presents technological advantages and it is used as a thickening, gelling agent and stabilizer. In bread making it is used for loaf volume increase.

Thermal techniques find application in food science and technology, where heat treatments are very frequent. Starch gelatinization and gluten reticulation are the main transformation in flour systems undergoing baking and they could be a reliable parameter to describe the progress of baking. Differential Scanning Calorimetry (DSC) may provide a useful diagnostic tool for indicating the stability of each phase formed in a mixed system, like a dough. Classical Thermogravimetric Analysis (TGA) can provide information about the gross partition of water between the dough phases, especially for what concerns the water trapped within the different phases that occur, because of phase separation caused by thermodynamic incompatibility. The behaviour of a dough is directly related to the role played by the macromolecules, which induce phase separation and govern the water partition. Blends of flours from cereals and pseudocereals or legumes allow dough preparations, in which many interactions are expected. The flour of some gluten - free cereals, such as carob, buckwheat and amaranth can trap water because of different proteins but cannot form stable dough, because the protein chains do not arrange themselves in a tight web.

The scope of this work is the investigation and better understanding of the behavior of carob flour and its components (namely carob protein and LBG), separately, as well as in mixed systems with wheat and rice flour using thermal analysis.

B. Introduction

B.1 Studied flours

B.1.1 Carob

B.1.1.1 History and origins of carob tree

Carob (*Ceratonia siliqua* L.) is a leguminous shrub native to the Mediterranean region, mainly in Spain, Italy, Greece, Portugal and Morocco (Dakia et al., 2007), being an important component of the Mediterranean vegetation (Bengoechea et al., 2008). The scientific name of carob tree derives from Greek *keras*, horn and Latin *siliqua*, alluding to the hardness and shape of the pod. It is also known as St. John's bread or locust bean (Wang et al., 2001) in reference to the presumed use of its 'locusts' as food by St. John the Baptist. Jewelers used its uniform seeds as a unit of weight (200 mg), the carat (Batlle and Tous, 1997).

Although the exact origins of the carob tree are unknown, the genesis of the wild carob tree took place somewhere in the Mediterranean, Arabian Peninsula, or the horn of Africa. The questionable origin is due to the widespread cultivation of carob for food, feed and animal bedding in pre-historical times. Through observation of wild varieties and archeological records, the first cultivations of carob probably took place in the areas of Turkey, Cyprus, Syria, Lebanon, Israel, Jordan, Egypt, Arabia, Tunisia, and Libya. It is generally accepted that the Greeks cultivated the crop in Greece and Italy from seeds taken from the Mediterranean and the crop eventually arrived to regions of southern France and Portugal, where climates permitted. In more recent times, carob was introduced into the United States in 1854 where it was primarily grown in California for ornamental purposes (Smith, 2009). The global carob crop production was estimated to be 310.000 tons in 1997 and declining (Batlle and Tous 1997). The main carob producing countries are Spain (42% of total production), Italy (16%), Portugal (10%), Morocco (8%), Greece (6.5%), Cyprus (5.5%) and Turkey (4.8%) (FAO, 1995). This data suggest the importance of carob derivatives as food ingredients.

B.1.1.2 Description

Carob tree is a legume from the family Leguminosae and the order Rosales. It is an evergreen, which takes 20 years to reach maturity. Trees start to bear fruit at the age of 5-7 years, yielding 10 kg/tree. At maturity they will yield 250-500 kg/tree and will continue to produce for up to 200 years. After flowering, the pods take about 6-8 months to mature, turning from green to chocolate brown in late summer (FAO, 1995). The fruit consists of long pods of which 90% is pulp and 10% seed (Wang et al., 2001). Each pod is about 10 cm to 30 cm long and 1.5 cm to 3.5 cm wide (Fig. 1). The straighter pods are considered more desirable because of the ease of harvest. Pods are filled with several seeds arranged in a linear non overlapping manner separated by the mesocarp. Seeds are compressed and slightly oblong with dimensions of 8 to 10 mm long x 7 to 8 mm wide x 3 to 5 mm thick (Batlle and Tous, 1997).

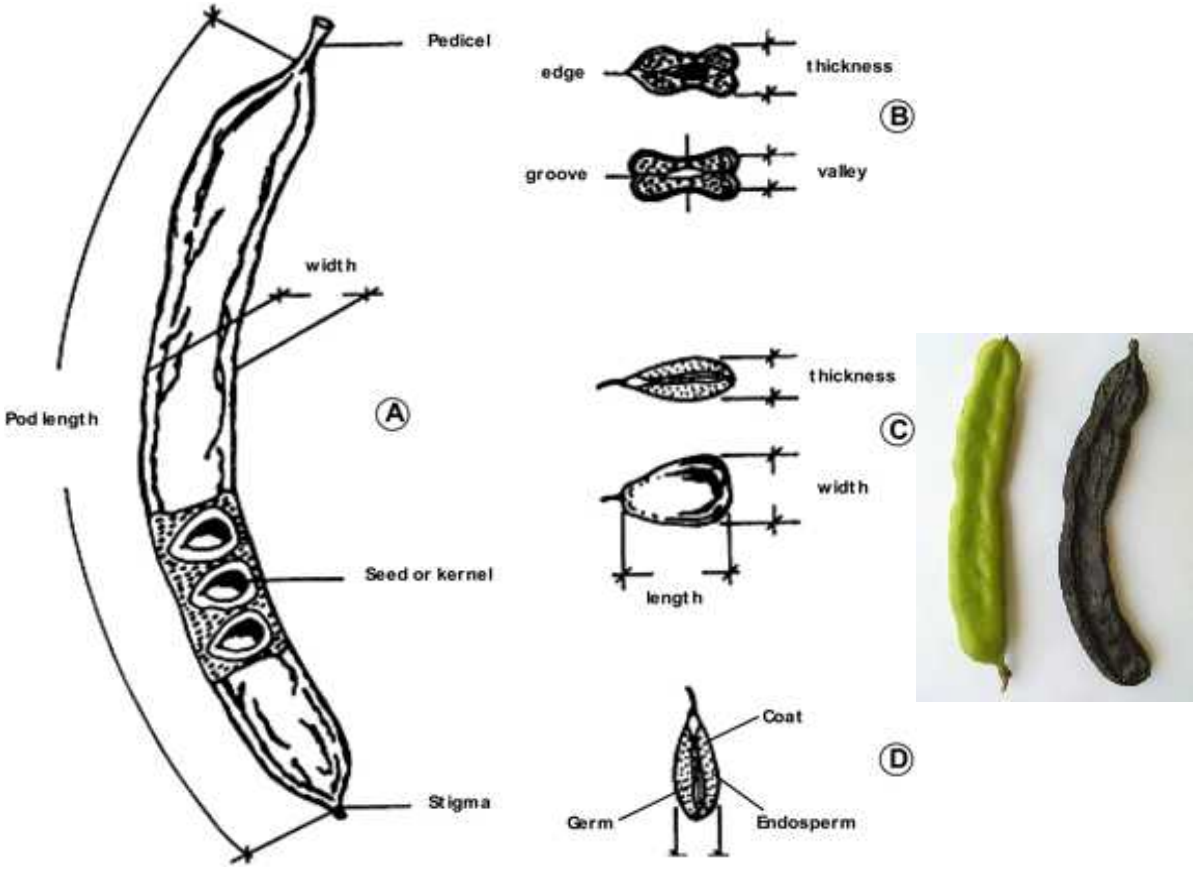


Figure 1: Carob fruit and seed descriptors used (Batlle and Tous, 1997): pod (A), cross section of pod (B), kernel (C), and cross-section of seed (D).

B.1.1.3 Processing

The harvested carob pods, which are characterized by high sugar content (more than 50%- mainly composed of sucrose), are brought to the processing plant. The pulp from the pods can be extracted to make syrup. The unique characteristics of the syrup make it ideal food flavoring (Wang et al., 2001).

When carobs arrive, moisture content is variable (10-20%) depending on harvesting conditions and autumn rainfall. Pods require further drying and thus are stored under shelter in dry and ventilated places to reduce moisture to around 8% and to avoid rotting. Insects in stored carobs, mainly carob moth, can be controlled by fumigation. Pods are kibbled to separate the two main components: pulp and seeds. Carob pods are crushed mechanically using a kibbler, then are separated from the kernels. The processing of the carob pods and the products obtained are shown in Fig. 2. This first coarse grinding can be followed by fine grinding of the pod pieces (kibbles) either at the same plant or at the feed or food factories. The feed factory grinds the deseeded pulp to different sizes in relation to the kind of livestock to be fed. The food industry processes the pulp further by roasting and milling to obtain a fine powder which is traded as carob powder. Carob powder is a natural sweetener with flavor and appearance similar to chocolate; therefore it is often used as cocoa substitute. It is mostly used in baking, cereal bars, chocolate confectionery, ice creams and light

products. The advantage of using carob as a chocolate substitute resides in that carob is an ingredient free from caffeine and theobromine (Bengoechea et al., 2008).
The carob seeds are transported in bulk by lorry to the gum factories. The kernels are difficult to process, since the seed coat is very hard. Kernels are peeled without damaging the endosperm and the embryos (germs).
The two main procedures applied to remove the tight-fitting brown seed coat are: a chemical that uses acid treatment (seeds treated with sulphuric acid to carbonize the coat) or a thermo-mechanical treatment namely roasting (kernels roasted in a rotating furnace to peel off the coat). If the acid pre-treatment is prolonged, the acid may reach the germ between the two endosperms and partially hydrolyse it. So, it is important to follow the acidic attack cautiously (Dakia et al., 2007). After the peeling process the white and translucent endosperm can be split from the cotyledons because of their different friability. When the peeled seeds are forced through a splitting machine the brittle embryos turn out as a fine powder (germ meal) and can be separated from the unbroken endosperm scales by a sifting operation. Subsequently the endosperm is ground on roller mills to the desired particle size (gum). The carob bean gum or Locust Bean Gum (LBG) is the ground endosperm and as a result the carob germ meal is co-produced and marketed as a by-product of seed processing and especially gum production (Batlle and Tous 1997; Smith et al., 2010). Carob flour derives from the milling of the whole seeds either roasted or fresh, containing both the germ meal and Locust Bean Gum and sometimes the coat as well. It is widely found in shops with organic food. The approximate composition of the seed is presented in table 1. In the following part, carob germ meal flour and LBG fraction are described.

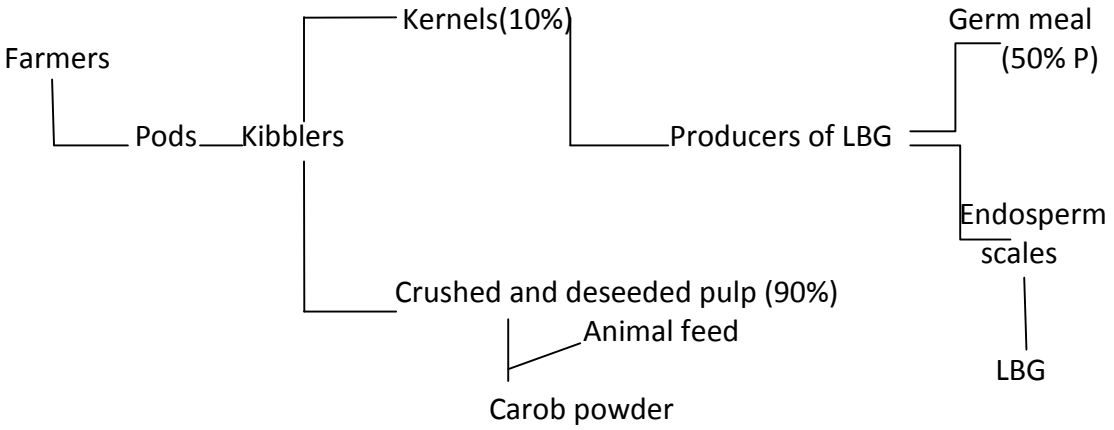


Figure 2: Carob pod processing.

Table 1: The approximate composition of the seed (by weight) (FAO, 1995).

Endosperm	40 - 50%
Hull	30 - 33%
Germ	20 - 25%

B.1.1.4 Carob germ

Dakia et al. (2007) stated that the germ is the smallest fraction in carob seed and the germ yields from acid (17–23%) and boiling (18–25%) treatment were not significantly different and close to those reported (23–25% of germ) by Herald (1986) and Neukom (1988). According to FAO (1995), the germ accounts for 20 to 25% of the seeds weight. It is composed primarily of protein and fiber with low to moderate amounts of water, lipid, ash, polyphenols, and soluble carbohydrates. The internal of the carob seed is shown in Fig. 3.

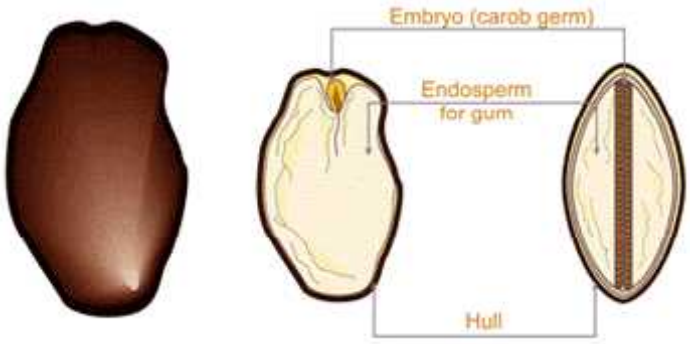


Figure 3: Carob seed with major anatomical features outlined (Smith, 2009).

Table 2: Chemical characterization of defatted carob germ flour, modified from Bengoechea et al. (2008).

Flour component	% of Flour
Protein content	48.2 ± 0.24
Lipids	2.26 ± 0.13
Moisture	5.76 ± 0.32
Ash	6.34 ± 0.15
Polyphenols	0.45 ± 0.01
Soluble carbohydrates	2.92 ± 0.03
Total fiber	24.3 ± 0.09

Maza et al. (1989) have reported that protein consists almost of 50% of the weight of the embryo. A chemical characterization of defatted carob germ flour is shown on table 2 (Bengoechea et al., 2008), while another analysis of carob germ meal (containing fine fragments of husk and endosperm), which could be really obtained industrially, showed the following composition: moisture 8.3%, ash 6.5%, lipids (neutral and polar) 6.6%, containing 21% of polar lipids, crude proteins 54.7% and energy value 17.5 kJ/g. Whatever the technique of extraction used (acid or boiling treatment) , the carob germ meal showed a high content of proteins; 54– 67% (Dakia et al., 2007). The protein content of carob germ flour obtained from seeds is higher than those observed for other beans, such as pea (18.83%) and soybean (34.35%) (Marcone et al., 1998). The protein system from carob germ has been called “caroubin” by Feillet and Roulland (1998).

Some studies have been carried out to investigate if large polymeric proteins of caroubin might have functional properties similar to those of wheat gluten.
Wang et al. (2001) reported that hydrated caroubin was capable of forming sheets and fibrils, but caroubin fibrils were finer in appearance than those formed by gluten. Caroubin was found to be more hydrophilic than gluten (it absorbs ~3 g water/g dry solid at 25^{o}C in contrast to ~2 g water/g at 25^{o}C for gluten). This fact may be related to the observations of the differences in spreading behaviours observed by microscopy (Fig. 4). When exposed to water, caroubin exhibited fewer changes to its secondary structure than did gluten. It was also found that there are significant differences between caroubin and gluten after cooking (Wang et al., 2001).

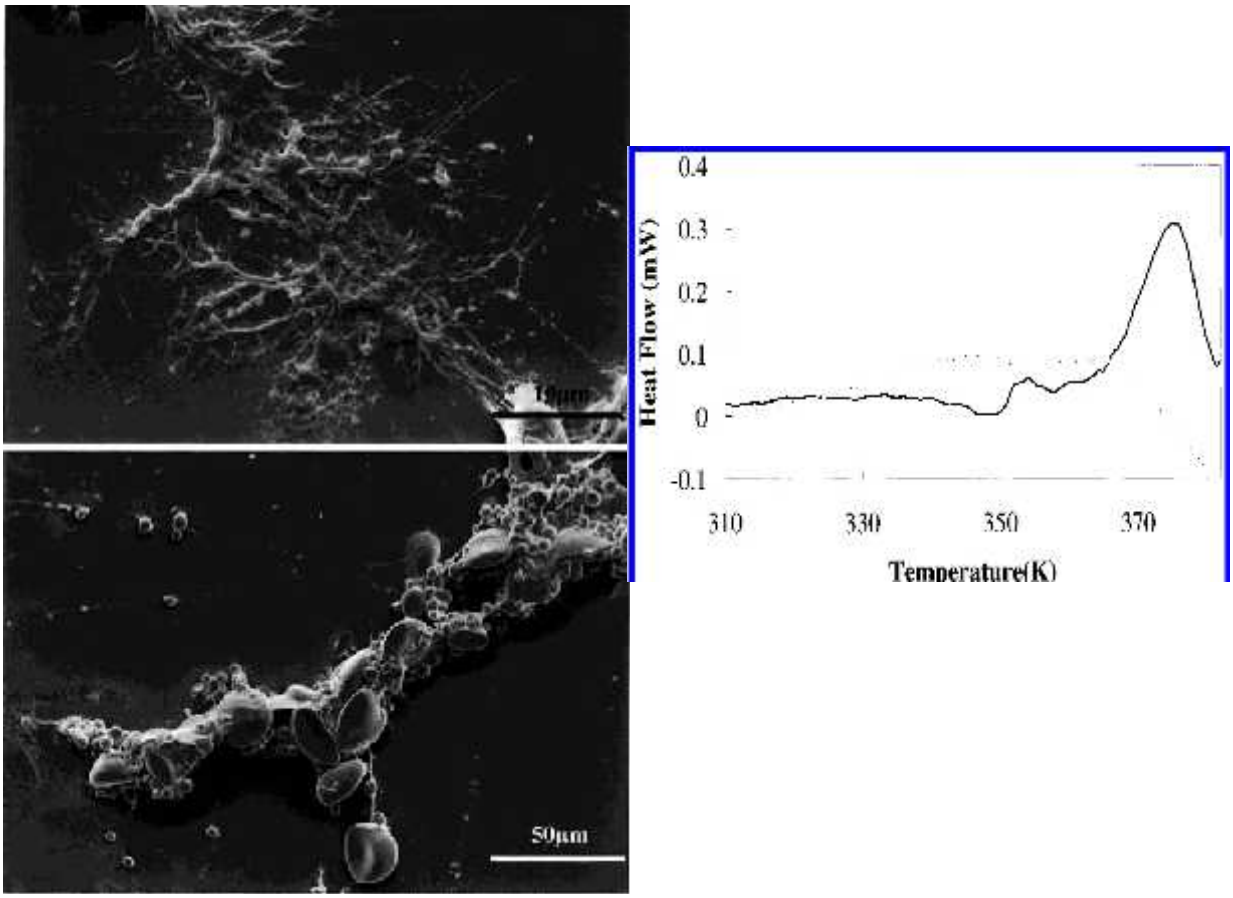


Figure 4: (left) Scanning electron micrographs of spread caroubin (above) and gluten (below) on a water surface. The particles associated with gluten fibrils are starch granules; (**right) DSC thermograms at 10 °C/min for fully hydrated caroubin (bold solid line) and gluten (Wang et al., 2001).**

Wang et al. (2001) also reported that the DSC curve of hydrated caroubin showed a broad asymmetric endothermic peak starting from 93° C, with Tm at 101°C and enthalpy 11 J/g. The origin of the transition observed on the caroubin sample is likely to be due to some partial denaturation and aggregation. Fig. 4 shows the difference in

the DSC scan of the fully hydrated caroubin and gluten when heated over a temperature range of 10-120° C.

Bengoechea et al. (2008) found that carob germ proteins were composed of aggregates formed both by disulfide bonds and through non-covalent interactions. They also studied carob germ flour's thermal behaviour, noting that there is an endotherm present at 105.7°C with a denaturation enthalpy of 16.6 ± 4.1 mJ/mg flour.

Smith et al. (2010) characterized the proteins of carob germ flour and compared them to similar proteins in wheat. Carob germ flour proteins were found to contain ~32% albumin and globulin and ~68% glutelin, with no prolamins detected. They also found that caroubin contained ~95% soluble proteins with maximum M_w up to ~$0.5x10^7$ Da and only ~5% insoluble proteins. As in wheat, it was found that, the insoluble proteins had a greater M_w than the soluble proteins and ranged up to $8x10^7$ Da, but wheat has been reported to contain 30-50% insoluble proteins. The lower level of insoluble proteins in carob germ flour may be one reason that carob proteins are only able to form weak dough, but still these polymeric proteins appeared to play a critical role in protein network formation.

Whereas gluten-like properties of carob germ protein have been reported (Wang et al., 2001; Bengoechea et al. 2008), Smith et al. (2010) proved that caroubin is quite different from gluten. The M_w distribution of carob germ proteins was shifted to lower M_w protein and was present in relatively smaller quantities than that of wheat gluten. Furthermore, in the Osborne extractions caroubin was found to contain no measurable amounts of prolamin, a protein fraction that is attributed to gluten functionality. These major biochemical differences may be the causative factor in the rheological differences reported also by Feillet and Roulland (1998). These authors found that caroubin had viscoelastic properties; however, due to caroubin's low levels of cysteine, the mechanism of this viscoelastic behavior may be different from that of wheat gluten.

Rice and Ramstad (1953) found that there were significant differences in the amino acid composition between the two proteins, with carob germ proteins having less cysteine, glutamic acid, and phenylalanine but more of the charged amino acids, arginine, aspartic acid, and lysine. A slightly lower level of glutamic acid content was found in caroubin (32%) compared to gluten (38%), although the glutamic acid levels are very high in both proteins (Feillet and Roulland, 1998; Bengoechea et al., 2008).

B.1.1.4.1 Nutritional aspects

The literature data on chemical composition of carob seed germ from Del Re-Jiménez and Amadò (1989), Maza et al. (1989), Dakia et al.(2007) and Bengoechea et al. (2008) showed that its nutritive value is high, due to its high protein content (≥50%). Furthermore, carob germ proteins have a well-balanced amino acid composition. Already since 1950 carob germ proteins were analyzed for use in high-protein cereal products for diabetics (Rice and Ramstad, 1953). The high content in glutamic acid and arginine aminoacids (table 3) makes carob germ products fairly attractive for special dietary needs (i.e. high performance sports and medical

nutrition). For example, carob proteins can be used as a suitable ingredient for functional foods that can be included in the nutrition of sportspeople, as they increase muscular matter, collagen synthesis, and glycogen production (Bengoechea et al., 2008).

Table 3: Amino acid composition (g amino acid/100 g protein) of carob germ flour (modified from Bengoechea et al., 2008).

Amino acids	Carob germ flour
Aspartic acid	8.75 ± 0.07
Glutamic acid	28.1 ± 0.07
Arginine	11.5 ± 0.21
Serine	5.05 ± 0.07
Glycine	5 ± 0
Alanine	4.4 ± 0.0
Proline	8.2 ± 0.3
Histidine	2.3 ± 0.0
Threonine	3.5 ± 0.0
Valine	3.05 ± 0.07
Isoleucine	2.3 ± 0.0
Leucine	5.9 ± 0.0
Lysine	5.5 ± 0.0
Tryptophan	0.9 ± 0.0
Phenylalanine	2.9 ± 0.0
Tyrosine	2 ± 0
Methionine	0 ± 0
Cysteine	0.8 ± 0.0

Carob germ flour can also be used as a potential ingredient in cereal-derived foods for celiac people (Feillet and Roulland, 1998). With the ever-increasing awareness and diagnosis of gluten intolerance from wheat, rye, barley and possibly oats, gluten-free food alternatives that contain proteins replacing gluten, are needed to enhance the quality of life of individuals with celiac disease. In order to address the gluten-free initiative food ingredients with functional and quality attributes similar to those of wheat and associated proteins can be identified.

As it can be seen, carob's potential as a food ingredient is high and it could be considered a low cost competitor to other food proteins like dairy or soy proteins creating new opportunities for exploitation (Tsatsaragkou et al., 2012). More research with respect to the nature of carob flour and the carob germ proteins as well as their thermal properties would be important from the point of view of the application of this crop as ingredient in formulated foods.

B.1.1.5 Locust bean gum

Locust bean gum (LBG), also known as carob gum, carob bean gum or additive E410, is obtained from the endosperm of the seed, which comprise the 40-50% of the seed's

weight. It occurs as a white to yellow-white, nearly odorless powder and it consists chiefly of high molecular weight hydrocolloidal polysaccharides (approximately 50-3.000 kDa), composed of galactomannans. Galactomannans are polysaccharides consisting of a mannose backbone with galactose groups (more specifically a-1,4 linked β-D-mannopyranose backbone with branch points from their 6- positions linked to α-D-galactose via 1,6 linkages) (Fig. 5). The galactose/mannose ratio of LBG is ~1:4 with a galactose appearing on about every fourth unit of the mannose chain (Iijima et al., 2012).

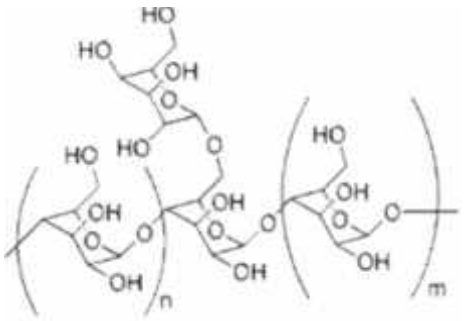


Figure 5: A segment of galactomannan showing mannose backbone (below) with a branching galactose unit (top) (http://en.wikipedia.org/wiki/File:Galactomannan.png).

LBG is insoluble in most organic solvents including ethanol. It is only partially soluble in water at ambient temperature and soluble in hot water. LBG typically needs heating to above 85°C for 10 min for complete solubility. This is due to the strong hydrogen bonding that occurs on the long mannose chain (Kawamura, 2008). It is utilized in food and non-food industries for its ability to bind water and form a very viscous solution at relatively low concentration (Iijima et al., 2012). It is also exploited for its synergy property with carrageenan, agar and xanthan to form stronger and more elastic gels (Dakia et al., 2007), having a wide application in foods as a thickening, gelling agent and stabilizer (Bengoechea et al., 2008). LBG, like other additives, is currently authorised under Directive 95/2/EC for a variety of uses and it has been allocated an acceptable daily intake (ADI) "not specified" by the Scientific Committee on Food (SCF) and therefore does not present any hazard to the health of consumers (Commission Directive 2010/69/EU). It is employed in a wide range of products, among the most important of which are ice cream, baby foods and pet foods. In these applications its texturizing properties are of great value and hard to replicate using other gums; in ice cream the gum slows the rate of melt-down and improves its storage properties (because of its capability of freeze-thaw resistance of gels). LBG is an important constituent of many soups, where its property of fully dissolving and thickening only at high temperatures is critical. In sausage products, such as salami and bologna, it acts as a binder and lubricant. Other food uses include the manufacture of soft cheeses, bakery products, pie fillings, powdered desserts, sauces and salad creams and dairy products other than ice cream. Hydrocolloids commonly named gums, like LBG, are capable to control both the rheology and

texture of aqueous systems throughout the stabilisation of emulsions, suspensions and foams and are also able to modify starch gelatinisation (FAO, 1995).

B.1.2 Wheat

Common wheat is a member of the wild grasses (Gramineae family) native to parts of Western Asia. About 600 genera of grasses have evolved, the main ones of interest being forms of the genus *Triticum*. Common or bread wheat or *Triticum aestivum* has been cultivated for about 10.000 years with extensive interbreeding by human endeavor. The wheat germ represents only 2-3% by weight of the kernel, but is rich in protein (25%) and lipid (8-13%). The endosperm represents the major part (80-85%) of the kernel and consists of an intimate mixture of proteins and starch (Atwell, 1997).

B.1.2.1 Main components of wheat flour

B.1.2.1.1 Lipids

Non polar lipids in cereals are dominated by triglycerides and as they are liquid at room temperature, they are described as oils. They occur as a storage reserve mainly in the embryo and as emulsion droplets (spherosomes) in the endosperm and aleurone layer. Polar lipids originate from the cell membranes and are dominated by phospholipids (phosphatidylserine and phosphatidylinositol) and glyco- or galactolipids. Wheat contains about 2% lipids (Eliasson and Larsson, 1993; Atwell, 1997).

Table 4: Average gross composition of wheat and rice flour (Data given in percent of dry weight) (Eliasson and Larsson, 1993).

cereal	*protein*	*fat*	*starch*	*fiber*	*ash*
wheat	12.2	1.9	71.9	1.9	1.7
rice	8.1	1.2	75.8	0.5	1.4

B.1.2.1.2 Proteins

Proteins are split into 4 main fractions based to Osborn classification system: albumins (water soluble), globulins (soluble in salt solutions), gliadins (soluble in aqueous ethanol) and glutenins (soluble in dilute acid or alkali).

The group of soluble proteins (albumins and globulins) is very heterogeneous in composition. The group contains enzymes, both metabolic enzymes from the developing grain and hydrolytic enzymes necessary for the germination of the seed; and also enzyme inhibitors. Other proteins are storage globulins in protein bodies, similar to globulins in legumes and lipoproteins.

The gluten proteins (gliadins and glutenins) constitute the main storage proteins in wheat endosperm. They are rich in glutamine and proline but poor in lysine and tryptophan. Gluten is what we get when we wash a wheat flour dough in water to

remove soluble components and starch–a mixture of proteins, lipids and carbohydrates.

The gliadin group is heterogeneous and it is divided in α-, β-, γ-, and ω-gliadins. The molecular weight of α-, β-, γ-, gliadins is in the range of 30-40 kDa and they belong to sulphur-rich prolamins, but the ω-gliadins have a molecular weight around 60-80 kDa and belong to sulphur-poor prolamins. The content of ionic amino acid residues is very low, whereas the content of hydrophobic residues is high, resulting in a very low water solubility of the proteins. Glutenins present low solubility because of their high molecular weight. Gliadin exists as single chains. Disulfide linkages exist, but they link cysteine R groups in the same chain. Because of the high level of proline, only about 20% of gliadin chains exist in a helical structure, and there is little evidence of pleated sheets. The tertiary structure is thought to be compact, with many binding interactions occurring between R groups within gliadin molecules. Glutenin molecules, in contrast, are larger than gliadin, because of the high number of disulfide bonds connecting subunits of the entire molecule, leading in fact to a linear molecule. The tertiary structure is thought to contain repetitive β-turns, which form a β-spiral structure that is stabilized by hydrogen bonding and may explain the elastic nature of glutenin (Eliasson and Larsson, 1993).

