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**GLUTEN-FREE BREAD:
OPTIMIZATION OF FORMULATION
AND PROCESS CONDITIONS**

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Coordinatore del dottorato: Prof. Roberto PRETOLANI
Dipartimento di Economia e Politica Agraria Agro-Alimentare e Ambientale
(DEPAAA)

Tutor: Prof.ssa Mara Lucisano
Co-tutor: Dott.ssa Manuela Mariotti
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DiSTAM)

Tesi di Dottorato di:
Carola CAPPA
Matricola n. R08133

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ABSTRACT

The aim of this PhD research project was to identify the formulation and to define the process conditions that most influence the quality and the shelf-life of gluten-free (GF) baked products. Particularly, it was focused on GF bread, aiming to get an innovative and nutritionally balanced product able to satisfy the consumers demand and whose technology could be adapted to other GF baked products.

In GF bread production, the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final product. Critical are the rheological properties of the dough that, lacking in gluten, shows a limited expansion due to the inadequate CO₂ retention during leavening, factors that lead to breads with reduced loaf volume and low crumb softness. Moreover, the presence of starches and flours from different origin in GF formulations inevitably increases the staling rate of the product, reducing its shelf-life.

Due to the challenging purposes of the PhD project, many factors were considered during the three years of research. Firstly, different GF raw materials were selected and characterized, focusing the attention on their amylose content (crucial for the shelf-life of the final product) and on their gelatinization/retrogradation behaviours. Both flours (from corn and rice) and proteins (from soy, pea and lupine) were considered. In addition to their chemical characterization, particle size distribution, water binding capacity and pasting and foaming properties were investigated. In particular, the evaluation of this last property proved to be very useful to screen the different proteins and to predict their technological aptitude.

A second phase of the research involved the study of the mechanical and rheological properties of GF gels, prepared starting from different starchy materials, during a short term ageing. This investigation was performed to better understand - and possibly decrease - starch retrogradation rate, mainly involved in bread staling. For this purpose GF gels from corn starch (CS), rice flour (RF), waxy rice flour (WRF), rice bran (RB) and their mixtures - in order to mimic the starchy component of a GF bread formulation - were prepared in a Brabender® Micro-Visco-Amylograph (Brabender OHG, Germany). The rheological properties of the gels were evaluated after 30min at 25°C (t₀) and after 1 (t₁), 2 (t₂), 3 (t₃), 4 (t₄) and 7 (t₇) days of storage at 4°C both by empirical (compression test; TA-HDplus Texture Analyzer, Stable Micro Systems, UK) and fundamental (strain sweep and frequency sweep tests;

MCR 300 Rheometer, Physica, Germany) rheology. Both WRF and RB came out to be very effective in reducing gel stiffness and storage modulus (G'). In particular, both WRF and RB, at 25% and 50% level of substitution, strongly reduced G' values of the mixtures containing CS or RF and, for the same gels, G' curves overlapped up to 7 days, indicating very slow hardening kinetics. Therefore, WRF and RB seem to be potentially effective in enhancing the shelf-life of GF baked products when included into a starchy matrix.

Afterward, the possibility of modifying the structuring properties and the technological aptitude of CS, RF and WRF by means of non-conventional physical treatments, such as High Hydrostatic Pressure (HHP), was investigated. The samples were previously mixed with water (up to a moisture content of 40g/100g of sample) and then treated. Different variables were considered: pressure holding time (5min and 10min), pressure (400MPa and 600MPa) and temperature applied (20°C and 40°C; both these temperatures were below the gelatinization temperature of the raw materials at atmospheric pressure). The pressurized samples were mainly evaluated for their pasting properties and solvent retention capacity (SRC). Pressure holding time and processing temperature were not discriminating parameters, whereas according to the pressure applied a different organization of the macromolecular compounds was obtained. In fact, observing the viscoamylographic profiles of the untreated and treated RF, it was evident how starch was partially gelatinized at both the pressures applied (400MPa and 600MPa): lower peak values (about 650BU and 620BU, respectively) were measured in comparison to the unpressurized sample (756BU). Also a higher solvent retention capacity was observed: untreated RF had a WRC of 103%, whereas it reached 121% for samples treated at 400MPa and was higher than 130% when 600MPa pressure was applied. Few differences were found for WRF samples treated at the different conditions, indicating that this sample was less influenced by pressure. On the contrary CS samples, pressurized at 600MPa, presented a slower gelatinization trend compared both to the untreated CS and to the sample treated at 400MPa; in fact, even if all the samples began to gelatinize at the same temperature (70°C), a clear shift of the viscoamylographic curves was evidenced for CS treated at 600MPa, suggesting the formation of a more compact structure. HHP could thus be used to modulate the technological behaviour of starch.

After these studies, different activities related to the optimization of the recipe and of the various steps of bread production (mixing, leavening and baking) have been carried out. Among them, the effects of the presence of two

different fibres, from Psyllium (Psy) and sugar beet (SB), on dough and bread properties have been evaluated. In particular, doughs having 200 Brabender Unit (BU) or 500BU consistencies were considered. The presence of 2.5% Psy determined for doughs having a 200BU consistency an increase of water absorption, dough height and CO₂ production during the leavening phase. On the contrary, doughs having a 500BU consistency were characterized by reduced development and gas retention during proofing. GF doughs thus required a lower consistency, if compared to wheat dough, to give good breadmaking performances. Furthermore, breads obtained from 200BU doughs showed, as expected, higher moisture content, specific volume and height as well as a good softness. As regards the two fibres investigated, a higher anti-staling effect was found for Psy fibre in comparison to SB fibre after 3 days of bread storage in paper bags at 20°C and 60% RH.

The final step of the PhD research was aimed at improving GF bread quality and shelf-life by using different leavening agents. Firstly, a GF bread formulation was created, on the basis of data reported in the literature, considering commercial recipes and through the expertise acquired from preliminary studies carried out at DiSTAM-Food Technology Section. The investigation also included the evaluation of the foaming stability of different pea proteins (Cream Tester CT II, Gerber Instruments, Switzerland). The isolate pea protein that resulted more appropriate for the GF breadmaking process presented a high protein content (90% vs. 88% and 82% of the other samples), a pH of 7.6 (vs. 6.6 and 6.3), an overrun equal to 103% (vs. 67% and 0.77%) and a foaming stability, after 60min, of 68.5% (vs. 58.2% and 0%). After the optimization of the recipe, a GF sourdough (Type I) with selected bacteria and yeasts was set up. The microorganisms of interest (*Lactobacillus sanfranciscensis* and *Candida humilis*) were isolated from a traditional sourdough used in Panettone production ($1.3 \cdot 10^8$ CFU/g bacteria and $2.3 \cdot 10^7$ CFU/g yeasts). The pure strains were then added into a GF-matrix to produce a GF starter (having a population of $5.2 \cdot 10^6$ CFU/g yeasts and $2.2 \cdot 10^9$ CFU/g bacteria after 22h and 30min of fermentation at 25°C). Part of this dough was then used to produce the GF sourdough that was constantly refreshed and monitored for pH, capability to produce/retain CO₂ (Rheofermentometer F3 Chopin, France) and number and type of microorganisms. A stable association between microorganisms, measured both in terms of microbiological population and technological properties, was obtained after few refreshments. As the GF sourdough microbial population was constant (*Lb. sanfranciscensis* and *Candida humilis* around 10^9 CFU/g and

10^7 - 10^8 CFU/g, respectively) and the leavening performance was satisfactory, the breadmaking trials were performed. Three leavening agents were compared: compressed yeast (CY), sourdough (SD) and compressed yeast + sourdough (CY/SD). Fresh and stored breads were evaluated for specific volume, crust and crumb color, moisture, a_w and crumb texture. The breadmaking trials evidenced that the produced GF sourdough could be used as an alternative leavening agent to improve GF bread quality and shelf-life; in particular, if used in combination with compressed yeast a synergic effect of SD and CY was highlighted.

RIASSUNTO

Lo scopo di questo progetto di dottorato è stato quello di identificare la formulazione e di definire le condizioni di processo che maggiormente influenzano la qualità e la *shelf-life* dei prodotti da forno *gluten-free* (GF). In particolare l'attenzione si è concentrata sul pane senza glutine, al fine di ottenere un prodotto innovativo e nutrizionalmente equilibrato in grado di soddisfare le richieste dei consumatori e la cui tecnologia possa essere adattata ad altri prodotti da forno GF.

Nella produzione di pane GF, l'assenza del reticolo viscoelastico del glutine rende l'intero processo problematico e penalizza la qualità sensoriale del prodotto finito. Critiche sono le proprietà reologiche dell'impasto GF che, privo di glutine, dimostra limitate capacità di espansione a seguito della ridotta ritenzione della CO₂ prodotta durante la lievitazione; tali fattori portano inevitabilmente ad un pane con un ridotto volume ed una scarsa sofficità della mollica. Inoltre, la presenza nelle formulazioni senza glutine di amidi di diversa origine e di farine contenenti elevate quantità di amido rende il prodotto più sensibile al raffermimento, riducendone la *shelf-life*.

A causa della complessità del progetto di dottorato, sono stati considerati diversi fattori sia legati alla formulazione che al processo produttivo. In primo luogo, sono state selezionate e caratterizzate alcune materie prime GF focalizzando l'attenzione sul loro contenuto in amido, in particolare in amilosio (fondamentale per la *shelf-life* del prodotto finito), e sulle proprietà di gelatinizzazione/retrogradazione dell'amido. Oggetto di questa fase della sperimentazione sono state sia farine di mais e riso che proteine di soia, pisello e lupino. Oltre alla composizione chimica, alla distribuzione granulometrica e alla capacità di legare acqua delle diverse materie prime, sono state studiate le loro proprietà viscoamilografiche (*pasting properties*) durante cicli di riscaldamento e raffreddamento in condizioni controllate. Inoltre è stato eseguito uno *screening* di diversi isolati proteici sulla base delle loro proprietà schiumogene, parametro che può senza dubbio condizionare la quantità di aria inglobata nell'impasto durante la fase di impastamento.

Una seconda fase della ricerca ha coinvolto lo studio delle proprietà reologiche dei gel ottenuti a partire da materie prime *gluten-free* durante un invecchiamento a breve termine. Tale studio è stato condotto con lo scopo di individuare eventuali ingredienti GF in grado di rallentare la retrogradazione dell'amido, il principale fenomeno coinvolto nel raffermimento del pane. A tal fine, utilizzando il Micro-Visco-Amilografo Brabender® (Brabender OHG,

Germany), sono stati prodotti gel a base di amido di mais (CS), di farina di riso (RF), di farina di riso waxy (WRF), di fibra di riso (RB) e di loro miscele, per simulare la componente amidacea presente nelle formulazioni da pane GF. Le proprietà reologiche dei gel sono state valutate dopo 30min a 25°C (t0) e dopo 1 (t1), 2 (t2), 3 (t3), 4 (t4), 7 (t7) giorni di conservazione a 4°C, sia mediante test di reologia empirica (test di compressione; dinamometro *TA-HDplus Texture Analyzer*; Stable Micro Systems, UK) che fondamentale (*strain sweep test* e *frequency sweep test*; Reometro MCR, Physica, Germany). Questo studio ha sottolineato come sia la farina di riso waxy che la fibra di riso siano efficaci nel ridurre la *stiffness* e il modulo elastico (G' , *storage modulus*) dei gel. In particolare, sia l'aggiunta di WRF che di RB a livelli del 25% e del 50% hanno ridotto fortemente i valori di G' delle miscele contenenti CS o RF; inoltre per gli stessi gel si è osservata una cinetica d'indurimento molto più lenta durante la conservazione (7 giorni), con una completa sovrapposizione delle curve del modulo elastico (*frequency sweep*). WRF e RB rappresentano quindi degli ingredienti potenzialmente utilizzabili nei prodotti da forno GF per estenderne la *shelf-life*.

In seguito, è stata investigata la possibilità di modificare le proprietà strutturanti e l'attitudine tecnologica di CS, RF e WRF - precedentemente miscelati con acqua fino a raggiungere un tenore di umidità pari a 40g/100g di campione - per mezzo di trattamenti fisici non convenzionali, come i trattamenti ad alta pressione idrostatica (HHP). Le diverse variabili considerate sono state: il tempo di mantenimento della pressione (5 minuti e 10 minuti), la pressione applicata (400MPa e 600MPa) e la temperatura (20°C e 40°C; si noti che entrambi i valori di temperatura considerati sono inferiori alla temperatura di gelatinizzazione delle materie prime a pressione atmosferica). I campioni trattati alle alte pressioni sono stati quindi valutati per le loro proprietà di *pasting* e di capacità di ritenzione del solvente (SRC). I risultati hanno dimostrato che il tempo di mantenimento della pressione e la temperatura di processo non risultano parametri discriminanti, mentre la pressione applicata (400MPa e 600MPa) determina una diversa strutturazione delle macromolecole presenti. Infatti, i profili viscoamilografici di RF trattato nelle diverse condizioni evidenziano una parziale gelatinizzazione della frazione amido ad entrambe le pressioni utilizzate (400MPa e 600MPa), rilevabile dal picco di viscosità inferiore rispetto al campione non trattato (650BU e 620BU, rispettivamente, vs. 756BU). Si è inoltre riscontrato un incremento della capacità di ritenzione del solvente-acqua (WRC): 103% per RF non trattato, fino a 121% per i campioni trattati a 400MPa e valori compresi tra 130-140%

per le farine di riso trattate a 600MPa. Poche differenze sono state rilevate tra i campioni di farina di riso waxy (WRF) diversamente trattati, ad indicare che questo campione risulta poco influenzato dai trattamenti ad alta pressione. Contrariamente, CS sottoposto ad una pressione di 600MPa, ha evidenziato un *trend* di gelatinizzazione più lento rispetto al campione non trattato e al campione trattato a 400MPa; infatti, anche se l'inizio della gelatinizzazione è avvenuta alla stessa temperatura (70°C), si è evidenziato uno spostamento delle curve viscoamilografiche verso temperature più elevate, ad indicare la formazione di una struttura più compatta. Si può quindi concludere che i trattamenti alle alte pressioni sono potenzialmente utilizzabili per modificare il comportamento dell'amido.

Dopo questi studi preliminari, si è focalizzata l'attenzione sull'ottimizzazione della formulazione e delle fasi principali del processo (impastamento, lievitazione e cottura) per la produzione di pane GF. Dapprima sono stati valutati gli effetti dell'aggiunta di due fibre (Psyllium e fibra di barbabietola) su un impasto da pane GF (avente una consistenza di 200UB - Unità Brabender e di 500UB) e sulle proprietà del prodotto finito. I risultati ottenuti hanno evidenziato come la presenza del 2.5% di Psy determini, in particolare per gli impasti che presentano una consistenza di 200UB, un aumento dell'assorbimento d'acqua durante l'impastamento ed un incremento dello sviluppo in altezza dell'impasto durante la lievitazione. Diversamente gli impasti a 500UB hanno presentato un ridotto sviluppo in altezza dovuto ad una scarsa capacità di ritenere il gas prodotto durante la lievitazione. Inoltre il pane ottenuto dall'impasto a 200UB ha mostrato, come atteso, un alto contenuto di umidità, una buona sofficità della mollica (bassa resistenza alla compressione) ed un elevato volume specifico. Questi risultati suggeriscono che per la produzione di pane senza glutine è preferibile lavorare un impasto più morbido (*liquid-like*) rispetto al convenzionale impasto da pane (500BU). Le prove di conservazione accelerate (3 giorni a 20°C, 60% UR, in confezione di carta) hanno inoltre evidenziato come la fibra di Psyllium risulti più efficace rispetto alla fibra di barbabietola nel rallentare il rafferimento del pane.

La fase finale della ricerca di dottorato si è incentrata sul miglioramento della qualità e della *shelf-life* del pane GF mediante l'utilizzo di diversi agenti lievitanti. Per raggiungere questi obiettivi, è stata definita in un primo momento la formulazione da pane GF sulla base dei dati riportati in letteratura, considerando alcune note ricette commerciali e attraverso le conoscenze acquisite da studi preliminari effettuati presso la Sezione di Tecnologia Alimentare del DiSTAM. In questo contesto si è svolto anche uno *screening* di

tre diverse proteine di pisello valutandone le proprietà schiumogene (*overrun*) e la stabilità della schiuma attraverso un utilizzo non convenzionale del Creme Tester CT II (Gerber Instruments, Switzerland). La proteina di pisello ritenuta opportuna per il processo di panificazione ha presentato un elevato contenuto proteico (90% vs. 88% e 82%), un pH di 7.6 (vs. 6.6 e 6.3), un *overrun* del 103% (vs. 67% e 0.77%) ed una stabilità della schiuma dopo 60 minuti a temperatura ambiente del 68.5% (vs. 58.2% e 0%).

Dopo l'ottimizzazione della formulazione è stata sviluppata una madre acida, o lievito naturale, GF (Tipo I) con batteri e lieviti opportunamente selezionati al fine di produrre un pane GF a lievitazione naturale. I microrganismi di interesse (*Lactobacillus sanfranciscensis* e *Candida humilis*) sono stati isolati da un lievito naturale usato per la produzione di Panettone (popolazione microbica pari a $1.3 \cdot 10^8$ UFC/g per i batteri e $2.3 \cdot 10^7$ UFC/g per i lieviti). I ceppi puri sono stati poi addizionati ad una matrice GF al fine di sviluppare uno *starter* GF che è risultato avere, dopo una fermentazione di 22h e 30min a 25°C, una popolazione microbica di $2.2 \cdot 10^9$ UFC/g batteri e $5.2 \cdot 10^6$ UFC/g lieviti. Parte dello *starter* è stato poi utilizzato per produrre il lievito naturale GF che è stato costantemente rinfrescato e monitorato in termini di pH, capacità di produrre/trattenere CO₂ (Rheofermentometer F3 Chopin, France) e numero e tipo di microrganismi presenti. Una associazione stabile - in termini di popolazione microbiologica e proprietà tecnologiche - è stata ottenuta già dopo pochi rinfreschi ed è rimasta tale fino al termine della sperimentazione (25° rinfresco). La popolazione microbica della madre GF utilizzata per le prove di panificazione è risultata contenere 10^9 UFC/g di *Lb. sanfranciscensis* e di 10^7 - 10^8 UFC/g di *Candida humilis*. Nell'ultima parte del progetto sono state allestite tre panificazioni confrontando diversi agenti lievitanti: lievito compresso (CY), madre acida (SD) e madre acida + lievito compresso (CY/SD). La qualità del pane fresco e conservato in condizioni accelerate è stata valutata in termini di volume specifico, colore della crosta e della mollica, umidità, a_w e consistenza della mollica. Le prove di panificazione hanno evidenziato che il lievito naturale GF può essere utilizzato come agente lievitante per migliorare la qualità del pane GF e la sua *shelf-life*, in particolar modo se usato in combinazione con il lievito compresso (effetto sinergico).

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AIM AND STRUCTURE OF THE THESIS

The aim of this PhD research project was to identify the formulation and to define the process conditions that most influence the quality and the shelf-life of gluten-free (GF) baked products. Particularly, it was focused on GF bread, aiming to get an innovative and nutritionally balanced product able to satisfy the consumers demand and whose technology could be adapted to others GF baked products.

The thesis is organized in 7 chapters, each one referring to a different topic linked to the main aim. Chapter 1 is a “General Introduction”, in which a survey on celiac disease (CD) and on the problematic issues related to GF bread production and storage are presented.

In Chapter 2, the topic “Characterization of Gluten-Free Raw Materials” is faced. Flours and starches (mainly from corn and rice), proteins (mainly from soy, pea and lupine), fibres and hydrocolloids have been characterized, and their technological aptitude has been evaluated by different techniques.

In Chapter 3, the “Rheological Properties of Gels Obtained from Gluten-Free Raw Materials During a Short Term Ageing” have been investigated. A manuscript related to this topic has been submitted to the *Journal of Cereal Science*. The rheological properties of corn starch and rice flour gels were investigated. In particular, the capability of waxy rice flour and rice bran to reduce the retrogradation rate of different starchy systems was studied, aiming to increase the shelf-life of starch-containing foods, such as GF baked products.