The functionality of gluten is largely related to the physical properties of its component, glutenin and gliadin. When hydrated, gliadin is viscous and can be stretched to a thin strand or made to flow easily with gravity. This propertiy is called extensibility. Hydrated glutenin, however, is very elastic; there is a considerable resistance when a mass of glutenin is stretched. Combined these 2 properties yield the gluten complex, which is said to have viscoelastic properties (Fig.6). Prolamin is known to contribute significantly to wheat gluten functionality, while the large polymeric glutenins are directly correlated to dough strength. When mixed in a dough with water and other components of flour, gluten forms a 3-dimensional continuous network, which has the ability to encapsulate gas cells and ultimately form a stable foam structure (Atwell, 1997).

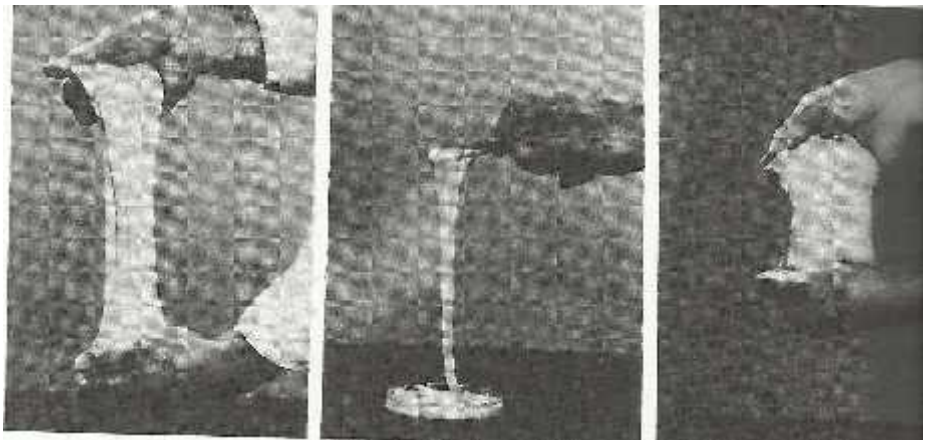


Figure 6: Photographs demonstrating the extensibility of gluten (left) and its components gliadin (center) and glutenin (right) (Atwell, 1997).

B.1.2.1.3 Starch

Whereas the role of lipids and proteins might be described as that of contributing gas-holding capacity and viscoelastic behavior to the dough, the role of starch is manifold. It provides the yeast with fermentable sugars and it contributes to the structure of crumb and the structure and color of the crust.

Starch is laid down in the shape of particles in special cells -called amyloplasts- in the plant. These particles are called granules, and they are the means by which the plant stores energy for the developing seed. The starch granule is a very efficient way not only to store the carbohydrate in a space-saving way, but also to make the energy easily accessible when the seed germinates. The size and shape of a starch granule is typical of its botanical origin (Fig. 7). Rice and wheat starch granules are pentagonal and angular; and spherical and lenticular–shaped, respectively (Singh et al., 2003).

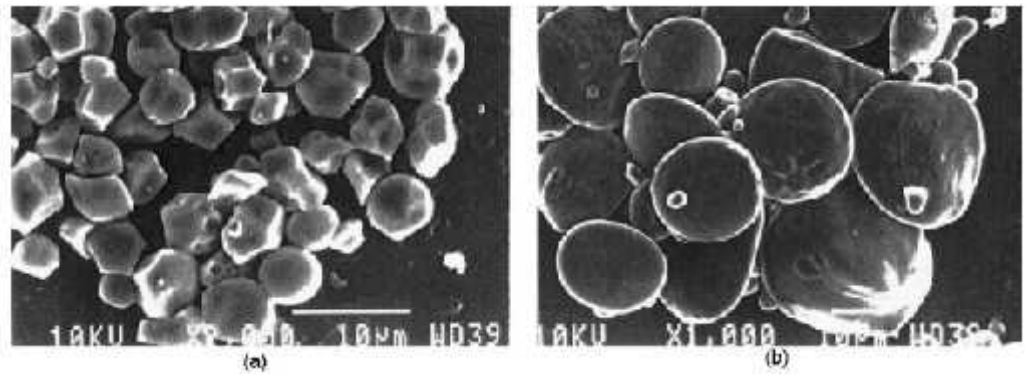


Figure 7: Scanning electron micrographs (SEM) of starches separated from: (a) rice and (b) wheat (Singh et al., 2003).

Starch is semicrystalline in nature with varying levels of crystallinity. The crystallinity is exclusively associated with the amylopectin component, while the amorphous regions mainly represent amylose. Amylose is a linear polymer composed of glucopyranose units linked through a-D-(1,4) glycosidic linkages, while the amylopectin contains also a few percent (4-5%) of a-D-(1,6) glycosidic linkages, leading to a branched molecule (Fig.8). Common wheat starch is about 25% amylose. There are 2 features of amylose in solution that are of special interest in relation to baking. The first is the great tendency to form intramolecular hydrogen bonds, which means a strong tendency toward crystallization (also referred as retrogradation). The second feature is its ability to form helical inclusion complexes, like amylose-lipid complexes. Amylopectin solutions do not show such a strong retrogradation tendency. An intriguing feature of amylose and amylopectin, when one tries to picture their distribution in the starch granule, is their incompatibility. The incompatibility is observed when solutions of amylose and amylopectin are mixed. With time a two-phase system will develop, with an upper amylose-rich phase and a lower amylopectin-rich phase. This result is also consistent with the starch structure (separately located in starch granule) (Eliasson and Larsson, 1993).

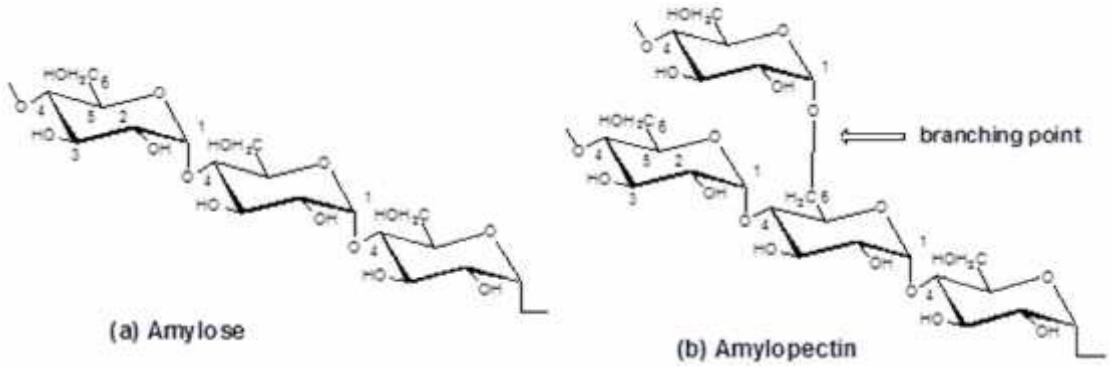


Figure 8: Structures of amylose and amylopectin (http://www.carbolea.ul.ie/composition.php).

B.1.2.1.4 Non-starch polysaccharides

Flour contains other polysaccharides in addition to starch. They can be divided into 3 groups: cellulose, β-glucans and pentosans. The cellulose content of white flour is only 0.6g/100g. Pentosans are made up of the 5-carbon sugars arabinose and xylose. The arabinoxylans are composed of chains of xylose units linked with a-(1,4) bonds to side chains of a single arabinose unit, usually attached to carbon 3 of xylose. Pentosan solubility is related to the molecular size and the degree of branching (number of arabinose side chains), splitting them into water-soluble and insoluble pentosans.
β-Glucans are another type of non starchy polysaccharide found in wheat flour. Their amount is only about one third the amount of the pentosans described above. They are composed solely of glucose. The linkages are β-1,3 and β-1,4 and there are no branch points. Arabinoxylans and β-Glucans are both natural components of the starchy endosperm, being concentrated in cell walls. They are present in very large amounts in the aleurone layer. Although present in low concentrations, they can affect the quality of the flour in significant ways, because of their ability to bind large amounts of water and increase the loaf volume of the bread, because they loose the dough structure (Eliasson and Larsson, 1993; Fessas et al., 2008).

B.1.3 Rice

Rice (*Oryza sativa* L.) is one of the most important cereal foodstuffs in the world. The need for non-gluten, low sodium, or low protein breads could be satisfied by using rice flour in yeast leavened breads. Rice has properties such as, the absence of gluten, low levels of sodium, protein, fat and fiber, and a high amount of easily digested carbohydrates, which are desirable for certain special diets combined with a natural, hypoallergenic, colorless and bland taste (Sivaramakrishnan et al., 2004; Demirkesen et al., 2010).
Starch is the major component of the carbohydrates in rice. Its main repository is also the endosperm, where it is deposited in the form of granules and it represents about 90% of grain matter.

Rice has a similar amount of lipids to wheat with glycolipids to be dominated by sterolglucosides and digalactosyldiglycerides and phospholipids by phosphatidylethanolamine. Table 4 shows the average gross composition of wheat and rice flour. Rice flour has different storage protein ratio than the one found in wheat. In rice, the major storage proteins are glutelins (oryzenin) (65-85%), while prolamins are the minor fraction (Eliasson and Larsson, 1993) (Table 5).

Table 5: Protein Content (%) of wheat and rice and distribution of proteins in solubility classes according to Osborne (Eliasson and Larsson, 1993).

cereal	*total protein of flour*	*albumins*	*globulins*	*prolamins*	*glutelins*
wheat	11.0	14.7	7.0	32.6	45.7
rice	7.3	10.8	9.7	2.2	77.3

The level of glutenins is important for the baking performance of wheat flour. However, glutenins from non-wheat cereals evidently lack the properties of wheat glutenins that are necessary for a good baking performance. Therefore, rice proteins do not process the viscoelastic properties typically found in gluten (which is the most important structure forming protein for making bread), thus leading rice flour to present considerable technological difficulty in the production of yeast - leavened products (Marco and Rossel, 2008; Eliasson and Larsson, 1993). On the other hand, rice flour is an ideal raw material for the production of gluten free products that are good alternatives for celiac people.

B.2 Principles of thermal analysis

Thermal analysis means the record of any physical property during a given thermal treatment under strict temperature control (Schiraldi et al. 2009), finding application in food science and technology, where heat treatments are very frequent. Thermal analysis encompasses a wide variety of techniques, such as (Riva and Schiraldi, 1992):

- differential thermal analysis, DTA
- differential scanning calorimetry, DSC
- thermogravimetry, TG
- thermal mechanical analysis, TMA

From these techniques, emphasis will be given in DSC and TG, since they are methods used in this study.

B.2.1 Calorimetry

Detection and determination of the amount of heat released or absorbed by a system is the main purpose of Calorimetry. It helps investigating processes connected with the generation or consumption of heat, such as chemical reactions, changes of state, protein denaturation, "gel" formation, polymerization, metabolic processes, etc. (Höhne et al., 2003). To measure heat means to exchange heat. The heat is a form of energy transient, which occurs only in the presence of a temperature gradient.
$Q=f(\Delta T)$

B.2.1.1 Differential Scanning Calorimetry

Differential Scanning Calorimetry means the measurement of the change of the difference in the heat flow rate to the sample and to a reference sample while they are subjected to a controlled temperature program; that means heating or cooling at a constant speed (scanning temperature) within a defined temperature range. DSC measures the change of the heat content-enthalpy of a sample (specifically of a heat flow rate difference, which normally is released due to an alteration of the sample temperature) or in other words the specific heat as a function of temperature. That means that a distinct temperature program or in general a mode of operation is always part of a DSC measurement (Höhne et al., 2003).
The DSC can be used in the food field for characterizing the first-order transitions (melting, protein denaturation, starch gelatinization, melting fat) or of transitions more complex (for example the glass transition, which is a heat capacity drop with no transition enthalpy(Levin and Slade,1991), to study the stability of fat's oxidation, the progress of enzymatic reactions, microbial growth, or to simulate thermal treatments and develop models to predict the stability of foods (Riva and Schiraldi, 1992)

B.2.1.2 Instrumentation

The calorimeter used in this study (PERKIN ELMER DSC 6) is described in Fig. 9. The instrument is made up of a furnace. The sample and reference are placed on individual bases inside the furnace, which contain a thermocouple and a heater. The two cells, arranged symmetrically with respect to the surrounding environment with which there is exchange of heat, are heated (or cooled) at controlled rate. The surrounding environment is made of a material with high heat capacity so as to not be influenced by the reactions that occur in the sample. The cells are hermetically sealed and the system can be considered at a constant pressure. The calorimeter is, in turn, isolated from the external environment. A thermocouple measures the heat flow.
Sample and reference are both maintained at temperature predetermined by the program even during a thermal event in the sample. The amount of energy which has

to be supplied to or withdrawn from the sample to maintain zero differential temperature between the sample and the reference is the experimental parameter displayed as the ordinate of the thermal analysis curve. ((Tsample - Treference) = 0) The electrical power supplied to the heater is adjusted so that the temperatures of both sample and reference remain equal to the programmed temperature, i.e. any temperature difference which would result from a thermal event in the sample is 'nulled'. The ordinate signal, the rate of energy absorption by the sample (e.g. J/sec.), is proportional to the specific heat of the sample, since the specific heat at any temperature determines the amount of thermal energy necessary to change the sample temperature by a given amount (Signorelli, 2004)

B.2.1.3 Acquisition of data

Any transition accompanied by a change in specific heat produces a discontinuity in the power signal, and exothermic or endothermic enthalpy changes give peaks detached from the baseline, whose areas are proportional to the total enthalpy change (Biliaderis, 1983). The direction of the peak corresponds to the nature of the transition, being heat absorbing (endotherms) or heat releasing (exotherms). While melting of solids and denaturation of proteins display endotherms, crystallization of carbohydrates and aggregation of proteins manifest themselves as exotherms. Inflection points are indicative of glass transitions; that is, reversible transitions of materials from a hard and relatively brittle state into a molten or rubber-like state or in other words, transitions from a glassy to rubbery state. The transition temperatures (*Tg*) reflect the thermal stability of the phase or state going through the transition (Schiraldi et al. 2009, pp6-8).

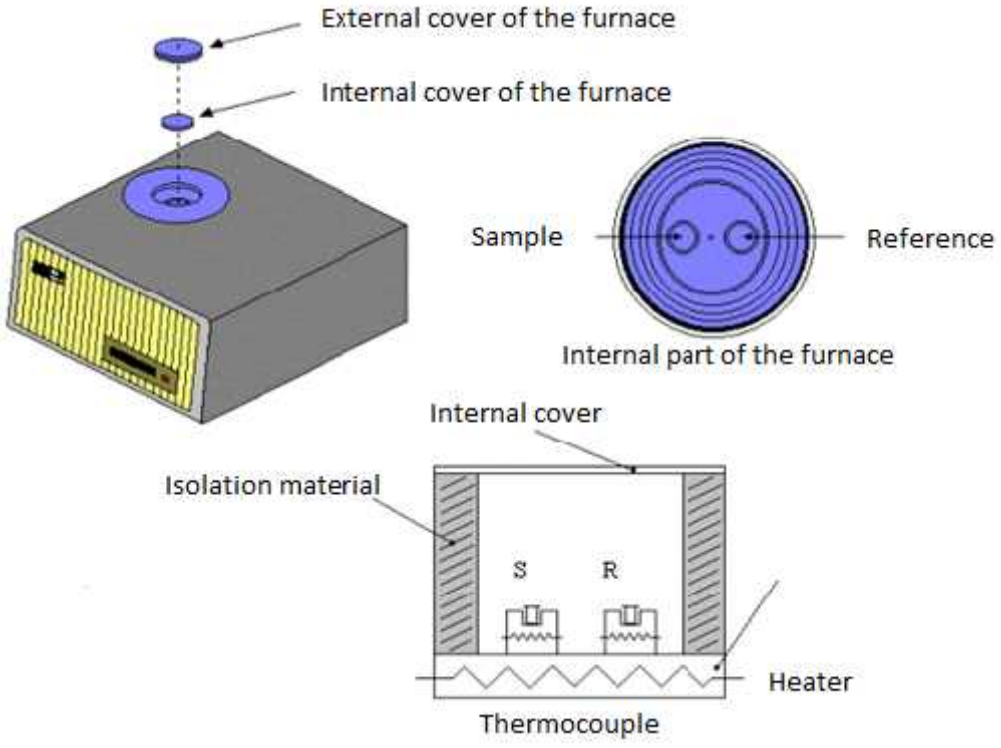


Figure 9: Description of Perkin Elmer DSC 6 (Signorelli, 2004).

In Fig. 10 typical DSC transitions can be seen, where T_g is the temperature where the glass transition takes place, T_c the crystallization temperature, T_m the melting temperature, T_o the temperature where cross-linking (cure) takes place and T_d the decomposition or oxidation temperature.

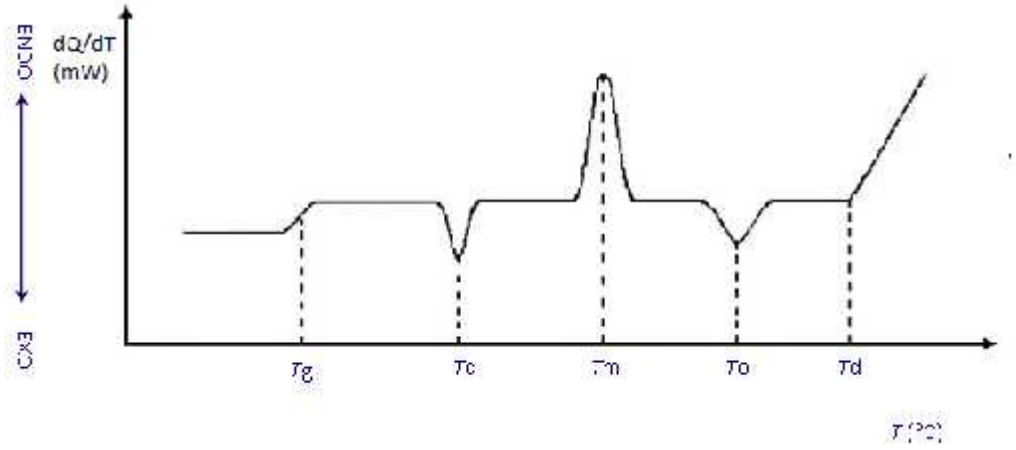


Figure 10: A schematic DSC curve demonstrating the appearance of typical phase transitions (Signorelli, 2004).

In other words, in DSC, the measuring principle is to compare the rate of heat flow to the sample and to a reference material which are heated or cooled at the same rate. Changes in the sample which are associated with absorption or evolution of heat cause a change in the differential heat flow which is then recorded as a peak. The area under the peak is directly proportional to the enthalpic change and its direction indicates whether the thermal event is endothermic or exothermic.
Examining a track we can detect the temperature (or temperature range) at which a significant change is undergone by the system. In the absence of thermal effects the route coincides with the base line.

B.2.1.4 Examples of DSC trace

A typical DSC plot, obtained from bread dough of wheat with standard formulation (44.2% w/w moisture) at 2°C/min heating rate is presented in Fig.11. To get this trace the output of the instrument dQ/dt (mW) was converted to specific heat by the equation : $Cp = \frac{dQ/dt}{m \cdot}$, where Cp is the specific heat, m is the mass of the sample and is the scanning speed.

The relevant signal in Fig. 11 shows an on-set temperature T_0, at about 45°C and three endothermic peaks at about 65, 90 and 120°C, respectively. This signal corresponds to starch conformational and phase transitions, the effects due to the other dough components, like gluten (residual) aggregation and denaturation of soluble proteins giving negligible contributions. Gluten proteins fail to produce denaturation endotherms when they are heated in the calorimeter (Eliasson and Larsson, 1993; Wang et al, 2001); caseins in milk are another example. The DSC technique requires

considerable cooperativity to produce detectable heat flow, which may not be possible with gluten proteins-the regions of identically ordered structure may be too small- and appears to be more like an amorphous polymer. Another explanation could be that the gluten proteins are unusually stable (Eliasson and Larsson, 1993).

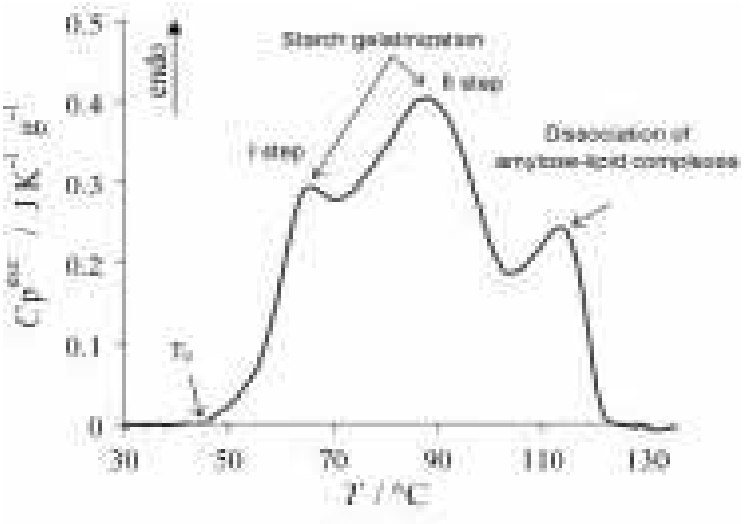


Figure 11: DSC trace of wheat dough sample at 44.2% w/w moisture content (Fessas and Schiraldi, 2000).

Most of the authors suggest that the first two peaks would correspond to the starch gelatinization, while the third peak would be related to the decomposition of amylose-lipid complexes in the course of the starch gelatinization (Fessas and Schiraldi, 2000). Amylose can form complexes with fatty acids, mono- and diglycerides. At around 100–120°C the lipid molecule settled along the axis of the amylose helix slips out, leading to the dissociation of the complexes. Defatted or lipid free starches do not exhibit this endotherm (Wasserman et al., 2007).

The same DSC trace was found by Biliaderis et al. (1986a) for intermediate water contents. It has been established that loss of birefringence is associated with the completion of the second peak of gelatinization. Regarding the nature of these two starch gelatinization endotherms, two models have been proposed. First, it was suggested (Donovan, 1979; Biliaderis et al., 1980) that, upon hydration/swelling of the amorphous parts of the granule and due to their coupling with the crystallites, melting of the latter occurs cooperatively as long as excess water is present in the system (first peak), a process known as gelatinization. However, when the amount of water becomes insufficient for this process to be completed, the remaining crystallites melt at higher temperatures (second peak), making the second peak dependent on the water content and the thermal stability of the remaining unmelted crystallites. This hypothesis focuses attention at the level of starch crystallite. An alternative explanation for starch gelatinization at intermediate water levels was given by Evans and Haisman (1982). They reported that the biphasic endothermic transition reflects two types of melting. Granules that contain the least stable crystallites melt first cooperatively, giving the first peak at low temperature. Upon melting, the

polysaccharide chains absorb more water and thus make it unavailable for the remaining ungelatinized granules. This means that the effective water concentration is further reduced by repartitioning of the water. Consequently, the ungelatinized granules will melt at even higher temperatures and thus give rise to the second transition. The attention here is shifted to the whole starch granule. Biliaderis et al. (1986a) proposed a new three-phase ((1) fully ordered crystalline phase, (2) non-ordered inter-crystalline phase, and (3) bulk amorphous phase) model, as opposed to traditional two-phase (amorphous and crystalline phases) model, for starch structures to help explain the gelatinization process. They argued that their three-phase model better described the multiple melting profiles observed during starch gelatinization at low moisture levels and that the gelatinization process involved partial melting, recystallization and final complete melting of crystallites.

Inspection of Fig. 12 allows one to notice that the onset of the signal, T_0, and the temperature corresponding to the maximum of the first peak are independent on the water content (about 45 and 64°C, respectively, in all cases): this can be easily explained by reminding that the onset of the signal conceals the starch glass transition endothermic shift and therefore depends on the water content within the native starch granules (about 12%), no matter the overall composition of the sample investigated. The rest of the signal is instead shifted toward high temperatures when the dough moisture decreases. At the high moisture level, the double endotherm transforms into a single, narrow endotherm, as it can be shown in figure 5 for 82.8% water content (Fessas and Schiraldi, 2000).

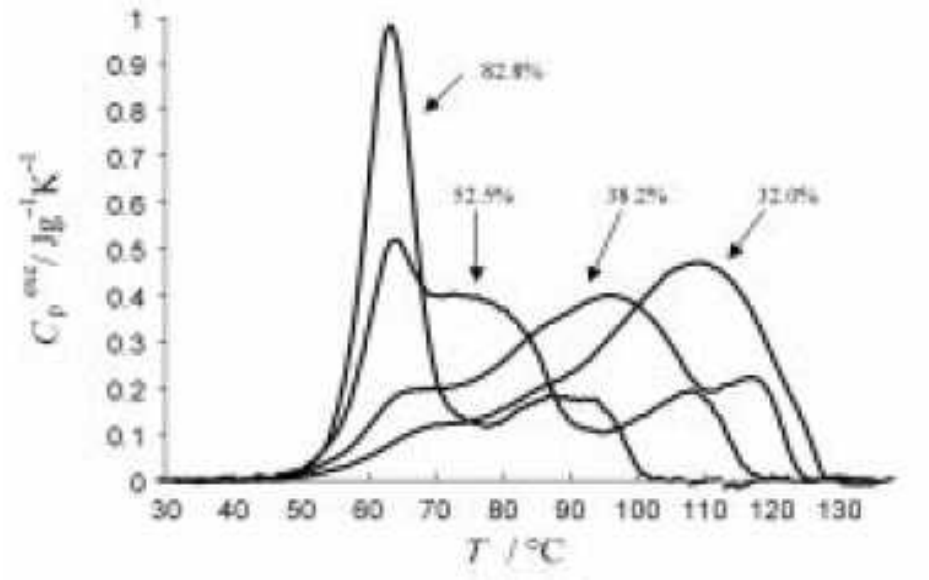


Figure 12: DSC traces of wheat dough samples with various moisture contents (82.8, 52.5, 38.2, 32.0% w/w) (Fessas and Schiraldi, 2000).

This result is consistent of that of Biliaderis (1983), where at a low starch/water ratio (more water), the double endotherm transforms into a single, narrow endotherm. For the lowest water contents, the second peak tends to be overlapped to the third one (Fessas and Schiraldi, 2000). The gelatinization is irreversible, whereas the transition

of the amylose- lipid complex is reversible and during cooling there will be thus one exothermic transition due to it (Biliaderis, 1983).
Different starches give rise to endotherms of different sizes (with different gelatinization enthalpies, ΔH values) and at different temperatures. Rice starch gelatinizes at higher temperature. Different temperature ranges have been recorded: Tm in the range of 63.6-78.2^{o}C for rice starch (Eliasson and Larsson, 1993) and also 68.4-73.9 °C for various rice varieties (Correa et al., 2013).

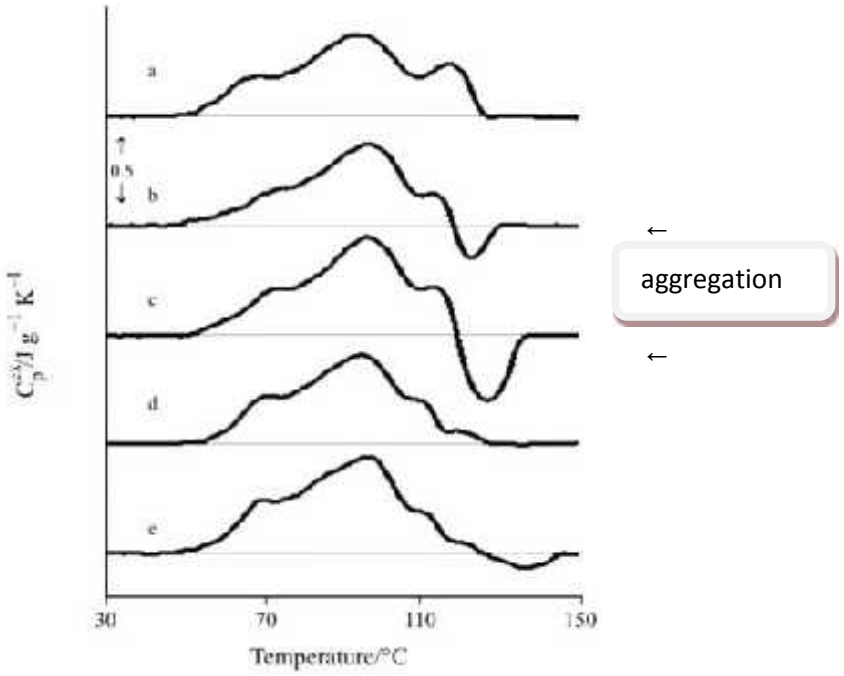


Figure 13: DSC traces of dough samples prepared with a – wheat b – integral buckwheat (BWI), c – de-hulled buckwheat flour (BWD) and 50 mass/mass% mixed flour, namely, d – wheat + integral buckwheat and e – wheat +de-hulled buckwheat. Doughs had 41% moisture content (Fessas et al., 2008).

Furthermore, in the above DSC trace the aggregation of buckwheat proteins as an exothermic peak can be observed (Fig. 13), since globular proteins (albumins and globulins) give rise to detectable peaks. Major differences related to wheat trace appear at higher temperature, where BWI and BWD traces show a large exothermic effect that can be mainly related to the aggregation of proteins (about 50% of the total proteins in buckwheat are albumins) that are much more abundant in buckwheat than in wheat flour (Fessas et al., 2008).

B.2.2 Thermogravimetry

Thermogavimetry is used to record the decrease of a sample's mass that corresponds to the water released, in relation to the temperature, allowing the investigation of water states and displacements (like the strength of water binding within a structure).
The TG trace of a sample undergoing dehydration is the mass-vs-T plot (thermogravimetric curve) and describes how much and how fast water is released

when the system is heated up at a constant rate β, so as to have T = To + β t (where t stands for time). The TG trace shows a sigmoid descending trend with a flexus at some intermediate temperature where the water loss rate is maximum (Fig. 14a). A more direct impact is offered by the trace of the time derivative, DTG (derivative of thermogravimetry), which shows a well defined peak, the maximum of which corresponds to the flexus point in the TG trace (Fig. 14b) (Fessas and Schiraldi, 2001).