Chapter 4 is focused on the possibility of using a non-conventional physical treatment, such as the High Hydrostatic Pressure (HHP), to modify the structuring properties and the technological aptitude of corn starch and rice and corn flours. This research was carried out (from September 2010 to February 2011) at the Washington State University (Pullman, USA), under the supervision of Prof. Gustavo V. Barbosa-Cánovas.

Chapter 5, named “Effect of Different Amounts of Fibre and Water on Dough Properties and Bread Quality”, refers the results related to the evaluation of the effects of the presence of two different fibres - Psyllium (Psy) and sugar beet fibre (SB) - on GF doughs and on the resulting breads.

Chapter 6, titled “Gluten-Free Breadmaking Trials: Compressed Yeast *versus* Sourdough”, reports the results related to the development of a GF sourdough (Type I), containing selected bacteria and yeasts, and to the breadmaking trials

performed using the developed GF sourdough as leavening agent, in comparison with a traditional compressed yeast (*Saccharomyces cerevisiae*). In Chapter 7, “General Conclusions and Future Perspectives” of this study are reported.

At the end, an Appendix collects all the publications and the other works presented to national and international congresses.

CHAPTER 1

GENERAL INTRODUCTION

1.1 CELIAC DISEASE

Celiac disease (CD) is a permanent intolerance to “gluten”, an affection of the small intestine that can appear both in children and adults. Since 2005 this disease has been recognized in Italy as a social illness (G.U. n.156, July 7 2005) and is more prevalent than previously believed: it is estimated that 0.5 to 2.0% of the population in most European countries and in the United States suffers from CD (Rewers, 2005).

The **cause** of the outbreak of this disease are storage proteins found in many common cereals such as wheat, rye or barley and hybrids of these grains (e.g. triticale), that are so considered harmful for the sensitive consumers; specific toxic amino acid sequences have been identified in α -gliadin, the alcohol-soluble extract of wheat protein.

Celiac disease results, in genetically susceptible individuals, from an abnormal T cell-mediated immune response and inflammatory injury to the mucosa of the small intestine, against ingested storage proteins: the gliadin fraction is mainly responsible for this intestinal damage. The importance of genetic factors is supported by the approximately 10 percent prevalence of the disease among first-degree relatives.

Over 95 percent of patients with celiac sprue express the human leukocyte antigen HLA-DQ (a1*501, b1*02) heterodimer (HLA-DQ2), which preferentially presents gluten-derived gliadin peptides on its antigen-presenting groove to stimulate intestinal mucosal T cells. As can be observed in Figure 1-1 (Farrell and Kelly, 2002), gliadin is absorbed into the lamina propria and presented in conjunction with HLA-DQ2 or DQ8 cell-surface antigens by antigenpresenting cells, probably dendritic cells, to sensitized T cells expressing the a/b T-cell receptor. Tissue transglutaminase deamidates gliadin peptides, generating acidic, negatively charged residues of glutamic acid from neutral glutamines (inset). Because negatively charged residues are preferred in positions 4, 6, and 7 of the antigen-binding groove of HLA-DQ2, deamidated gliadin elicits a stronger T-cell response. These lymphocytes then activate other lymphocytes to generate cytokines, such as interferon, interleukin-4, and tumor necrosis factor α (TNF α), which damage the villi, resulting in enteritis. Induction of aberrant HLA class II cell-surface antigens on the enterocytes may permit these cells to present additional antigens to the sensitized lymphocytes.

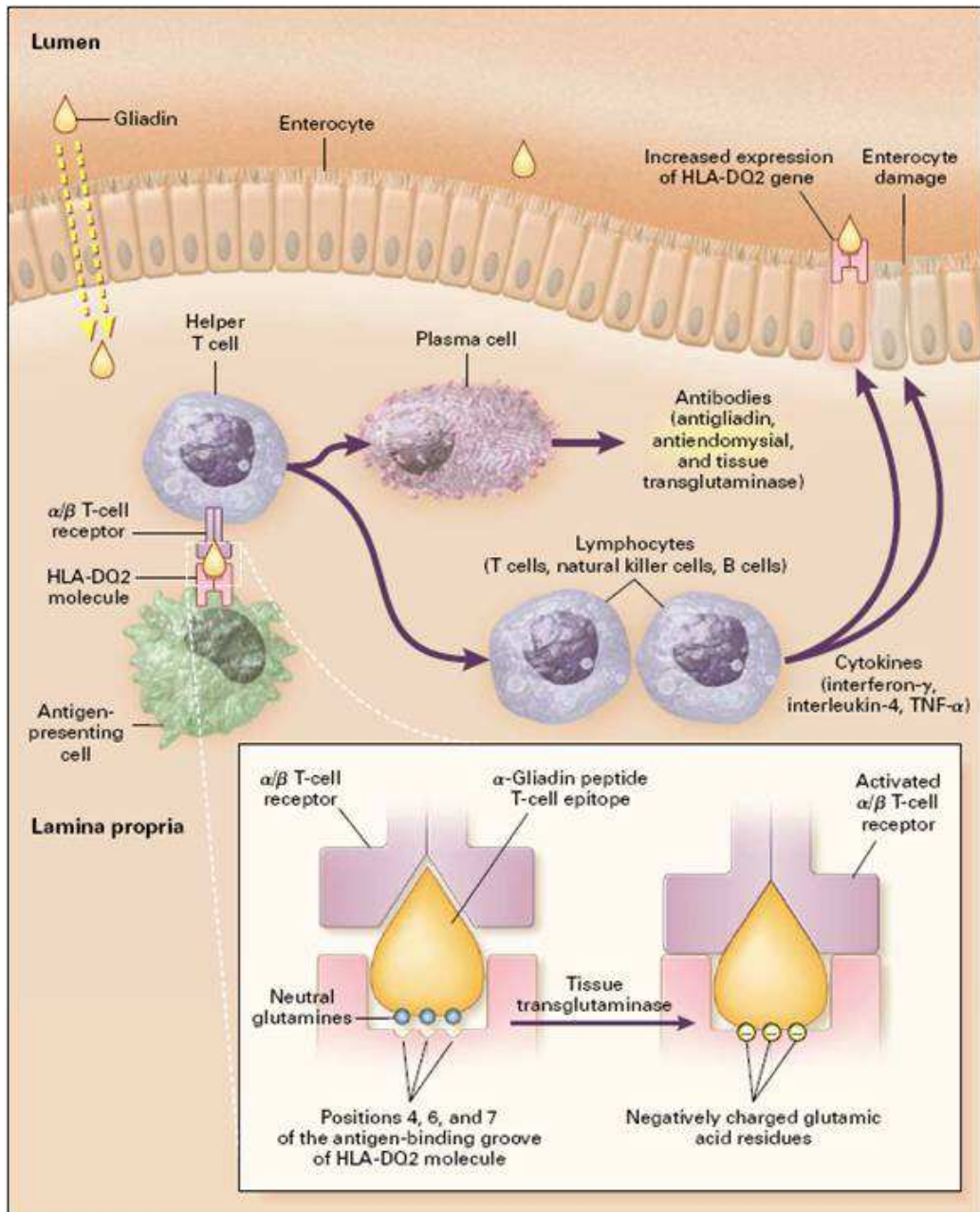


Figure 1-1. Pathogenesis of Celiac Sprue (Farrell and Kelly, 2002).

Symptoms of CD can range from the classic features, such as diarrhea, weight loss, and malnutrition, to latent symptoms such as isolated nutrient deficiencies. The clinical manifestations are mild weakness, bone pain, and aphthous stomatitis, chronic diarrhea and abdominal bloating and anomalies of

the intestinal mucosa with partial or total atrophy of the villi (Figure 1-2). In Figure 1-2 (on the left) there are three images taken by Farrell and Kelly (2002): in Panel A, a duodenal-biopsy specimen from a patient with untreated celiac sprue shows a flat mucosal surface, severe enteritis, crypt hyperplasia, disarray of enterocytes, and extensive inflammatory infiltration of the lamina propria and epithelial-cell layer (hematoxylin and eosin, x100). In Panel B, the epithelial cells in a patient with untreated celiac sprue are cuboidal and vacuolated and are infiltrated by numerous intraepithelial lymphocytes and plasma cells (hematoxylin and eosin, x200). In Panel C, a duodenal-biopsy specimen from a normal person shows tall villi, shallow crypts, and sparse infiltration of the lamina propria and epithelial-cell layer with lymphocytes and plasma cells (hematoxylin and eosin, x100).

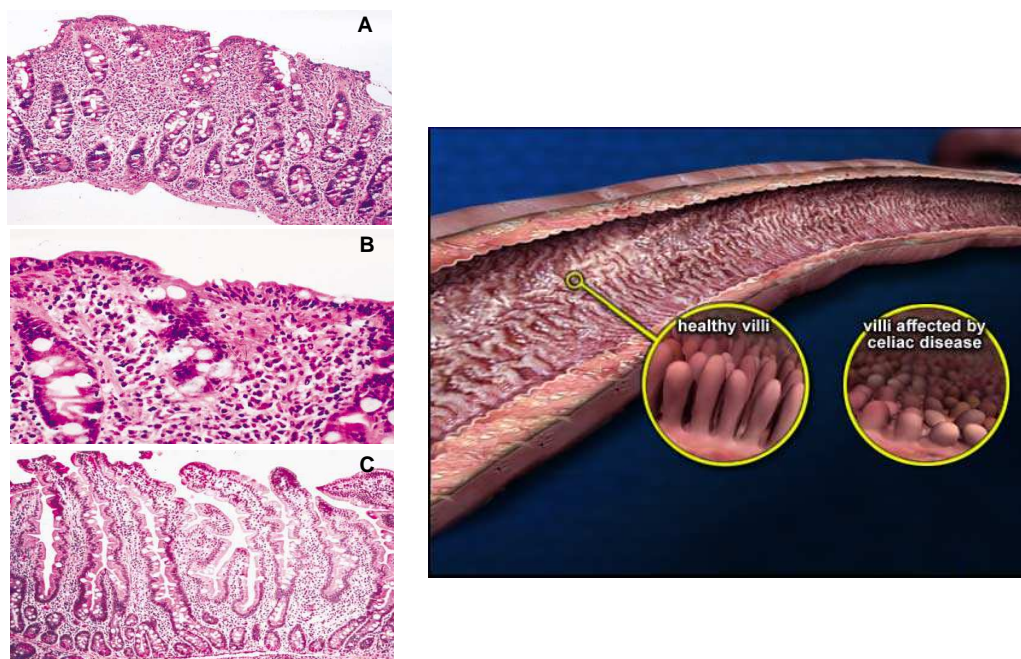


Figure 1-2. Mucosal histopathological findings in Celiac sprue (left, from Farrell and Kelly, 2002; right from <http://mydoctor.kaiserpermanente.org>).

Because of the broad range of symptoms, the diagnosis of celiac disease may result arduous. Recognized associated disorders include dermatitis herpetiformis, hyposplenism, IgA nephropathy, primary biliary cirrhosis, sclerosing cholangitis, Sjögren's syndrome, and insulin-dependent diabetes mellitus (Goggins and Kelleher, 1994) and malabsorption of several important nutrients including iron, folic acid, calcium and vitamins (Feighery, 1999).

These malabsorptions may cause clinical complications such as anemia, osteopenia and moderate, reversible elevations in serum aminotransferase concentrations with minimal histopathologic changes in the liver (Goggins and Kelleher, 1994). Besides, different studies have shown that for celiacs there is an increase of gastrointestinal cancer chances by a factor of 40 to 100 times compared to the normal population (Goggins and Kelleher, 1994; Trier, 1991).

The conventional approach for an **accurate diagnosis** includes an initial serologic screening followed by endoscopic biopsy of the duodenal mucosa. Depending on the severity of the celiac disease, the sensitivity of a positive test for antigliadin antibodies can be greater than 90 percent, and the specificity of positive tests for IgA antigliadin and antiendomysial antibodies greater than 95 percent, but mucosal biopsy remains the gold standard (Halsed, 1996).

In patients suspected to suffer from celiac disease but that are seronegative or do not have the typical biopsy findings, it may be helpful to test for IgA and IgM antibodies to gliadin in jejunal secretions, for increased intestinal permeability to large sugar molecules, or for intraepithelial-cell lymphocyte infiltration in the rectal mucosa after topical gluten challenge. The validation of the celiac disease diagnosis requires the evidence of improvements in the mucosal lesions of the small intestine in response to a gluten-free diet.

Anyway many epidemiological studies have shown that the prevalence of celiac disease has been significantly underestimated (Ascher and Kristiansson, 1997; Hovdenak *et al.*, 1999; Fasano and Catassi, 2001) due to the large number of symptoms that celiacs present and to the subjective response to the screening tests.

The iceberg model is commonly used to explain the **prevalence** of celiac disease (Visakorpi, 1997; Figure 1-3) and the prevalence can be estimated as the overall size of the iceberg. Cases which have been properly diagnosed make up the visible section (A) of the iceberg in quantitative terms (Fasano and Catassi, 2001). Patients who have been recently diagnosed, and are now following a gluten-free diet and show a normal mucosa form the lower part of the this section. Below the waterline there is a group of “silent” cases (B), which have not yet been identified and have flat small intestinal mucosa. They may remain undiagnosed because the symptoms are not linked to the celiac disease. At the bottom of the iceberg (C), there is a small group of patients with latent celiac disease; these people show a normal mucosa while taking gluten, yet still have the potential to develop the disease (Feighery, 1999).

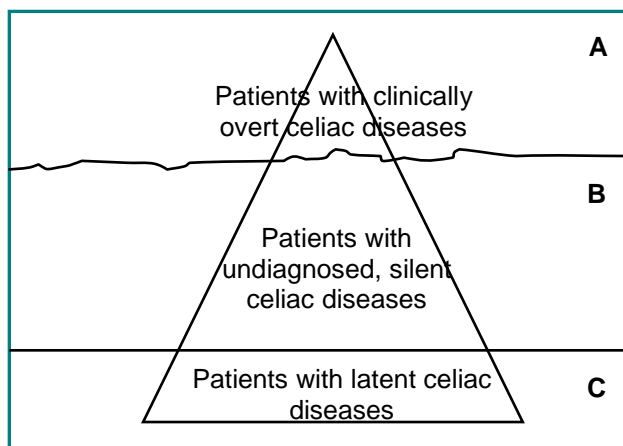


Figure 1-3. Iceberg model depicting prevalence of celiac disease (Feighery, 1999).

At present the only acceptable treatment is a total lifelong avoidance of gluten ingestion, so celiacs must strictly follow a **gluten-free diet** (GFD), eating only dedicated foods. A gluten-free diet means avoiding all products that contain wheat, rye and barley, or any of their derivatives. It is evident that many typical Italian foods like bread and pasta cannot be consumed by celiac people. Besides, there are many hidden sources of gluten found in the ingredients of many processed foods or cross-contaminations.

Due to the increase of CD people, several **alternatives** to a lifelong GDF are now being studied. Shan *et al.* (2002) proposed the use of a prolyl endopeptidase (PEP) from *Flavobacterium meningosepticum* to accelerate the breakdown of gluten. Oral supplementation with such a post-proline cutting enzyme, administered just prior or together with a gluten-containing meal, might be an effective way to remove gluten toxicity because it would degrade gluten into fragments that can no longer bind to HLA-DQ2 or HLA-DQ8. Indeed, the enzyme was shown to cut toxic gluten-derived peptides *in vitro*. *In vivo* application, however, the action was hampered by the fact that the enzyme was inactivated by low pH and pepsin, both present in the stomach. Additional limitations to this enzymatic action result from relatively low enzyme activity and its preference for small peptides as substrates (Shan *et al.*, 2004). Bacterial PEP alone would therefore be unable to degrade gluten before it reaches the small intestine, the site where gluten sensitivity is expressed. In a recent article, Siegel *et al.* (2006) suggest a combined therapy to overcome this problem: the authors in fact demonstrated that the combination of EP-B2

and bacterial PEP could bring to a fully gluten degradation under simulated gastrointestinal conditions.

An alternative to this approach relies on the application of a single enzyme such as prolyl endoprotease from *Aspergillus niger*, a food-grade organism (Edens *et al.*, 2005). This enzyme, termed AN-PEP, is unrelated to the above-mentioned bacterial PEP, it works optimally at acid pH and it is also resistant to pepsin. In a recent study Stepniak *et al.* (2006) have shown that the AN-PEP enzyme effectively degrades all T-cell-stimulatory gluten peptides tested under conditions that mimic those in the stomach. Moreover, the enzyme breaks down intact gluten molecules into fragments that can no longer bind to HLA-DQ2 and HLA-DQ8. Finally they concluded that, the enzyme AN-PEP is food grade, extremely stable and can be produced at acceptable cost so it is a potential prime candidate for testing in clinical trials.

Furthermore treatments with a mixture of selected sourdough lactobacilli and fungal proteases to eliminate the toxicity of wheat flour during long-time fermentation (Rizzello *et al.*, 2007) or with inhibitors of tTGase (Hoffmann *et al.*, 2009) have been studied. In addition, mechanisms of competition to prevent T-cell stimulation or biotechnological treatments on cereals to avoid the expression of the toxic sequences (GMOs) have been suggested by the scientists.

As underline by Stepniak and Koning (2006), the problem is now if the oral supplementation work *in vivo*. It should be pointed out that the above-mentioned studies have been carried out with gluten peptides, recombinant gluten molecules and crude gluten, which are not the same as the gluten present in our day-today foodstuffs (where gluten is mixed with other food components and it has often been cooked). Therefore, gluten in real food might be less readily accessible to enzymes and harder to degrade. Furthermore, because there are no good animal models for celiac disease, clinical trials will ultimately have to demonstrate if an oral enzyme supplementation can be developed into an effective medical treatment.

In conclusion, even if researchers are studying alternative solutions, more investigations and clinical trials are required before these new approaches could be used for celiacs.

1.2 LEGISLATION AND LABELLING

There are problems around the world on the issue of labelling GF products because the exact amount of toxic prolamins that individuals with CD may consume without damaging the mucosa of the small intestine has not been defined (Thomson, 2000).

Worldwide, there is a major debate regarding the accepted definition of what constitutes “gluten-free”. Products labelled “gluten-free” in Canada must meet standards of less than 20mg gluten per kg (Arendt *et al.*, 2008), whereas other countries use 200mg/kg and still others prefer a double standard for those products rendered GF and those naturally GF. The Codex Alimentarius Commissions of the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO UN) have approved the Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (Codex Stan 118-1979) on July 1, 2008 (Adopted in 1979, amended in 1983 and revised in 2008). The following distinctions have been made:

1) Gluten-free foods:

- *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, oats¹ or their crossbred varieties, and the gluten level does not exceed 20mg/kg in total, based on the food as sold or distributed to the consumer, and/or*
- *consisting of one or more ingredients from wheat (i.e., all Triticum species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, which have been specially processed to remove gluten, and the gluten level does not exceed 20mg/kg in total, based on the food as sold or distributed to the consumer.*

2) Foods specially processed to reduce gluten content to a level above 20 up to 100mg/kg:

These foods consist of one or more ingredients from wheat (i.e., all Triticum species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, which have been specially processed to reduce the gluten content to a level above 20 up to 100mg/kg in total, based on the food as sold or distributed to the consumer.

As oats can be tolerated by most, but not all, people who are intolerant to gluten, the Codex Standard 118-1979 indicates that the allowance of oats (not

contaminated with wheat, rye or barley) in foods covered by this standard may be determined at national level.

In the Codex Standard 118-1979 it is indicated that, in addition to the general labelling provisions contained in the General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985) and the General Standard for the Labelling of and Claims for Prepackaged Foods for Special Dietary Uses (CODEX STAN 146-1985) and any specific labelling provisions set out in a Codex standard applying to a particular food, the following provisions for the labelling of “gluten-free foods” shall apply:

1) The term “gluten-free” must be very close to the name of the product in the case of the gluten-free products previously described. Whereas, the labelling information of specially processed products to reduce gluten content should be stated at national level. However, these products can not be named “gluten-free”. The labelling information for these products should indicate the true nature of the food, and they must be printed in the immediate proximity of the name of the product.