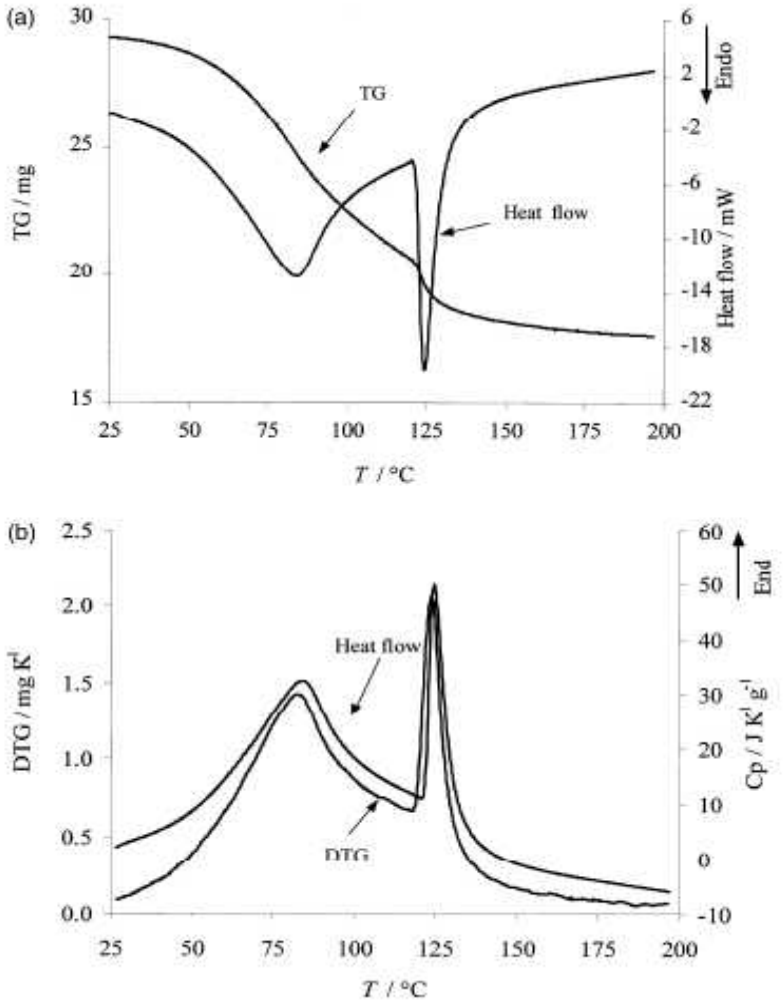


Figure 14: (a) Raw TGA trace (TG) of manually mixed wheat dough with 42% moisture content (30 mg sample, 2°C/min heating rate) and related heat flow. (b) DTG and Cp traces of the same sample (Fessas and Schiraldi, 2001).

Widely available are nowadays TG-DSC instruments that combine TG with DSC and provide both the relevant traces, namely mass loss (*m*) and heat flow *HF* (d*Q*/d*t*), *vs. Temperature (T)*, the former being also in the time derivative form, DTG (d*m*/d*t*). In this combined case, signal allows determination of the enthalpy drop relevant to the process that produces the mass loss. The ratio between the heat flux and the related mass loss rate (*HF*/DTG) allows a simple check of the enthalpy drop related to the mass loss, since *HF*/DTG=d*Q*/d*m*= ΔH_{vap} (in J/g units) (Schiraldi, 2012; Schiraldi and Fessas, 2003). In the case of water vaporization from food samples, it is very easy to

verify that the corresponding enthalpy is close to 2.3 kJ/g (referred to the mass released), namely, the vaporization enthalpy of pure water in this temperature range. This evaluation cannot be of help to single out the enthalpies of the different water fractions of a given food system, since the differences between them are some order of magnitude smaller than the vaporization enthalpy. However one can recognize different states of water because of the different temperatures at which water is released during an experimental run (Fessas and Schiraldi, 2001).

When the system contains water in different conditions, either because of different molecular mobility or because of tertiary force links of different strength with the substrate, DTG trace shows either several peaks or shouldered peaks which can be mathematically singled out. For each of them the corresponding enthalpy drop is accessible to ensure that the underlying process still deals with water vaporization.

One has therefore to sketch reality using thermogravimetry with a simplistic scheme. First, water partition in the system is supposed to be reasonably stable before the experimental run; second, water mobility rises with rising temperature and produces only non-chemical diffusion-limited effects related to the reversible formation of hydrogen bonds; third, structural water remains fixed at its positions until a sufficiently high temperature is reached where its links are loosened and it can move away. In spite of these oversimplifications, many processes that occur in food systems, like starch gelatinization, protein denaturation, formation of physical gels and networks, can be satisfactorily described according to this scheme (Schiraldi and Fessas, 2003).

B.2.2.1 Instrumentation

In general a thermobalance consists of a few basic components: a scale, a heating device, a device for temperature's control and measurement, an apparatus for recording changes in sample's mass and temperature, a system of controlling the atmosphere around the sample and a calorimeter.

In this work a thermobalance Setaram TG-DSC111 was used, which is described in Fig. 15. There are two cells, housed in two cylindrical cavities of a differential calorimeter type Calvet. The ability of the cells (one of which contains a counterweight inert) allows handling samples of mass of 50 mg weight. The sensitivity of the balance is ± 0.001mg. As discussed above, in TG there is a mass measurement as a function of temperature. The initial and final temperatures, as well as the trend of the curve, depend on many factors such as the speed of heating, the heat of reaction, the atmosphere of the oven, the amount of sample, the material of the sample container, the size and the packing of the sample.

During the heating process there is a temperature difference between the sample and the oven. One can generalize that this difference is proportional to the speed of heating of the sample. Taking into account, that the T of the sample is lower than the T of the oven, the difference between the two temperatures will be greater the greater is the rate of heating. The atmosphere inside the cavities is the variable that most influences the TG curve, because if the atmosphere is enriched with the volatile

product of the reaction, the thermal equilibrium shifts. The control of the atmosphere allows separating simultaneous reactions in case of different gases production (Signorelli, 2004).

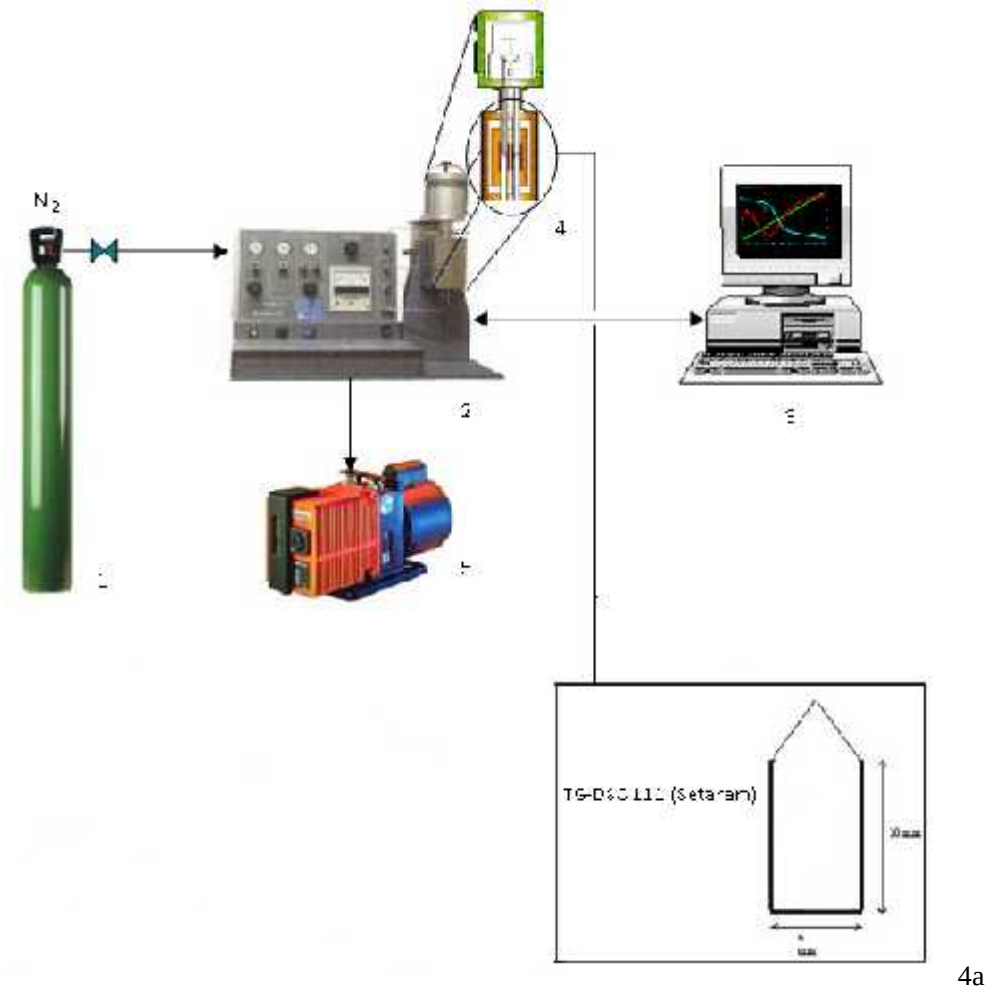


Figure 15: Thermobalance Setaram TG-DSC 111
1: nitrogen bottle, 2: thermobalance, 3: computer, 4: calorimetric cell, 4a: open pan, 5: pump used for controlling the instrument's vacuum (Signorelli, 2004).

B.2.2.2 Examples of DTG trace of starch and gluten systems

Below 45°C water can freely vaporize according to its partial pressure and it can be referred to as imbibing water (a_w=1). Above 45°C, water vaporization is controlled by simple diffusion through two main media, namely, a starch (amylose + amylopectin) gel and a gluten network which is undergoing reticulation. This accounts for the first DTG peak observed. A tightly bound water fraction that mainly deals with gluten is released only at higher T with a maximum rate occurring at 125°C and produces the second DTG peak. This fraction can be referred to as structural water, in the sense that it is an element of the structure of the substrate. In the presence of excess water, this fraction is less tightly bound and can be released at lower T, since water in the gluten phase would include a poorly linked fraction which is able to flash off when the gluten network is not yet too tight. The temperature gap between the two maxima

is related to the looseness of the gluten network. The position of the low-T peak remains practically unaffected (Fig. 16) (Fessas and Schiraldi 2001; Schiraldi and Fessas, 2003).

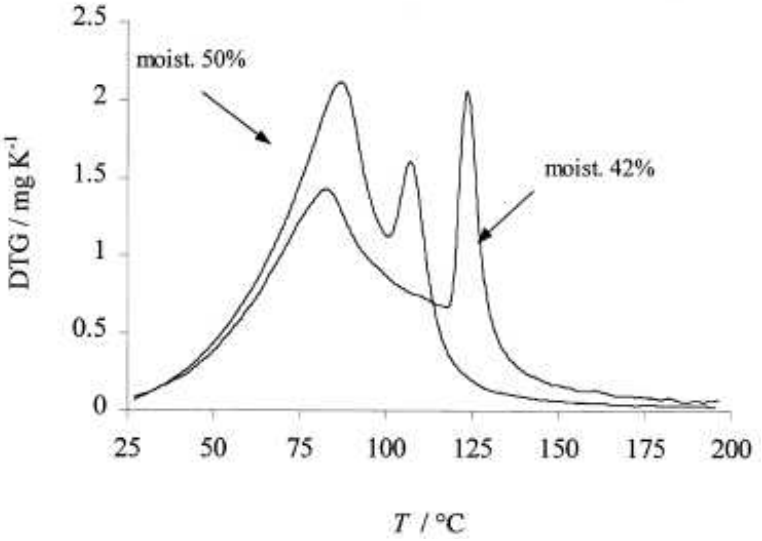


Figure 16 (right): DTG traces (30 mg samples, 2°C/min heating rate) of manually mixed dough with 42% and 50% moisture content (Fessas and Schiraldi, 2001).

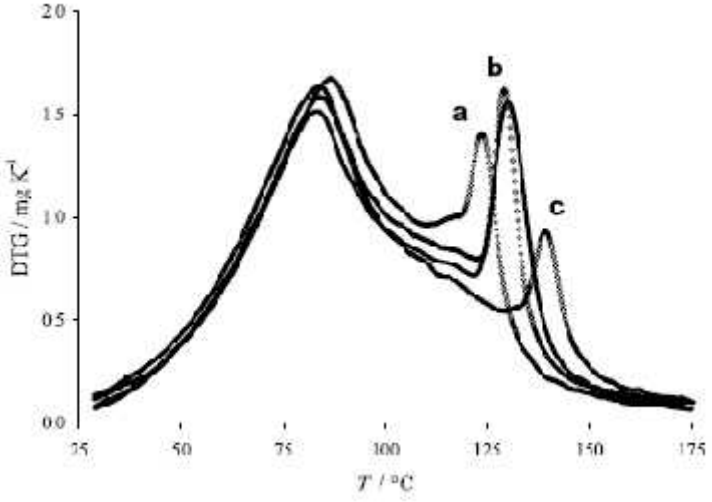


Figure 17 (right): DTG traces obtained from dough samples with initial moisture content, 47%, 43.5% and 40% w/w, (dotted curves a, b and c, respectively) and from a dough sample enriched with 1% (w/w with respect to the flour mass) water-extracted pentosans and an overall 40% (w/w) moisture (thick line) (Fessas and Schiraldi, 2005).

The presence of water-extracted pentosans within the gluten phase affects the gluten structure (high-T peak-b in contrast to c- occurs at lower temperature) so as to reduce its trapping strength for water (Fessas and Schiraldi, 2005). It is well demonstrated (Fessas et al., 1998) that in the presence of these polysaccharides a weaker gluten network is formed (Fig. 17).

Furthermore, DTG traces (Fig.18) obtained from dough samples prepared with cereals, pseudocereals and legumes which do not contain gluten show a single broad peak. This finding has to be interpreted as follows: the aqueous phases that are separated because of the thermodynamic incompatibility of their solutes (carbohydrates and proteins) can easily exchange the solvent between one another. The water that evaporates from one aqueous phase is quickly replaced by the water

migrating from any neighbouring aqueous phase. As a result, the dehydration of the samples looks like a single process governed by the core-to-surface diffusion of moisture (Fessas and Schiraldi, 2008).

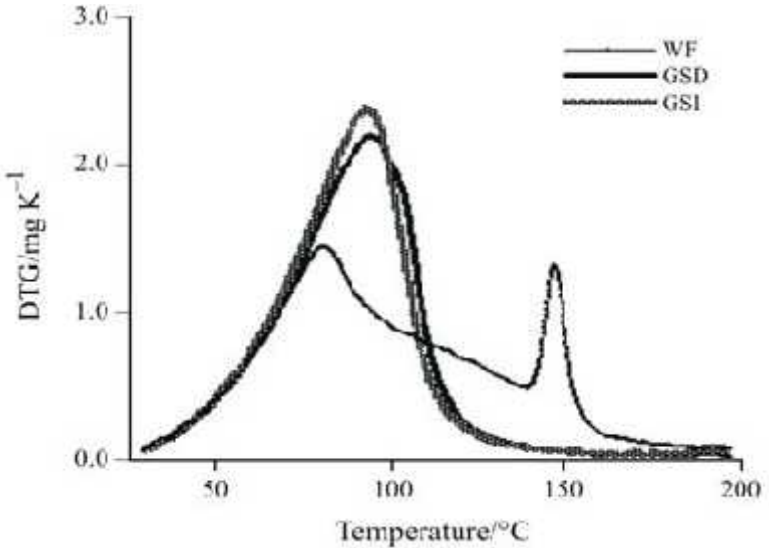


Figure 18: DTG traces from dough samples of wheat flour (WF), commercial integral buckwheat flour (BWI) and buckwheat flour obtained by milling dehulled grains (BWD) with 41% moisture content (Fessas and Schiraldi, 2008).

B.3 Thermodynamic incompatibility of biopolymers of flours

Thermodynamic approaches are highly promising for analysing the formation of food structures, providing information about the possible state and potential behaviour of a multicomponent food system. Biopolymer incompatibility is a thermodynamic phenomenon typical of foods (Tolstoguzov, 1997; Polyakov et al., 1997). It determines the heterophase nature of many food systems. Moreover, it has been shown that *phase separation* in biopolymer mixtures is a key parameter determining food structural hierarchy (Tolstoguzov, 1997). The main parameter governing this phase separation is the difference between excluded volumes of polymer components: the resulting *immiscibility* reflects the so-called *thermodynamic incompatibility* between different polymers (Grinberg and Tolstoguzov, 1997).

Dough is a heterogeneous system, since it is composed of thermodynamically incompatible polymers (Closs et al., 1999), which therefore form separate aqueous phases, each of which is richer in a given polymer with respect to the nominal dough composition. Driven by a chemical potential gradient, water is exchanged between these phases during mixing and baking (Larsson & Eliasson, 1993; Tolstoguzov, 1997). Proteins of different classes, according to the Osborne classification, are incompatible in aqueous media, with the phase separation to normally take place only at high protein concentrations. Proteins of the same class are incompatible when they differ in their conformations, e.g. the native and denatured forms of the same protein (Polyakov et al, 1997). Moreover, aaccording to Grinberg and Tolstoguzov (1997)

there is thermodynamic incompatibility between starch carbohydrates and pentosans and between these and flour soluble proteins. Gliadin and glutenin fractions of wheat flour are not miscible with albumins, globulins, starch and non-starch polysaccharides. It was found (Schiraldi and Fessas, 2003) that aggregation of gliadins, that takes place at a lower temperature when their concentration is increased, occurred at lower temperature in the presence of arabinoxylans, as indeed expected in the case of a phase separation produced in aqueous mixtures of incompatible polymers. Furthermore, Closs et al. (1999) have examined starch polysaccharides /galactomannan mixtures indicating their thermodynamic incompatibility. Alloncle et al. (1991) described the starch/hydrocolloid system as a suspension of swollen starch particles dispersed in a solution of hydrocolloid.

Additional literature reports (Tolstoguzov, 1997; Tolstoguzov 2003; Fessas and Schiraldi, 2008) support the expectation that starch carbohydrates and flour proteins are thermodynamically incompatible and therefore may not be involved in specific direct interactions with each other. The phase separation driven by the thermodynamic incompatibility implies that conformational and structure transitions experienced by carbohydrates and proteins of a given dough are not chemically correlated, the only allowed interactions being those related to physical properties of the relevant phases and interfaces, namely, steric hindrance, surfactant effects, water displacements, phase viscosity, etc. The same can be said for the interaction between starch carbohydrates and gluten proteins. These inter-phase interactions govern the formation of the macroscopic structure and the texture of the final baked product.

Therefore, blends of flours from cereals and pseudocereals or legumes allow dough preparations in which many interactions are expected. The relevant DSC traces should therefore be interpreted by taking into account the possible interactions between different dough components (Fessas et al., 2008).

B.4 Role of water in mixed systems

Water is ubiquitous in food products, where, because of its small molecular mass, is the major mobile component. As it easily forms hydrogen bonds with a number of substrates, water can either solvate ions and/ or polar molecules (or functional groups) keeping them apart from one another, or become a structure component of supra-molecular clusters. Water that occupies intermediate sites between solvated molecules, thanks to its high mobility, acts as a plasticizer of the whole system (Slade and Levin, 1995). For these reasons, water is responsible for many physical properties of food systems, as well as for the microbial growth, which can produce degradation processes and texture changes. Usually, when a true equilibrium is attainable, three regions can be recognized (monolayer water, multilayer and capillary linear region, and solvent or “free” water region) along a given adsorption/desorption isotherm. Unfortunately this description does not match many food systems. In practice since in most food products water is partitioned among different phases, either as a result of the preparation process or as a consequence of the thermodynamic incompatibility between the polymer components of the system, a single a_w value may have a reliable

physical meaning only if a true equilibrium has been attained, i.e., when the system is thermodynamically stable. However, almost every food can be referred to as a system far from the true thermodynamic equilibrium (Slade and Levin, 1995). This means that water in a food system can be found in various states, each with its own water activity (Fessas and Schiraldi, 2005).

Water found throughout the dough, can be used as a reliable marker of the changes that take palce in the various stages of preparation, acting as a "probe compound" (Schiraldi et al., 2009). So, the following questions provide the basis for comprehensive overview of the evolution of a mixed flour system (Schiraldi, 2012):

- ✓ Where is water primarily conveyed once it comes into contact with the flour powder?
- ✓ Which molecules or molecular aggregates compete for water?
- ✓ What is the result of this competition?
- ✓ Finally, how is the partition of water within the system related to the state of the dough before and after leavening and after baking?

As it was mentioned above, a dough is a heterogeneous system, since it is composed of thermodynamically incompatible polymers. Water in freshly mixed dough is shared between these different phases (mainly starch granules, gluten, globular proteins, pentosans, etc.) and occupies the inter-phase regions (Sahi, 1994). When the dough is heated up, separated phases are still present in the system and water accordingly rearranges its partition over the phases, being subjected to different driving forces. The water in the inter-phase regions can be supposed to move freely and generate a vapor phase with a given partial pressure; the rest of the water has to pass through phase boundaries and reach the inter-phase region to contribute to the overall water activity of the system, a_w. This evidence supports the description of a bread dough as a metastable dispersed system with a huge interphase surface across which water can move from one phase to another (Fessas and Schiraldi, 2001).

Table 6: Water uptake and distribution in wheat dough (Atwell,1997).

Constituent	Amount in 100 g Flour	Water Uptake (g/g, dry basis)	Water Uptake (g/100 g flour)	Water Distribution (%)
Starch (gelatinized)	68	...	...	...
Granular		0.44	25.4	26.4
Damaged		2.00	18.4	19.1
Proteins (gluten)	14	2.15	30.0	31.2
Pentosans	1.5	15	22.5	23.4

Water influences starch gelatinization and protein denaturation during the baking of dough. Starch and proteins mainly involve the next neighboring water molecules to undergo gelatinization and networking, respectively.

Starch is a supramolecular substance that nature assembles within the seeds of cereals and pseudocereals, legumes, and tubers. Starch has neither a definite molecular mass nor a fusion point, and it does not react with other substances until its granular structure is rotten and its glucose polymers, amylose and amylopectin, are exposed to the surrounding environment. Starch chemistry starts with surface processes that take place at the pores and defects of the granule structure, which remains practically unaffected at temperatures below 45 ° C, even with excess water. Starch granules are relatively dense and insoluble and hydrate only slightly in cold water. Starting from an onset temperature that depends on the vegetal origin of the starch investigated (e.g., 45°, 50°, and 65° C, for potato, wheat and rice, respectively), water enters the granule, making it swell to several times its initial size. This leads to the disaggregation of the internal crystal regions that are mainly formed by the side branches of amylopectin molecules. The whole starch granule is transformed in a swollen jelly ghost of the original hard and birefringent body. A gentle stirring turns the starting suspension into a dispersion of amylopectin gel and amorphous insoluble amylase that has previously leached out of the granules. The two glucose polymers are mutually incompatible which means that they may not stay in the same phase, being competitors for the available moisture. Because of this, the system is rather heterogeneous and unstable. On further heating, the amylopectin gel turns into a sol, while around 90°C, nucleation of amylose crystals can take place. The whole process (loss of birefringence in polarized light, absorption of water and swelling, change of shape and size of starch granules, leaching of amylose from the granules and formation of a gel or a paste), currently dubbed "***starch gelatinization***" is therefore a multistep, irreversible transformation of the starting suspension of starch granules, mainly dependent of the water content. (Singh et al., 2003; Fredriksson et al., 1998; Schiraldi et al., 2009)

When the system is cooled to room temperature (and below), the amylopectin gel becomes the matrix of growing crystals that entrap water and cause the system to harden. Although the structure of the original granules is by no means restored, the term "***starch retrogradation"*** is currently used to indicate this process.

In most cereals, both globular and networking proteins are present. The former, dubbed albumins and globulins can be either enzymes or carriers, are soluble in aqueous media, and are therefore easily extractable. The latter tend to form wide three-dimensional meshes that entrap aqueous phases and separated bodies, like starch granules. Gluten is the most important representative of this family: it is not soluble in water and therefore can be separated by washing a dough loaf with hot water to wash away starch carbohydrates and globular proteins. When a dough is prepared from a cereal flour, globular proteins play mainly a surfactant role that is crucial in stabilizing the air bubbles formed in a leavening loaf, whereas gluten is responsible for the overall rheological behaviour of the system. Both globular proteins and gluten fix water molecules, although in rather different ways. The former are normally solvated at the surface polar groups and modify their own solvation shell when unfolding and ***denaturation*** take place. Gluten instead uses water molecules as bridges between the next neighbouring chains and develops an extended network, due

to a large number of hydrogen bonds. Because of this, gluten can entrap large amounts of interstitial water within its meshes. Some disulphide bonds provide more robust inter - and intra-chain links and affect the overall extensibility of the network.
The flour of some gluten-free cereals and pseudocereals, such as buckwheat, soy, amaranth, and carob can trap water because of different proteins but cannot form stable dough because the polymer chains do not arrange themselves in a web. This water fraction is therefore much more mobile than the moisture trapped within gluten meshes.
Because of the thermodynamic incompatibility (Tolstoguzov, 2003) between different proteins and between carbohydrates and proteins (Grinberg and Tolstoguzov, 1997), a flour dough is indeed a dispersed system in which several aqueous phases coexist and can exchange the solvent between one another.

B.5 Bread making

B.5.1 Basic procedures for making bread

Three ingredients constitute the minimum needed to make a loaf of bread: flour, yeast and water. In the dough some other components may be added, like salt, sugar, redox agents, gluten, enzymes, fats and emulsifiers. In order to convert the mix ingredients into a bread structure a number of processing operations are performed. Three objectives are sought in the processing operations (Scanlon and Zghal, 2001):

1. Mixing and development of the dough
2. Formation of a foam structure in the dough (moulding, proofing and baking); and
3. Stabilization of a porous structure by altering the molecular configuration of the polymeric components in the cell walls through the application of heat (baking).

1. Proteins, starch and non starch-polysaccharides are hygroscopic components of flour that they demonstrate their functionality only when hydrated. Once flour is mixed with water to prepare a dough, the system undergoes substantial macroscopic changes: the flour powder forms an apparently sticky wet paste that, when kneading correctly, takes up the residual flour powder and gradually develops a rubber-like consistency. These transformations take place over a 5-minute period of mixing (Schirladi, 2012). At this stage the dough has a decreasing tendency to stick to the mixer and eventually the whole piece of dough will wind around the mixer blade. Specifically, mixing of a wheat dough helps the ingredients to be blended into homogeneous mass, proteins are developed into a 3-dimensional structure that has the capacity to hold gas and air cells are included. The quality of the final loaf of bread is strongly dependent on the mixing, and for each combination of flour and mixer it is possible to find an optimum stage of dough development.
The wheat dough can be illustrated as an aqueous continuous medium of gluten and starch (a) containing the dispersed gas phase (b), where there are also interfaces, such as the air/water interface (c) (Fig. 19) (Eliasson and Larsson, 1993). The continuum of the wheat dough is essentially the gluten gel, but starch also forms a continuous

starch-water phase. Water is necessary for all types of interactions and reactions that occur during the bread making process and influences the rheological behavior of the dough, as it is described above.

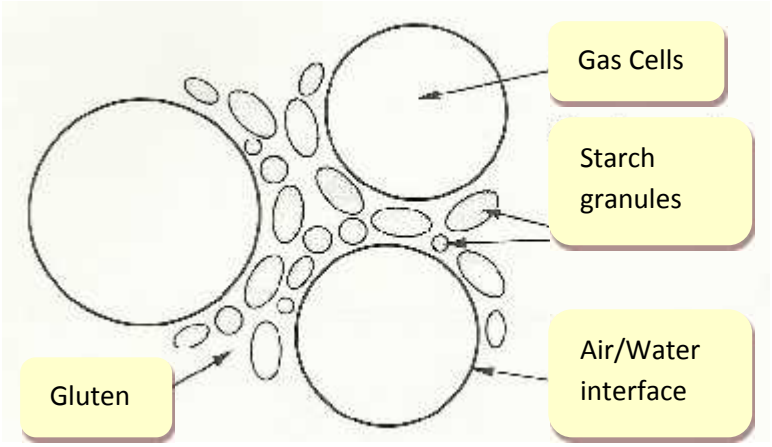


Figure 19 : A simplified model of wheat dough structure (Eliasson and Larsson, 1993).

If water content is not at the optimum level, the dough is difficult to handle and the loaf volume is also affected. The water content of a standard wheat bread dough is about 40%. However the ingredients in the formula are usually expressed as a percentage of the flour by weight and the water content in a bread dough will then be around 65%. The optimum level of water addition is related to the composition of the flour and therefore it is necessary to determine this optimum level for every flour or mixed flour system. This may be done in test baking, but water absorption can also be determined by the use of the Barbender farinograph. The water absorption of a flour is described as the amount of water necessary to bring the dough to a specified consistency (normally 500 Brabender units, BU) at the point of optimum development. Quantity and quality of proteins, damaged starch and content of pentosans and β-glucans affect water absorption.

Electrical conductivity measurements of the dough have shown that there must be more than 35% water in the dough to achieve a continuous conducting phase. This water appears to be a phase of “free” water. The “free” water may be present in the dough at the beginning of mixing as large regions of bulk water, as small droplets in the protein network or surrounding the starch granules. The distribution of water among these three types of locales is influenced by additives such as salt and sugar. It is important, however, to keep in mind that the conductivity of the dough shows that the free water phase must be continuous. This indicates that the dough is biscontinuous- the gluten being one continuous phase interpenetrated by the other free water phase. The structural result of wheat dough mixing is the formation of biscontinuous networks of two water-containing phases, the gluten phase and the “free” water phase. In the bread no free water is observed; presumably starch absorbs all the water during gelatinization. At the dough stage the temperature never

approaches the gelatinization temperature of the starch and therefore we do not need to be concerned with the gel-forming behavior of starch (Eliasson and Larsson, 1993).