2) A food which, by its nature, is suitable for use as an ingredient of a gluten-free diet, shall not be designated as “special dietary”, “special dietetic” or any other equivalent term. However, such a food may bear a statement on the label like “this food is by its nature gluten-free” provided that it complies with the essential composition requirements for a gluten-free product.

To make easier the identification of gluten-free product by the consumers there are some graphical images that appear on the package. In Italy there are two generally recognized GF certification:



“Associazione Italiana Celiachia”



“Ministero della Salute”

Worldwide, in the last years, many other label indications have been put on the market as follows:



1.3 GLUTEN AND STARCH

The Codex Alimentarius Commissions of the World Health Organization (WHO) defines **gluten** as “*a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant and that is insoluble in water and 0.5M NaCl*”. Whereas the scientist generally defined gluten as a proteinaceous material that can be separated from flour when the starch and other minor components of the flour are removed by washing out with running water. The resulting gluten contains approximately 65% water. On a dry matter basis, gluten contains 75-86% protein, the remaining part being carbohydrate and lipid, which are held strongly within the gluten-protein matrix (Bloksma and Bushuk, 1998).

Two functionally distinct groups of “gluten proteins” can be distinguished: prolamins (from wheat are named gliadin, from rye secalin, from barley hordein and from oats avenin) defined as the fraction from gluten that can be extracted by 40-70% aqueous ethanol and glutenins. In wheat, gliadins and glutenins (Figure 1-4) are generally found in more or less the same amount. Gliadin is extremely sticky when hydrated and it has been reported to contribute to the viscous properties and dough extensibility, whereas glutenins have a prominent role in the elasticity and tenacity (strengthening) of dough (Pomeranz, 1988 and MacRitchie, 1980). Gliadins represent a highly heterogeneous mixture of monomeric gluten proteins. Three structurally distinct groups of gliadins can be distinguished: α -, γ - and ω -types. The α -types have six cysteine residues, γ -types have eight cysteine residues and ω -types lack of cysteine residues and also have a very low level of methionine. On the contrary, wheat glutenin is a heterogeneous mixture of disulfide-linked polymers of glutenin subunits that can be liberated upon treatment with reagents that promote thiol-disulfide exchange reactions. The glutenin is a polydisperse protein complex that has essentially a linear structure and its physical properties depend on their molecular weight. Small polymers result in a viscous liquid; as the molecular weight increase, the polymer becomes an elastomer with low strength and high extensibility. Above a molecular weight of about 10^5 , the polymers are subject to molecular entanglements and show rubbery properties. This explains the elastic properties of glutenin and therefore gluten.

By properly mixing wheat flour and water, gliadins and glutenins form a viscoelastic networks, named gluten (Figure 1-4), from which dough properties strongly depend. The resulted dough, cohesive and viscoelastic, is hence

capable of holding gas produced during proofing and oven-rise resulting, after baking, in the typical open foam structure of bread. Due to the unique structure and functional properties of gluten is clear that it is extremely challenging, from a technological point of view, to find alternative ingredients that could mimic gluten properties in breadmaking.

Table 1-1. Amino acid composition of wheat gluten, gliadin, and glutenin (mol/105g of protein; Kasarda *et al.*, 1971).

Amino acid	Gluten	Gliadin	Glutenin
Arginine	20	15	20
Histidine	15	15	13
Lysine	9	5	13
Threonine	21	18	26
Serine	40	38	50
Aspartic acid	22	20	23
Glutamic acid	290	317	278
Glycine	47	25	78
Alanine	30	25	34
Valine	45	43	41
Leucine	59	62	57
Isoleucine	33	37	28
Proline	137	148	114
Tyrosine	20	16	25
Phenylalanine	32	38	27
Tryptophan	6	5	8
Cysteine	14	10	10
Methionine	12	12	12
Ammonia	298	301	240

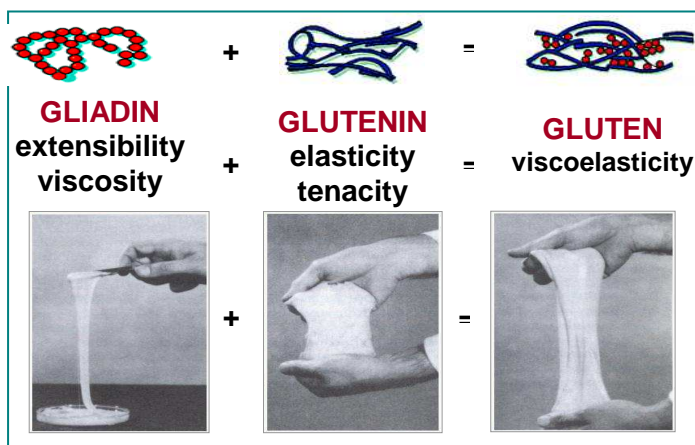


Figure 1-4. The main gluten components and its unique properties.

Starch is the most important vegetable reserve polysaccharide and it is organized in semi-crystalline granules - a relative dense and insoluble structure - that can show significant variations in size and shape when view by scanning electron microscopy (SEM; Figure 1-5). Most starch granules are composed of a mixture of two types of polymers, amylose and amylopectin, having D-glucose as a basic constituent unit (Figure 1-6). These polymers differ in their glucose linkages: amylose is a straight chain polymer characterized by α -1 \rightarrow 4 whereas amylopectin is a branched polymer linked in α -1 \rightarrow 4 and α -1 \rightarrow 6 conformations. The amylose molecules are small (molecular weight varying from 10^5 to 10^6 Da) and can arrange themselves in a helix conformation that make easier the formation of complex with iodine, lipids, and other polar substances. For it particular conformation, amylose is the key component involved in water absorption, swelling and gelation of starch in food processing. Instead amylopectin is a much larger polymer (molecular weight varying from 10^7 to 10^8 Da) and highly branched; the branched chains are short therefore they cannot form the helix. For its structure-conformation the amylopectin is bigger than amylose and has different properties: the gels formed are more flexible and resistant. Amylopectin is also much more resistant to retrogradation therefore it is commonly used to extend the self-life of the starch-based products.

Depending on its biological origin, starch can contain a different ratio of amylose and amylopectin (Table 1-2): typical levels are 25-28% and 72-75%, respectively. However the starch of some mutant genotypes of corn, barley and rice contain either an increased amylose content (up to 70%) or an increased amylopectin content (waxy starches with 0% to <5% amylose content). Generally, waxy starches swell rapidly at low temperature, reach much higher peak viscosity than normal starches, but thereafter waxy pastes quickly disintegrate and form low consistency gels due to the absence of amylose (Abdel-Aal *et al.*, 2002).

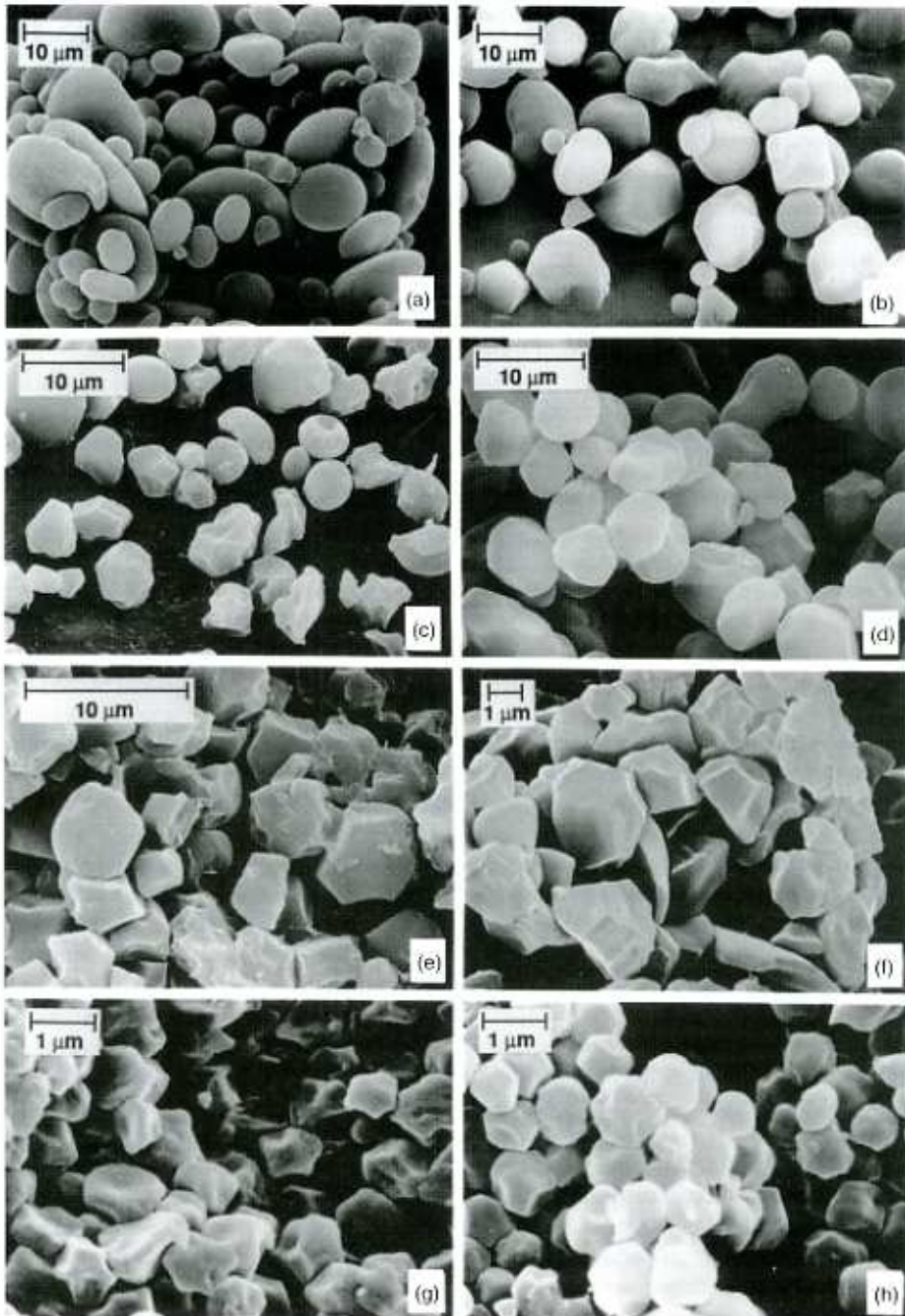


Figure 1-5. SEM images illustrating shape and size of different starches: (a) wheat, (b) corn, (c) oat, (d) buckwheat, (e) rice, (f) canary seed, (g) quinoa and (h) amaranth (Abdel-Aal, 2009).

Table 1-2. Properties of common starches and their pastes (from Abdel-Aal *et al.*, 2002; Chaisawang and Supphantharika, 2006; Thomas and Atwell, 1999).

Property	Corn	Waxy corn	Wheat	Waxy Wheat	Rice	Potato	Tapioca
Shape	Round, polygonal	Round, polygonal	Round, lenticular	Round, lenticular	Polygonal, spherical compound granules	Oval, spherical	Oval, truncated
Diameter (mm)	3-25	3-25	2-35	2-35	1-3	5-100	4-35
Amylose (%)	21.0	2.9	26.9	3.2	16.4	22.0	17.0
Crystallinity (%)	27	28	20	27	25	24	24
Peak viscosity (BU)	500	1200	210	1100	315	770	620
DSC transition temperature (T_o - T_p - T_c , °C)	57-70-84	60-72-84	55-63-73	56-66-80	64-68-74	60-66-76	63-71-81
Paste viscosity	Medium	Medium high	Medium low	Medium high	Medium high	Very high	High
Paste texture	Short	Long	Short	-	-	Long	Long
Paste clarity	Low	Medium high	Low	-	-	Very high	High
Retrogradation rate	High	Very low	High	Very low	Medium low	Medium low	Low

BU= Brabender unit; T_o , T_p and T_c = onset, peak and completion temperature, respectively.

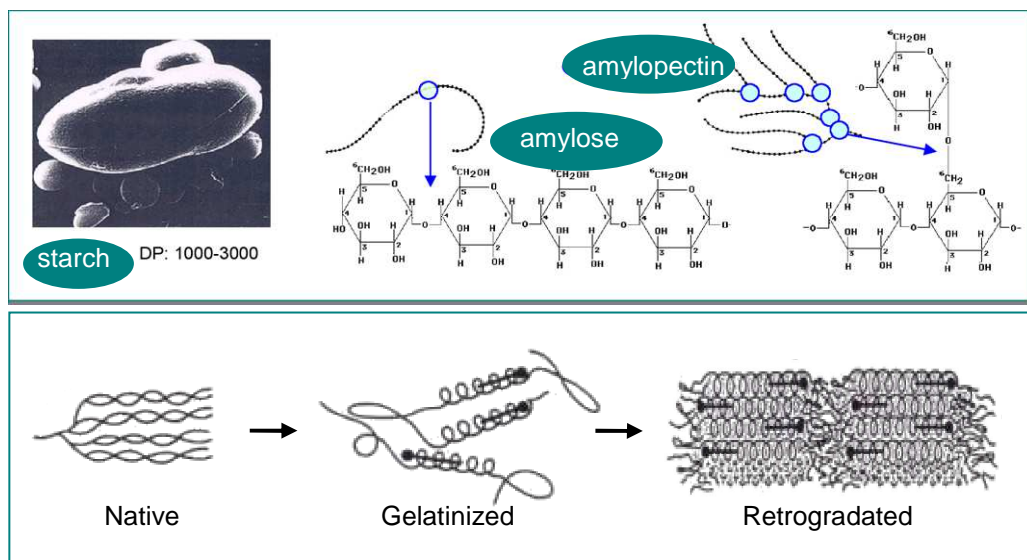


Figure 1-6. Starch components and molecular order.

Starch has unique organization and properties that determine its functionality in many food application (Figure 1-6): when starch granules are heated in the presence of sufficient water and above a specific temperature (the glass transition temperature), it adsorb water undergoing towards a disruption of the molecular order, loss of crystallinity and granular swelling. This process is termed gelatinization. Heating and hydration of non-crystalline regions facilitate molecular mobility in these regions and disassociation of the amylopectin double helices and melting of crystallites (Tester and Debon, 2000). The gelatinization process is also associated with amylose leaching, which increases the viscosity of the starch suspension. During further heating, swelling and leaching continue and a continuous phase of amylose solubilised and a discontinuous phase of swollen, amorphous starch granules or remnants are formed. When the current slurry is cooled the starch polysaccharides re-associate to a more compact state: this process is termed retrogradation. The gelatinization and retrogradation of starch-water systems can be investigated by many tools such as Rheometer, Micro-Viscoamylograph, Differential Scanning Calorimetry (DSC) or enzymatic assays. The kinetics of the starch retrogradation is influenced by a number of conditions and substances such as the starch origin and concentration, the pH of the paste and the presence of salts, sugars and lipids (Eliasson and Gudmundsson, 2006). Jacobson *et al.*

(1997) found that the starch retrogradation rate followed the order of wheat, common corn > rice, tapioca >> waxy corn.

1.4 BREAD

Bread has been a staple food for many cultures across the world; it consists of a dough, made from flour and water with or without other ingredients, which has been fermented by yeast or otherwise leavened and subsequently baked or partly baked.

There are varieties of bread different in sizes, shapes, colors, textures and flavours. Bread is generally characterized by two main parts: the crust, that is the surface of a loaf, generally browner and harder and the crumb, white, soft and with an alveolar structure. The processes involved in producing bread include three basic operations: mixing and dough formation, fermentation, and baking. These operations have to be properly modulated to obtain the large variety of bread presented on the market.

One of the most important step of breadmaking is the leavening phase during which an anaerobic fermentation occurs: the oxygen is rapidly consumed by the yeast and bacteria and glucose is transformed as followed: $C_6H_{12}O_6 \rightarrow 2 CO_2 + 2 CH_3CH_2OH + 66.5kJ/mol$.

The major product of yeast fermentation thus are carbon dioxide and ethanol. As carbon dioxide is produced, the dough's pH decreases. Indeed, dough just out of the mixer usually has a pH of about 6.0 and during fermentation drops to about 5.0. or less (sourdough fermentation). A rapid drop is caused at first by carbon dioxide dissolving in water; a second factor is the slow production of organic acids by the bacteria in the dough. At the end of fermentation, most of the leavening gas is present as carbon dioxide (CO_2) and little of it is bicarbonate (HCO_3^-) or carbonate (CO_3^{2-}). The carbon dioxide is produced in the aqueous phase and when the water will saturate, newly produced CO_2 must migrate into the pre-existing air bubbles. This determines an increase of bubbles pressure. The dough's viscosity-flow properties allow the bubbles to expand and thereby equalize the pressure. The results are an increase of the total volume of the dough's mass and the formation of the typical aerated matrix that will become the bread crumb (with a typical alveolar structure) after baking. A useful tool to study the development of dough and the gas production and retention by the dough during proofing, crucial phase of the breadmaking, is the Chopin Rheofermentometer. A second increase of the dough volume could appears during baking due to the water evaporation.

There are different **processes** around the world to produce bread. The main differentiation is between “discontinuous” and “continuous” processes: the former require different steps carried out in different plants, whereas the latter are performed on the whole ingredients and without any stops among the different operations (continuously). The traditional European breadmaking technology is the discontinuous process (Figure 1-7) that is farther distinguished in “straight dough” and “sponge and dough”. The most used is the “straight-dough system”, characterized by only one mixing phase during which all ingredients are added and a leavening step performed with compressed yeast. As reported by Delcour and Hosenev (2010), the first step is to weigh the ingredient (flour, water, salt, fat, yeast, others), mix for 15-20min at 25-30°C to obtain an homogeneous and developed dough, rest at 80-90% RH “punch phase”, divide and intermediated proof for 25min to increase the dough size. Then the dough is moulded into the loaf shape and place into the baking pan, proofed (at 25-30°C, 85-90% RH, for around 50-60min) and baked. This process give bread with a coarser cell structure and the quality of the product obtained is quite sensitive to the timing between individual process steps.

In North America and in some part of Italy is preferred a “sponge-and-dough procedure” in which the leavening agent is a part of a dough just proofed by yeast (Figure 1-7, middle flow sheet). In this process two-thirds of the flour, part of the water, yeast and yeast food are mixed just enough to form a loose dough, which is referred to as a “sponge”. The sponge is allowed to fermented for up to 5 hours. It is then combined with the rest of the formula ingredients and mixed into developed dough. Then there is a intermediate proof (20-30min) allows the dough to relax. The following operations are as in the straight-dough system.

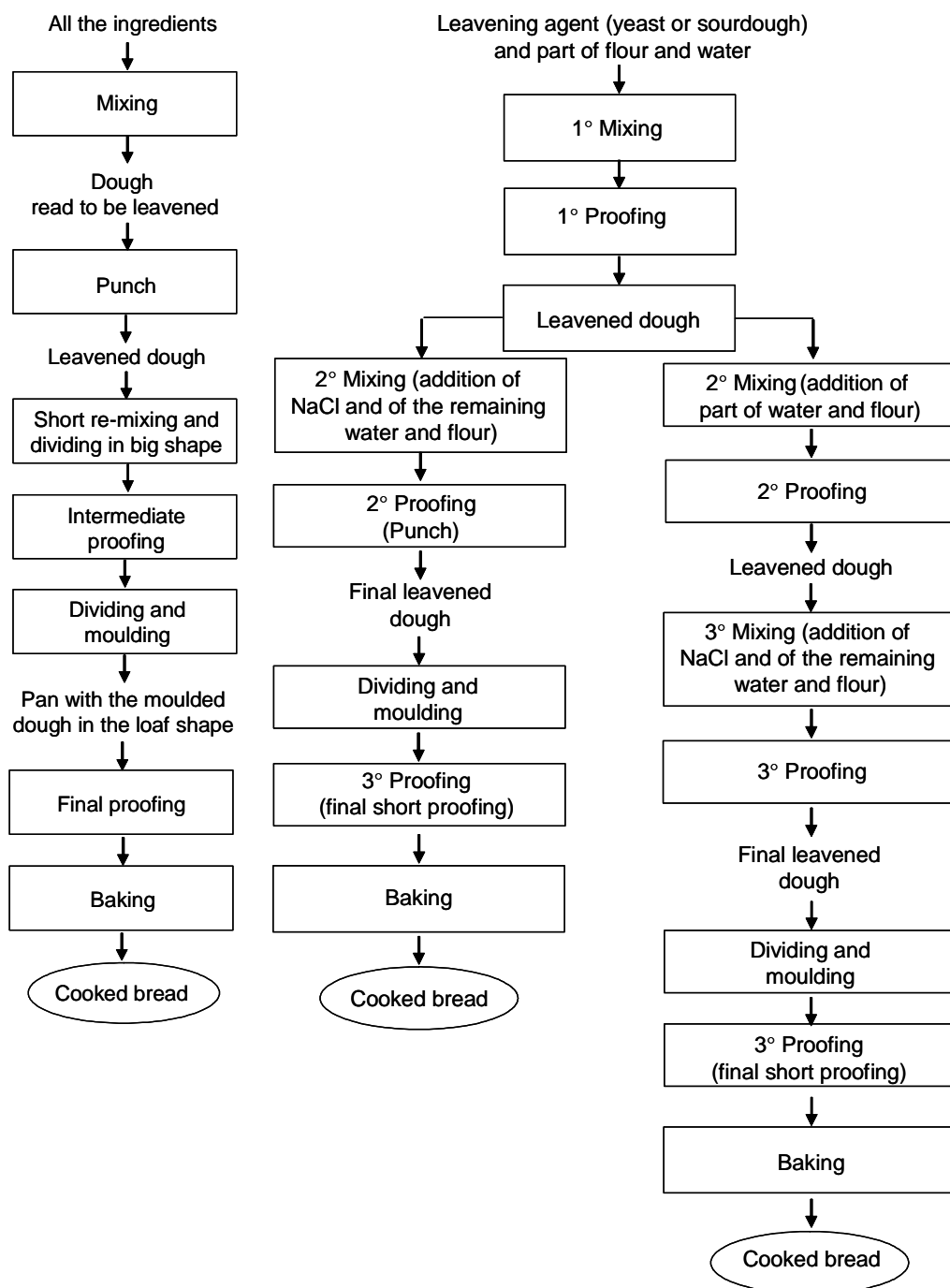


Figure 1-7. Discontinuous process: (left) straight-dough; (right) sponge-and-dough systems.

A similar discontinuous process with further mixing and proofing steps, that develops sourdough (also called “seed sour”, “mother dough” or “starter dough”) as leavening agent (Figure 1-7, flow sheet on the right), is also widespread; in this case many refreshments are required to develop and maintain the microbial species typical of the sourdough. As reported by Pagani *et al.* (2006) the sourdough procedure takes its name from the sharp acidification that occurs due to the microorganisms present in the dough. The microbial groups are linked in a noncompetitive and often mutualistic equilibrium that is quite stable to external perturbation (Foschino *et al.*, 1995; Gobetti 1998).

Generally, sourdough contains yeast (about 10^6 - 10^7 CFU/g) belonging to *Saccharomyces* and *Candida* species, which are responsible for the alcoholic fermentation and, consequently for the development of the volume of the dough. The yeast are in association with the lactic acid bacteria (LAB) of the genera *Lactobacillus*, *Pediococcus* and *Leuconostoc* in a yeast:lactobacilli ratio of 1:100 (Ottogalli *et al.*, 1996; Stolz, 2003). Even if the composition of the microflora of sourdough samples from different bread producers shows considerable variability (Tables 1-3a and 1-3b), between the LAB, *Lb. sanfranciscensis* can be considered the key bacterium in the sourdough process. In fact, it represents, with *Lb. pontis*, the major part of the microbial flora (till 10^8 - 10^9 CFU/g), and it establishes interesting trophic relationships with the sourdough yeasts (*S. cerevisiae* and *S. exiguus*) due to its effective maltose metabolism (Stolz *et al.*, 1993). Generally, LAB perform an intense acidifying activity, produce lactic and acetic acids in quantities related to the species present in the sourdough (obligate homofermentative, facultive heterofermentative and obligate heterofermentative) (Foschino and Galli, 1997; Stolz, 2003), and capable, in any case, of producing both a significant increase in the total titratable acidity and a pH decrease. These changes positively influence not only the sensorial properties of the dough and of the finished product, but also their consistency, thus providing an extended shelf life (physical and microbiological) for the bread obtained using this process.

The “sponge-and-dough system” is more time consuming than “the straight dough system” and it gives soft bread with fine cell structure. The bread obtained using sourdough is also characterized by a typical sour taste and has a longer shelf-life.

The “liquid-sponge system” that involves a fermentation in a liquid medium is also widespread. In this case, part of the flour is held out of the fermentation step and added in a second time. In United Kingdom is more popular a “short-time breadmaking system” that used the Chorleywood procedure consisting of a mixing under a partial vacuum, with a strong mixing and a physical leavening.

Table 1-3a. Characteristics of lactic acid bacteria and yeasts isolated from sourdough of Italian typical baked products (Pagani *et al.*, 2006).

Genus	Species	Fermentation	FQ ^a	Optimal temperature °C (F)	Recovery rate
Lactic acid bacteria					
<i>Lactobacillus</i>	<i>acidophilus</i>	obligate homofermentative	> 20	37-42 (98-108)	low
	<i>amylophilus</i>				low
	<i>amylovorus</i>				low
	<i>delbrueckii</i>				low
	<i>faracinis</i>				medium
<i>Lactobacillus</i>	<i>alimentarius</i>	facultative heterofermentative	10-20	30-35 (86-95)	low
	<i>casei</i>				medium
	<i>curvatus</i>				low
	<i>paracasei</i>				
	<i>plantarum</i>				high
<i>Lactobacillus</i>	<i>brevis</i>	obligate heterofermentative	1-5	25-30 (77-86)	high
	<i>buchneri</i>				medium
	<i>fermentum</i>				high
	<i>fructivorans</i>				high
	<i>hilgardii</i>				low
	<i>pontis</i>				medium
<i>sanfranciscensis</i>	high				
<i>Enterococcus</i>	<i>spp.</i>	homofermentative	>20	37-42 (98-108)	medium
<i>Pediococcus</i>	<i>spp.</i>	homofermentative	>20	25-30 (77-86)	low
<i>Leuconostoc</i>	<i>spp.</i>	heterofermentative	1-5	20-25 (68-77)	medium
	<i>citreum</i>				low
<i>Lactococcus</i>	<i>lactis</i>	homofermentative	10-20	25-30 (77-86)	low

Abbreviations: ^aFQ, fermentation quotient=lactate/acetate molar ratio.

Table 1-3b. Characteristics of **lactic** acid bacteria and yeasts isolated from sourdough of Italian typical baked products (Pagani *et al.*, 2006).

Genus	Species	Fermentation	FQ ^a	Optimal temperature °C (°F)	Recovery rate
Yeast					
<i>Debariomyces</i>	<i>hansenii</i>			25-30 (77-86)	medium
<i>Hansenula</i>	<i>anomala</i>			25-30 (77-86)	low
	<i>subpelliculosa</i>				low
<i>Pichia</i>	<i>guilliermondii</i>			25-30 (77-86)	low
	<i>saitoi</i>				high
<i>Saccharomyces</i>	<i>cerevisiae</i>	alcoholic		25-30 (77-86)	high
	<i>exiguus</i>			20-25 (68-77)	high
<i>Torulaspora</i>	<i>delbrueckii</i>			25-30 (77-86)	medium
<i>Candida</i>	<i>holmii</i>				high
	<i>krusei</i>			20-25 (68-77)	high
	<i>milleri (humilis)</i>				medium
	<i>robusta</i>				medium
	<i>stellata</i>				low
<i>Rhodotorula</i>	<i>glutinis</i>			20-25 (68-77)	low

Abbreviations: ^aFQ, fermentation quotient=lactate/acetate molar ratio.

The mechanism of **bread staling** has been studied since a long time. It is a complex phenomenon, sensitive to flour types, additives, processing conditions, and storage conditions. Proposed theories of bread staling mechanisms include physical and chemical changes in bread during storage, such as taste, aroma, firmness, opacity, crystallinity, crumbliness, water absorption capacity and susceptibility to attack by β -amylase. Numerous studies have been performed and several possible theories have been proposed.

Schiraldi *et al.* (1996) proposed a water migration model (Figure 1-8), which attributed bread firming to moisture migration. In this model, water molecules form a bridge between each-other-facing binding sites. A direct bond between

chains can easily displace these bridge bindings. Then water molecules can diffuse to the next neighbouring sites and promote the formation of a new direct inter-chain link along polymer chains. Water molecules, which act as sliders of an inter-chain zipper, promote an extension of cross-link networks throughout the bread crumb, such as starch crystallinity. As a result, water migration increases crumb firmness (Schiraldi *et al.*, 1996). Water binding compounds such as sugar, alcohol, pentosan, and hydrocolloid can reduce the bread firming rate. According to this model, those compounds compete with large biopolymers for water and reduce water activity, thus reducing water redistribution and starch retrogradation, and slowing the overall crumb firming.

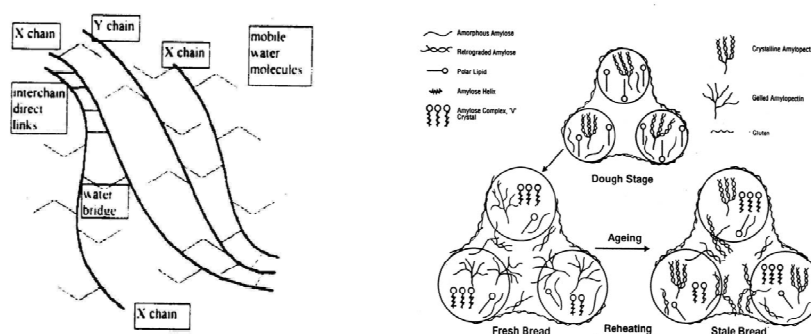


Figure 1-8. Bread staling: a) Moisture migration model (Schiraldi *et al.*, 1996); b) Starch retrogradation model (Zobel and Kulp, 1996).

In 1996, Zobel and Kulp (1996) proposed a model that attributed bread firming to starch (Figure 1-8). Many of the staling mechanisms proposed so far have been accounted for and integrated into this model to some extent. As previously said, starch has different physical states during the bread baking and aging stages. During baking, starch granules are swollen and gelatinized. Crystallinity of branched amylopectin (AP) is disrupted, and some parts of the AP expand into the inter-granular space. At the same time, amorphous and single-helical amylose is released from starch granules. As the bread cools, amylose exists as retrograded double helix and forms juncture points that gel within the inter-granular space. It gives the initial loaf firmness to the fresh bread. During storage, the AP reforms into double helical structure and crystallizes again. It provides rigidity to both the swollen granules and the inter-granule materials. As a result, it firms bread crumb. Retrograded AP is more sensitive to heat than retrograded amylose. If bread is reheated, AP crystallinity is disrupted again and bread is re-freshened. In this model, gluten

plays a secondary role during firming because it is relatively inert to changes with time.

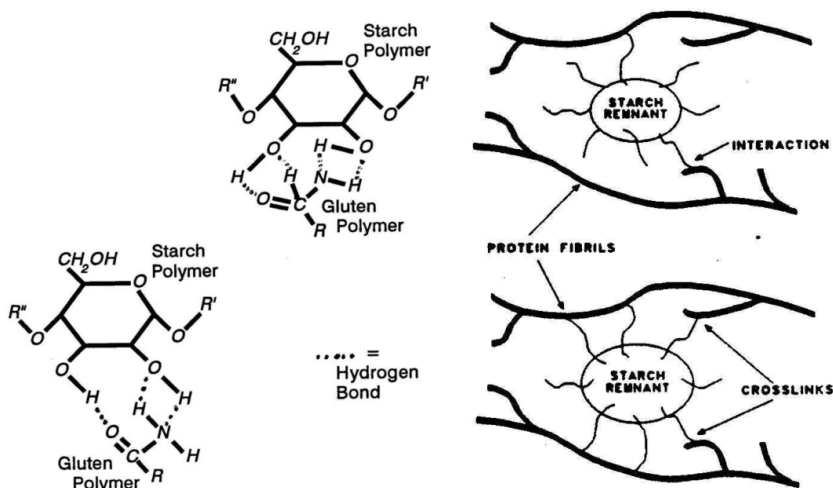


Figure 1-9. Bread staling: a) Hydrogen bonds between gluten and starch (Erlander and Erlander, 1969); b) Gluten-starch interaction model (Martin and Hosney, 1991).

Even if, starch retrogradation is accepted to be the major responsible for the bread staling some points are not explained by this theory, such as starch and protein changes during staling, and water redistribution. Researchers have been aware that protein probably plays an essential role in bread firming since 1954 (Ovadia, 1994). Later, a number of researchers studied the nature and significance of protein in bread hardening. Erlander and Erlander (1969) studied bread made from whole wheat flour and white wheat flour characterized by different protein contents. They concluded that protein inhibited starch retrogradation by forming a complex with starch, and that the ratio between starch and protein in the dough was critical in determining the rate of staling. They suggested that the amide group of glutamine could interact with a glucose unit belonging either to the amylose or to the amylopectin chain (Figure 1-9).

An other model attributing bread firmness to starch-gluten interaction (Figure 1-9) was proposed by Martin and Hosney (1991). In this model, the continuous gluten network in the crumb is cross-linked (entanglements and/or hydrogen bonds) with the remnants of starch granules. The cross-linked structure makes bread firm. After starch gelatinization, the partially soluble starch molecules and swollen granules may entangle with the gluten protein. During aging, the number and strength of the interactions between starch and gluten increase.

The stiffened network makes bread firmer. Surfactants and starch fragments interfere with cross-linking and lead to softening.

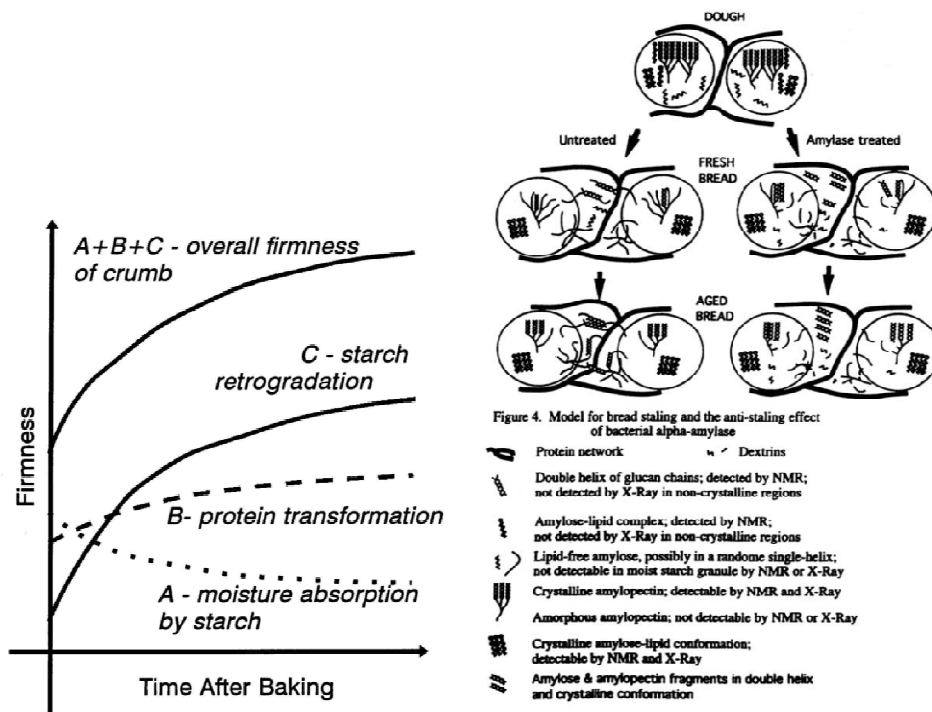


Figure 1-10. Bread staling: a) Multi-component model (Willhoft, 1973); b) Starch and gluten model (Every *et al.*, 1998).

Willhoft (1973) proposed a “multi-component model” that attributed bread firming to starch, gluten, and moisture migration (Figure 1-10). During staling, moisture is released by gluten as part of the staling process. This makes gluten become more rigid. The released moisture is subsequently taken up through starch retrogradation. The overall crumb firmness depends thereof by starch retrogradation rate, protein changes, and softness of starch granules caused by the increase in their moisture level.

Every *et al.* (1998) proposed a model that assigns bread staling to both starch and protein (Figure 1-10). During baking, glucose chains of amylopectin and amylose protrude from the starch granule. They cross-link with the amylose-amylopectin network via double helices to form an increasingly rigid crumb structure in the inter-granule space. During aging, glucose chains also interact with protein fibrils. This model doesn’t explain how gluten-starch interactions develop during staling.

In conclusion it is clear that bread staling is a complex process: no single factor can explain the whole mechanism. Starch and proteins are in close contact in all steps of bread making. In bread, starch is embedded in a continuous three-dimensional gluten network and surrounded by continuous gas cells. Presumably staling is mainly due to changes occurring in the solid phase of crumb and is little influenced by the air cells (Fearn and Russell, 1982).

It may be reasonable to consider that bread staling is caused by changes in starch, gluten, and moisture together. Starch retrogradation is probably a more important phenomenon for bread staling than the interactions between gluten and starch.

1.5 GLUTEN-FREE BREAD

Although in the last years many studies have been made (Gallagher *et al.*, 2004; Guarda *et al.*, 2004; Lazaridou *et al.*, 2007), the gluten-free products present on the market are often characterized by a low nutritional value and unsatisfactory sensory quality, particularly when compared to their wheat counterparts. Why?

- The first reason is the absence of gluten that has unique properties and plays a central role in giving a peculiar texture: soft and aerated (Dobraszczyk *et al.*, 2001).
- Furthermore, due to the lack of gluten and the presence of starch in a large amount, the onset of staling is more rapid than in gluten-containing baked products.

Indeed, as said before, in gluten-free bread production the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensory quality of the final products. Critical are the rheological properties of the dough that, lacking in gluten, shows limited abilities of gas expansion and retention during leavening, factors that inevitably lead to bread with a reduced volume and a low softness of the crumb (Mariotti, 2004). Moreover, the significant presence of starch from different origin (mainly corn, rice and potato) and of flours containing high amounts of starch (rice and corn flour) in GF formulations makes the product more sensible to staling and reduces its shelf-life (Arendt *et al.*, 2008). During staling, water migrates from crumb to crust and leads to a glass to rubber transition of the two main components: the crust becomes soft and leathery and the crumb become more firm and less elastic. The technological approach for the production of gluten-

free bakery products with satisfactory structure, mouth-feel, acceptability and shelf-life includes:

- 1) recipe changes to meet for the gluten absence;
- 2) optimization of the breadmaking process conditions to fit it to the new formulation.

1.5.1 *Raw materials*

Several flours have been used in the development of gluten-free products, alone or in combination; the most used cereals are corn and rice.