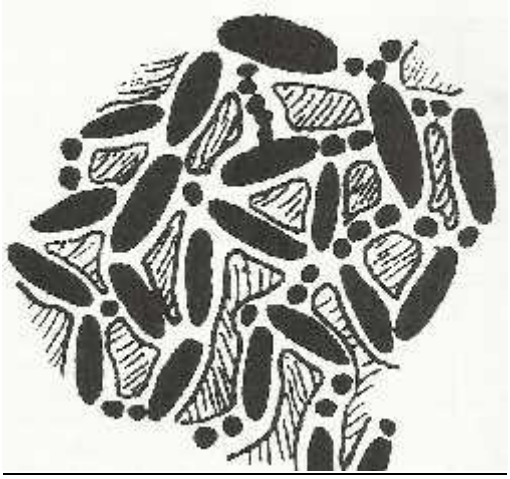


Figure 20: Two phase systems in the aqueous region of the dough consisting of two interpenetrating water phases. Starch granules with a surface coat of "free" water, are indicated in the figure. These granules are associated into a network interpenetrating the continuous gluten gel. The gluten gel is indicated by the shaded area. The gluten gel is thus considered to fill the space between the water-fused starch granules (Eliasson and Larsson, 1993).

2. When the dough is put in the oven after fermentation, a considerable increase in volume occurs; the oven spring. The increase in volume induces considerable stress on the air/water interfaces in the dough. This is the most critical point in the bread making process. All mistakes made earlier in the process will now be revealed. The dough may have fermented to the same size, but during oven spring the extent of its ability to produce a bread of satisfactory loaf volume is displayed. This is a consequence of the increase in temperature, which induces changes in both the dispersed and continuous phases of the dough, which in their turn result in the volume expansion and the setting of the crumb. Carbon dioxide is soluble in the aqueous phase of the dough, but to create the leavening effect and the porosity it must be transformed to the gaseous phase. This can occur only if the dissolved carbon dioxide diffuses to the already existing gas cells in the dough, since these cells are nuclei for all gas produced. Porosity can be affected in several ways. The dough can be allowed to ferment and if it is then punched or remixed, the large gas cells are divided into many small ones. Also mixing under reduced pressure creates large gas cells that can be divided into many small cells with punching.

3. The gelatinization of starch puts an end to the oven spring, but at the same time it inhibits the collapse of the bread crumb. During gelatinization the starch granules absorb water and swell to a degree that depends on the availability of water (as the "free" water in insufficient). There is clearly a redistribution of water from gluten to starch during baking. The gelatinization of starch is the most obvious prerequisite for the formation of bread crumb. It is not possible to use any starch for baking. Waxy

maize starch for example, results in dough that ferments properly, but the loaf's crumb structure collapses after baking. If rice starch is used, differences in volume are observed during proofing. The best result is obtained with wheat starch, but rye and barley starches perform almost as well.
The dough at the end of the fermentation was modeled as a foam. The loss of expansion during heating after starch gelatinization is clearly related to the water transport from the gluten phase to the starch-water compartment. This volume change in combination with the transition from gel to coagel results in the rupture of the gluten gel surface zone that we saw in the dough. The gas cells will fuse into an open pore system during these changes.
The gross structural changes and the transition from gel to coagel induce the crucial steps in the metamorphosis from dough to bread.
The main difference between the crumb and the crust is the difference in the temperatures they attain during baking. The high temperature in the crust causes the evaporation of water, so the water content of the crust is very low compared to the crumb. From a structural point of view, wheat crust is a hard, vitreous surface layer formed of collapsed crumb pore walls. It is a continuum of dried starch gel with dispersed protein and lipid aggregates (Eliasson and Larsson, 1993).

B.5.2 Role of salt, sugar, fat and yeast in bread making

Salt (NaCl) is present in the aqueous phase either because it is added as such or because it is dissolved in the added water. Salt adds flavor and it influences gas retention. The optimum loaf volume is obtained with 1.5-2% NaCl (based on flour weight).
Sugar adds sweetness to the end product, serves as an easily accessible carbohydrate for the yeast, contributes brown color to the crust through the Maillard reaction (enzymes from the yeast will hydrolyze sucrose to fructose and glucose, which can react with proteins) and through caramelization. At the levels of addition used in bread making, the effect on starch gelatinization may not be observable, but in other products with a high sucrose/flour ratio this effect can cause the gelatinization temperature range to be shifted to higher temperature due to competition of water.
A few percent by weight of *fat* is often added to dough to improve the crumb structure and also to obtain a larger volume. The texture is changed toward finer and more uniform pores with thinner walls, which gives an improved softness. The probable role of the fat crystals is to mechanically strengthen the pore walls, whereas the oil will act as a lubricant for the solid particles of the flour during dough mixing.
One ingredient not mentioned earlier, but which (in volumetric terms) is a significant component of the dough is *air* (bread properties and crumb structure). The dispersed gas phase in the dough consists of the gas cells. The inclusion of gas cells in the aqueous gluten phase is a thermodynamically unstable situation and with time it is expected the cells to collapse. The gas in the gas cells is obtained from 2 sources: from air included in the dough mixing process and from the carbon dioxide (CO_2) produced by yeast. The most common leavening agent in bread is *yeast*

Saccharomyces cerevisiae. Besides its contribution to bread volume by production of CO_2, the role of yeast fermentation is to influence the texture of the dough and to contribute to flavor and aroma. The level of yeast is usually 3.5-5.5% calculated in the amount of flour. There is a physical work on the dough due to the expansion of the air cells. However a large volume is not enough for a bread of high quality; the porosity must also be acceptable. Pores of uniform and rather fine size are preferred. Porosity is established during mixing and subsequent punching and molding of the dough (Eliasson and Larsson, 1993).

B.5.3 Celiac disease

If wheat has such a superior baking performance it does not seem very reasonable to use other cereals in bread making. However, there may be very good reasons for doing exactly this. The incorporation of other cereal flours gives the bread a new taste and may improve its keeping qualities. It may be convenient to use locally produced crops instead of imported wheat (Eliasson and Larsson, 1993).
Finally, some people suffer from celiac disease or dietary wheat intolerance. That is why, there is nowadays an increasing interest for gluten-free products as the number of the celiac patients grows. Celiac disease is a digestive disorder which damages the villi, tiny hair-like projections in the small intestine that absorb nutrients due to an immunological reaction to the gliadin fraction of wheat gluten (and the prolamins of rye (secalins), barley (hordeins) and possibly oat (avidins)). This leads to the malabsorption of several important nutrients including iron, folic acid, calcium and fat-soluble vitamins. This gluten-sensitive enteropathy can be caused by genetic, immunologic or environmental conditions. The only way to overcome this problem is to follow a strict gluten-free diet throughout the life-span (Demirkesen et al., 2010; Gallagher et al., 2004; Fasano and Catassi, 2008).

B.5.4 Non-gluten products -Carob in bread making

According to Codex Alimentarius, gluten free foods are dietary foods:
a) consisting of or made only from one or more ingredients which do not contain wheat (i.e., all Triticum species, such as durum wheat, spelt and kamut), rye, barley, oats or their crossbred varieties, with a gluten level not exceeding 20 mg/kg in total, based on the food as sold or distributed to the consumer, and/or
b) consisting of one or more ingredients from wheat, rye, barley, oats or their crossbred varieties, which have been specially processed to remove gluten, with a gluten level not exceeding 200 mg/kg in total.
Gluten-free doughs are closer to cake batters, concerning their rheological properties (Cauvain, 1998). They cannot be satisfactorily leavened, since the dough structure collapses. For this reason no real crumb can be obtained from the dough of a gluten-free cereal flour, unless the collapse of the structure is avoided with a careful adjustment of the viscosity of the continuous carbohydrate-rich phase and a selection of surfactants that stabilize the liquid/air interface (Fessas and Schiraldi, 2008). In recent years there has been significantly more R&D on gluten-free products,

involving a diverse approach, which has included the use of starches, dairy products, gums and hydrocolloids, other non-gluten proteins and combinations thereof, as alternatives to gluten, to improve the structure, mouth feel, acceptability and shelf-life of gluten free bakery products (Gallagher et al., 2004). Hydrocolloids are widely used in the bakery industry to impart texture and appearance properties to cereal-based foods like bread. Rice starches are widely available and offer potential in the formulation of gluten-free baked products. Gums and thickeners are used in gluten-free formulations for a variety of purposes including gelling and thickening, water retention and texture improvement.

Ács, Kovacs, and Matuz (1996a, 1996b) investigated the use of different binding agents (LBG, xanthan, guar gum) as a substitute for gluten in gluten-free bread formulations based on corn starch. They found that the binding agents resulted in a highly significant increase in loaf volume and loosening of the crumb structure. Schwarzlaff et al. (1996) found that LBG can partially replace flour in bread leading to an increased height of wheat bread loaves and retarding bread staling. Optimum levels for locust bean gum are 2–4%. Mi et al. (1997) examined the effects of gums on the quality of rice bread and they founded that all the gum type additives studied (1– 4.5% HPMC, 1.5% locust bean gum, and 1.0% each of guar gum, carrageenan, xanthum gum and agar) resulted in successful formation of rice bread showing optimum volume expansion.

A few studies have also been carried out on carob's behaviour in baked goods. Tsatsaragkou et al. (2012) used carob flour in a gluten-free bread batter with rice. They reported that carob flour-rice flour mixed system requires a high amount of water in order to be fully hydrated and able to form bread with acceptable quality, because carob flour is rich in dietary fiber and creates a tight structure difficult to handle. They also stressed the fact that, carob could be used as protein source, enhancing the overall nutritional value of gluten-free products.

Miñarro et al. (2012) found that carob germ flour batter structure was thicker compared with other batters (from chickpea flour, pea isolate, soya flour), probably due to the different protein behaviour. Also carob germ flour bread obtained the lowest specific volume values. Although the reported ability of hydrated carob germ protein to form a network structure, the use of carob germ flour at 1.5% in the bread did not result in a network able to expand as much as the other formulations, when proofed or baked.

Smith et al. (2010) after the mixing of carob germ flour-maize starch mixed system in a farinograph, they found that no mixing curve could be produced, indicating that the proteins of carob germ flour were not able to form as strong of a dough as those of wheat. They also reported that carob germ meal's proteins are mainly globular proteins and glutenins, with no prolamins detected. As the extensibility of the gluten network is attributed to the prolamin fraction, its lack in carob germ flour would account for the low volume of carob germ bread. Furthermore, volume impairment may also be due to an excess of hydrocolloid content due to residual gum content present in carob germ flour, as LBG can form a gel when heated, which would not be able to expand (Miñarro et al., 2012).

C. Scope of the work

Although most of the chemistry and biochemistry of carob (and related nutritional and health advantages) have been investigated, much of the physics underlying the technological properties and potentialities of this legume has not yet been thoroughly described. One way to approach this issue is based on the evidence that the behaviour of a flour dough is directly related to the role played by the macromolecules which induce phase separation and govern the water partition.

The objective of this study is to investigate starch and protein (both carob protein and gluten) thermal transitions through Differential Scanning Calorimetry and understand what happens with the available water that comes out from the competition between starch, gluten, carob proteins and LBG, through Thermogravimetric Analysis. Two different mixed systems were investigated, one with carob-wheat flour and another with carob-rice flour. The latter is also appropriate for celiac sufferers.

Therefore, the scope of the work is the investigation and better understanding of the behavior of carob flour and its components (namely carob protein and LBG), separately and in mixtures with wheat and rice flour. The performance of carob protein as structural ingredient in formulated foods and its potential as a gluten substitute is another issue to be investigated.

Breads from mixed flour systems were also produced to confirm the results, from the technological point of view, since thermal characteristics of the mixed flour systems explain their behavior during baking.

D. Materials and Methods

D.1 Materials

The materials used were:

1) Carob flour with moisture content 9.35%, protein 22.96%, dietary fiber (including LBG) 51.8%, lipid 1.79%, ash 4.85%. The particle size was between 250 and 315 μm. Carob seeds were received from Cypriots local producers, milled in a laboratory mill. As a result carob flour consists of germ, endosperm (LBG) and coat.
2) Carob germ flour or carob protein flour, isolated from carob seeds, with moisture content 8.03%, protein 64.07%, lipid 10.22%, dietary fiber 13.25% and ash 6.27%.
3) Locust Bean Gum (Sigma-Aldrich Chemie GmbH, Munich, Germany).
4) Commercial wheat flour with moisture content 12.26%, protein 9.08% and ash 0.39%. The particle size was between 150 and 250 μm.
5) Rice flour with moisture content 13.10%, protein 7.39%, dietary fiber 0.5%, lipid 0.39%, and ash 0.8% (Kaplanidis mill group S.A., Serres, Greece).
6) Distilled water.

For the bread making part the following materials were also used: moist yeast (L'hirondelle, S.I. Lesaffre, France), sugar, salt (iodised sea salt, Kallasgroup S.A., Katerini Branch, Greece), shortening (Vitam, Unilever S.A, Athens, Greece) and tap water.

D.2 Methods

D.2.1 Thermal analysis

D.2.1.1 Sample preparation

The dough was prepared with flour and distilled water, without adding any salt and yeast for the sake of reducing the number of variables that could affect water partition within the dough. The recipe was modified by changing the water content and the kind of flour or mixture of flours. The dough was mixed with manual kneading for 4 minutes. Then a sample of 30 mg of dough was inserted in a cell and put in the calorimeter DSC 6 or was given a ball shape and inserted in the thermogravimeter. The following types of dough were accordingly considered:

1. Dough with overall 40% moisture,
2. Dough with overall 55% moisture,
3. Dough with overall 32% moisture.
4. An overall 70% humidity, where no dough was created. In this case, the flour or the mixture of the flours was added as a powder in the cell and the appropriate amount of distilled water was added, so that the cell contained 30mg of sample.

The following types of samples were considered:

A. controls: 1) wheat flour, 2) rice flour, 3) carob flour, 4) carob protein flour.

B. mixed systems: 1) wheat flour + carob flour, 2) wheat flour + carob protein flour, 3) wheat flour + LBG, 4) rice flour + carob flour, 5) rice flour + carob protein flour, 6) rice flour + LBG, 7) LBG + carob protein flour.

D.2.1.1.1 DSC

A Perkin Elmer DSC-6 with 60mL cells was used. The reference cell contained aluminium. DSC runs were performed from 25 to 150°C with 2°C/min scanning rate. Indium was used for calibration. Each run was repeated at least twice and the typical sample mass inside the cell was 30 mg. Some pictures of the sample preparation are shown in Fig. 21.The relevant moisture content within the sample was determined after the DSC run. The cover of the measure pan was pierced and the cell was kept in an oven at 105°C for 24 h; the moisture loss was therefore determined as the cell mass change. To obtain the correct correspondence between heat flow and the detected signal, the instrument was calibrated using indium as standard substance with known parameters: Tfus 157 ° C and ΔH_{fus} = 28.45 J/g. Before each measurement the instrument was calibrated at the scanning speed used, which is in this case 2° C/min. The heat flow traces were expressed as apparent specific heat Cp (J $K^{-1}g^{-1}$), dividing the instrument output (in mW units) by the product [sample mass x scanning rate] (in mg K s^{-1} units).

Three temperatures associated with the gelatinization process could be defined, the one where gelatinization began (To), the peak temperature (Tm) and that at which gelatinization ceased (Tc). The significance of the ΔH value is that it represents the amount of thermal energy involved in the transition.

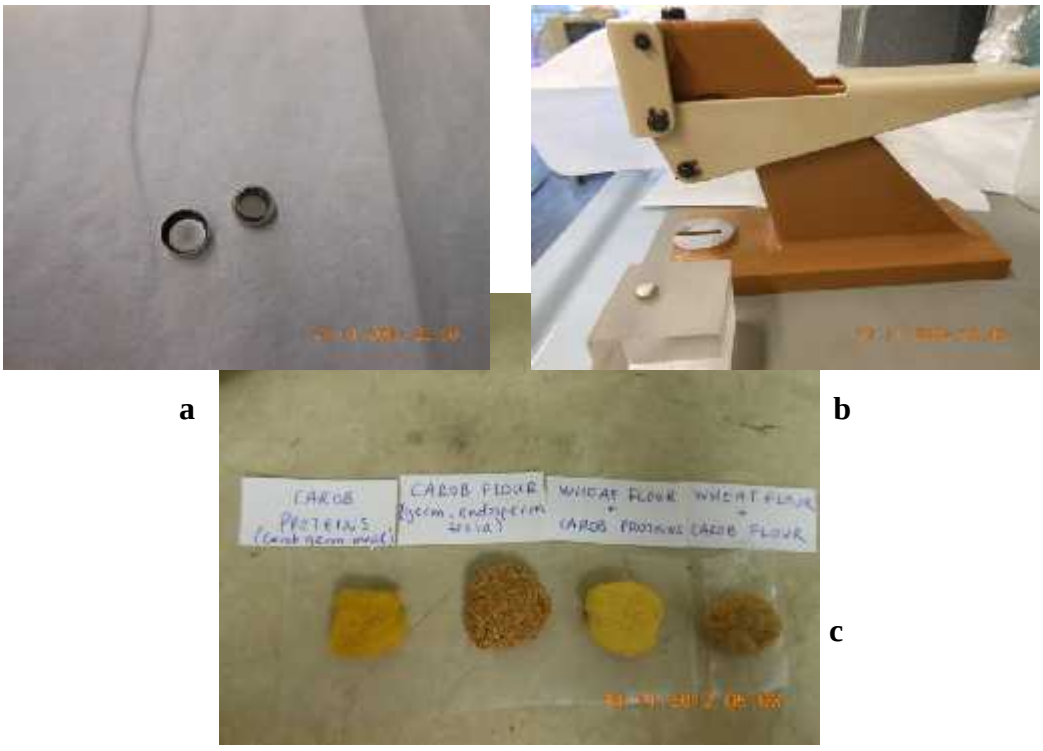


Figure 21: a: cells, b: illustration of sealing of the cells, c: dough samples.

D.2.1.1.2 TGA

The TG instrument was a Setaram TG-DSC111 (Lyon, France) with the simultaneous output of the thermal effect (heat flow vs. T), TGA trace (mass loss vs. T) and its time derivative DTG. The typical sample mass was 30 mg. Each run was repeated at least twice. The typical TGA run for the present work was carried out at 2^{o}C/min heating rate starting from 28^{o}C and ending at 200^{o}C. All TGA records were normalized to 100 mg water content. The DTG traces were expressed as mg of lost water per degree K (with reference to the scanning rate used, 2°C/min).Only doughs with 40 and 55% moisture were used in TGA analysis. The ratio between the heat flux and the related mass loss rate was found equal to the enthalpy of water evaporation in whole temperature range. This check confirmed that the mass loss was substantially related to water evaporation only. Possible losses of volatiles therefore were meaningless in our case.

D.2.1.2 Analysis of the results

The raw data from both instruments (TGA, DSC) were worked out with the dedicated software IFESTOS, which was assembled by Fessas and Schiraldi for handling raw calorimetric data, according to the suggestions by Barone et al. (1993).

D.2.2 Bread making

D.2.2.1 Bread making procedure

The basic recipe for the dough (based on the weight of the flour) was 4% yeast, 3.5% shortening (margarine), 3% sugar and 2% salt. LBG was integrated at 2 and 4% and carob protein at 5% (based on the weight of the flour) in both wheat and rice dough. The amount of water added to wheat and rice dough was 55% and 90% (based on the weight of the flour) respectively. During the conduction of preliminary experiments for rice breads, three levels of moisture content (55, 70 and 90%) were selected based on the weight of the flour. In the cases of 55 and 70% moisture content (MC), the amount of water was too low for the rice dough to be fully hydrated, resulting to poor bread proofing and porosity development. So, the amount of 90% water was selected for rice breads.

For the dough preparation, dry ingredients (wheat or rice flour, LBG or carob protein, sugar, salt) were first mixed in a mixer (Hobart N50, Hobart Co., Troy, OH, USA). Then, the yeast mixed with the appropriate water amount was added progressively, followed by the addition of melted shortening to the final blend. All the ingredients were mixed for 5 min using a mixer at a speed of 475 rpm. After complete mixing, 420 g of dough was placed in oiled pans (20 x 10 x 6cm) and was fermented at 35^{o}C and 85% RH for 50 min. Following fermentation, samples were baked at 180^{o}C for 45 min in a convection oven. The loaves were cooled to room temperature, to ensure that condensation does not form on the inside of the package and placed in polyethylene bags for 24 h before determination of their physical properties. Rice flour and wheat

flour dough samples without any gum or carob protein were used as controls and all breads were made in duplicate.

Table 7: Percentage of water and added ingredients (in flour basis) for different formulations of dough.

wheat	*55% water content*	*rice*	*90% water content*
	control		control
	+ 2% LBG		+ 2% LBG
	+ 4% LBG		+ 4% LBG
	+ 5 %carob protein		+ 5 %carob protein

D.2.2.2 Bread analysis

D.2.2.2.1 Yield in baked product

Yield in baked product was estimated as the ratio of the sample weight after and before baking using Equation 1:

$$\% \text{ yield in baked product} = (Wb/Wd) \times 100 \quad (1)$$

By the end of the baking process, duplicate bread loaves were left for 1 h to cool in room temperature and their final weight (Wb) was calculated (initial weight, Wd=420g).

D.2.2.2.2 Bread moisture

The evaluation of moisture in the bread was performed using AOAC method 935.36.

D.2.2.2.3 Crumb texture

The firmness of bread crumb was estimated with the 74-09 method of the American Association of Cereal Chemists (2000) in an Instron (Universal Testing Machine, Model 1100, Massachusetts, USA) equipped with a 50 N load cell. A slice of 2.5 cm (thickness) from the centre of the loaf was compressed to 40% of its initial height with a 4 cm diameter probe coming down with a speed of 101 mm/s. The force (N) reading, measured at 40% of compression, expressed the resistance of the crumb to the penetrating probe and represented the crumb firmness. The crumb firmness was measured in duplicate after 24 h of bread preparation.

The crumb's relative elasticity testing was carried out in crumb cubes of 2 x 2 x 2 cm (length x width x height). The cubes were always cut from the same (almost middle) part of the loaf. The measurements were done in triplicate. A uniaxial compression test with subsequent relaxation phase that lasted 4 min was applied in order to determine the textural and viscoelastic properties of the bread crumb. Crumb's relative elasticity was calculated at 25% compression, within the linear viscoelastic region. The relative elasticity of the crumb (the force with which the crumb resisted

the defined mechanical stress during compression) was derived from the recorded force-time diagram. The calculation was done according to Equation 2:

$$REL\ \% = (Fres / Fmax) \times 100 \quad (2)$$

D.2.2.2.4 Porosity determination

For porosity measurements, samples of 1.5 x 1.5 x 1.5cm (length x width x height) from the geometric centre of the crumb were taken for all breads. The volume of solids (V_s, m^3) was measured with gas pycnometer (Stereopycnometer SPY-3, Quantachrome, Syosset, N.Y., USA) based on Archimedes principle of fluid displacement. The displaced fluid is a gas which can penetrate the finest pores to assure maximum accuracy. For this reason helium was used, since its small atomic dimension assures penetration into crevices and pores approaching one Angstrom (10^{-10} m). Its behavior as an ideal gas is also desirable.

For each measurement, three different samples were used, each measured three times. The solid density (kg/m^3) is the ratio of the mass of dry solids to the volume of dry solids and is expressed by Equation 3:

$$p_s = m_s / V_s \quad (3)$$

The bulk density is estimated from measurement of the actual geometrical characteristics of the bread sample by Equation 4:

$$p_b = m_s / V_b \quad (4)$$

The total porosity was calculated from Equation 5:

$$\varepsilon = 1 - p_s / p_b \quad (5)$$

D.2.2.2.5 Crumb grain measurements

Two slices of 1.5 cm thickness from each of the 2 breads were cut, so that four slices were used in total for each case. Images of the slices were captured using a flatbed scanner (HP scanjet 4370, HewlettePackard, USA). Image analysis of bread slices was carried out using Image analysis software (ImageProPlus 7, Media Cybernetics, USA). Values of scanned images were obtained in pixels and converted into cm by using known length values. Area of cells, average cell diameter and measured cells/cm were determined. The surface porosity was calculated according to Equation 6:

$$\varepsilon_s = \text{area of cells/total area} \quad (6)$$

D.2.2.3 Statistical analysis

Analysis of variance (ANOVA) was performed using STATGRAPHICS (Centurion XV.II.). LSD test was used for comparison of sample data, and evaluations were based on a significance level of $p < 0.05$. JMP 8 was used for conducting the principal component analysis. The purpose of this analysis is to obtain a small number of linear combinations of the measured variables, which account for most of the variability in the data and to put bread samples in different categories depending on their similarity.

E. Results and Discussion

E.1 Thermal analysis

E.1.1 DSC analysis

E.1.1.1 Carob

E.1.1.1.1 Carob components in relation to moisture content

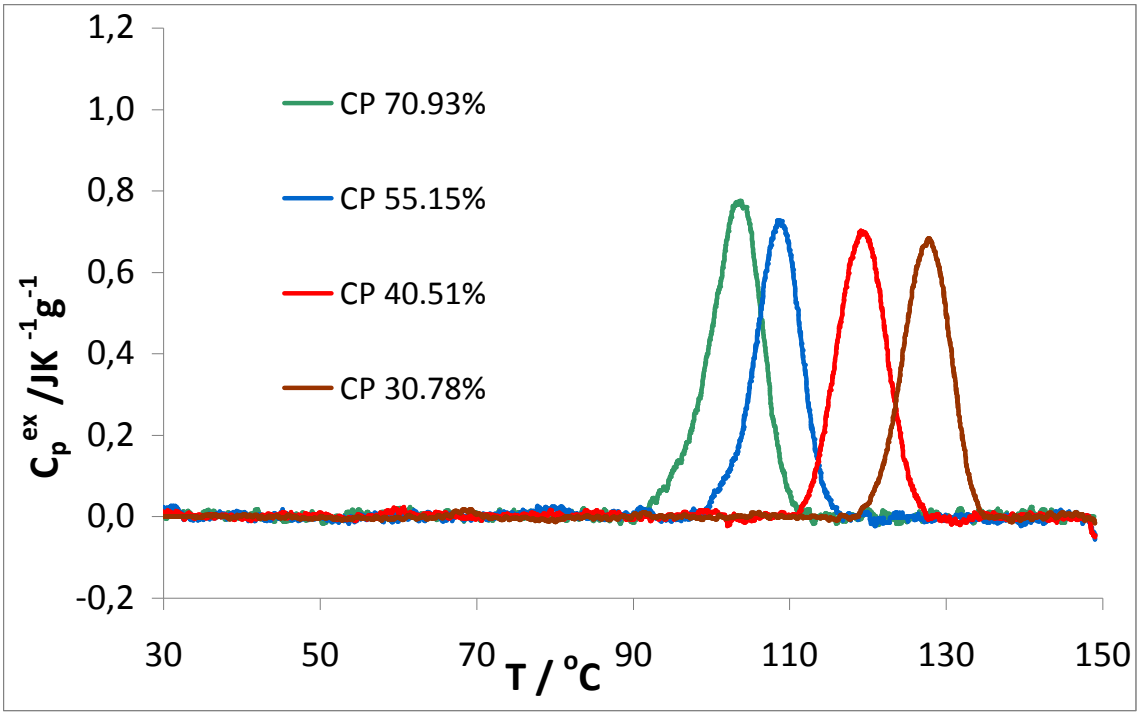


Figure 22: DSC traces of carob protein in different moisture contents.

Carob germ meal or carob protein and LBG, were studied separately in relation to moisture content. LBG does not undergo thermal transitions that are detected by DSC (Data not shown).

Carob protein (CP) denaturation significantly depends on the moisture content (MC) (Fig. 22), showing that humidity of the sample plays an important role in its stability. As the overall water content of the system decreases, the endothermic peaks corresponding to carob proteins' denaturation are shifted toward higher temperature. Denaturation (maximum of the endothermic peak-T_m) occurs at 103.8, 108.4, 119.1, 127.7 ^{o}C at 70.93, 55.15, 40.51 and 30.78% moisture content respectively. DSC trace at 70% water content is consistent with results of Wang et al. (2001) for fully hydrated caroubin as well as those of Bengoechea et al. (2008).

E.1.1.1.2 Carob flour in relation to moisture content

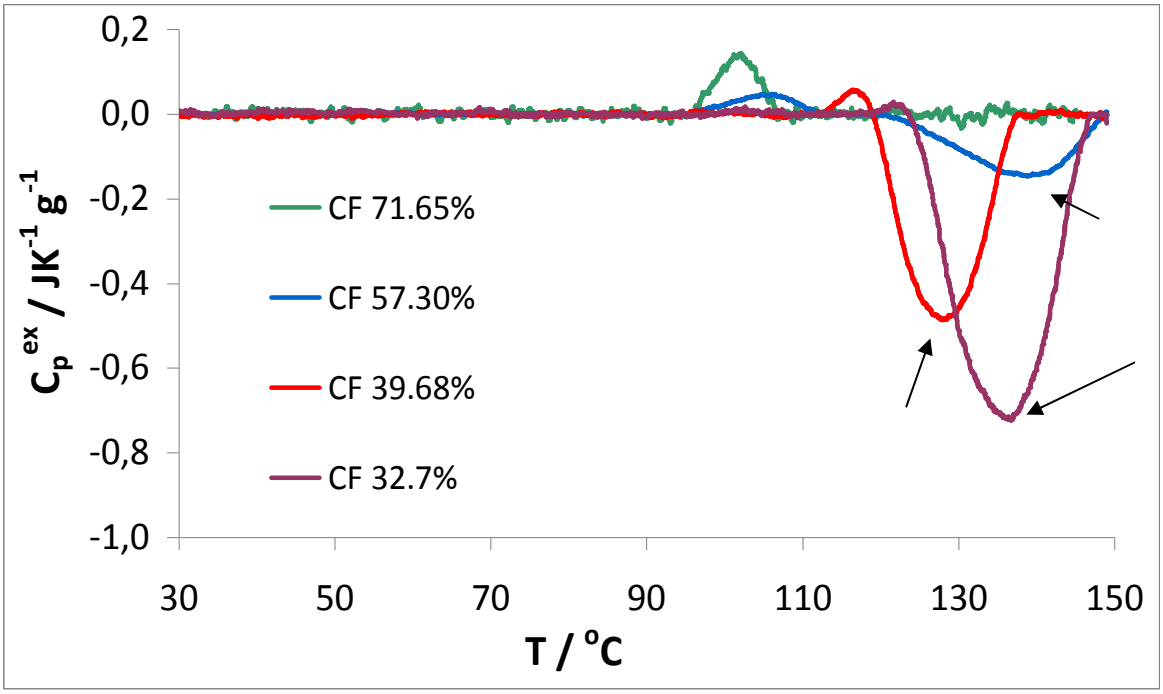


Figure 23: DSC traces of carob flour in different moisture contents.