Many types of maize (*Zea mays L.*, also referred to as “corn”) are grown around the world but the dent type is generally preferred for flour. The colour of corn kernel can be quite variable. It may be solid or variegate and can be white, yellow, red, blue, dark brown or purple. Yellow is the most common colour, followed by white. The kernel is made up of four principal parts: bran or hull that includes pericarp, epidermis and seed coat (5-6% of the kernel), germ (10-14% of the kernel), tip cap, and the remaining part is endosperm from which flour is obtained (Delcour and Hosney, 2010). The corn flour generally contains between 75% and 87% starch and 6-8% protein (Shukla and Cheryan, 2001). In the corn kernel there is a vitreous part of the endosperm, near the aleurone, that is tightly compact and an opaque part near the centre of the kernel referred as “soft” endosperm. Chemical analysis of the separated opaque and translucent parts of the endosperm have shown that the two portions have similar protein concentration but that proteins are different in terms of distribution and amino acid composition. The starch granules in the two parts of the endosperm are different: polygonal in shape and held together by a protein matrix in the translucent part, and spherical and with many air spaces, leading to opacity, in the opaque endosperm part. One possible explanation for two different starch-granule shapes in a single kernel is that, during the natural drying process, the protein loses water and shrinks. The adhesion between protein and starch is strong enough to pull the starch granules closer and closer together. At this stage the granules are pliable and, as they are tightly packed, they become polygonal in shape. In the opaque endosperm, protein-protein bonds rupture during drying, giving intergranular air spaces and maintaining spherical starch granules (Delcour and Hosney, 2010). This theory is confirmed by the fact that if maize is harvested before it drying, granules are more spherical.

As regards the corn proteins they are made mainly of a prolamin called “zein”. The endosperm contains about 5% albumins and globulins, about 44% zein, about 28% glutelins and the remaining protein is a zein fraction cross-linked by disulfide bonds. Maize proteins have a good level of glutamic acid (but half in comparison with wheat), a high level of leucine (amino acid implicated in the onset of pellagra: B-vitamin deficiency disease) and, in a cross-linked zein fraction, an interesting level of praline (18%).

An other important cereal for the GF market is rice (***Oryza sativa L.***) that possesses unique attributes such as bland taste, white colour, ease of digestion and hypoallergenic properties. Many studies on gluten-free bread have been carried out considering rice flour as the main ingredient: for example Gallagher *et al.* (2002b) investigated the application of novel rice starches, manufactured from low to high degrees of starch hydrolysis, as replacements for wheat starch in GF bread formulations, concluding that the optimum level of rice starch inclusion is 6%. Whereas, Cato *et al.* (2002) found that fine white and ground rice flours gave GF breads of good quality when used in combination with hydrocolloids.

Rice is harvested with the hull (or husk) attached and is called “paddy” or “rough rice”. The hull is about 20% of the weight of rough rice and contain cellulose (25%), lignin (30%), arabinoxylans (15%) and ash (21%). The rice caryopsis varies from 5 to 8 mm in length and weighs about 25mg. Brown rice (rice after hull removal) consist of a pericarp (about 2%), seed coat, nuclear epidermis and aleurone (about 5%), germ (2-3%) and endosperm (89-94%). The aleurone is removed with the pericarp and seed coat during abrasive milling, to produce white rice.

Many varieties of rice are grown throughout the world. The most diffuse are: the sticky, short grained *japonica*, and the non-sticky, long-grained *indica* variety. *Japonica* are usually cultivated in dry fields, in temperate East Asia, upland areas of Southeast Asia and high elevations in South Asia, while *indica* are mainly lowland rices, grown mostly submerged, throughout tropical Asia. A third subspecies, which is broad-grained and thrives under tropical conditions, was identified based on morphology and initially called *javanica*, but is now known as *tropical japonica*.

The rice variety differs for the morphology of the plants and grains (e.g. there are white, brown, black, purple, and red rice), resistance to falling, precocity, ramification, productivity, as well as resistance and tolerance to biotic factors

(weeds, diseases and insects) and non-biotic factors (cold, drought, soil acidity, lack of mineral components, *etc.*).

Rice starch granules have a polygonal shape maybe due to the compression of the starch granules during grain development. They are considered composite granules (large granules made up of many small granules) as the granules of oat starch. The individual rice starch granules are very small, averaging 2-4 μ m. As regards the rice proteins, low content (about 7%) is generally showed, compare to other cereals. The glutelin represent the major fraction of total protein (around 80%), whereas the prolamins accounts for only 3-5% of the total proteins. However the amino acid composition is relatively well-balanced, with lysine (limiting amino acid) constituting about 3.5% of the total protein, whereas the level of glutamic is relatively low (less than 20%). The rice endosperm proteins, occurs mainly as protein bodies varying in size from 1 to 4 μ m. A small proportion of the rice protein is associated with the starch granules. Rice has two types of proteins bodies: spherical and irregular shaped.

Other flours used in GF breadmaking to increase the protein content and/or the taste of the product are those from pseudocereals. There are two major subclass of flowering plants, that is, monocots (one seed leaf) and dicots (two seed leaves). Wheat, rye and barley are monocots whereas buckwheat, amaranth and quinoa are dicots and very distantly related to grains in the monocot subclass; for that reason they are classified as pseudocereals.

Gambus *et al.* (2002), highlighted an increase in protein and fibre levels by 32% and 152% respectively, by replacing 10% of corn starch with **amaranth** flour. In fact the protein content of amaranth (*Amaranthus spp.*) (11.7-18.4%) is generally higher than that of wheat (Berghofer and Schoenlechner, 2002) and contains acceptable levels of essential aminoacids (particularly lysine, tryptophan, and methionine), which are found in low concentrations in cereals and leguminous grains of common usage; structural characteristics of these proteins influence their functional properties (Avanza *et al.*, 2005).

Not only protein enrichment is an important issue, but also the technological improvements that may be connected with the use of amaranth. In this regards, Mariotti *et al.* (2009) studied the influence of the replacement of corn starch with amaranth on dough rheological properties. The authors found that a replace of 40% of corn starch with amaranth flour gives a dough with a less

liquid-like behaviour and a better structure, this is more evident in presence of 2% of Psyllium.

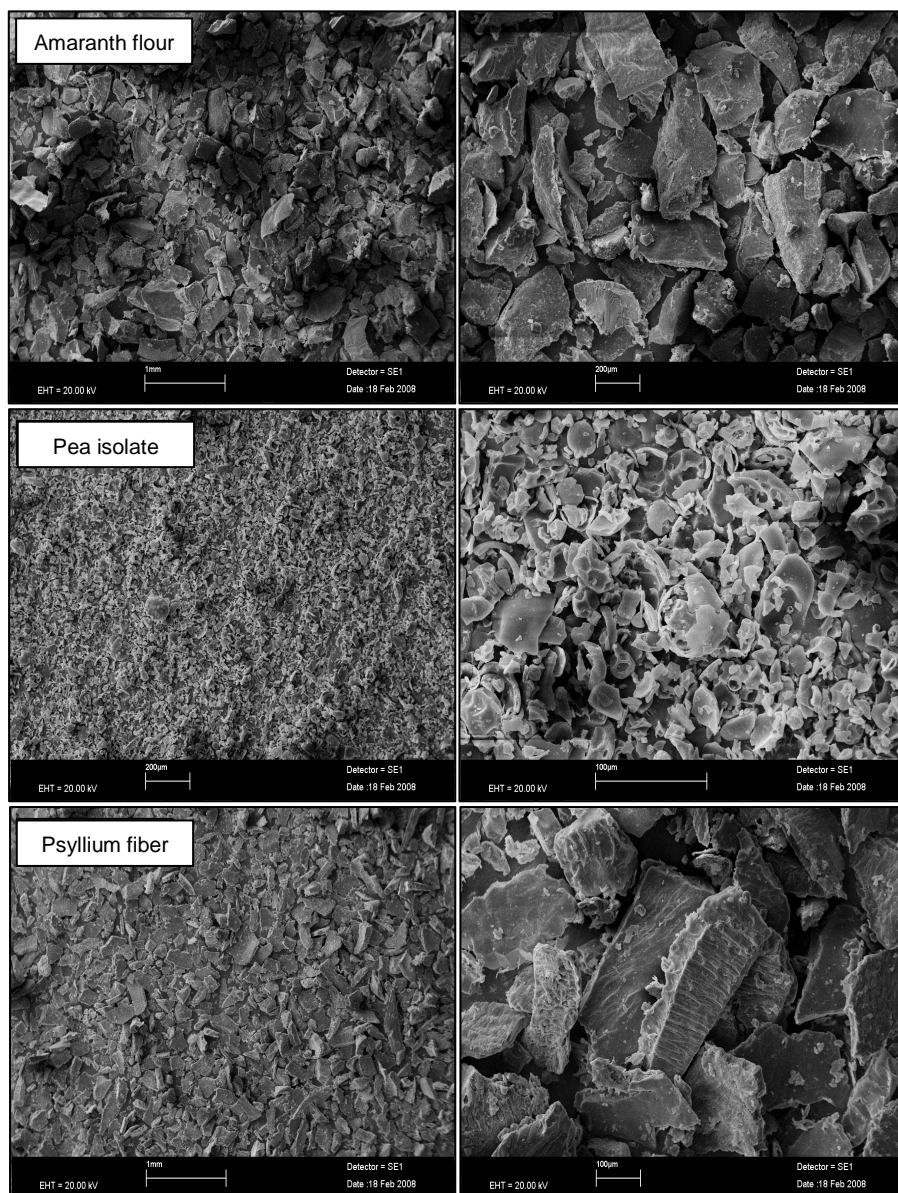


Figure 1-11. SEM images of amaranth flour, pea isolate and Psyllium fibre - images are at different magnifications to optimize views of raw material particles - (Mariotti *et al.*, 2009).

As mentioned, others vegetable ingredients useful to enrich in protein the GF bread and to improve the texture of the final products are Psyllium flour and protein isolated.

Psyllium, or *lispaghula*, (*see also the following section about hydrocolloids*) is a natural hydrophilic mucilloid of the genus *Plantago* and an excellent source of natural soluble fibre. For years its hydrocolloidal property has made it a popular bulk laxative, and more recent studies have shown it has a lipid-lowering property as well (Bell *et al.*, 1989); Psyllium has also a potential role in the treatment and prevention of many diseases and disorders (constipation and diarrhoea, bowel diseases, hypolipaemic activity, diabetes control, hypertension, body weight control) and could even play a protective role in the prevention of colon cancer (Warnberg *et al.*, 2009). In addition Mariotti *et al.* (2009) reported a study on the influence of different ingredients on the rheological properties and on the ultrastructure of doughs containing pea isolate (to increase the protein content) and Psyllium fibre (as thickening agent and fibre source). Psyllium showed interesting technological properties that could be explained not only by Psyllium's extremely strong gelling and water-absorbing abilities, but also by the creation of a thin network (composed by protein and hydrocolloid) able to limit starch swelling and gelatinisation.

Pea (*Pisum sativum*) is a natural gluten-free, hypoallergenic and highly digestible seed; these properties make it an interesting ingredient in food for susceptible people, such as celiacs. The variety mostly used in food industry is the yellow peas (*Canadian Yellow Pea*) from which isolated pea proteins are produced to be used as thickening agent or to increase the protein content of the final product (Mariotti, 2009). Marco and Rosell (2008) found a cross-linking reaction catalyzed by transglutaminase between rice protein and different protein isolated (pea, soybean, egg albumen and whey proteins); The elastic modulus (G') recorded in the oscillatory tests was affected by the protein origin: pea and soybean significant increased the elastic modulus, whereas egg albumen and whey proteins decreased it. Furthermore the Authors showed that pea proteins were able to change the pasting properties of rice flour, decreasing the setback viscosity.

Soybean is another interesting ingredient, but it has some problematic aspects. Soy flour has been used to increase protein quality and quantity as

well as to improve the structural properties of GF products. It is rich in protein but it lacks of S-containing amino acids. Sanchez *et al.* (2002) found that the inclusion of 0.5% soy in a gluten-free formulation enhanced the crumb grain score, bread volume and overall bread score. However the Codex Alimentarius Commission, the EU Commission and other international organisations, have designed soya beans (as well as peanuts, tree nuts, crustacean, fish, cow's milk, eggs, and wheat, sesame) as a high allergenic ingredient and therefore its use has to be carefully evaluated, specially for particular products such as GF.

Moore *et al.* (2004) produced good quality bread by incorporation of buckwheat flour in a GF formulation. Bread had low specific volume and high crumb hardness but the staling rate was lower than in wheat bread. **Buckwheat** flour comes from the achenes of buckwheat (two main species: *Fagopyrum esculentum* and *Fagopyrum tataricum* that generally contain 55% starch, with a ration of 24% amylose and 76% amylopectin, and 11-15% proteins).

The application of **quinoa** (*Chenopodium quinoa*) in GF production has still to be extensively investigated but its addition could be a good way to increase the protein content of the final product as the protein content of quinoa is slightly higher than that of most other cereal grains, while the starch content is lower (ranging from 52% to 69%).

Due to the low intake of fibre attributed to their GF diet (Thompson, 2000) recent studies have also investigated the possibility to enrich gluten-free bread with animal and dietary fibre (Gallagher *et al.*, 2004).

The supplementation of GF product with **dairy protein**, even if it could give technological improvements, is not appropriate for CD suffering people who have significant damages to their intestinal villi, thus lacking in the lactase enzyme, which is normally generated by the villi itself (Ortolani and Pastorello, 1997). According to Murray (1999) approximately 50% of people with CD have in fact a lactose intolerance. Nevertheless several studies have addressed the inclusion of dairy proteins in GF products with good results such as the research by Gallagher *et al.* (2003) where an improvement in the volume, appearance and sensorial quality was obtained upon addition of dairy ingredients, demineralised whey and skim milk powders, sodium caseinate or milk protein isolate. The type of dairy ingredients added is a key factor in

determining the success of their use, as was evidenced by Nunes *et al.* (2007). Despite the encouraging results obtained using dairy products, seems more appropriate and safer in gluten-free products to use for celiacs lactose-free dairy ingredients.

Due to the fact that GF products are frequently made with refined flour or starch, they may not contain the same levels of nutrients of gluten-containing counterparts. To ensure a nutritionally balanced diet GF products have to be fortified with **dietary fiber**. Inulin, that is a non-digestible polysaccharides consisting of chain of $\beta(2-1)$ -linked fructose units with a terminal glucose molecule, is classified as dietary fibre and its addition on GF recipes has been studied in recent years. Inulin also acts as a probiotic by stimulating the growth of “healthy” bacteria in the colon (Gibson and Roberfroid, 1995). Gallagher *et al.* (2002a) incorporated inulin (8% inclusion level) into a wheat starch-based gluten-free formulation by increasing the content of dietary fibre from 1.4% (control) to 7.5% (control+inulin) and by enhancing the crust colour. Besides Silva *et al.* (1996) found that inulin and hydrocolloids show a synergistic effect, with a significant increase in viscosity. Korus *et al.* (2006) used two different levels of inulin (3.5 and 8%) in the production of a GF bread that was stored for 48 hours. They found that 5% inulin increased loaf volume, reduced rate of crumb hardening and had a positive sensory effect. Other studies have shown that the addition ingredients with high-fibre content can bring texture, gelling, thickening, emulsifying, and stabilizing properties to gluten-free foods (Sharma, 1981 and Dreher, 1987).

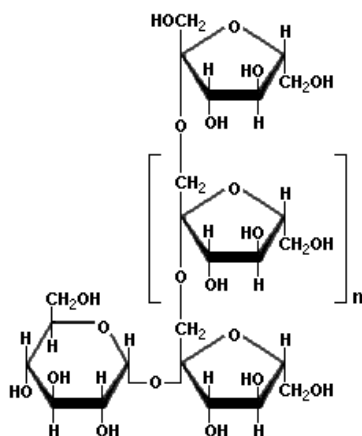


Figure 1-12. Chemical structure of inulin.

Currently, to compensate for the lack of gluten and to simulate its viscoelastic behaviour, the addition of gums and **hydrocolloids** (Figure 1-13) is a quite common practice because these ingredients have a strategic role in making dough workable and in improving the texture of the final product (Gallagher *et al.*, 2004; Kobylański *et al.*, 2004; Lazaridou *et al.*, 2007).

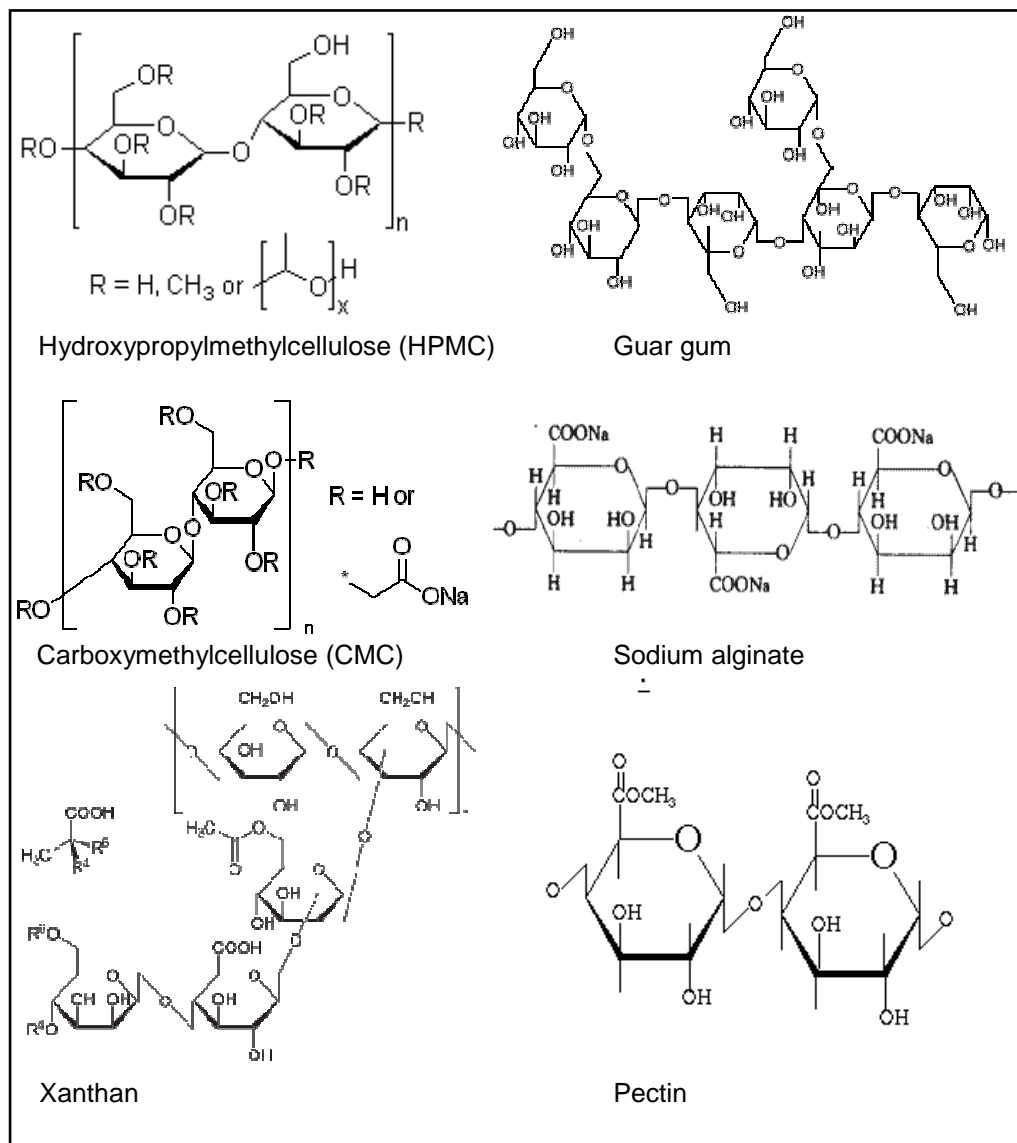


Figure 1-13. Chemical structure of some hydrocolloids used in food industry.

Besides improving the viscoelastic characteristics of dough (Armeo and Collar, 1996), hydrocolloids increase the gas retention capability during proofing (Rosell *et al.*, 2001). Indeed the hydrocolloids are able both to bind water by reducing its migration during bread storage, and to slow down starch retrogradation rate. Although hydrocolloids are generally present at concentration less than 1-2%, they have a significant effect on final product quality and their use can also lead to a reduction in fat use. The mostly used hydrocolloids in bakery are: Hydroxypropylmethylcellulose (HPMC), Carboxymethylcellulose (CMC), guar gum, sodium alginate, k-carrageenan. A synergic effect between HPMC and Psyllium on loaf volume and a no influence of xanthan incorporation in rice bread properties was evidenced by Haque and Morris (1994). Lazaridou *et al.* (2007) studied the effects of different hydrocolloids and their optimum amount in bread recipes concluding that a supplementation level of 1% (w/w) improved volume, whereas with a further increase up to 2% (w/w) a volume reduction was evidenced. Besides, they showed that the elasticity and the resistance to deformation of doughs prepared with the different hydrocolloids followed the following order: xanthan > CMC > pectin > agarose > β -glucan. Also Collar *et al.* (2001) evidenced different performance for the different types of hydrocolloids used; particularly they found that CMC has a preferred interaction with proteins, while HPMC preferentially binds to starch. The results show that the overall effect of hydrocolloids on bread quality undoubtedly depends on the source of hydrocolloids, their structure and concentration, extraction process, chemical modification, and their interaction with GF dough constituents.

Furthermore, also enzymes are used as technological aids in food processing. In the baking industry it is quite commune to use **enzymes** that decrease the ability of amylose/amylopectin to retrograde during storage, thus delaying the bread staling. Gujral *et al.* (2003) successfully used α -amylase of intermediate thermostability and cyclodextrin glycosyl transferase (CGTase) in rice bread formulation. The enzyme are also used as improving agents during the breadmaking process: Diez Poza (2002) studied the activities of transglutaminase as a new tool in the manufacture of baked good and Moore *et al.* (2006) evaluated the impact of transglutaminase at different levels in conjunction with different protein sources such as soya, skim milk or egg powder. This enzyme acts by modifying proteins of different origin by amine incorporation, cross-linking or deamidation. The authors concluded that

transglutaminase can be successfully applied to GF flours to improve their breadmaking potential by promoting network formation.








The glucose oxidase (GO) is an enzyme that catalyzes the oxidation of β -D-glucose in presence of O_2 with the production of D-gluconic acid and a molecule of hydrogen peroxide that can cause the oxidation of free sulfhydryl units from gluten protein giving disulfide linkages, or the gelation of water-soluble pentosans. These reactions promote important changes in the rheological properties of wheat dough (Hoseney and Faubion, 1981). More recently, Gujral and Rosell (2004) demonstrated the ability of GO to modify also rice flour proteins with an increase in the number of disulfide bridges.

1.5.2 *Breadmaking process*

Besides the formulations that contains - as already mentioned before - hydrocolloids to increase dough viscosity and emulsifiers to enhance the formation of a starch-continuous system, also the breadmaking process must be changed appropriately. Most gluten-free doughs contain high water levels and have a more fluid-like structure. In addition, they require short mixing and proofing times than their wheat counterparts so “straight dough method” by using compressed yeast as leavening agent is generally preferred (Figure 1-7).

Moore *et al.* (2004) developed a process with a very short mixing time (only 2 minutes), followed by a scaling to 500g and molding into the baking pan before a fast leavening (30°C, 85% RH, for 30min) and baking. On the contrary, Mariotti (2004) increased the kneading time up to 10min (plus 5min of pre-mixing) with a slow mixing (60rpm) in the first period and a higher speed later (Hobart N-50 mixer, Troy, Ohio). A wire whip was used to mix and foam the liquid dough (Table 1-4, attachment D). At the end of the mixing period the liquid-like doughs (150g) were divided into the baking pans before leavening at 30°C and 80% RH for 35min. Afterwards, they were baked (at 230°C the bottom and 200°C the top of the oven, for 30min), cooled (1h at room temperature) and removed from the baked pan.

Table 1-4. Agitators that could be used with Hobart N-50 mixer (from www.hobartfood.com).

ATTACHMENT	APPLICATION	FOOD PRODUCTS	RECOMMENDATIONS
"B" FLAT BEATER 	<ul style="list-style-type: none"> Multi purpose agitator 	<ul style="list-style-type: none"> Mashing potatoes Mixing cakes Icings 	<ul style="list-style-type: none"> Use 1st speed for starting Medium speed for finishing
"D" WIRE WHIP 	<ul style="list-style-type: none"> Maximum blending of air into light products 	<ul style="list-style-type: none"> Whipping cream Beating egg whites 	<ul style="list-style-type: none"> 2nd & 3rd speeds for 3 speed mixers 3rd & 4th speeds for 4 speed mixers
"E" DOUGH ARM 	<ul style="list-style-type: none"> Mixing Folding Stretching dough in 5-40 qt. mixers 	<ul style="list-style-type: none"> Breads Pizza dough 	<ul style="list-style-type: none"> 1st & 2nd speeds for 2 & 3 speed mixers 1st, 2nd, 3rd speeds for 4 speed mixers
"ED" DOUGH ARM 	<ul style="list-style-type: none"> Mixing Folding Stretching dough in 20-140 qt. mixers 	<ul style="list-style-type: none"> Breads Pizza dough 	<ul style="list-style-type: none"> 1st & 2nd speeds for 2 & 3 speed mixers 1st, 2nd, 3rd speeds for 4 speed mixers
"C" WING WHIP 	<ul style="list-style-type: none"> Heavy whipping 	<ul style="list-style-type: none"> Potatoes Butter Mayonnaise Light icing 	<ul style="list-style-type: none"> 1st & 2nd speeds
"I" HEAVY DUTY WIRE WHIP 	<ul style="list-style-type: none"> Heavy whipping applications 	<ul style="list-style-type: none"> Sponge cakes Light marshmallow 	
"P" PASTRY KNIFE 	<ul style="list-style-type: none"> Cutting action for combining ingredients 	<ul style="list-style-type: none"> Pastry dough Pie dough 	<ul style="list-style-type: none"> For stirring use low speeds For cutting use medium speeds

As alternative leavening agent, the use of **sourdough** represents an attractive alternative to increase the quality of gluten-free breads. Many studies demonstrated the improvement of bread volume and crumb structure (Corsetti *et al.*, 2000 and Clarke *et al.*, 2002), flavour (Thiele *et al.*, 2002) and mold free shelf-life (Lavermicocca *et al.*, 2000; Dal Bello *et al.*, 2006).

The flavour obtained during sourdough fermentation is mainly due to the starter cultures used and to the organic acids and amino acids released during fermentation. Besides, the gas-holding seems to be positively influenced by the acidification of the dough. Incorporation of 20% sourdough proved to have remarkable effects on the final bread quality, delay the bread staling and the growth of spoilage organisms, as showed by Moore *et al.* (2008).

Even if the breadmaking process with sourdough is more time consuming in comparison with the other processes, available data indicate that this procedure may represent an attractive tool to increase the quality (e.g. flavour and shelf-life) of gluten-free bread. It should be noticed that the sourdough process, applied to a gluten-free matrix, has to be further studied in order to optimize processing conditions in terms of formulations and number/conditions of refreshments.

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CHAPTER 2

CHARACTERIZATION OF GLUTEN-FREE RAW MATERIALS

2.1 INTRODUCTION

As reported in Chapter 1, in GF bread production, the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final product. Critical are the rheological properties of the dough that, lacking in gluten, shows a limited expansion due to the inadequate CO₂ retention during leavening, factors that lead to breads with reduced loaf volume and low crumb softness. Moreover, the presence of starches and flours from different origin in GF formulations inevitably increases the staling rate of the product, reducing its shelf-life. Unlike the traditional bread, that is principally made with only one flour (wheat), GF breads contain a mixture of flours and starches from different cereals and pseudocereals. Rice and corn are generally the fundamental raw materials, that can be combined with other ingredients (*i.e.* fibres, vegetable proteins, *etc.*) that are present in GF formulation at lower percentage. Recent studies have investigated the possibility to enrich GF bread with proteins and dietary fibres (Gallagher *et al.*, 2004), to improve the low intake of fibres generally attributed to the GF diet (Thompson, 2000).

In this phase of the PhD research, a chemical and technological characterization of some of the most used GF raw materials was performed. Due to the large variability among the samples, for each of them the most appropriate analytical technique has been used and, in some cases, appropriate changes have been made.

2.2 MATERIALS AND METHODS

2.2.1 *Materials*

The raw materials investigated (Table 2-1) were collected from the market or from other internal laboratory studies on GF topics. Flours and starches from corn and rice, and proteins from pea were mainly considered. Also a waxy rice flour was included, as the amylose content of the raw material is a critical parameter for the shelf-life of the final GF bread.

2.2.2 *Chemical characterization*

The raw materials were characterized for moisture (AACC 44-15A, 2000), total nitrogen content (AOAC 920.87, 1999) and protein content (calculated

adopting 6.25 as conversion factor). The amounts of total starch (TS) and damaged starch (DS) were determined using the “Total Starch Assay Kit” and the “Starch Damage Assay Kit”, respectively (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). The amylose content (UNI ISO 6647, 1991) was determined only on specific raw materials (used also for the trials reported in Chapters 4, 3 and 6). Measurements were performed at least in duplicate ($n \geq 2$).

Table 2-1. Raw materials.

Samples	From
Rice flour (RF)	Beneo-Remy NV (Leuven-Wijgmaal, Belgium)
Rice flour (RF-b)	Internal laboratory studies
Waxy rice flour (WRF)	Beneo-Remy NV (Leuven-Wijgmaal, Belgium)
Rice starch (RS)	Remy Industries NV (Leuven-Wijgmaal, Belgium)
Rice protein (RP)	Remy Industries NV (Leuven-Wijgmaal, Belgium)
Rice bran with germ (RB)	Beneo-Remy NV (Leuven-Wijgmaal, Belgium)
Corn starch (CS)	Roquette Frères (Lestrem, France)
Psyllium fibre (Psy)	Roeper GmbH (Hamburg, Germany)
Locust bean and guar gum (Caremix)	Caremoli SpA (Nova Milanese, Italy)
Guar gum	Cesalpina food Spa (Bergamo, Italy)
Sugar beet fibre (SB)	Danisco Sugar AB (Malmö, Sweden)
Oat (thermo treated)	Internal laboratory studies
Lupine protein (LP)	NaproFood GmbH&Co.KG (Westermosweg, Germany)
Pea protein isolated (IPP)	Nutri-Pea Limited (Manitoba, Canada)
Pea protein isolated (IPP-F9)	Cosucra (Warcoing, Belgium)
Pea protein isolated (IPP-C9)	Cosucra (Warcoing, Belgium)

2.2.3 Water affinity

The methods outlined by Anderson *et al.* (1969), opportunely adjusted, were used to determine the water absorption index (WAI, defined as the grams of water absorbed per grams of dry solids) and the water solubility index (WSI, defined as the water-soluble fraction expressed as a percent of the sample) of CS, RF, WRF and RB. To determine the WAI, 1.67g of sample were suspended in 20mL of distilled water at room temperature in a 50mL centrifuge tube. The content was stirred intermittently (every 10min) over a 30min period and centrifuged at 3.000xg for 10min at 25°C. The supernatant was poured

carefully into an evaporating dish. The remaining gel was weighed and the WAI calculated. WSI was determined from the amount of dried solids recovered by evaporating the supernatant from the water absorption test, and was expressed as a percentage of solids in the sample extract.

For hydrocolloids and fibres, that presented a higher affinity for water, the water binding capacity (WBC) was evaluated, according to the procedure described by Medcalf and Gilles (1965) opportunely adjusted: the sample (0.2g for Caremix, Psy and guar; 0.5g for SB) was added to 50mL distilled water in a 50mL centrifuge tube. The tube was capped, the dispersion was shaken for 60min, and then centrifuged for 10min at 2200xg. After the removal of the supernatant, the sediment was weighed and the amount of water held by the sample was calculated by subtracting the initial weight of the sample. The WBC of the sample was expressed as a percentage, referring to the initial weight of the sample. All the determinations were made in triplicate (n=3).

2.2.4 *Pasting properties*

To study the gelatinization and retrogradation behaviour of starch, the pasting properties of the raw materials containing more than 20% db of total starch were measured using a Brabender_Micro-Visco-Amylograph (MVA) (Brabender OHG, Duisburg, Germany), that provides an accurate control of time-temperature and shear profiles. Fifteen or twelve grams of sample, depending on the sample tested, were dispersed in 100mL of distilled water, scaling both sample and water weight on a 14% flour moisture basis. The suspensions were subjected (stirring at 250min⁻¹ and using a 300 cm³g cartridge) to the following standard temperature profile: heating from 30°C up to 95°C, holding at 95°C for 30min, cooling from 95 °C to 50°C, holding at 50°C for 30min and, cooling to 30°C. For some samples (C S, RF, WRF, RB) the holding phase at 50°C was not carried out. A heating/cooling rate of 3.0°C/min was always applied.

From the resulting viscosity profiles, the following indices were considered (Figure 2-1): gelatinisation temperature (GT, °C; temperature at which an initial increase in viscosity occurs), peak viscosity (PV, Brabender Units, BU; maximum paste viscosity achieved during the heating cycle), breakdown (BD, BU; index of viscosity decrease during the first holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95°C); final viscosity (FV, BU; viscosity achieved at the end of the cooling cycle), and setback (SB, BU; index of the viscosity increase during cooling, corresponding

to the difference between the final viscosity and the viscosity reached after the first holding period). Measurements were performed at least in duplicate ($n \geq 2$).

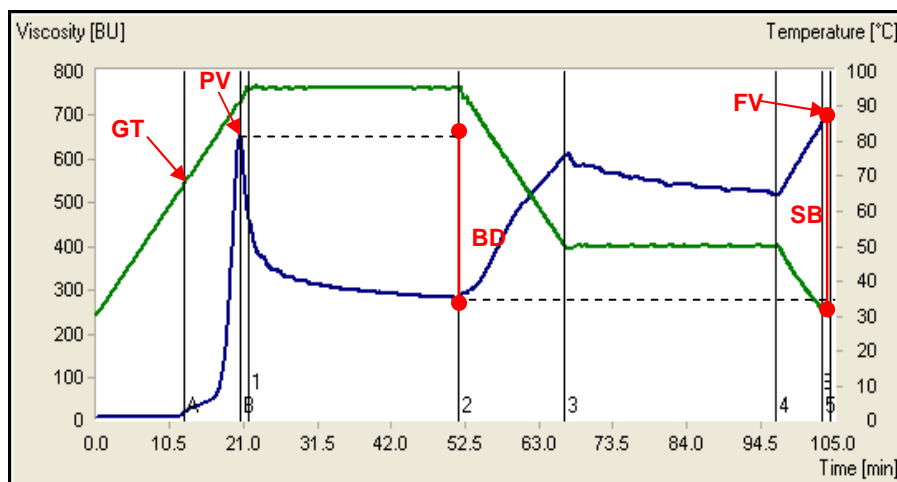


Figure 2-1. Viscoamylographic profile and indices.

2.2.5 Foaming properties and foam stability

The foaming properties of protein isolates were investigated using a Cream Tester CT II (Gerber Instruments, Effretikon, Switzerland) in a non-conventional way. This instrument, generally used for dairy creams, mixes very fast the liquid-like sample up to a fixed consistence or time, permitting air incorporation into the liquid system to form a foam. During mixing, the temperature of the mixing chamber can be regulated (heated or cooled) by a water system.

To study the foaming properties of pea proteins, a diluted suspension was made: 2.5g of the protein isolate was carefully mixed with 50mL of distilled water up to obtain an homogeneous suspension, and immediately poured into the container for analysis (measuring the initial height of the mix), and then into the chamber thermostated at 30°C (Figure 2-2). The suspension was mixed for 4min; after this period, the height was measured again and the suspension was carefully poured into a plastic graduated container with a conic shape, to evaluate the volume of the liquid and of the foam, as well as foam stability, up to 1h at rest at room temperature (t_0 , immediately after mixing; t_{30} and t_{60} , after 30 and 60min, respectively). The following indices were considered: Overrun Properties (OP, %; percentage height increase due to mixing); Foam Stability (FS, %; ratio between the foam volume at each considered time and the t_0 volume). After 1h at room temperature the foam and the liquid phases

were again mixed by hand and the pH value of the suspension was measured. Results are the average of two determinations (n=2).

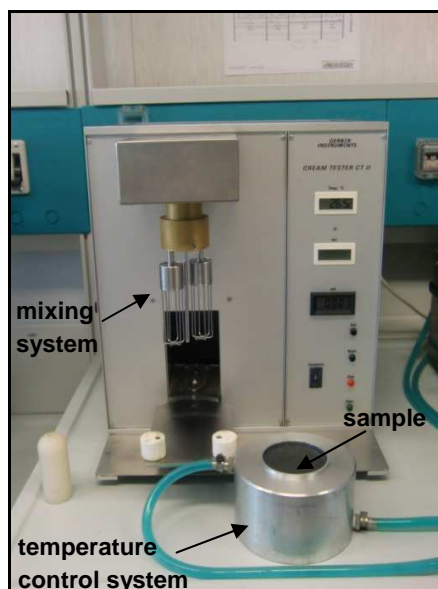


Figure 2-2. Cream Tester CT II (Gerber Instruments, CH).

2.2.6 Statistical analysis

Analytical results were processed by STATGRAPHIC_Plus for Windows 5.1. Analysis of variance (ANOVA) was performed using the Least Significant Differences (LSD) test to compare sample means; differences were considered significant at $P < 0.05$.

2.3 RESULTS AND DISCUSSION

2.3.1 Chemical evaluations

The samples were firstly investigated for their chemical properties (Table 2-2). The moisture content ranged from 5.03 ± 0.05 g/100g db for rice bran (RB) to 12.31 ± 0.01 g/100g db for waxy rice flour (WRF). RF and WRF were significantly ($P < 0.05$) different also for amylose (26.2 g/100g db and 0.7 g/100g db, respectively), protein and damaged starch, while they had the same starch content. RF_b was more similar to WRF than to RF, as regards moisture and protein content.

Table 2-2. Chemical and physical characteristics of raw materials.

Sample	Moisture (g/100g)	Protein (N*6.25) (g/100g db)	TS (g/100g db)	DS (g/100g db)	DS/TS (%)
RF_b	11.47±0.26 ^h	7.86 ± 0.03 ^{bc}	85.16±1.91 ^e	5.80±0.28 ^d	6.73
CS	11.05±0.03 ^g	nd	97.97±1.3 ^h	0.75±0.02 ^a	0.77
RF	9.24±0.01 ^d	8.47 ± 0.05 ^{bc}	86.94±1.97 ^f	11.64±0.07 ^f	13.39
WRF	12.31±0.01 ^j	7.58 ± 0.03 ^{bc}	88.61±2.18 ^f	11.19±0.05 ^e	12.63
RB	5.03±0.05 ^a	15.16 ± 0.05 ^d	20.35±0.25 ^c	4.47 ± 0.05 ^c	21.97
RP	6.9±0.3 ^b	87.44 ± 2.34 ^f	4.58±0.19 ^b	nd	nd
RS	10.35±0.13 ^f	nd	90.73±1.53 ^g	12.00±0.37 ^g	13.24
Psy	8.46±0.05 ^c	3.69 ± 0.11 ^a	nd	nd	nd
SB	9.58±0.25 ^e	9.30 ± 0.32 ^c	1.32±0.04 ^a	nd	nd
Oat	9.82±0.06 ^e	13.26 ± 0.27 ^d	59.92±0.66 ^d	3.52 ± 0.04 ^b	5.87
LP	7.07±0.21 ^b	49.74 ± 2.62 ^e	1.62±0.07 ^{ab}	nd	nd
Guar gum	10.44±0.05 ^f	nd	nd	nd	nd
Caremix	11.84±0.03 ⁱ	6.79 ± 0.14 ^b	nd	nd	nd
IPP*	6±1	82 ± 2	°<12%	nd	nd
IPP-F9*	5±1	90 ± 2	°Max 3%	nd	nd
IPP-C9*	5±1	88 ± 2	°Max 3%	nd	nd

Abbreviations: TS, total starch; DS, damaged starch; nd, not determined; * as stated in the product leaflet; carbohydrates. *Note:* Values followed by same letter in the same column are not significantly different ($P<0.05$).