In Fig.23, the endothermic peaks characterize CP denaturation. Almost the same temperatures are recorded for the proteins' denaturation peak maxima in the DSC trace of carob flour (T_m at 102.1, 106.1, 117 and 121.6^oC for the moisture contents shown in the figure). This indicates that in carob flour (CF - where both LBG and carob protein naturally exist), LBG does not influence the stability of carob protein phase. The areas under the peaks, as it is mentioned, are proportional to the total enthalpy change and they depend on the amount of protein that the sample contains. Peaks of CP denaturation are much lower than peaks of Fig. 22, because carob flour contains about 25% carob germ, from which 64% is protein, in contrast to carob protein sample which contain 64% protein. At 39.68% and 32.7% MC, protein denaturation is immediately followed by an exothermic peak that can be mainly related to the aggregation of the unfolded carob proteins and is noted by the arrows (T_m at 128 and 137^oC, respectively). This exothermic peak is lower in the case of 57.3%, that means that the aggregation is not so intense and does not immediately follow the protein denaturation (T_m at 140 ^oC), whereas it disappears at about 70% MC (Fig. 23).

E.1.1.1.3 Mixed systems of LBG and carob protein

A simulation of carob flour was studied, with much more carob protein than that of carob flour, as the selected LBG:CP ratio was 50:50%. The results are shown in Fig. 24. Specifically, CP's denaturation peaks are higher than those in Fig. 23, since CP has been added in higher proportion. The exothermic peak at around 40% MC is again

evident. It seems that LBG leads to a local concentration of CP inside the mixed system (dough), which then leads to their aggregation at low water contents. Results are summarized in Fig. 25 at ~40% water content. The higher the protein content of the flour the larger the peak height .

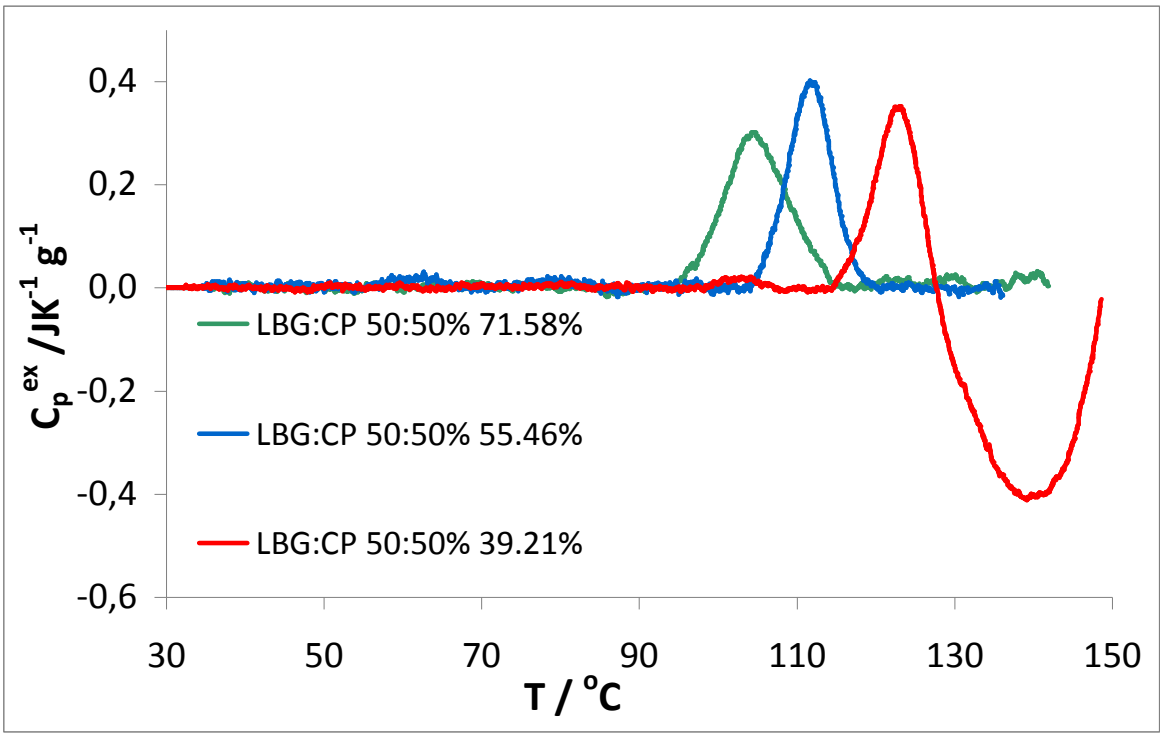


Figure 24: DSC traces of LBG:CP 50:50% in different moisture contents.

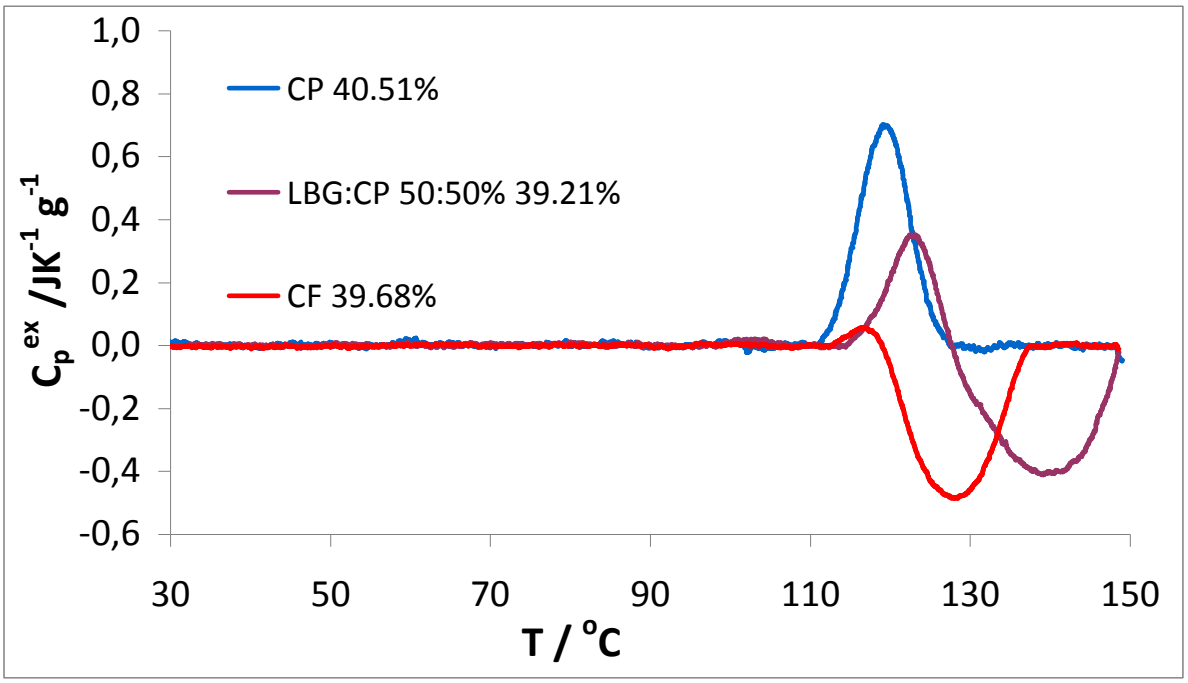


Figure 25: DSC traces of CP, LBG:CP 50:50% and CF at ~ 40% moisture content.

Various ratios of CP and LBG were also examined at around 33% moisture content (Fig. 26), where differences are more striking, because the competition for available water is stronger. Fig. 26 is not worked out with the software IFESTOS. The baseline chosen to work out all the other DSC traces was the DSC record of the immediate re-heating run. In this figure baseline has not been worked out and the row data are presented, allowing one to see the differences. That is why the y-axis is expressed as apparent C_p. In ratio of 60%CP: 40%LBG and 70%CP: 30%LBG, CP aggregation is observed, while in ratios, where there is a bigger percentage of LBG than CP, the aggregation is not observed. Denaturation of proteins occurs in the same temperature (128-130^{o}C), regardless of the amount of added LBG. That means that the phases created by carob proteins and LBG while mixing the dough and baking it, are thermodynamically incompatible; they act as "the one does not see the other".

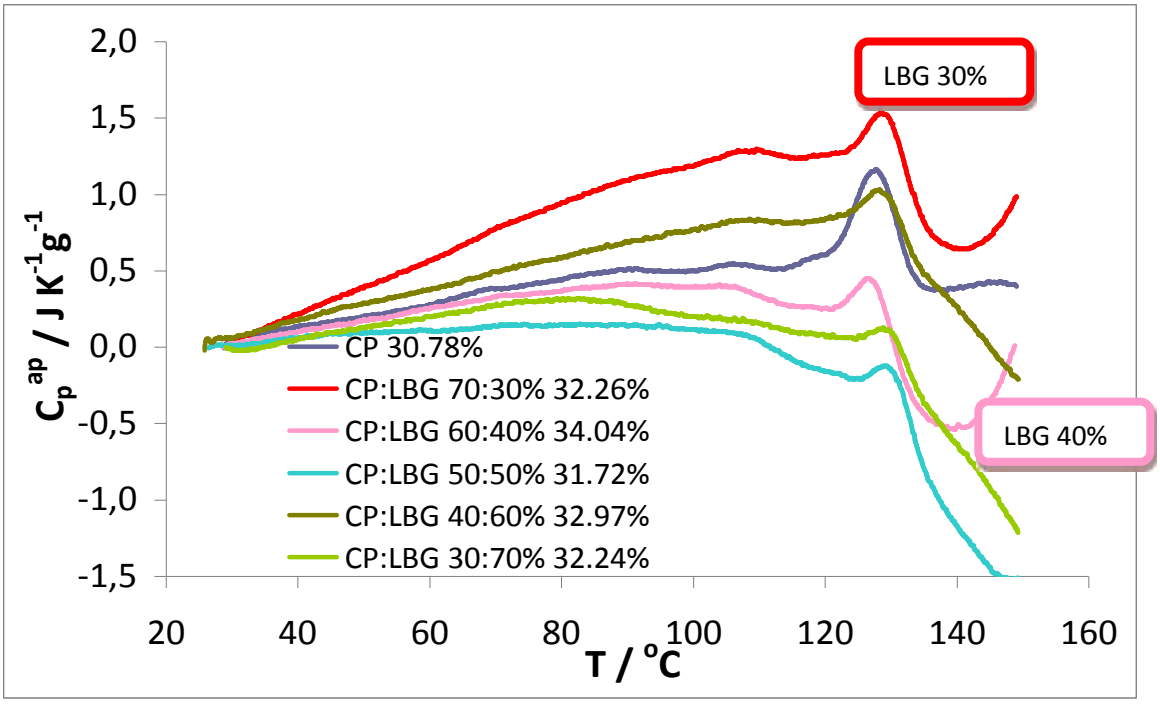


Figure 26: DSC traces of dough samples prepared with various ratios of carob protein and LBG at ~33% moisture content.

E.1.1.2 Mixed systems of carob and wheat flour

E.1.1.2.1 40% moisture content

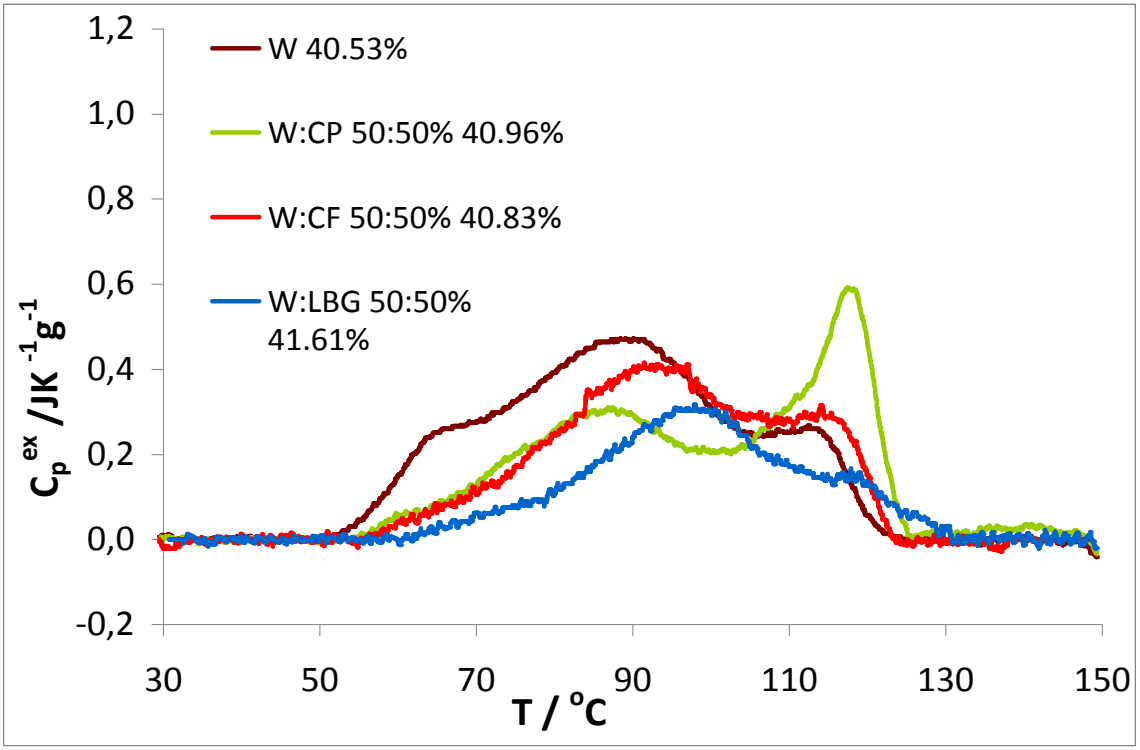


Figure 27: DSC traces of wheat dough sample and its mixed systems at ~ 40% moisture content.

The trace of wheat (W) in Fig.27 shows the trend expected (Fessas et al., 2000). Starch gelatinization process presents 2 endothermic peaks at about 66 and 90°C, followed by a third one at about 116°C, which is related to the decomposition of amylose-lipid complexes. The transition's origin observed on the mixture of wheat:CP at around 118°C is due to the denaturation of proteins. The fact that CP denturation appears at the same T as Fig. 22, indicates the stability of the protein phase in its mixture with wheat flour. It is difficult to determine the exact temperature of amylose-lipid fusion in this curve, because of the overlapping endotherm of the amylose-lipid complex and CP denaturation. In W:CF mixed system the CP denaturation peak is not detectable, because of the low percentage of protein in carob flour.

As far as the gelatinization process is concerned, the 1st gelatinization peak is disappeared in W:CP, W:CF and W:LBG mixed systems. The 2nd gelatinization peak is observed at about the same temperature of that in the trace of wheat, except for the W: LBG mixed system, where it is shifted toward higher temperature (around 99°C).

E.1.1.2.2 55% moisture content

At intermediate moisture content, starch's 2nd gelatinization peak presents a shoulder at around T_m=73 °C. The 1st gelatinization peak again does not appear in the 3 flour

mixtures. One more time, LBG leads to a shift of the second gelatinization peak toward higher temperature (around 80°C). CP denaturation occurs at 110 °C.

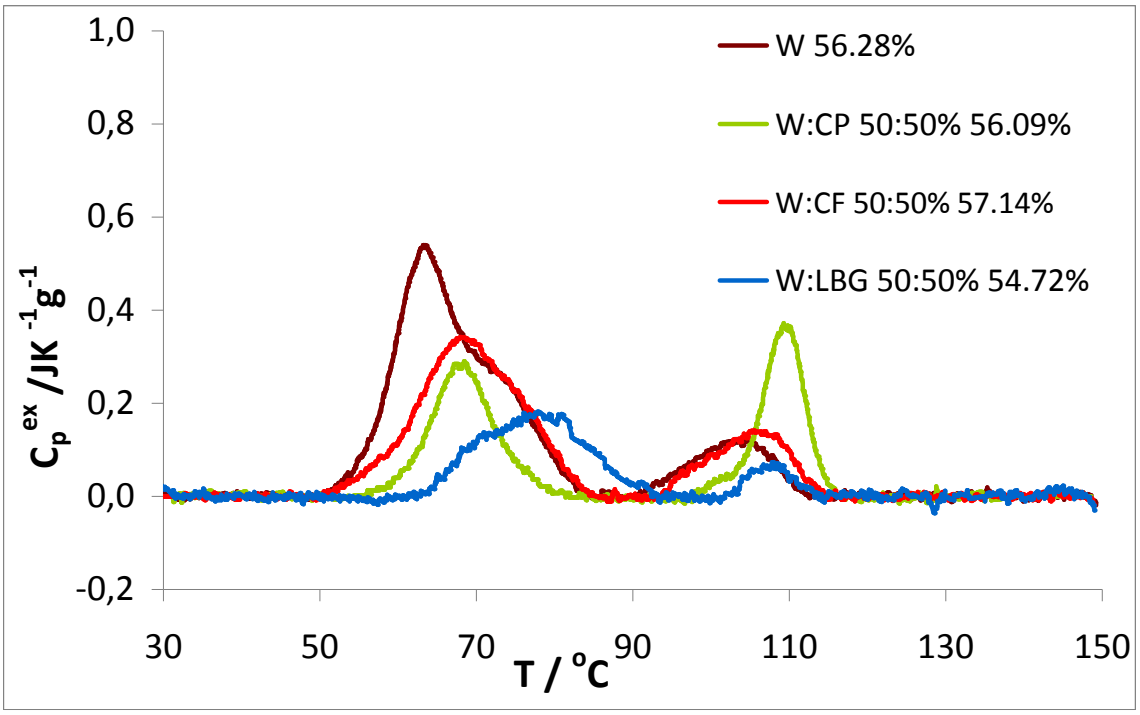


Figure 28: DSC traces of wheat mixed systems at ~55% moisture content.

E.1.1.2.3 70% moisture content

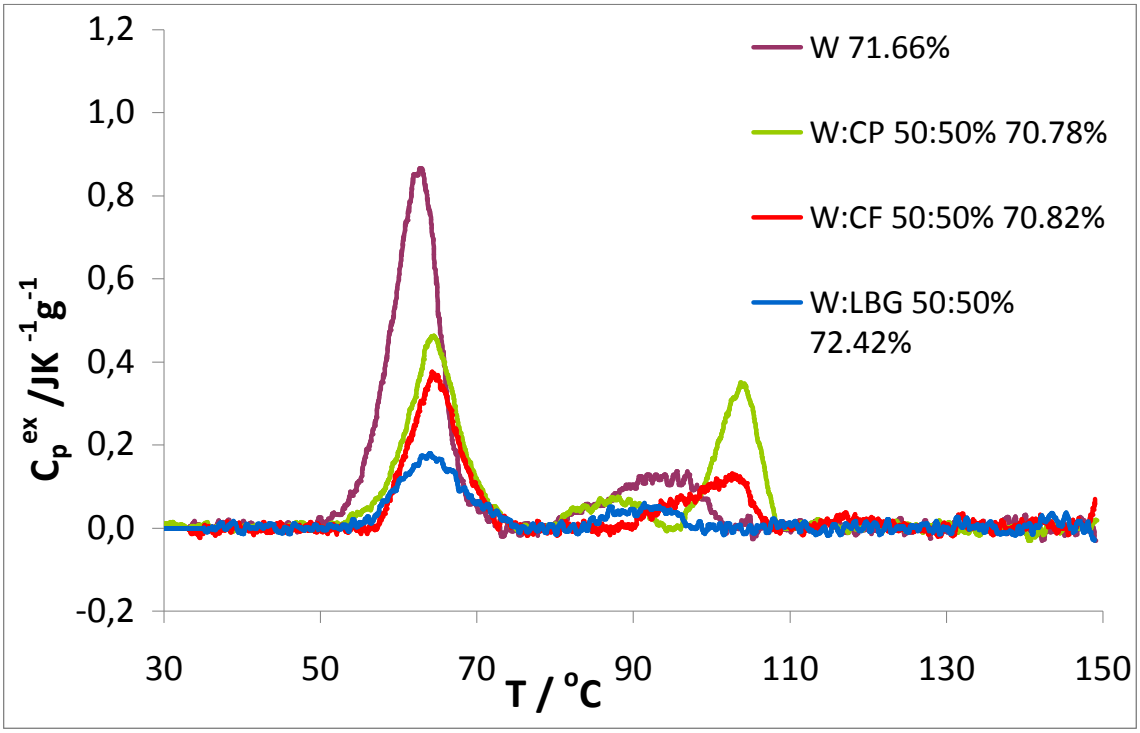


Figure 29: DSC traces of wheat mixed systems at ~70% moisture content.

In the presence of excess water all starch granules can undergo gelatinization in a narrow temperature range. The first peak (63^{o}C) in the sample of wheat is large and sharp, the second (around 94^{o}C) is reduced and the third is split into a couple of components, like Fessas and Schiraldi (2000) have reported.

The peak of CP denaturation is apparent at 104^{o}C in W:CP mixed system. The fact that both peaks (starch gelatinization and protein denaturation) are apparent means that in the constant conditions of DSC measurements, where no water is released, carob proteins and starch behave like thermodynamically incompatible biopolymers.

Due to the excess water the first gelatinization peak of starch is apparent in all mixed systems (Fig. 29) in contrast to ~40% and 55% water content (Fig. 27). However, the area under this peak is decreasing in the following order: W>W+CP>W+CF>W+LBG. Altough gelatinization occurs in the same temperature (63-65^{o}C) for all the samples of Fig. 29, the extent of gelatinization decreases, indicating that when adequate water exists, LBG has still an impact (altough smaller than that at ~40 and ~55% water content) on starch gelatinization.

E.1.1.2.4 Impact of LBG on wheat starch gelatinization

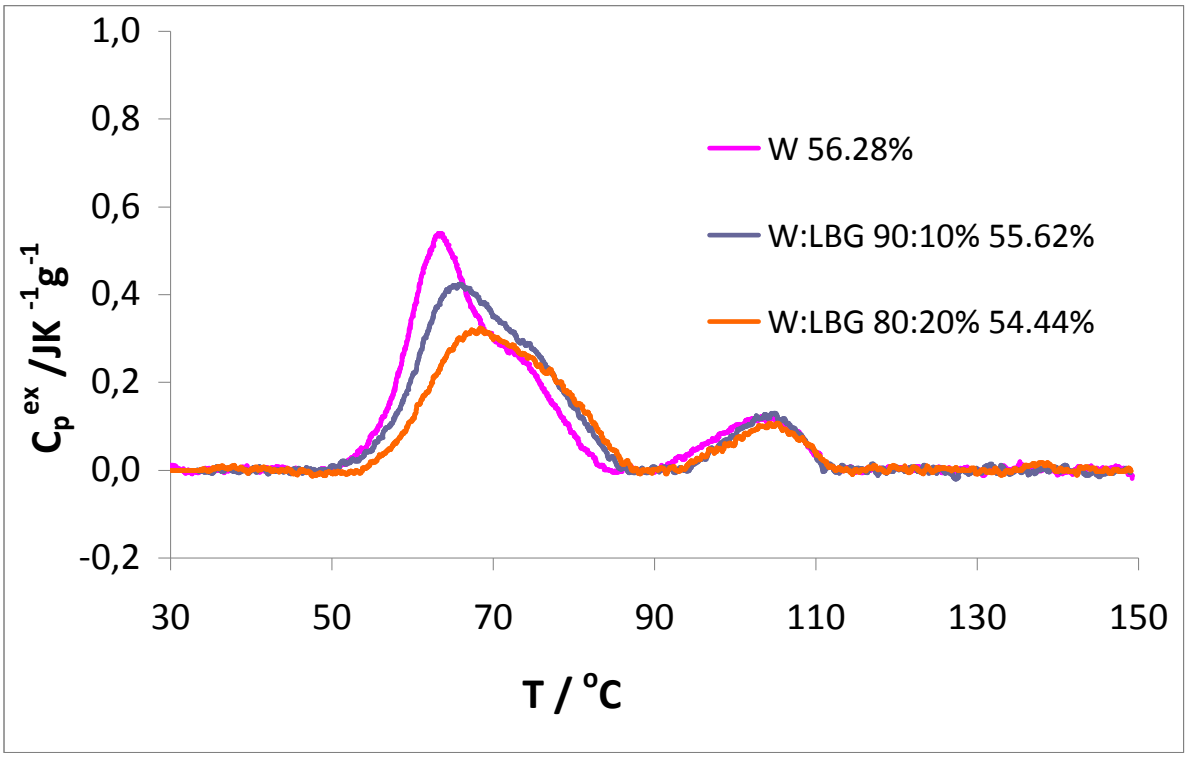


Figure 30: DSC traces of dough made of wheat, wheat 80%:LBG 20% and wheat 90%:LBG10% at ~55% moisture content.

Gradual addition of LBG influences starch gelatinization of wheat flour, as shown in Fig.30. With 10% LBG addition, starch gelatinization occurs, with both gelatinization peaks apparent, but with a smaller area under the peak. Addition of 20% LBG leads to an even smaller aera under the 1st gelatinization peak indicating the bigger influence on wheat starch. This explains why Tsatsaragkou et al. (2012) had to use a high

amount of water in a carob flour-rice flour mixed system in order to be fully hydrated and able to form bread with acceptable quality.

E.1.1.3 Comparison between DSC traces of rice and wheat flour

In Fig. 31 and 32, DSC traces of rice and wheat flour at the same moisture levels are shown. One can notice that the onset of the signal T_0, and the temperature corresponding to the maximum of the first peak are independent on water content, but Tm appears at around 76-79°C in rice flour and 63-66°C in wheat flour.

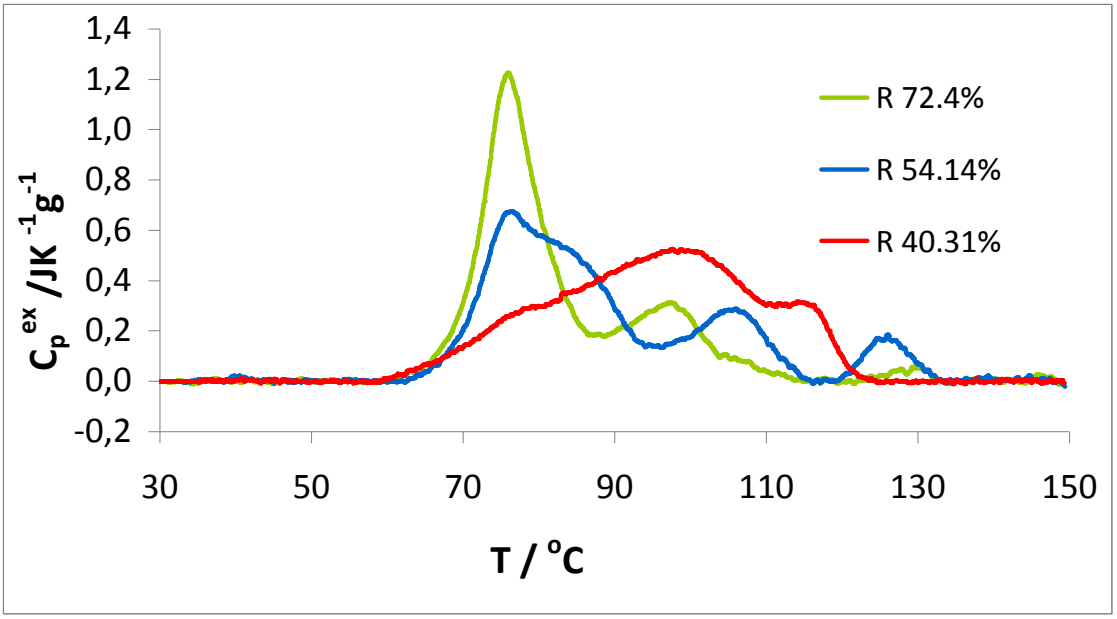


Figure 31: DSC traces of rice flour at three moisture contents.

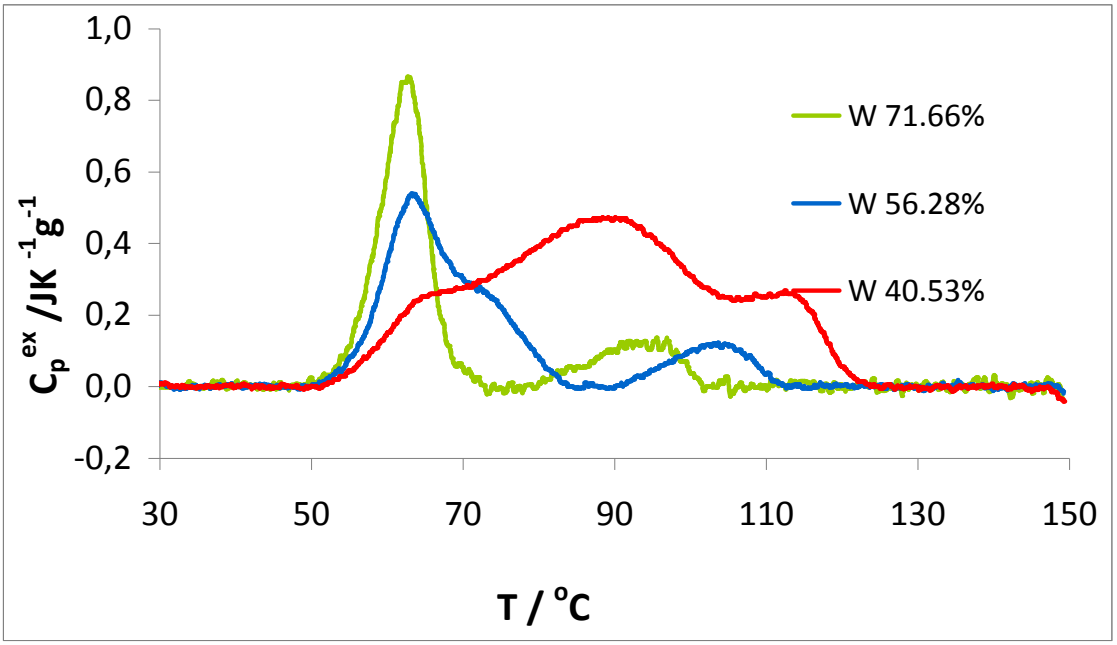


Figure 32: DSC traces of wheat flour at three moisture contents.

Rice flour at around 40% humidity presents 2 endothermic peaks at ~79 and 99°C, respectively due to starch gelatinization, followed by a third one at about 115°C, which is related to the fusion of amylose-lipid complexes. The DSC structure of rice flour is the same with wheat flour at ~55% and ~70% water content. In general, rice's starch gelatinization occurs at a higher temperature than that of wheat (Fig.31).
Another difference except the above, is that one more peak above 120°C other than the peak attributed to amylose-lipid fusion is apparent at ~55% and to a less extent at ~70% moisture content. In fact, Biliaderis et al. (1986b) have suggested that two high-temperature (100-140°C) endotherms have been attributed to amylose-lipid complex. This finding indicates that organization of amylose-lipid complexes occur during gelatinization. Observation of a second amylose-lipid complex was beyond the temperature range of the calorimeter in the study of Normand and Marhall (1989).

E.1.1.4 Mixed systems of carob and rice flour

E.1.1.4.1 40% moisture content

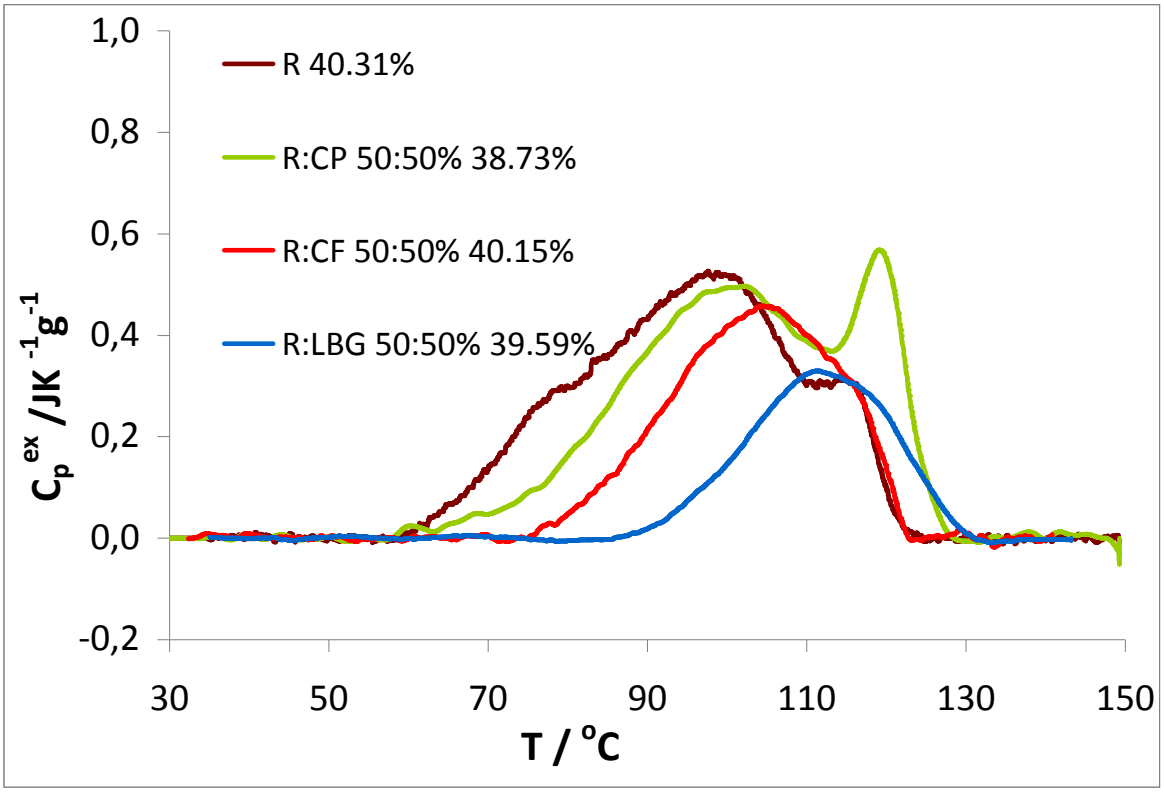


Figure 33: DSC traces of wheat dough and its mixed systems at ~ 40% moisture content.

Results are more or less the same with mixed wheat systems. The first gelatinization peak does not appear in the 3 mixed rice systems in contrast to the rice flour sample (Fig. 33). CP phase stability is one more time obvious (119.1 °C). The 2nd gelatinization peak of rice starch is shifted toward higher temperature in its mixture with LBG (112°C instead of 99°C). LBG phase has a strong impact to starch

gelatinization either wheat's or rice's flour. It seems that gelatinization of rice starch is influenced not only by LBG, but also by carob flour.

E.1.1.4.2 55% and 70% moisture content

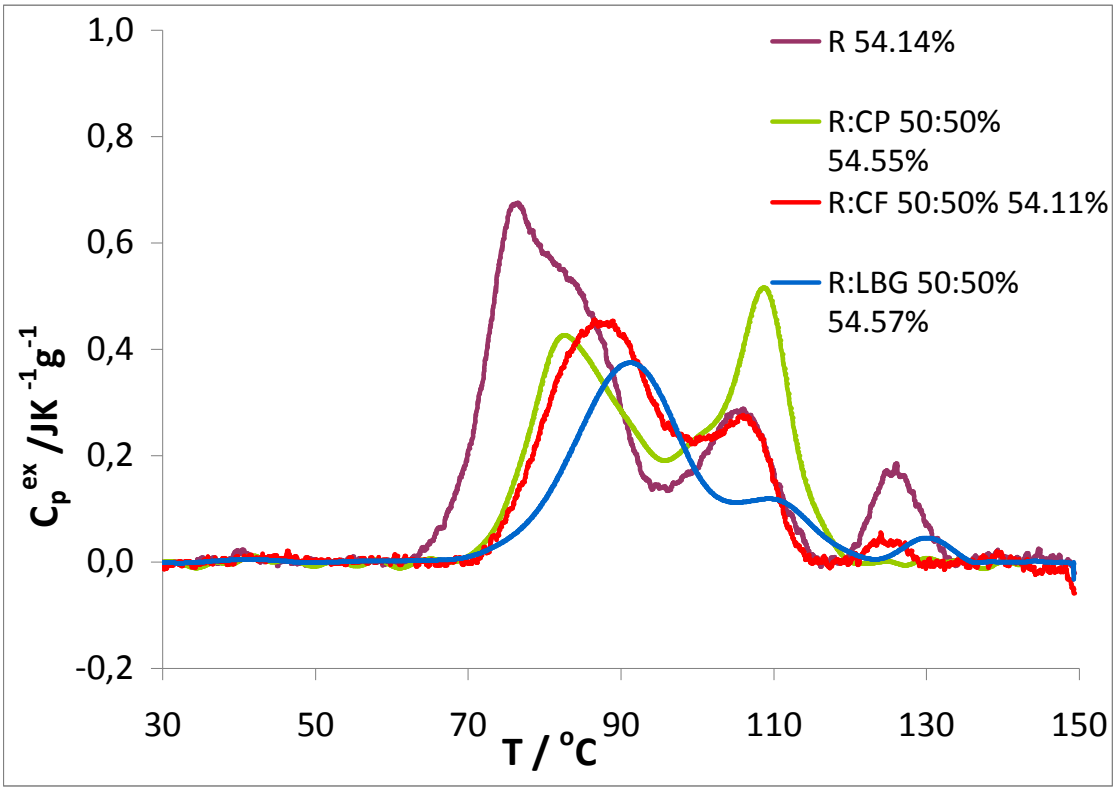


Figure 34: DSC traces of mixed rice systems at ~55% water content.

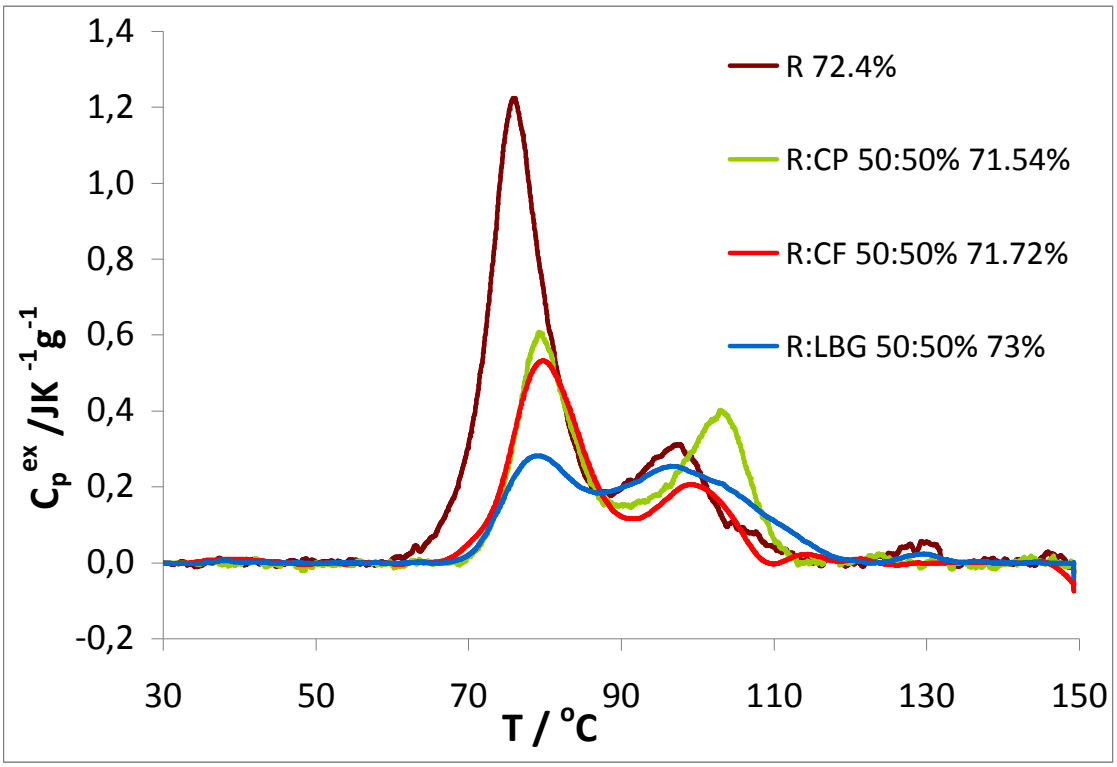


Figure 35: DSC traces of mixed rice systems at ~70% water content.

At ~55 and ~70% water content, results from rice and wheat mixed systems present a lot of similarities (Fig.28, 34 and 29, 35). CP denaturation peaks appear at almost the same temperature with the amylose-lipid complexes, as it was noticed before with wheat mixed systems. Furthermore, the peak that derives from CP denaturation in all water contents appears in the same position, a fact based once again on the incompatibility between CP and rice starch, as it was found to be between CP and wheat starch and between CP and LBG.

E.1.1.5 Carob protein's thermodynamic incompatibility with starch and LBG

As mentioned above, Fig. 36 shows that, CP whether it is in a blend with LBG, wheat flour or rice flour, denaturates almost in the same temperature for every moisture content studied, following the same trend.

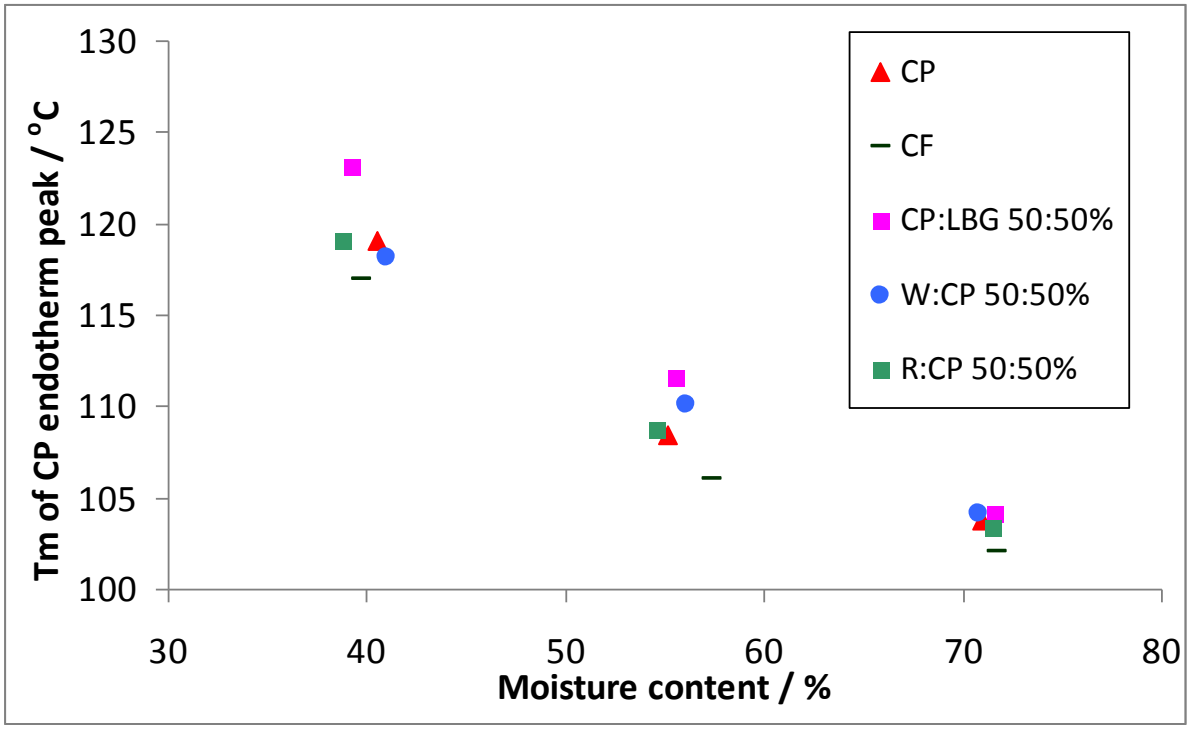


Figure 36:Trend followed by Tm of CP denaturation peaks in relation to water content of the samples. As moisture increases CP denaturation occurs at lower temperature.

E.1.2 TGA analysis

All the TG traces have been normalized to 100 mg water content.

E.1.2.1 Behavior of carob protein in relation to water content

Reminding the general considerations reported in the introduction, that water in a freshly mixed dough is shared between different phases and occupies the inter-phase

regions, it can be said that when dough is heated up, separated phases are still present in the system and water rearranges its partition over the phases, being subjected to different driving forces (Fessas and Schiraldi, 2001). In the DTG trace of the water richer CP dough, the two peaks (98 and 111°C) are closer to one another and it is almost wholly dehydrated at 131°C, while the dough with 38.87% water underwent the fastest release of its tightly bound water, as shown by the second peak of CP. As MC decreases, the phase created by carob proteins releases the water trapped in it at higher temperatures (Fig. 37). This means that carob proteins form a looser "network" with a poorly linked fraction which is able to flash off at lower temperature, when water content is higher. The vaporized water corresponding to the area under the second peaks could be the water released from the structure formed by aggregated carob proteins.

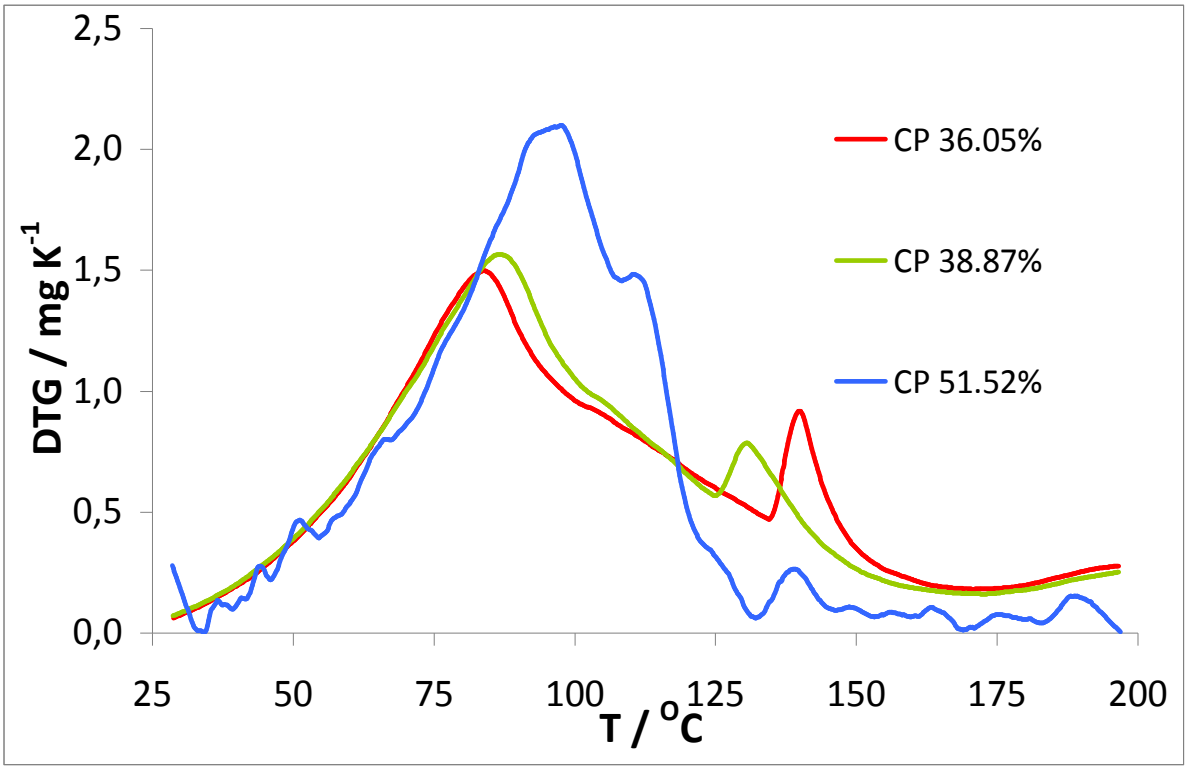


Figure 37: DTG trace of carob proteins in 36%, 38.87% and 51.52% moisture content.

E.1.2.2 40% moisture content

E.1.2.2.1 Mixed system of wheat and carob protein flour at 50:50% ratio

Fig. 38 shows traces with a first broad signal at around 85-87°C, which accounts for the easy to remove water through diffusion process. Water loss rate reaches a maximum at 115°C in the wheat flour sample. This water that is released abruptly above the boiling point is the water tightly bound to gluten network and can be

referred to as structural water, in the sense that it is an element of gluten stucture, according to Fessas and Schiraldi (2001).

The stronger the water is bounded, the highest will be the temperature that water is released. The water is stronger bounded in carob protein than in gluten. It can be observed that wheat dough was almost wholly dehydrated at 131^{o}C, while CP dough underwent the fastest release of its tightly bound water. Furthermore, the area beneath each part of the DTG record corresponds to the relevant amount of vaporized water. In W:CP mixed system, the gluten peak is moved toward lower temperature (inside the broad peak) and the CP peak is smaller, which means that the phase of carob proteins can bind a lower amount of water, but they bind it stronger, since it is released at a higher temperature (142-144 ^{o}C).

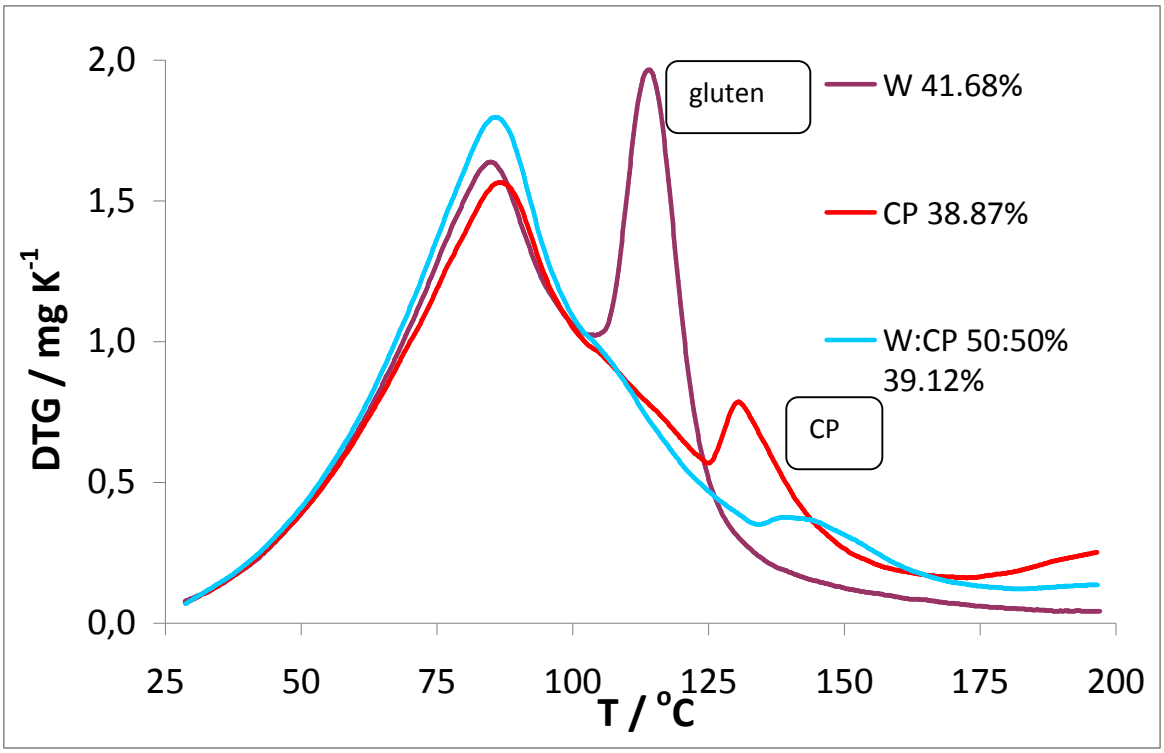


Figure 38: DTG trace of W, CP, W+CP doughs at ~40%moisture content.

E.1.2.2.2 Mixed system of rice and carob protein flour at 50:50% ratio

DTG record of rice flour dough shows a single broad peak. Similar DTG trace was obtained from buckwheat flour, which does not contain gluten (Fessas and Schiraldi, 2008). Carob protein network in its mixture with rice starch, release its water at a lower temperature (119 instead of 131 ^{o}C). The structure formed by carob protein in its mixture with rice flour is related to the looseness of its network. Therefore, this fraction of water is less tightly bound to CP and can be flash off at lower T (Fig. 39).

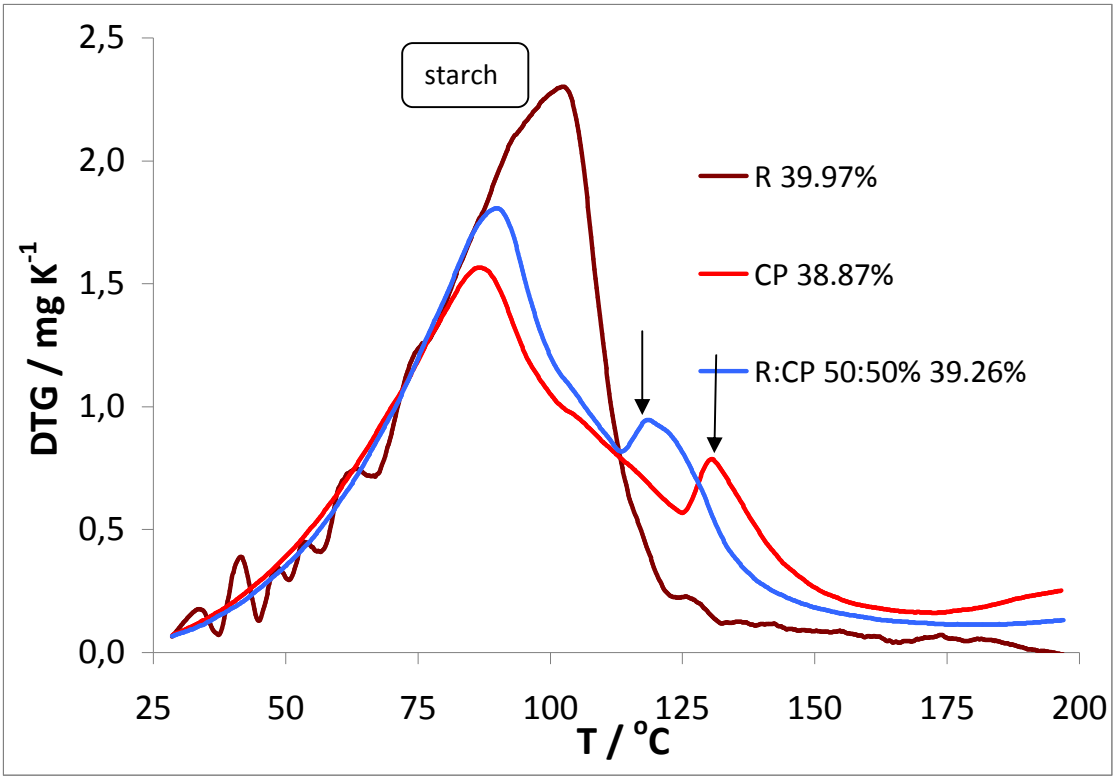


Figure 39: DTG trace of R, CP, R:CP doughs at ~ 40% moisture content.

E.1.2.2.3 Mixed system of wheat and rice flour at 50:50% ratio

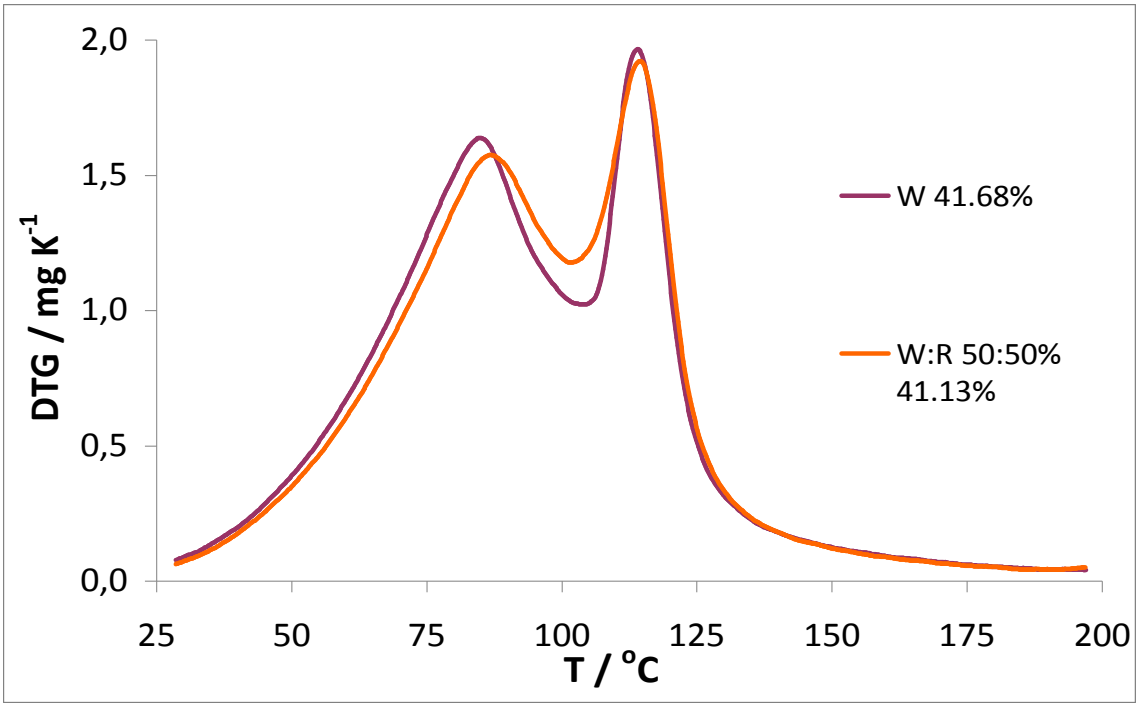


Figure 40: DTG trace at ~41% moisture content of mixed W+R system.

Even in the presence of "double" amount of starch (from both wheat and rice flour), gluten network releases its water in the same temperature (~115 °C), as expected

because of the thermodynamic incompatibility between gluten and starch (Fig. 40) (Fessas and Schiraldi 2001, Grinberg and Tolstoguzov, 1997).

E.1.2.2.4 Mixed systems of wheat: LBG and carob protein: LBG

By observing W:LBG and CP:LBG 50:50% mixtures (both light-blue curves on Fig. 41 and 42), one can notice that LBG has a big impact on both gluten and carob protein binding strength, when it is added at 50% of the mixed system. In this case, both gluten and CP peaks are shifted inside the broad peak (arrows indicate the shift).

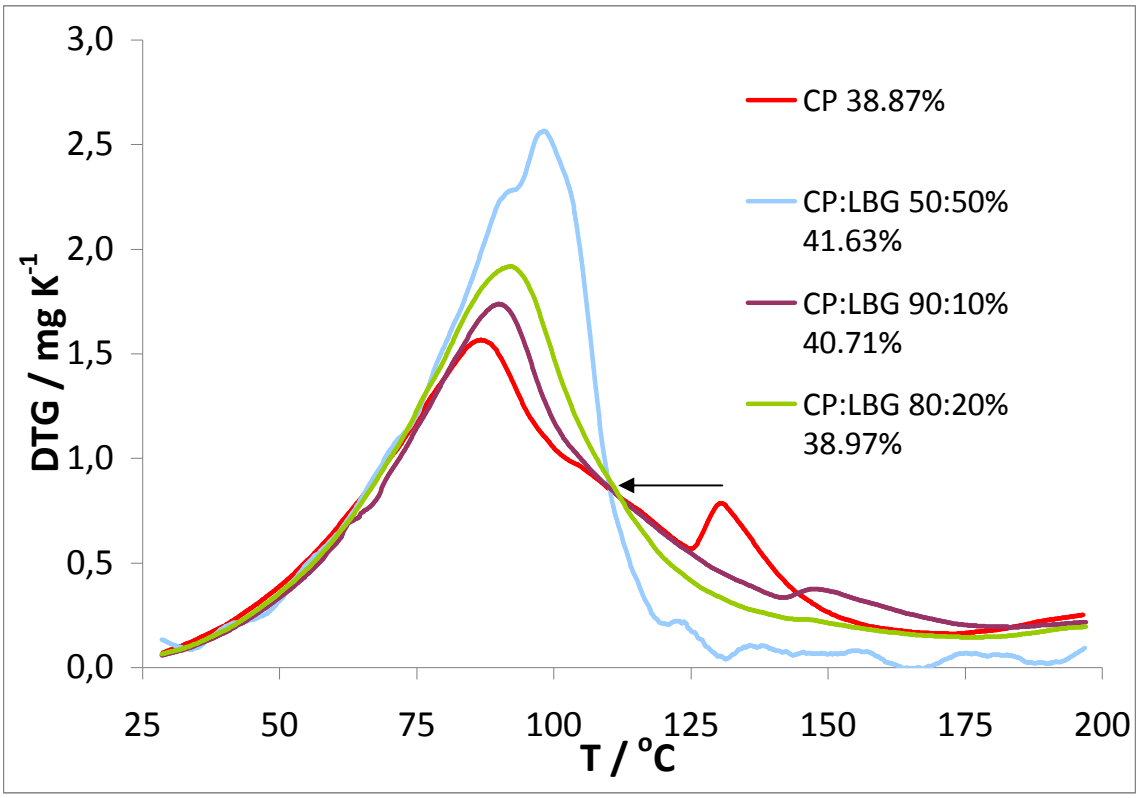


Figure 41: DTG trace of CP, CP:LBG 50:50%, CP:LBG 80:20% and CP:LBG 90:10% at ~ 40% moisture content.