Concerning total starch, samples could be divided into the following groups: starchy ingredients (in which starch was more than 90g/100g db), rice flours (around 85-88g/100g db), oat and rice bran (60g/100g db and 20g/100g db, respectively) and other ingredients (less than 4g/100g db). It was not possible to determine the total starch content of hydrocolloids such as Psyllium, guar gum and Caremix. In fact, during the test, a high temperature (100°C), in presence of ethanol (80% v/v) and Dimethyl Sulfoxide (DMSO), is achieved to ensure complete starch solubilisation (also of resistant starch) by a thermostable α -amylase enzyme; in these conditions, hydrocolloids create a strong gel that cannot be anymore destroyed and homogenized, making thus not possible the subsequent steps of the analysis. For the same samples, the same happened during the determination of the damaged starch content. As reported by Mariotti *et al.* (2005), the damaged starch content is an important parameter that influences the water absorption and the gelatinization behaviour of a sample. For most of the investigated raw materials, the damaged starch content was quite low. Only RB showed a high damaged starch value, when this index was expressed on the basis of total starch (21.97g/100g db); this has

probably to be related to the severe heat treatment applied for the stabilization of rice bran against lipid oxidation.

As regards proteins, lupine (LP) had a protein content of $49.74 \pm 2.62 \text{g}/100 \text{g db}$, while the isolates of pea proteins (IPP) showed values equal to or higher than $82 \pm 2 \text{g}/100 \text{g db}$. On the contrary, Psyllium fibre was characterized by a reduced protein amount (less than $4 \text{g}/100 \text{g db}$), RF_b, RF, WRF and SB proteins ranged from 7.58 to $9.30 \text{g}/100 \text{g db}$, while RB and oat showed protein contents equal to $15.16 \pm 0.05 \text{g}/100 \text{g db}$ and $13.26 \pm 0.27 \text{g}/100 \text{g db}$, respectively.

2.3.2 Water affinity

The affinity for water - expressed as water absorption index (WAI) or water binding capacity (WBC) - is an important parameter for predicting the water amount to be added during the breadmaking process. The chemical composition of the raw materials, in term of protein, starch, damaged starch and fibre, can certainly have an important impact on these parameters (Table 2-3).

Table 2-3. Water affinity of the samples.

Sample	WAI (g H ₂ O/g db)	WSI (g/100g)	Sample	WBC (g H ₂ O/g db)
CS	1.67 ± 0.24^{ab}	0.71 ± 0.12^a	Psy	48.29 ± 2.77^d
RF	1.90 ± 0.07^b	1.44 ± 0.05^b	SB	8.27 ± 0.48^a
WRF	1.51 ± 0.04^a	3.48 ± 0.01^c	Guar	26.39 ± 1.82^c
RB	2.27 ± 0.10^c	13.36 ± 0.05^d	Caremix	15.10 ± 1.67^b

Abbreviations: WAI, water absorption index; WSI, water solubility index and WBC, water binding capacity. *Note:* values followed by same letter, in the same table and column, are not significantly different ($P < 0.05$).

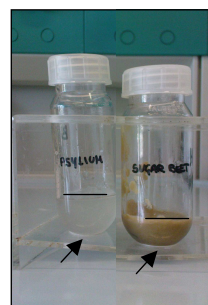


Figure 2-3. Water binding capacity of Psyllium (Psy) and sugar beet fibre (SB).

RB characterized by a high protein ($15.16 \pm 0.05 \text{g}/100 \text{g db}$) and fibre ($>20 \text{g}/100 \text{g}$) content - as stated in the product leaflet - absorbed a higher amount of water (WAI, $2.27 \pm 0.10 \text{gH}_2\text{O}/\text{g db}$) than corn starch and rice flours ($1.67 \pm 0.24 \text{gH}_2\text{O}/\text{g db}$, $1.51 \pm 0.04 \text{gH}_2\text{O}/\text{g db}$ and $1.90 \pm 0.07 \text{gH}_2\text{O}/\text{g db}$ for CS, WRF and RF, respectively) and showed the highest water solubility index (WSI). Even if RB absorbed more water than CS, RF and WRF, this amount was much less than those reached by gums and hydrocolloids. As sugar beet

fibre (SB) can be used in combination with Psyllium (Psy) as a water binding agent, its water affinity was investigated adopting the same procedure used for hydrocolloids and gums. Results are reported in Table 2-3: SB and Psy exhibited extremely different water affinities (WBC: $8.27 \pm 0.48 \text{gH}_2\text{O/g db}$ and $48.29 \pm 2.77 \text{gH}_2\text{O/g db}$, respectively). Furthermore Psy was able to form a gel, whereas SB was unable and appeared, after 1h shaking and centrifugation for 10min at 2200xg, as a weak-slurry (Figure 2-3); guar gum and Caremix (a mixture of locust bean and guar gum) had intermediate behaviours.

2.3.3 Pasting properties

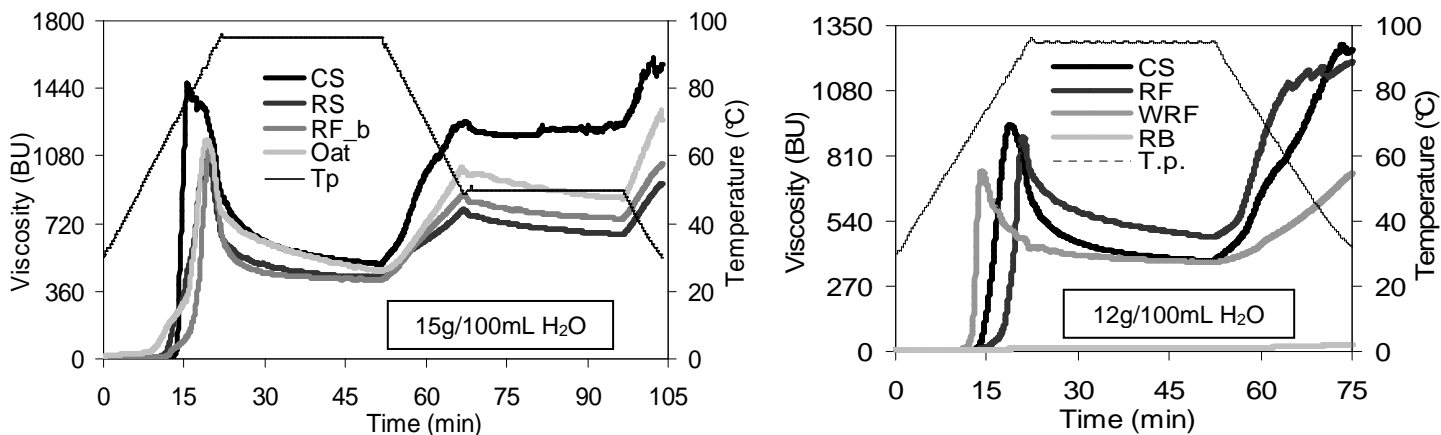
The pasting properties of the raw materials containing more than 20% db of total starch were investigated, adopting two different concentrations: 15g of sample were dispersed into 100mL H₂O for CS, RS, RF_b and Oat; for the other samples more dilute conditions were used (12g in 100mL H₂O) in order to observe a well defined peak viscosity (Figure 2-4).

The pasting properties of flours can be influenced by several factors, including the type and concentration of starch, the amylose/amylopectin ratio, the temperature profile applied to the paste, and the presence and concentration of other soluble and insoluble compounds such as protein, salts, lipids, and fibres (Lii *et al.*, 2004). As shown in Figure 2-4, all the slurries - except RB - increased their viscosity as the gelatinization started, due to starch swelling and rupturing and to the release of amylose outside the granules to form a three-dimensional network with the swollen granules embedded into the matrix (Bhattacharya *et al.*, 1982). Then, on cooling, a further viscosity increase was experienced as the hot paste turned into gel.

The pasting profiles were very different in shape and absolute values (Figure 2-4 and Table 2-4): corn starch (CS), at both the concentrations used, presented the highest peak viscosity (PV), breakdown (BD) and setback (SB) values, and a high final viscosity (FV), indicating that this raw material originated strong gels but very sensible to retrogradation (SB, $1062 \pm 1 \text{BU}$ and $877 \pm 47 \text{BU}$, respectively). This behaviour can certainly be ascribed to the high total starch ($97.97 \pm 1.30\% \text{ db}$) and low damaged starch content ($0.75 \pm 0.02\% \text{ db}$) of this sample. On the contrary, rice starch (RS) showed much lower PV and SB ($1144 \pm 22 \text{BU}$ and $496 \pm 23 \text{BU}$, respectively), even if its total starch content was very high ($90.73 \pm 1.53 \text{ g/100g db}$), to confirm that not only the starch amount, but also the origin of starch and the level of starch damage (DS/TS equal to 13.24% vs. 0.77% of corn starch sample) influences the

retrogradation phenomenon and as the shelf-life of the starchy based food. The oat flour showed a PV similar to RS and RF_b but it started to gelatinize at lower temperatures (54°C, compared to 62.6°C and 65.3°C for RS and RF_b respectively).

Considering the viscoamylographic behaviour of the rice flours having a similar starch content but a very different amylose content (RF, 26.2g/100g db and WRF, 0.7g/100g db), WRF showed lower gelatinization temperature (GT) and a steeper viscosity increase during heating, to indicate that WRF swelled rapidly as soon as gelatinization occurred, while RF exhibited a delay between gelatinization and pasting. This effect has to be related to the more crystalline nature of non-waxy starch (Fitzgerald, 2004), that slowed down starch water absorption and granule swelling. Despite the two rice flours were not significantly different ($P < 0.05$) in terms of starch amount, RF was characterized by a higher PV value. These results are in agreement with the findings of Klug Tavares *et al.* (2010), that evaluated the pasting properties of three rice cultivars having different levels of amylose. Opposite results were obtained by Jiranuntakul *et al.* (2011) and Varavinit *et al.* (2003). These authors found that PV was negatively influenced by the amylose content. Anyway, as reported by many authors (Champagne *et al.*, 1999; Singh, *et al.*, 2000; Zhou, *et al.*, 2003), other components different from amylose -such as proteins and lipids- can also affect the pasting behaviour of rice flours, and therefore they can be partially responsible for the conflicting results obtained by the different authors. As mentioned before, CS showed a high tendency to retrograde, while WRF exhibited a low increase of viscosity during the cooling phase (SB, 370 ± 11 BU), thus suggesting that it could be the most suitable ingredient to delay starch retrogradation rate in starchy products, consequently increasing their shelf-life. It was not possible to study the pasting properties of rice bran (RB): due to its low starch content (20.35 ± 0.25 g/100g db), it did not gelatinize in the experimental conditions adopted during the viscoamylographic test.



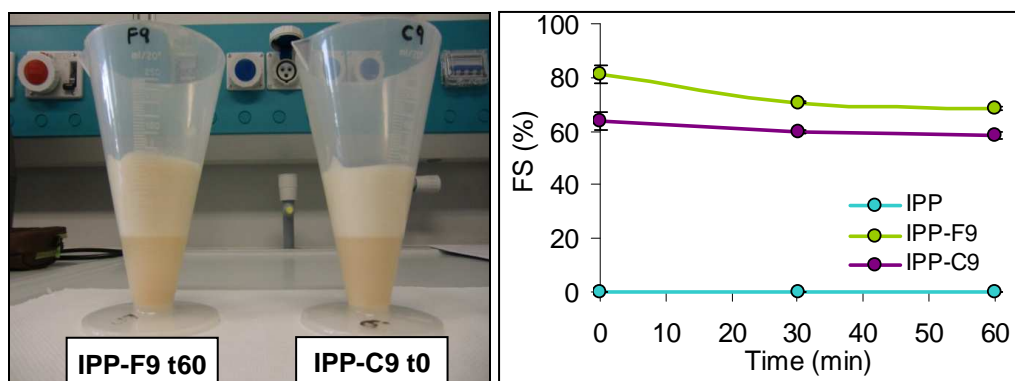
Sample	GT (°C)	PV (BU)	FV (BU)	BD (BU)	SB (BU)
CS	69.0 ± 0.1 ^d	1374 ± 15 ^b	1568 ± 9 ^d	400 ± 31 ^c	1062 ± 1 ^d
RS	62.6 ± 0.2 ^b	1144 ± 22 ^a	930 ± 19 ^a	259 ± 24 ^a	496 ± 23 ^a
RF_b	65.3 ± 0.4 ^c	1118 ± 18 ^a	1036 ± 8 ^b	309 ± 54 ^{ab}	616 ± 12 ^b
Oat	54.0 ± 1.4 ^a	1161 ± 20 ^a	1268 ± 35 ^c	343 ± 19 ^{bc}	800 ± 29 ^c
CS	70.1 ± 0.2 ^b	937 ± 15 ^c	1252 ± 34 ^d	563 ± 12 ^c	877 ± 47 ^c
RF	74.1 ± 0.2 ^c	887 ± 12 ^b	1199 ± 55 ^c	414 ± 9 ^b	726 ± 54 ^b
WRF	65.0 ± 0.1 ^a	748 ± 12 ^a	736 ± 29 ^b	382 ± 10 ^a	370 ± 11 ^a
RB	n.d.	n.d.	28 ± 1 ^a	n.d.	n.d.

Abbreviations: Tp, temperature profile; GT, gelatinisation temperature; PV, peak viscosity; FV, final viscosity; BD, breakdown; SB, setback; n.d., not detectable. *Note:* in each samples group, values followed by same letter, in the same column, are not significantly different ($P < 0.05$).

Figure 2-4 and Table 2-4. Pasting properties of the raw materials.

2.3.4 Foaming properties and foam stability

The evaluation of the foaming properties of proteins is of great interest for screening those more suitable to be used in a GF formulation and for predict their technological behaviour. In contrast to the pasting properties, that are mainly related to the starchy fraction, the foaming properties are connected to the presence of proteins and emulsifiers in the raw material. The ability to form a foam, as stable as possible, is a crucial point to obtain a sponge-dough system suitable for GF breadmaking (Chapter 1). Animal or vegetable proteins have been frequently used to improve the texture of a GF dough and to increase the protein content of GF products (Gambus *et al.*, 2002; Gallagher *et al.*, 2003; Moore *et al.*, 2004; Mariotti *et al.*, 2009). In this research, as well explained in Chapter 7, pea proteins have been used as they are a source of protein less allergenic than soy or dairy ingredients. Three different pea protein isolates, in particular, have been here investigated and compared for their Overrun Properties (OP, %) and Foam Stability (FS, %) immediately after mixing (t0) and after 30 and 60min of rest at room temperature (FS t0, FS t30 and FS t60, respectively).



Sample	OP (%)	FS t0 (%)	FS t30 (%)	FS t60 (%)	pH
IPP	0.77 ± 0.05 ^a	/	/	/	6.29 ± 0.01 ^a
IPP-F9	103.47 ± 5.97 ^c	81.10 ± 3.58 ^b	70.72 ± 0.26 ^b	68.47 ± 0.40 ^b	6.59 ± 0.01 ^b
IPP-C9	67.42 ± 0.36 ^b	63.78 ± 3.61 ^a	59.69 ± 0.72 ^a	58.16 ± 1.44 ^a	7.60 ± 0.01 ^c

Abbreviations: OP, overrun properties; FS, foam stability at the 3 considered times.

Notes: values followed by same letter in the same column are not significantly different (P<0.05).

Figure 2-5 and Table 2-5. Foaming properties of the 3 different pea protein isolates.

As it can be observed in Table 2-5 and Figure 2-5, no foam was produced by IPP, whereas the other two samples gave smooth and quite stable foams. These difference can probably be related to the protein content of the different samples (IPP, 82g/100g db; IPP-F9, 90g/100g db; IPP-C9, 88g/100g db) and to the pH of the solution obtained (pH of 6.30, 6.60 and 7.60, respectively). IPP-F9, that showed an OP of 103% and a FS t60 of 68.5% (Table 2-5) was considered the most appropriated for the subsequent GF breadmaking trials (Chapter 6).

2.4 CONCLUSIONS

Unlike the traditional bread, that is principally made with one flour (commonly wheat flour), the GF leavened products are developed using several flours and starches, alone or in combination with other ingredients (Sharma, 1981; Gallangher *et al.*, 2004; Moore *et al.*, 2004; Lazaridou *et al.*, 2007; Arendt *et al.*, 2008; Mariotti *et al.*, 2009). The absence of the viscoelastic gluten network, in fact, makes the whole breadmaking process problematic and penalizes the sensorial quality of the final product (Mariotti, 2004). To increase the mouth-feel, acceptability and shelf-life of the products, two technical approaches - frequently combined - can be used: enriching the recipe with specific ingredients such as hydrocolloids and proteins, or changing the breadmaking conditions.

In this research, the chemical composition and the technological properties of some of the raw material suitable for GF bread production were evaluated. Particularly useful information were obtained from the micro-visco-amylograph test, that allows to study the pasting properties of a starchy material and to screen it on the basis of its susceptibility to retrograde. CS showed a strong tendency to retrogradation, also higher than rice starch; whereas, as expected, WRF was the least sensible, indicating that it could be used to delay starch retrogradation rate of starchy products such as GF bread, consequently increasing their shelf-life. It is well known that starch re-organization during staling is mainly responsible for the increase of bread firmness, but also water migration from crumb to crust is a crucial point. To reduce the rate of this migration and to increase the water content of the final product the use of fibres and hydrocolloids is quite common (Rosell *et al.*, 2001; Kobylański *et al.*, 2004; Lazaridou *et al.*, 2007). The water absorption index (WAI) and the water binding capacity (WBC) came out to be useful tools for studying the water

affinity of the different ingredients; particularly, Psyllium - among the others - showed the highest WBC.

The technological properties of three pea protein isolates were also investigated: for this purpose, a new test to determine the foaming properties of the raw material and the stability of the foam was developed. This simple and quick test allowed to make clear which pea protein isolates was the most suitable for GF breadmaking.

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CHAPTER 3

RHEOLOGICAL PROPERTIES OF GELS OBTAINED FROM GLUTEN-FREE RAW MATERIALS DURING A SHORT TERM AGEING

*Carola Cappa, Mara Lucisano, Manuela Mariotti
(submitted to Journal of Cereal Science)*

3.1 INTRODUCTION

Many gluten-free (GF) foods, and baked goods in particular, contain a large amount of starch, whose behavior during process and storage greatly influences the final products quality and shelf-life. As reported by Lii *et al.* (1995), the formation of a gel or a paste is one of the principal events that controls the texture and quality of starch-containing foods. Gel structure depends on many factors, such as starch source and concentration, amounts and types of amylose and amylopectin leached out from starch granules, interactions among amylose, amylopectin and granules, besides heating and cooling conditions in terms of treatments temperature, length and rate (Mariotti *et al.*, 2005). During baked goods production, due to the presence of water and to the heat treatments applied, starch gelatinizes: granules swell, and amylose leaches out into the water phase, increasing the viscosity of the system. Upon cooling, if starch concentration is high enough, the leached-out amylose and the swollen granules can convert themselves into a gel. Both amylose and amylopectin are involved in this process, even if at different rates and extents: amylose aggregates at a much faster speed than amylopectin, arranging in a three-dimensional conformation by entanglement of the molecular chains, formation of junction zones and embedment of swollen granules. During this phase, named retrogradation, there is a progressive change from an elastoplastic behavior, typical of dispersions, to an elastic gel (Doublier *et al.*, 1992). As regards GF breads actually available on the market, often based on pure starches, this phenomenon results in low technological and nutritional quality, dry crumb, poor overall mouthfeel and quick staling. The rheological properties provided by the starchy matrix to the final products thus appear to be of great importance in GF breadmaking.