To investigate the role of LBG, different added ratios were examined (Fig. 41, 42). Addition either of 20% or 10% LBG (Fig. 42) has great impact to wheat gluten, because the peak of gluten is swifted toward lower temperature making DTG trace appear again as a single broad peak.

On the other hand, CP phase seems to be more resistant than gluten in releasing its water, when it is in mixture with 10% LBG. Fig. 41 shows that by 10% LBG addition, CP phase binds stronger the water (in contrast to wheat gluten), winning LBG hydrophilic nature, which tends to bind the available water. CP in the mixture binds less water but stronger, releasing it at 150 instead of 131^{o}C. From DSC trace (Fig. 26), denaturated proteins of the W:LBG mixed system have already been aggreagated at this temperature. Perhaps this structure of aggregated carob proteins have the ability to bind the water and release it at high T. As LBG ratio increases (20% - green

curve Fig.41), carob proteins start to lose their resistance to bind water and the DTG trace is transformed again to a single broad peak

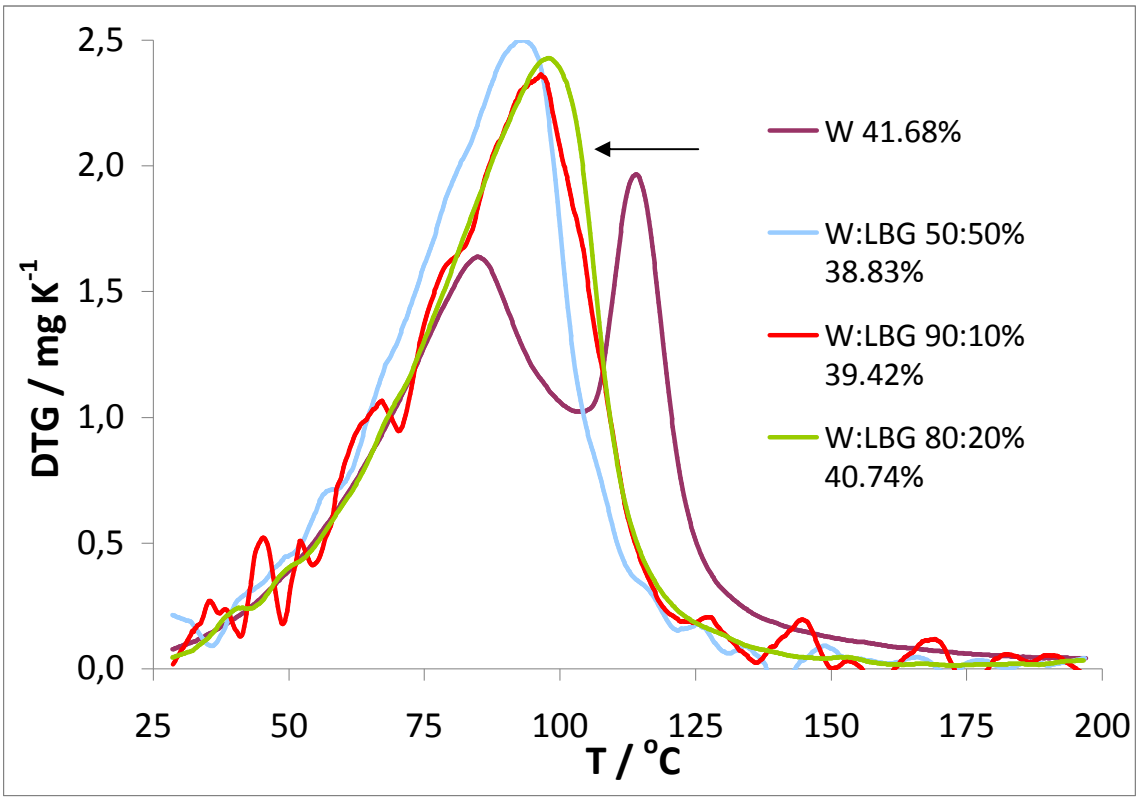


Figure 42: DTG trace of W, W:LBG 50:50%, W:LBG 80:20% and W:LBG 90:10% at ~ 40% moisture content.

E.1.2.3 Mixed system of wheat flour and LBG at 55% moisture content

Ratios of Fig. 42 were also studied at ~55% moisture content and results where quite different confirming that the overall dough moisture can modify water partition between phases and the way water is released. The trace of wheat (Fig. 43) shows the trend expected (Fessas and Schiraldi, 2001). The higher the sample moisture, the smaller the gap between the first and the second peak and the looser is the gluten network (its maximum water release at 107°C). At ~55% moisture content, where there is more available water for both LBG and gluten phases, it seems that the structure formed by gluten binds stronger the water than at ~40%, as LBG decreases (Fig. 42). This is the reason why 2 peaks which are referred to the water release from the gluten network are observed in the blue and green curve. The high-T peak is moved toward lower T as LBG addition increases (133, 124, 106 °C).

The dough recipe is modified by the addition of hydrophilic LBG. Its presence within the gluten network affects the gluten structure, reducing also its trapping strength for water. This phenomenon is more profound at ~40% moisture content than at ~55%, where gluten network is found to be more resistant to LBG addition.

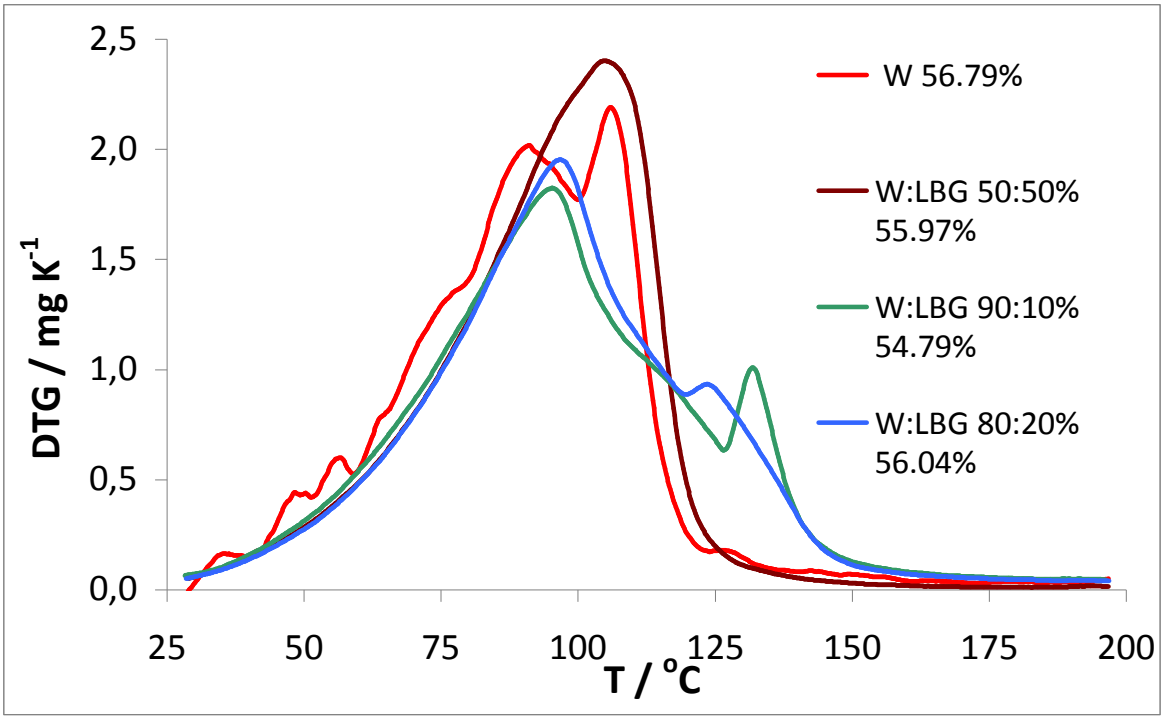


Figure 43: DTG trace of W, W:LBG 50:50%, W:LBG 80:20%, W:LBG 90:10% at ~ 55% moisture content.

E.2 Bread making

E.2.1 Yield in baked products

The yield in baked wheat breads is 82-83% and in rice breads 75-79% with the breads with 4% LBG representing the highest yield. The moisture loss is a measure of the water absorbing capacity of the bread, ascribed to the water holding capacity of bread's ingredients, that is carob protein and locust bean gum (Tsatsaragkou et al., 2012).

E.2.2 Bread moisture

Moisture content (MC) of wheat bread crumbs varies from 38.5 to 41% without presenting a statistically significant difference. Rice breads show a higher MC varying from 50 to 54.5%, because of the higher initial water content of the dough. Addition of 2 and 4% LBG in rice breads leads to a higher crumb moisture, because of the hydrocolloid's nature to bind water. Moisture content of rice bread crumb with 5% carob protein shows also higher water content in relation to control, without statistically significant difference (Fig. 44).

Results from measuring moisture content of bread crusts show that it varies from 19 to 22% and 19 to 25% for wheat and rice breads respectively. In both cases, crust moisture gradually increases from control bread to bread with 4% LBG (Fig. 45)

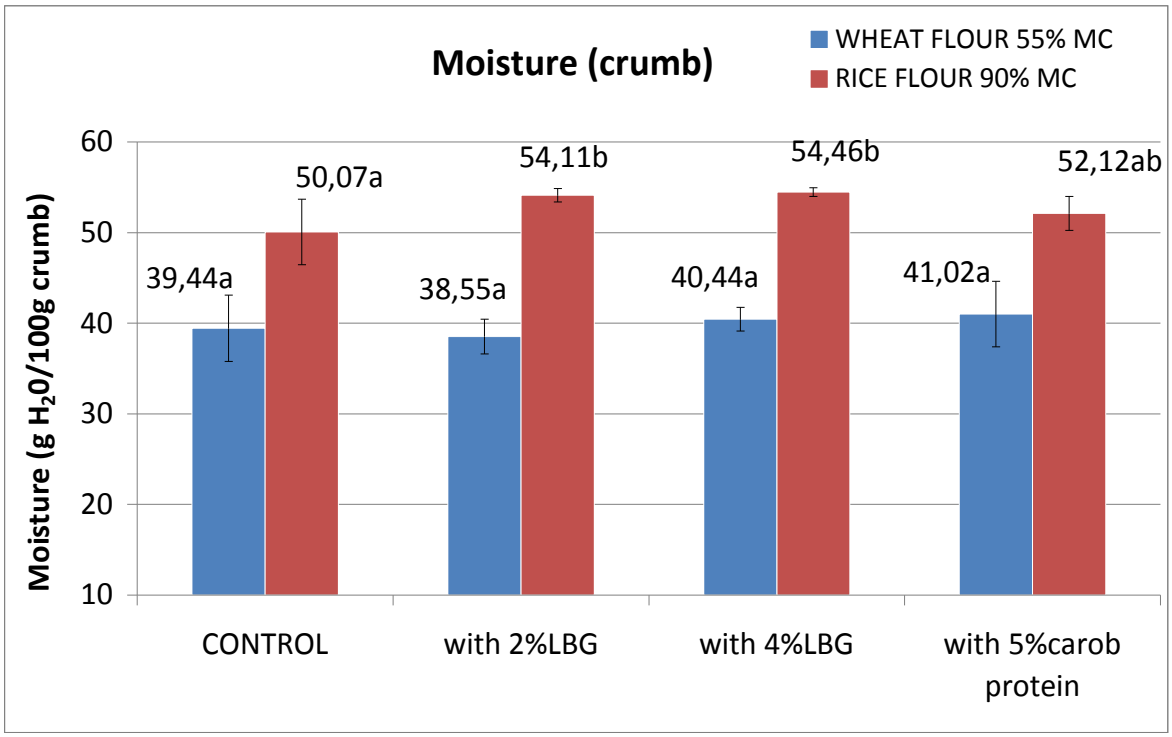


Figure 44: Crumb moisture content of the end products. Values are the averages of two replicates and error bars represent standard deviation.

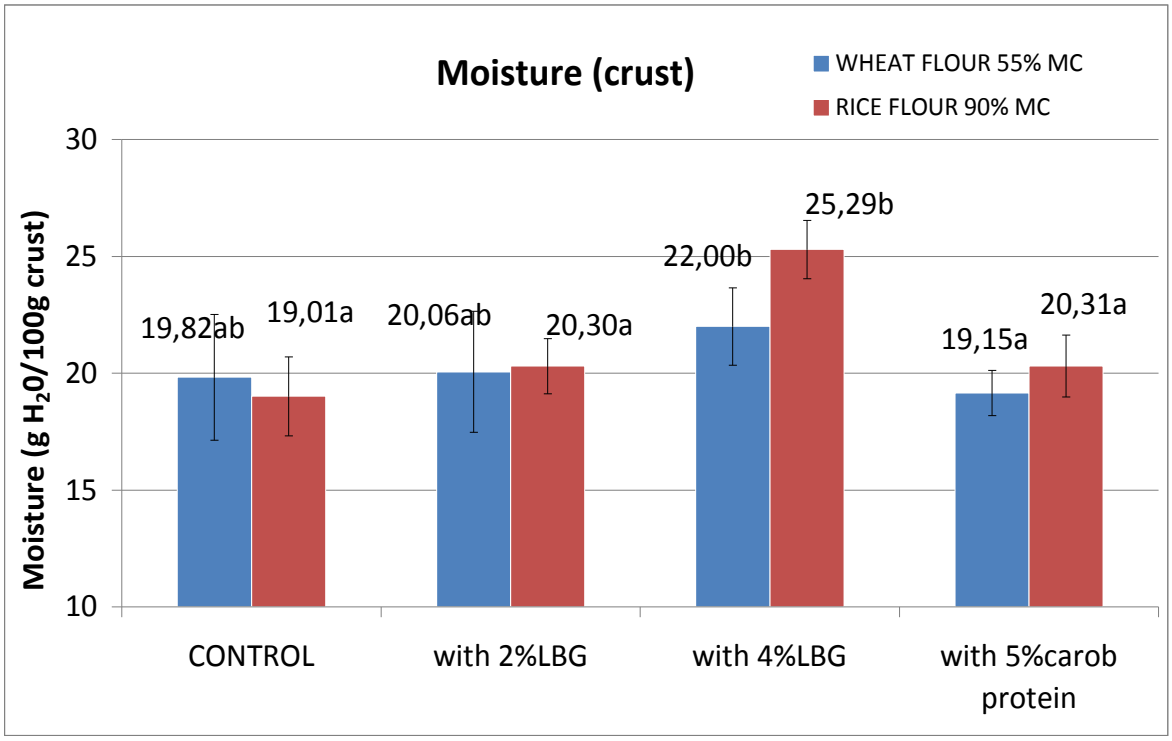


Figure 45: Crust moisture content of the final products.

Table 8 shows in detail the results of bread moisture contents, as far as means, standard deviation and experiment repeatability is concerned.

Table 8: Crumb and crust water content.

RICE	WATER CONTENT - CRUMB (g H_2O/100g crumb)			WATER CONTENT - CRUST (g H_2O/100g crust)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	50,07	3,63	7,26	19,01	1,69	8,89
with 2%LBG	54,11	0,75	1,39	20,30	1,18	5,82
with 4%LBG	54,46	0,49	0,90	25,29	1,24	4,91
with 5%carob protein	52,12	1,89	3,62	20,31	1,32	6,52

WHEAT	WATER CONTENT - CRUMB (g H_2O/100g crumb)			WATER CONTENT - CRUST (g H_2O/100g crust)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	39,44	3,66	9,28	19,82	2,69	13,56
with 2%LBG	38,55	1,92	4,98	20,06	2,59	12,89
with 4%LBG	40,44	1,32	3,27	22,00	1,66	7,53
with 5%carob protein	41,02	3,61	8,80	19,15	0,97	5,07

E.2.3 Texture analysis

Table 9 shows the results of crumb firmness and relative elasticity.

Table 9: Crumb firmness and relative elasticity of wheat and rice breads.

RICE	COMPRESSION-MAX LOAD(N)			RELATIVE ELASTICITY(%)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	47,96	2,43	5,07	30,01	7,07	23,57
with 2%LBG	20,56	8,16	39,70	32,86	7,23	21,99
with 4%LBG	49,66	0,95	1,92	34,06	12,52	36,77
with 5%carob protein	48,77	1,29	2,65	48,17	2,20	4,56

WHEAT	COMPRESSION-MAX LOAD(N)			RELATIVE ELASTICITY(%)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	15,09	5,56	36,87	46,45	5,96	12,82
with 2%LBG	20,81	11,77	56,60	43,27	1,90	4,40
with 4%LBG	27,97	7,42	26,52	50,93	4,19	8,23
with 5%carob protein	12,71	1,03	8,12	43,58	1,22	2,80

In crumb firmness measurements, a slice of bread is compressed and the force necessary to achieve the preselected compression is recorded. The greater the force, the harder is the texture of the bread.

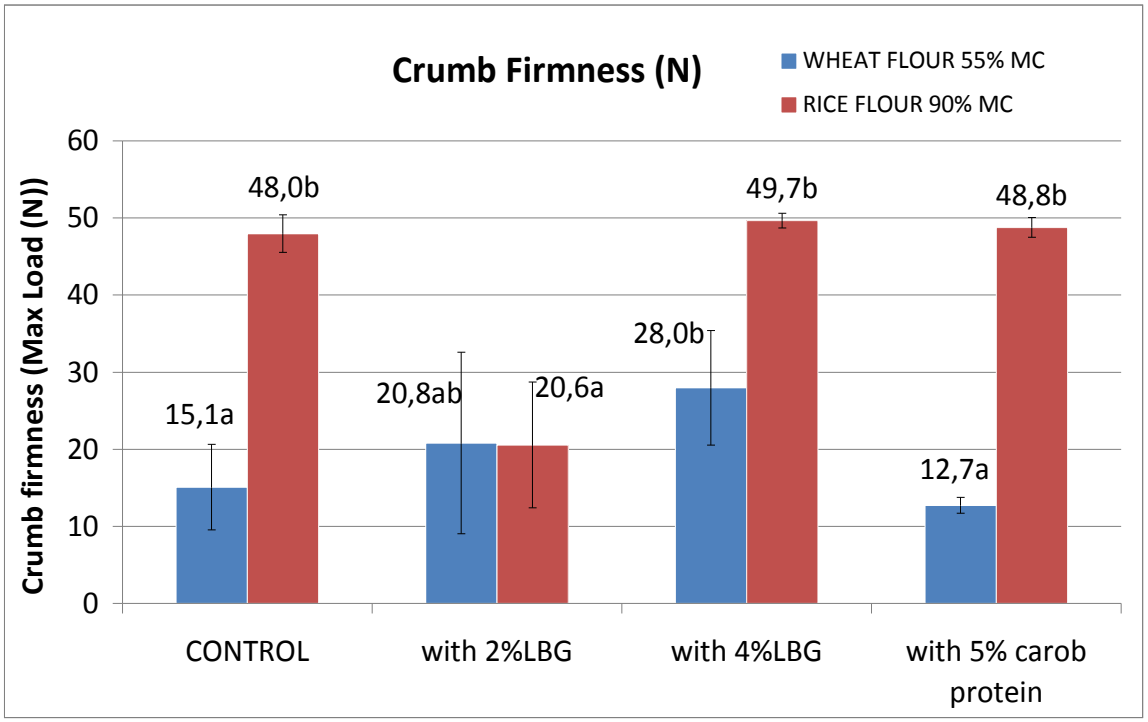


Figure 46: Influence of different ratios of carob ingredients on crumb firmness.

Fig. 46 shows that addition of 4% LBG gives firmer bread crumb in both rice and wheat breads. Bread firmness gradually increases with the addition of LBG in wheat breads, in contrast to rice breads, where addition of 2% LBG leads to lower crumb firmness, because it was fractured along the inside of the crust (detachment of the crumb from the crust- Fig. 52). The dough made of rice flour and 2% LBG may have fermented to the same size with that containing 4% LBG, but during oven spring its ability to produce a bread of satisfactory loaf volume was lost.

The results of wheat breads are consistent with those of Schwarzlaff et al. (1996), where 4% LBG replacements produced firmer textures compared with the other treatments. The greater the concentration the more viscous the gum becomes producing gumminess, which in turn may have actually produced a firmer texture. Also, it is found that he combination of starch and hydrocolloids can lead to a considerable viscosity increase (Closs et al., 1999).

Hard texture and compact structure, as well as general appearance scores seem to be the reasons that lead consumers to indicate carob germ bread as the least preferred in contrast to other legume breads (Miñarro et al., 2012). Also microscopy of carob germ breads showed a compact structure without spaces between starch granules. However, in this study comparing the control with the 5% carob protein bread, breads with 5% carob protein remained as soft as the standard recipe crumb (no statistically significant difference).

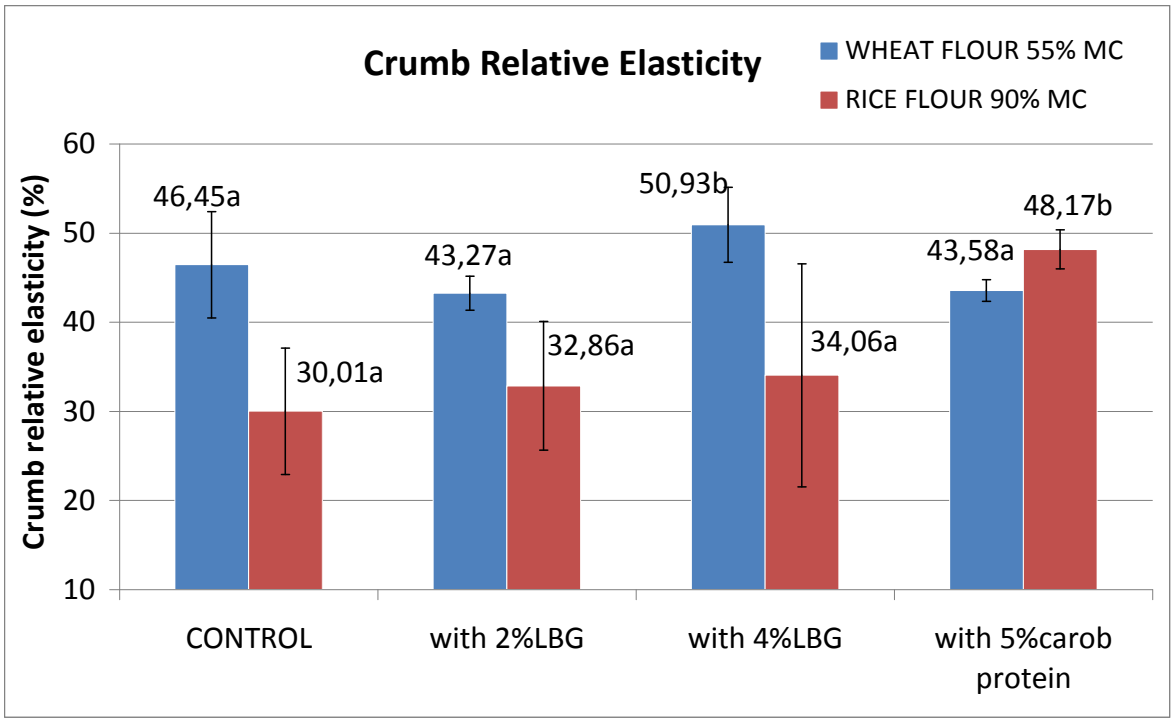


Figure 47: Influence of different ratios of carob ingredients on crumb relative elasticity.

In Fig. 47 it can be observed that wheat bread with 4% LBG and rice bread with 5% carob protein present the greatest elasticity. Carob's ingredients like LBG and CP can lead to a more elastic crumb and similar results were also found by other researchers (Wang et al., 2001; Tsatsaragkou et al., 2012).

E.2.4 Crumb structural characteristics

E.2.4.1 Total porosity and Surface porosity

Porosity is an important attribute for bread that significantly affects consumer's acceptance. As shown in Fig. 48, total porosity is influenced by LBG addition. Porosity is increased with increase of LBG to 2%, but is reduced with addition of 4% LBG. Carob protein addition up to 5% does not seem to have an important effect to bread porosity. It is also obvious, that rice breads present lower total porosity than wheat breads, with control and bread with 5% CP to reach almost the half of the value of wheat bread total porosity.

The results are consistent with the research of Fessas and Schiraldi (1998), who found that if the dough water is bind to hydrophilic non-gluten compounds, like arabinoxylans, (or the hydrophilic component locust bean gum in this case) it can neither allow adjustments of the polymer chains necessary to the extension of cross-linking, nor form an extended liquid film over the alveolar walls. This makes the gluten network to loose and fermentation gases can expand more easily yielding a coarse alveolar structure. Wheat bread with 2% LBG presents the lower surface porosity contrary to what happens with total porosity. In rice breads, the greatest surface porosity appears in addition of 2 and 4% LBG (Fig.49).

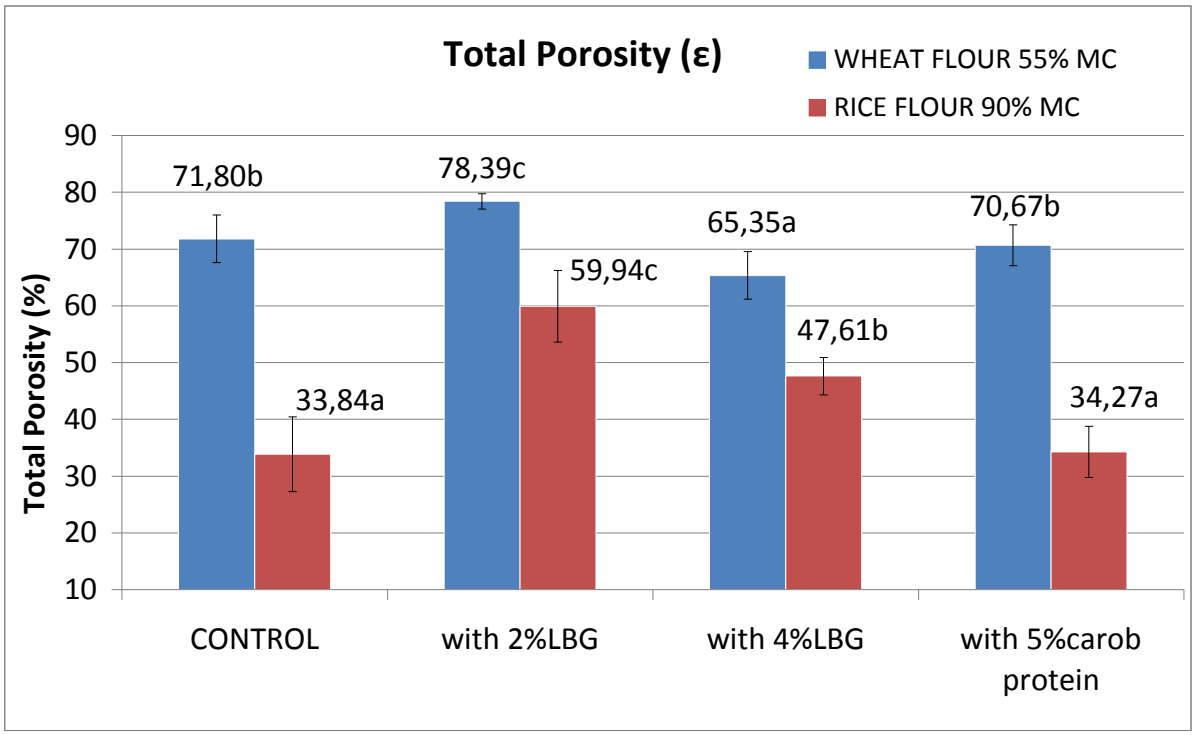


Figure 48: Influence of different ratios of carob ingredients on crumb total porosity.

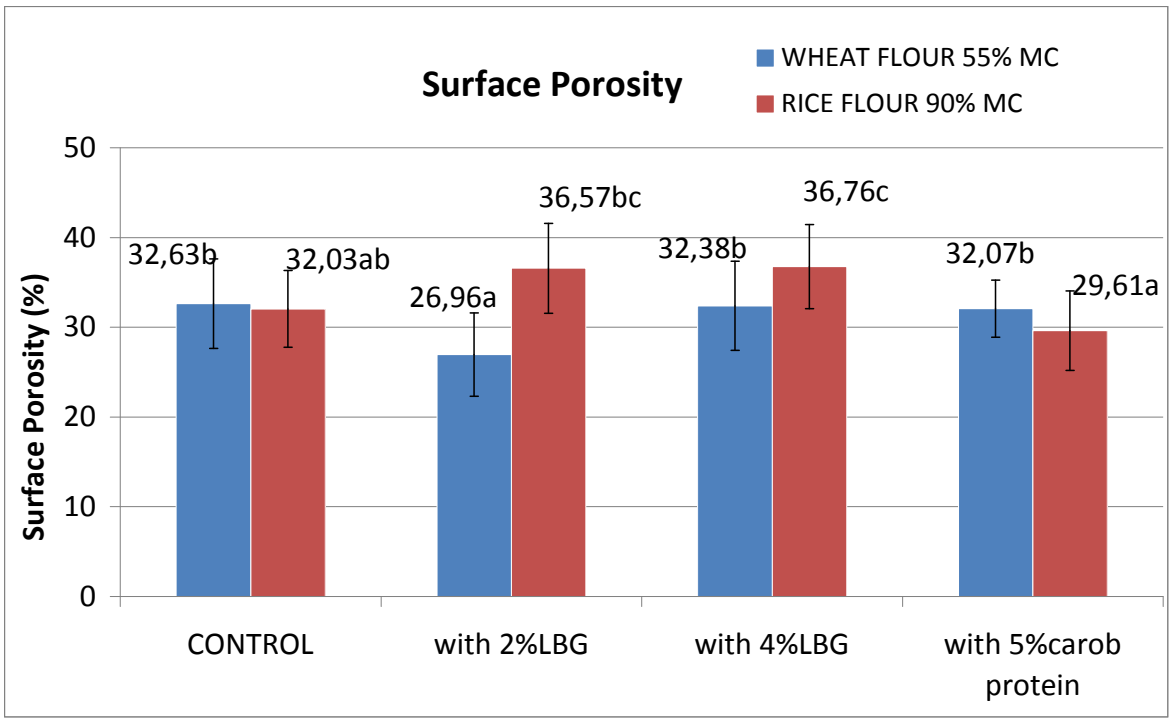


Figure 49: Influence of different ratios of carob ingredients on crumb surface porosity.

E.2.4.2 Number of crumb cells per cm and average cell diameter

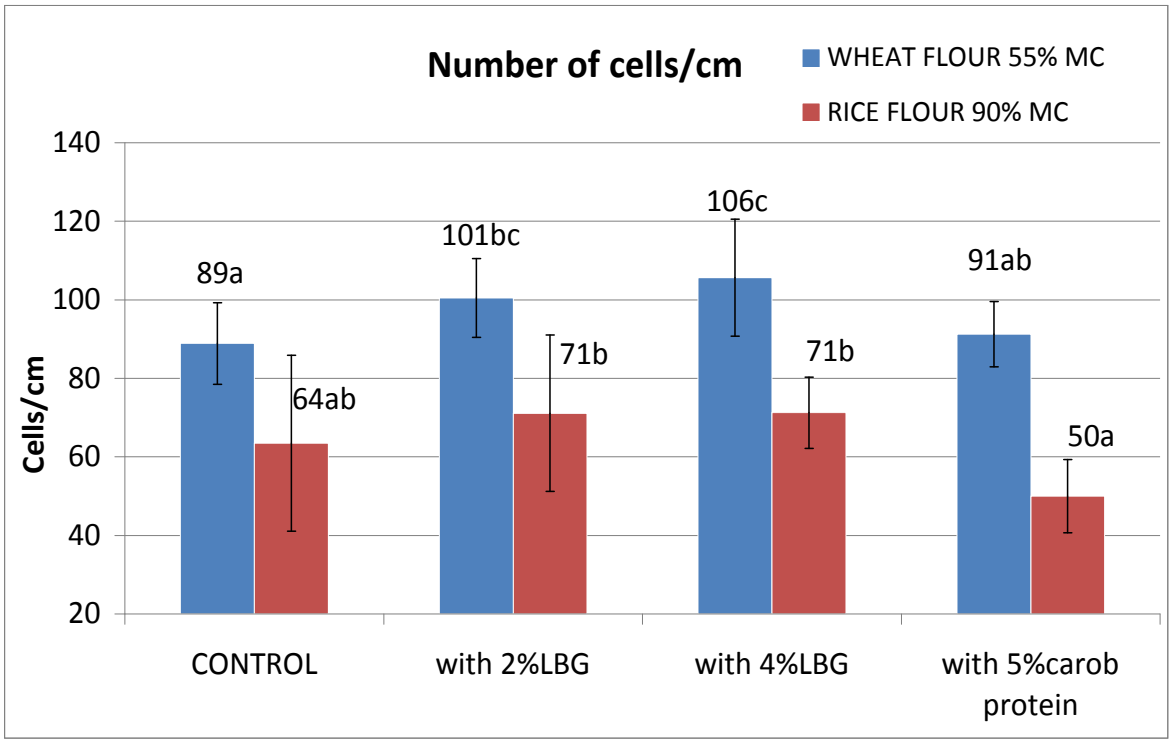


Figure 50: Number of cells/cm of bread crumb.

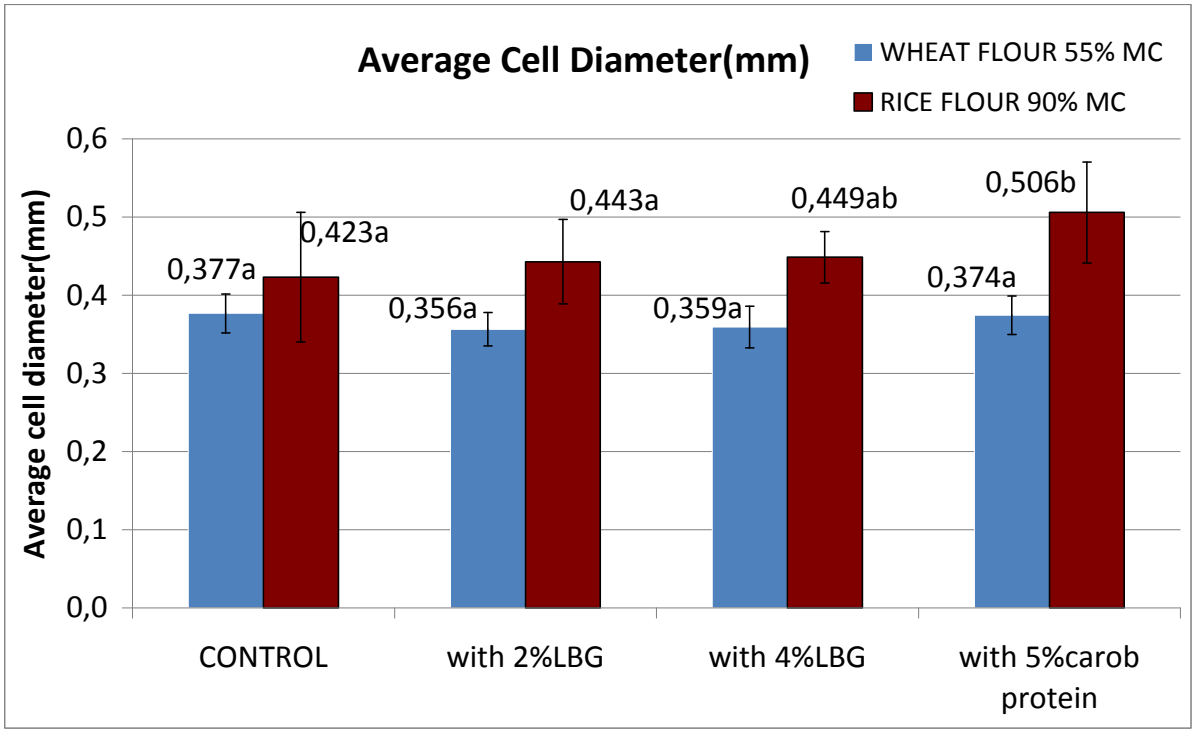


Figure 51: Average cell diameter in bread crumb.

In Fig. 50 and 51 one can see that, LBG addition leads to an increase of cells/cm of bread crumb, which doesn't differ significantly between 2 and 4% both in wheat and rice breads. Average cell diameter does not differ significantly between wheat breads,

but in general it decreases as the number of cells increases. Rice breads present lower number of cells/cm than wheat breads, but higher average cell diameter, which can also be observed in Fig. 52-54. Rice bread with 5% carob protein has the lowest but biggest cells. Table 10 presents the results from the crumb structural characteristics, while in Fig. 52, 53 and 54 images from bread slices of first and second replicate of bread making are presented. Photos of breads are not identical between the 2 replicates, as it is expected. There are some ingredients like yeast, which are inherently variable. Also, a baking procedure involves many steps and manipulations, some of these, such as molding are difficult to do in an exactly reproducible manner (MacRitchie, 1984).

Table 10: Crumb structural characteristics of wheat and rice breads

RICE	POROSITY(%)			SURFACE POROSITY(%)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	33,84	6,60	19,51	32,03	4,28	13,35
with 2%LBG	59,94	6,31	10,52	36,57	5,01	13,70
with 4%LBG	47,61	3,32	6,98	36,76	4,69	12,75
with 5%carob protein	34,27	4,53	13,21	29,61	4,43	14,95
	Cells/cm			Average Cell Diameter(mm)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	64	22	35,23	0,423	0,083	19,56
with 2%LBG	71	20	27,96	0,443	0,054	12,19
with 4%LBG	71	9	12,70	0,449	0,033	7,33
with 5%carob protein	50	9	18,67	0,506	0,065	12,77

WHEAT	POROSITY(%)			SURFACE POROSITY(%)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	71,80	4,18	5,82	32,63	5,00	15,32
with 2%LBG	78,39	1,38	1,76	26,96	4,65	17,26
with 4%LBG	65,35	4,19	6,42	32,38	4,97	15,35
with 5%carob protein	70,67	3,61	5,11	32,07	3,20	9,97
	Cells/cm			Average Cell Diameter(mm)		
	Average	STDEV	RSDr (%)	Average	STDEV	RSDr (%)
CONTROL	89	10	11,72	0,377	0,025	6,60
with 2%LBG	101	10	9,99	0,356	0,021	5,94
with 4%LBG	106	15	14,09	0,359	0,027	7,40
with 5% carob protein	91	8	9,09	0,374	0,025	6,58

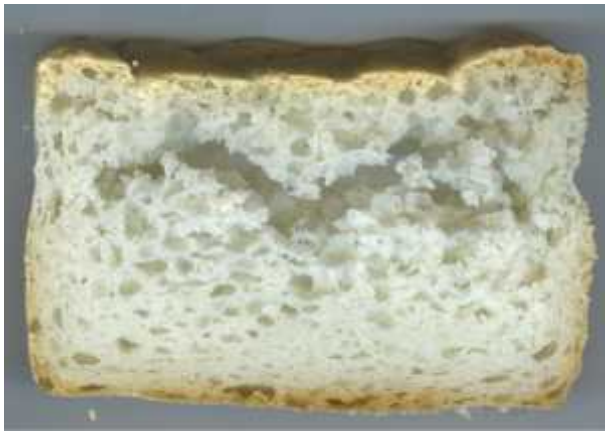
1a: rice control

2a: rice control

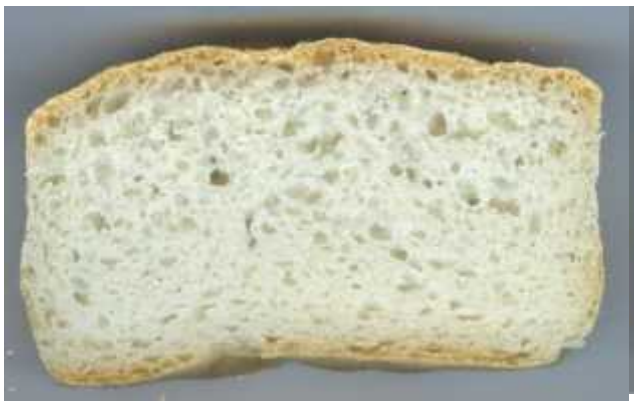
1b: rice- 5%carob protein

2b: rice-5%carob protein

1c: rice-2%LBG



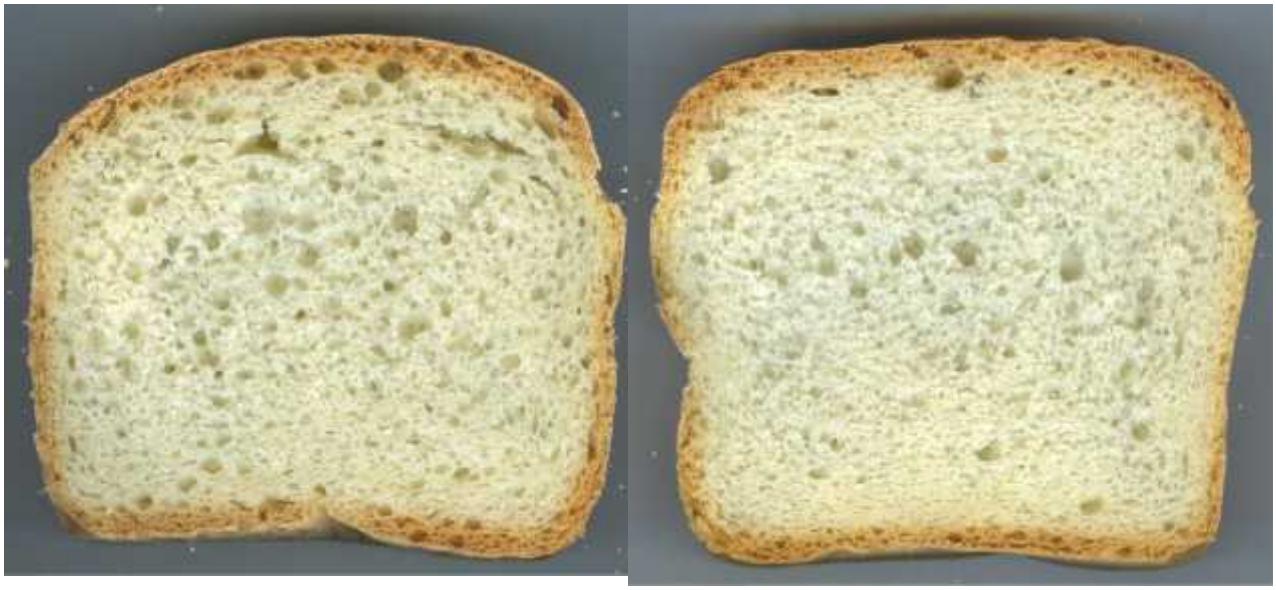
2c: rice-2%LBG



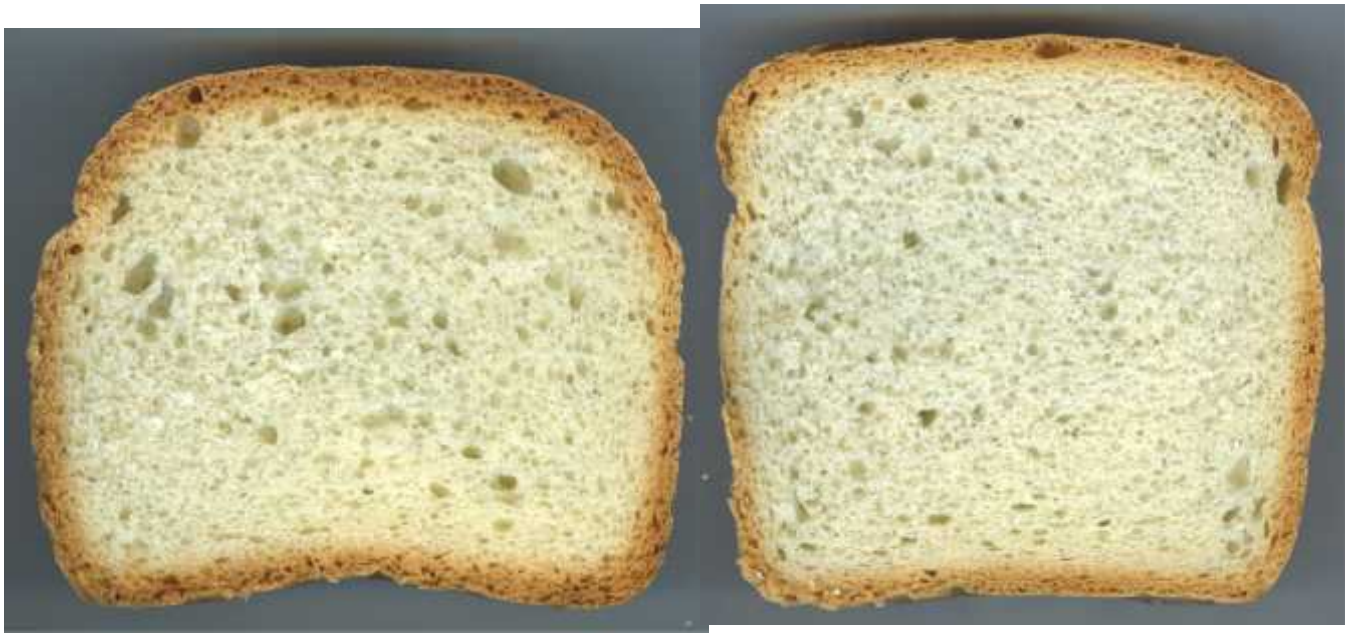
1d: rice-4%LBG

2d: rice-4%LBG

Figure 52: Surface crumb porosity of rice breads (1 represents the first replicate and 2 the second).

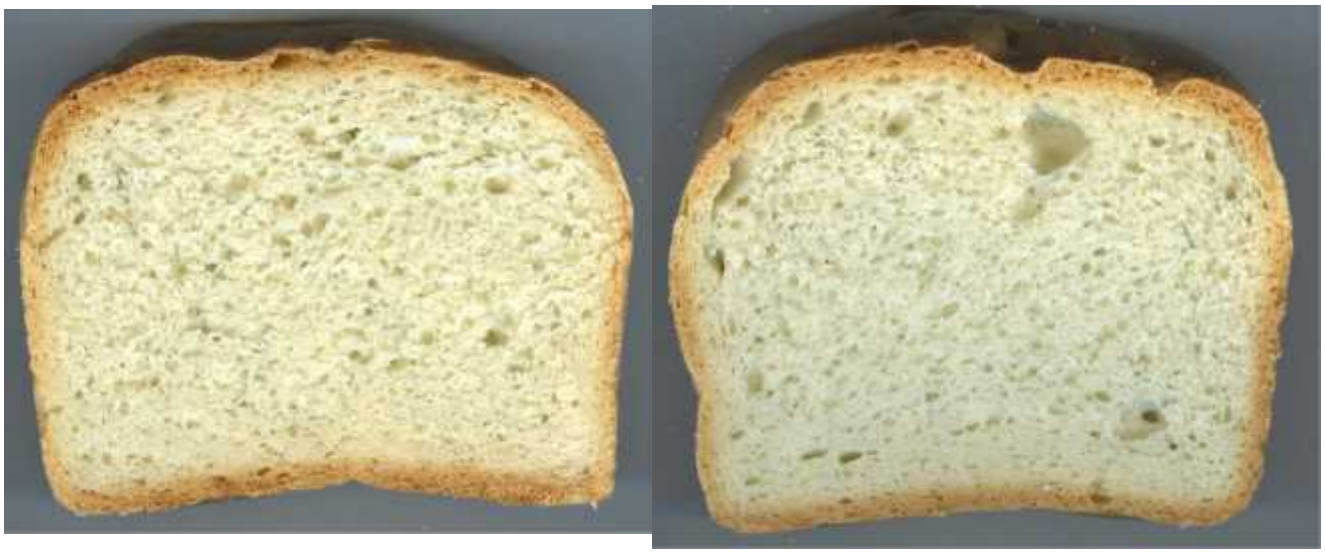


1a: wheat control 2a: wheat control

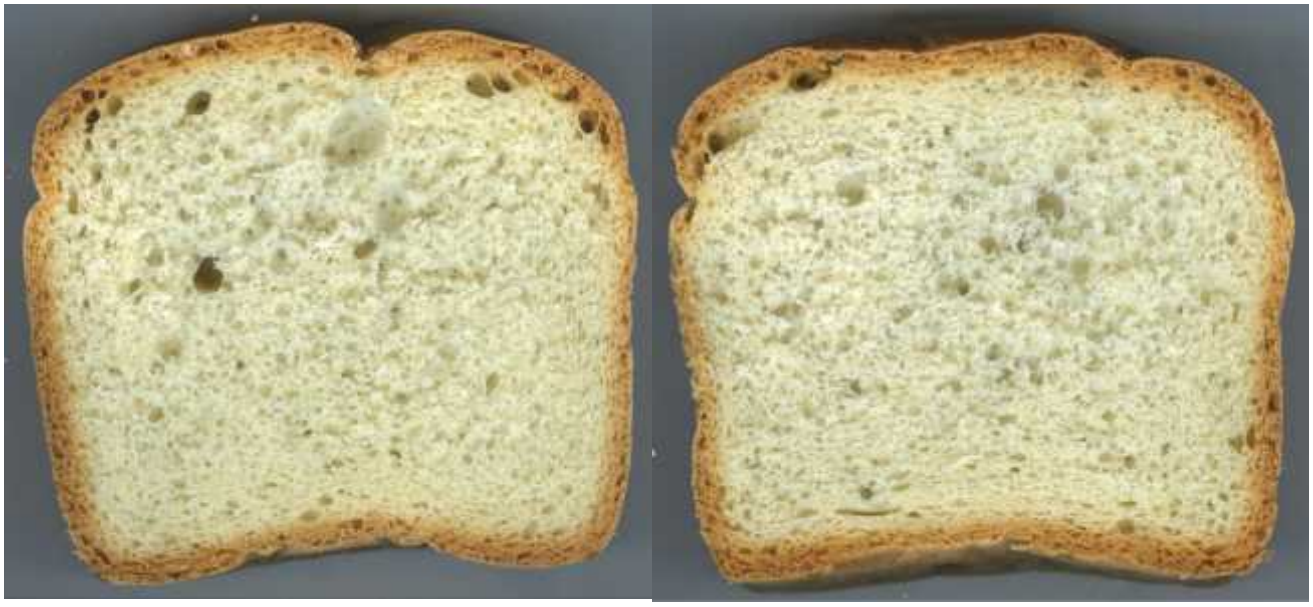


1b: wheat-2%LBG 2b: wheat-2%LBG

Figure 53: Surface crumb porosity of wheat breads (1 represents the first replicate and 2 the second).



1c: wheat-4%LBG 2c: wheat-4%LBG



1d: wheat- 5%carob protein 2d: wheat-5%carob protein

Figure 54: Surface crumb porosity of wheat breads (1 represents the first replicate and 2 the second).

As LBG concentration increases, competition of LBG with starch and gluten network for water is bigger (as shown in Fig. 30 and 42), resulting in height fall. On the other hand 2% LBG addition enhanced a little bit the standing height (Fig. 55), like Schwarzlaff et al. (1996) have reported.

Figure 55: Bread height (Left: control, in the middle: with 2%LBG and right: with 4%LBG in both wheat and rice breads).

Figure 56: Bread height (Left: control, right: with 5%carob protein for wheat breads and the opposite for the rice breads).

In Fig. 56 it can also be observed that breads with 5% carob protein do not show particular differences in comparison to control breads.

E.2.5 Principal Component Analysis (PCA)

In Fig. 57, breads are placed into groups according to the characteristics thay have in common, namely crumb and crust moisture, total and surface porosity, firmness, elasticity, average cell diameter and number of cells/cm. Wheat breads present more similarities than rice breads, because they are all very close to one another in PCA. Rice breads are split into 2 groups, which are in different quadrants. The addition of LBG gives rice breads different characteristics. Breads with 5% CP present a strong resemblance to control breads either for rice or wheat flour, with exception of the slight yellow tint in rice breads.

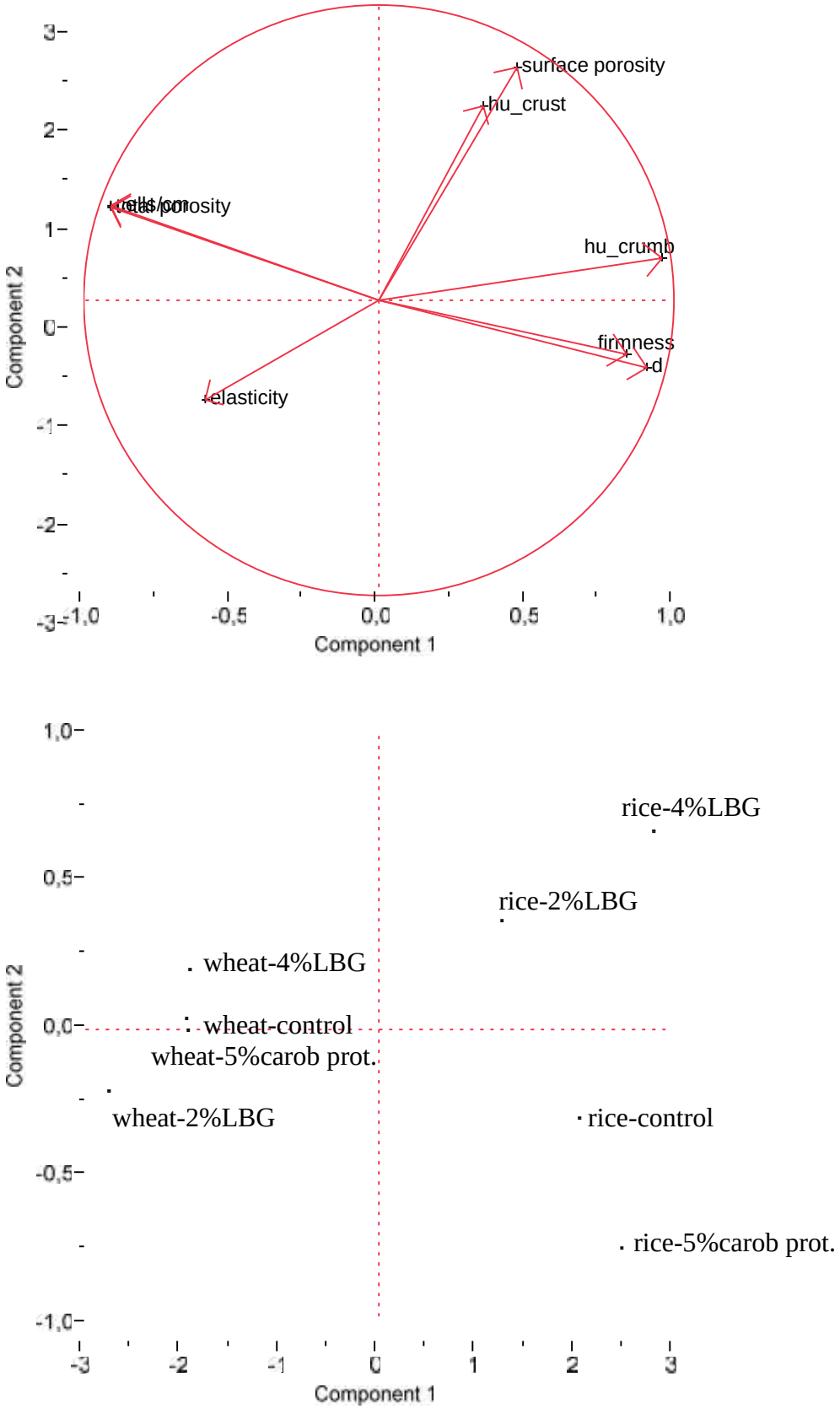


Figure 57: Results from principal component analysis (PCA) of bread samples

F. Conclusions

This study deals with the investigation of physicochemical and structural properties of carob protein and carob polysaccharide (LBG) and the influence of these components on starch and gluten in their mixtures with wheat and rice flour, using thermal analysis.
Through DSC analysis, starch gelatinization and denaturation and aggregation of CP were studied. Through TGA, the way the water is released by starch and gluten network, when carob components are added, was investigated.

One could conclude that:
1) Carob Protein due to its themodynamic incompatibility with starch, seems to affect starch gelatinization of both wheat and rice flour to a lesser extend than carob flour and LBG. A small influence in the first gelatinization peak of starch is observed, because of the residual LBG that exists in carob germ flour during the seperation of LBG and carob germ meal. The stability of CP phase is proved to be high from the resluts of DSC analysis. Whether it is in a blend with LBG, wheat flour or rice flour, CP denaturates almost in the same temperature for every moisture content studied. This means that in all cases makes it possible for starch to gelatinize.

2) CP's phase binds lower amount of water than gluten but much stronger, indicating that it creates a poor structure-network. That means that it is not a gluten-like protein, but it does not destroy the structure of bread either.
This is also confirmed by the bread making experiment, where in pricipal component analysis, breads produced adding 5% CP, present a strong resemblance to control breads either for rice or wheat flour, with exception of the slight yellow tint in rice breads. Therefore CP can be added to a bread to enhance its nutritional properties because of the high protein content and its high amount of glutamic acid and arginine, without influencing the bread structure.

3) CP seems to be thermodynamically incompatible also with LBG, like starch because its denaturation temperature is maintaned stable during DSC analysis. LBG leads to CP aggregation only in low dough water content and mainly when CP is in higher ratio than LBG.

4) LBG has the biggest impact on both wheat's and rice's starch gelatinization; its impact is independent of the starch nature. It binds water making it available for the starch gelatinization in higher temperature. Starch gelatinization is influenced by the following decreasing order: W+LBG < W+CF < W+CP.

5) LBG affects gluten network formation in its blends with wheat in different ratios. It seems to be involved within the gluten rich phase of the dough affecting the gluten structure and reducing its trapping strength for water. The overall dough moisture can modify water partition between phases and the way water is released. Increasing

dough moisture from 40 to 55%, the impact of LBG to gluten is lower, indicating that at 55% there is enough water available for both phases.

6) Results from bread making show that wheat breads present more similarities, as far as their studied characteristics is concerned, because the 4 samples are all very close to one another in the principal component analysis (PCA). Breads with 2%LBG present better characteristics, namely texture and porosity, than those with 4%LBG. It seems that addition of 2%LBG is the optimum LBG concentration for the enhancement of wheat bread. In concentrations of up to 4%, LBG starts to show its impact on starch gelatinization, confirming the thermal analysis results.
Rice breads are split into 2 groups, which are in different quadrants in the PCA; one group form rice bread and rice bread with 5%carob protein and the other group rice bread with 2 and 4%LBG. This shows that the addition of LBG gives rice breads different characteristics. If rice bread with 2%LBG could be enriched with protein either suitable for celiac sufferers or not, it could develop a more tight structure optimizing bread's appearance.

More research is necessary to fully exploit the use of CP as nutritious ingredient in the production of palatable products. Combination of different protein sources together with CP can also be conducted in order to optimize gluten-free bread formulations, integrating the nutritional properties of CP with the structural characteristics of the others.

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