Up to now, instruments such as the Rapid Visco Analyzer and the Brabender Viscoamylograph, have been used as standard analytical tools to assess the pasting characteristics of starch and flour suspensions during heating and cooling cycles (Shuey and Tipples, 1980; Mariotti *et al.*, 2008). As shown by Limpisut and Jindal (2002), the Brabender Viscoamylograph is useful in the development of predictive models for evaluating the hardness and the adhesiveness of rice flours and cooked rice. Dynamic oscillatory rheometry has also been frequently applied to study the viscoelastic properties of starch gels (Lii *et al.*, 1995; Tsai *et al.*, 1997; Hsu *et al.*, 2000) and to monitor gel development without breaking its structure (Kim *et al.*, 2006). Through dynamic rheological tests performed at small amplitude, for instance, Lii *et al.* (1996)

investigated the gelatinization of rice starch gels having various amylose contents. They highlighted how the rheological behavior of starch during heating was mainly dependent on granule properties and, at a lower extent, on the amount of amylose leached out from the granule during thermal processing. On the contrary, Hagenimana *et al.* (2005) indicated that the retrogradation tendency was low in common waxy rice starch, and more evident in high-amylose rice samples. In particular, they found that amylose content played the most important role in changing the elasticity of the starchy system upon cooling and ageing, and that mixtures containing 25% waxy rice flour were the most effective in reducing the rate of gel hardening. According to Lu *et al.* (2009), not only the amylose content but also the chain length distribution in amylopectin affects the viscoelastic properties of rice gels.

This paper investigates, both through empirical and fundamental rheology, the mechanical properties of corn starch and rice flour gels as well as the capability of waxy rice flour and rice bran to reduce the retrogradation rate of different starchy systems, aiming to increase the shelf-life of starch-containing foods, as GF baked products are.

3.2 MATERIALS AND METHODS

3.2.1 *Materials*

Rice flour (RF), waxy rice flour (WRF) and rice bran with germ (RB) were provided by Beneo-Remy NV (Leuven-Wijgmaal, Belgium). Corn starch (CS) was obtained from Roquette Frères (Lestrem, France). Gels were prepared from each raw material (100CS; 100RF; 100WRF; 100RB) and from seven different mixtures (50CS-50RF; 50CS-50WRF; 50CS-50RB; 50RF-50WRF; 50RF-50RB; 50CS-25RF-25WRF; 50CS-25RF-25RB, as percentage w/w sample based) in order to mimic the starchy component of a GF bread formulation.

3.2.2 *Raw materials characterization*

a) *Chemical composition.* The moisture content of the raw materials was determined according to the Official Standard Method AACC 44-15A (2000). The total nitrogen content was evaluated according to the Official Standard Method AOAC 920.87 (1999); the protein content was calculated adopting 6.25 as conversion factor. The amounts of total starch (TS) and damaged starch (DS) were determined using the “Total Starch Assay Kit” and the “Starch

Damage Assay Kit", respectively (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). The amylose content was assessed by the UNI ISO 6647 Method (1991) and expressed as the proportion by weight of amylose (g/100g db). All these determinations were made at least in duplicate ($n \geq 2$).

b) Color. The color of the different flours was measured by means of a Minolta Chroma Meter CR210 (Minolta, Osaka, Japan). Three random readings ($n=3$) were recorded on the leveled surface of each sample (about 40-50g) placed in a Petri dish (diameter: 9.7cm; height: 1.4cm). The color was expressed in the CIELAB space, as L^* (lightness; from 0=black to 100=white), a^* (+a=redness, -a=greenness) and b^* (+b=yellowness, -b=blueness).

c) Particle size distribution. The particle size distribution of the samples (50g) was evaluated by means of the analytical sieve shaker Octagon Digital (Endecotts L.t.d., England), by using 6 certified sieves (openings: 40, 90, 125, 250, 500 and 1000 μ m). Seven fractions were collected after continuously sieving for 10min at amplitude 6 in presence of 3 plastic spheres (diameter: 3.0cm) on each sieve, to make easier the sifting of the fine particles. Duplicate measurements were performed for each sample ($n=2$) and results were expressed as percentage.

d) Water absorption and water solubility. The methods outlined by Anderson *et al.* (1969), conveniently adjusted, were used to determine the water absorption index (WAI), defined as the grams of water absorbed per grams of dry solids, and the water solubility index (WSI), defined as the water-soluble fraction expressed as a percentage of the sample. In order to determine the WAI, 1.67g of flour were suspended in 20mL of distilled water at room temperature in a 50mL centrifuge tube. The content was stirred every 10min, over a 30min period, and centrifuged at 3.000xg for 10min at 25°C. The supernatant was carefully poured into an evaporating dish. The remaining gel was weighed and the WAI calculated. WSI was determined from the amount of dried solids recovered by evaporating the supernatant from the water absorption test and expressed as a percentage of solids in the sample extract. Measurements were made in triplicate ($n=3$).

3.2.3 *Pasting properties of the different raw materials and mixtures*

The pasting properties of the 4 raw materials and of the 7 mixtures previously indicated were measured using a Brabender® Micro-Visco-Amylograph (MVA) (Brabender OHG, Duisburg, Germany), that provides an accurate control of time-temperature and shear profiles. Twelve grams of sample were dispersed in 100mL of distilled water, scaling both flour and water weight on 14% flour moisture basis. The suspensions were subjected (stirring at 250min^{-1} and using a 300cm^3 cartridge) to the following standard temperature profile: heating from 30°C up to 95°C , holding at 95°C for 30min, cooling from 95°C to 30°C . A heating/cooling rate of $3^\circ\text{C}/\text{min}$ was applied. The following indices were considered: gelatinization temperature (GT, $^\circ\text{C}$; temperature at which an initial increase in viscosity occurs), peak viscosity (PV, Brabender Units, BU; maximum paste viscosity achieved during the heating cycle), breakdown (BD, BU; index of viscosity decrease during the holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95°C); final viscosity (FV, BU; paste viscosity achieved at the end of the cooling cycle), and setback (SB, BU; index of the viscosity increase during cooling, corresponding to the difference between FV and the viscosity reached after the holding period at 95°C). Results are the average of at least six replicates ($n \geq 6$).

3.2.4 *Empirical rheological measurements*

The pastes obtained at the end of the viscoamylographic test (see § 3.2.3) were immediately poured into plastic containers, as explained below, and the mechanical properties of the forming gels were evaluated after 30min at 25°C (t_0) and after 1 (t_1), 2 (t_2), 3 (t_3), 4 (t_4), and 7 (t_7) days of storage at 4°C . Before each measurement, gels were conditioned at 25°C for 30min. The mechanical properties of the gels were measured using a TA-HDplus Texture Analyzer (Stable Micro Systems, Surrey, UK), equipped with a 10N load cell. The Texture Exponent TEE32 V 3.0.4.0 Software (Stable Micro System, UK) was used to control the instrument and for data elaboration.

A defined mass of each slurry (18g) was transferred into a small cylindrical container (diameter: 6.0cm; height: 1cm), carefully and gently leveled, and submitted to compression with a cylindrical probe (diameter: 3.5cm) at a crosshead speed of 1mm/s. The sample was compressed up to 30% deformation, and maintained at this deformation for 60s, before releasing the force pulling the probe off the sample. The following parameters were

evaluated from the resulting curves: stiffness (N/mm; slope of the first linear trait of the compression curve), maximum force (F_1 , N), force after the relaxation period of 60s (F_2 , N), elastic index (EI; ratio between F_2 and F_1 , indicates the capability of the material to retain its structure), and, only for t_0 samples, adhesiveness (mJ; negative area measured while pulling the probe off the sample). At least five replicates ($n \geq 5$) were performed for each gel and for each storage time.

3.2.5 *Fundamental rheological measurements*

The fundamental rheological behavior of gels was studied by dynamic oscillatory measurements performed on a Physica MCR300 Rheometer (Anton Paar GmbH, Graz, Austria), supported by the Universal Software US200 (version 2.5) (Anton Paar, Ostfildern, Germany). Gels were prepared as described at § 3.2.4, and evaluated after 30min at 25°C (t_0) and after 1 (t_1), 2 (t_2), 3 (t_3), 4 (t_4), and 7 (t_7) days of storage at 4°C. Measurements were carried out at 25°C, using a corrugated parallel plate system (diameter: 2.5cm) at a gap of 1mm, and a special humidity cover (H-PTD 150) with a water trap and wet pads designed to saturate the water vapor inside, to prevent moisture loss during measurements. Before each trial, gels were conditioned at 25°C for 30min. After loading the sample between the parallel plates, the excess was trimmed off and the sample was allowed to rest at 25°C for 5min to relax stresses, before starting the test.

Dynamic shear data were determined within the linear viscoelastic region (LVR), as determined by preliminary amplitude sweep tests performed in the range of 0.01-300% strain, at a constant frequency of 1Hz. Frequency sweep tests were performed over the range 0.1-10Hz at 1% strain. From each trial, storage modulus (G' , Pa), loss modulus (G'' , Pa) and $\tan \delta$ (ratio between G'' and G') were computed. All measurements were performed in triplicate ($n=3$) with highly reproducible results (relative standard deviation <8%) for each sample.

3.2.6 *Statistical analysis*

Analytical results were processed by STATGRAPHIC®Plus for Windows 5.1. Analysis of variance (ANOVA) was performed using the Least Significant Differences (LSD) test to compare sample means; differences were considered significant at $P < 0.05$.

3.3 RESULTS AND DISCUSSION

The different raw materials studied in this research were chosen taking into account two aspects: which ingredients are actually the basis for GF bread production, and what could be done to reduce the staling phenomena that greatly affect this product, making simple changes to the formulation focusing on the starchy fraction. In this contest, corn starch (CS) and rice flour (RF) were considered as ingredients that are mainly responsible for the texture of many GF systems; whereas waxy rice flour (WRF) and a rice bran (RB) were selected as other raw materials that could be used in GF baked products. The physicochemical properties of these samples were first studied. The consequent step was to assess the technological performances of different starchy mixtures, and specifically the ageing of the systems.

3.3.1 Raw materials properties

The physicochemical properties of the samples are reported in Table 3-1. The moisture content ranged from 5.03 ± 0.05 g/100g db for RB to 12.31 ± 0.01 g/100g db for WRF. RF and WRF were significantly ($P < 0.05$) different for moisture, protein and damaged starch (DS), while they had the same total starch (TS) content, and - as expected - WRF was characterized by a very low amylose content (0.7g/100g db). Generally, rice flours exhibited a high level of starch damage. RB, in particular, showed the highest DS value when expressed on total starch. This result could probably be related to the severe heat treatment applied for bran stabilization against lipid oxidation. It is also interesting to underline the very low DS value of CS, both expressed as absolute value or on total starch basis. The DS content is one of the parameters that mostly affect flours water absorption and gelatinization-retrogradation phenomena (Mariotti *et al.*, 2005). RB was also characterized by a high protein content and a high level of fibre (>20g/100g, as stated in the product leaflet), that - together with the high DS to TS ratio - can be the reasons of the highest WAI of this sample. On the opposite, CS, even if characterized by the finest particle size (more than 90% of the particles smaller than 40 μ m), presented one of the lower WAI and the lowest WSI, given its negligible DS percentage. As regards particle size, RF and WRF had more than 50% of particles ranging between 40 μ m and 90 μ m and an important amount of medium size particles. On the contrary, RB presented very few particles smaller than 125 μ m (only 1.08%), while the majority (69.04%) had sizes included between 250 μ m and 500 μ m.

Table 3-1. Physicochemical properties of the different raw materials.

Sample	Moisture (g/100g)	Protein (g/100g db)	TS (g/100g db)	DS (g/100g db)	DS/TS (%)	Amylose (g/100g db)	L*	a*	b*
CS	11.05±0.03 ^c	-	97.97±1.30 ^c	0.75±0.02 ^a	0.76	26.3	98.4±0.1 ^c	- 1.5±0.1 ^b	3.0±0.1 ^a
RF	9.24±0.01 ^b	8.47±0.05 ^b	86.94±1.97 ^b	11.64±0.07 ^d	13.39	26.2	94.5±0.1 ^b	- 0.2±0.1 ^c	2.5±0.1 ^a
WRF	12.31±0.01 ^d	7.58±0.03 ^a	88.61±2.18 ^b	11.19±0.05 ^c	12.63	0.7	95.7±1.7 ^b	- 0.8±0.1 ^b	4.2±0.2 ^b
RB	5.03±0.05 ^a	15.16±0.05 ^c	20.35±0.25 ^a	4.47±0.05 ^b	21.95	1.5	70.6±0.1 ^a	- 0.2±0.2 ^c	23.5±0.8 ^c

Sample	WAI (gH ₂ O/g db)	WSI (g/100g)	Particle size distribution						
			X ≤ 40	40 < X ≤ 90	90 < X ≤ 125	125 < X ≤ 250	250 < X ≤ 500	500 < X ≤ 1000	X > 1000
			µm (%)	µm (%)	µm (%)	µm (%)	µm (%)	µm (%)	µm (%)
CS	1.67 ± 0.24 ^{ab}	0.71 ± 0.12 ^a	90.26 ± 2.33 ^c	4.09 ± 1.19 ^b	1.83 ± 0.17 ^a	1.60 ± 0.40 ^a	0.73 ± 0.16 ^a	0.61 ± 0.16 ^{ab}	0.33 ± 0.07 ^c
RF	1.90 ± 0.07 ^b	1.44 ± 0.05 ^b	14.25 ± 2.56 ^b	56.70 ± 0.87 ^d	13.10 ± 0.36 ^b	13.78 ± 0.93 ^{ab}	1.04 ± 0.14 ^a	0.43 ± 0.01 ^{ab}	0.27 ± 0.01 ^{bc}
WRF	1.51 ± 0.04 ^a	3.48 ± 0.01 ^c	2.89 ± 1.43 ^a	50.59 ± 0.76 ^c	20.58 ± 0.89 ^c	24.42 ± 0.95 ^{bc}	0.51 ± 0.21 ^a	0.30 ± 0.03 ^a	0.13 ± 0.07 ^{ab}
RB	2.27 ± 0.10 ^c	13.36 ± 0.05 ^d	n.d.	0.15 ± 0.16 ^a	0.93 ± 0.47 ^a	29.96 ± 9.17 ^c	68.24 ± 9.76 ^b	0.68 ± 0.17 ^b	0.12 ± 0.03 ^a

Abbreviations: TS, total starch; DS, damaged starch; WAI, water absorption index; WSI, water solubility index; n.d., not detectable.

Note: values followed by same letter in the same column are not significantly different (P<0.05).

The colorimetric indices (CIE $L^*a^*b^*$) are reported in Table 3-1, too. In fact, as the studied raw materials could be used in GF bread production, color - an important parameter for the consumer acceptability - was also evaluated. Statistically significant differences ($P<0.05$) were evidenced among the samples. CS, RF and WRF were characterized by low values of redness (a^*) and yellowness (b^*) (from -1.5 ± 0.1 to -0.2 ± 0.1 and from 2.5 ± 0.1 to 4.2 ± 0.2 , respectively) and by high lightness (L^* , more than 94.5 ± 0.1). On the contrary, RB presented a low L^* (70.6 ± 0.1) and a high b^* (23.5 ± 0.8); for this reason, the amount of RB to be used in a GF bread formulation should have to be carefully balanced to obtain a pleasant color of the final product.

3.3.2 *Pasting properties of the different raw materials and mixtures*

Generally, starchy slurries increase their viscosity as the temperature raises, due to starch swelling and rupturing and to the release of amylose outside the granules to form a three-dimensional network with the swollen granules embedded into the matrix (Bhattacharya *et al.*, 1982). Swelling is characterized by an initial phase of slight swelling, a second phase of rapid swelling and a final stage in which maximum swelling of starch granule is reached (Tester and Morrison, 1990) (peak viscosity, PV). When starch rupture becomes prominent, a decrease of viscosity (breakdown, BD), is observed. Then, on cooling, a further viscosity increase is experienced as the hot paste turns into gel (setback, SB).

The pasting profiles of the various raw materials and mixtures and their viscoamylographic indices are reported in Figure 3-1 and Table 3-2. Different shapes and absolute values were observed. CS presented the highest PV, BD and SB values and a high final viscosity (FV), indicating its ability to form strong gels but a high tendency to retrograde (SB, 877 ± 47 BU). This behavior could certainly be ascribed to the high total starch ($97.97\pm 1.30\%$ db) and the low damaged starch content ($0.75\pm 0.02\%$ db) of this sample. On the opposite, RB - characterized by the lowest total starch content and the highest level of starch damage - did not form a gel in the experimental conditions adopted during the MVA test. Thus, when RB was combined with CS or RF it was embedded into the starchy matrix and determined an important decrease of viscosity, due to its physical interference during gel networking. This decrease was even higher than expected: the experimental PV values of the blends, in fact, were much lower than those calculated theoretically starting from the raw

materials viscosities (215BU vs. 468BU for 50CS-50RB, 140BU vs. 443BU for 50RF-50RB, and 463BU vs. 690BU for 50CS-25RF-25RB). As regards the two rice flours, WRF showed a lower gelatinization temperature (GT) and a quicker viscosity increase during heating, suggesting a more limited range of GTs in comparison to RF. As reported by Kiribuchi-Otobe *et al.* (1998), when starch is composed exclusively of amylopectin, it exhibits low initial pasting temperature, high paste clarity, low syneresis, and high resistance to retrogradation. Despite the two rice flours were not significantly different ($P < 0.05$) in terms of starch amount, RF was characterized by a higher PV value. These results are in agreement with the findings of Klug Tavares *et al.* (2010), that evaluated the pasting properties of three rice *cultivars* having different levels of amylose. Opposite results were obtained by Jiranuntakul *et al.* (2011) and by Varavinit *et al.* (2003). These authors found that PV was negatively influenced by amylose content. Anyway, as reported by many authors (Champagne *et al.*, 1999; Singh *et al.*, 2000; Zhou *et al.*, 2002), other components different from amylose - such as proteins and lipids - also affect the pasting behavior of rice flours, and therefore they can be partially responsible for the conflicting results obtained by the different authors.

Table 3-2. Viscoamylographic indices of the raw materials and their mixtures.

Sample	GT (°C)	PV (BU)	BD (BU)	FV (BU)	SB (BU)
CS	70.1 ± 0.2 ^d	937 ± 15 ^h	563 ± 12 ⁱ	1252 ± 34 ^h	877 ± 47 ^g
RF	74.1 ± 0.2 ^h	887 ± 12 ^g	414 ± 9 ^h	1199 ± 55 ^g	726 ± 54 ^f
WRF	65.0 ± 0.1 ^a	748 ± 12 ^f	382 ± 10 ^g	736 ± 29 ^d	370 ± 11 ^c
RB	n.d.	n.d.	n.d.	28 ± 1 ^a	n.d.
50CS-50RF	72.2 ± 0.2 ^f	881 ± 13 ^g	445 ± 7 ⁱ	1300 ± 26 ⁱ	864 ± 17 ^g
50CS-50WRF	68.3 ± 0.1 ^c	716 ± 12 ^e	349 ± 12 ^e	916 ± 43 ^e	549 ± 47 ^d
50CS-50RB	72.8 ± 0.2 ^g	215 ± 1 ^b	89 ± 4 ^b	364 ± 14 ^c	239 ± 12 ^b
50RF-50WFR	67.6 ± 0.3 ^b	565 ± 16 ^d	153 ± 19 ^c	921 ± 5 ^e	509 ± 48 ^d
50RF-50RB	77.3 ± 0.4 ⁱ	140 ± 1 ^a	36 ± 2 ^a	273 ± 4 ^b	168 ± 4 ^a
50CS-25RF-25WRF	70.2 ± 0.1 ^d	750 ± 21 ^f	366 ± 18 ^f	978 ± 40 ^f	594 ± 40 ^e
50CS-25RF-25RB	71.8 ± 0.1 ^e	463 ± 18 ^c	232 ± 13 ^d	761 ± 20 ^d	529 ± 22 ^d

Abbreviations: GT, gelatinisation temperature; PV, peak viscosity; BD, breakdown; FV, final viscosity; SB, setback; n.d., not detectable. *Note:* values followed by the same letter in the same column are not significantly different ($P < 0.05$).

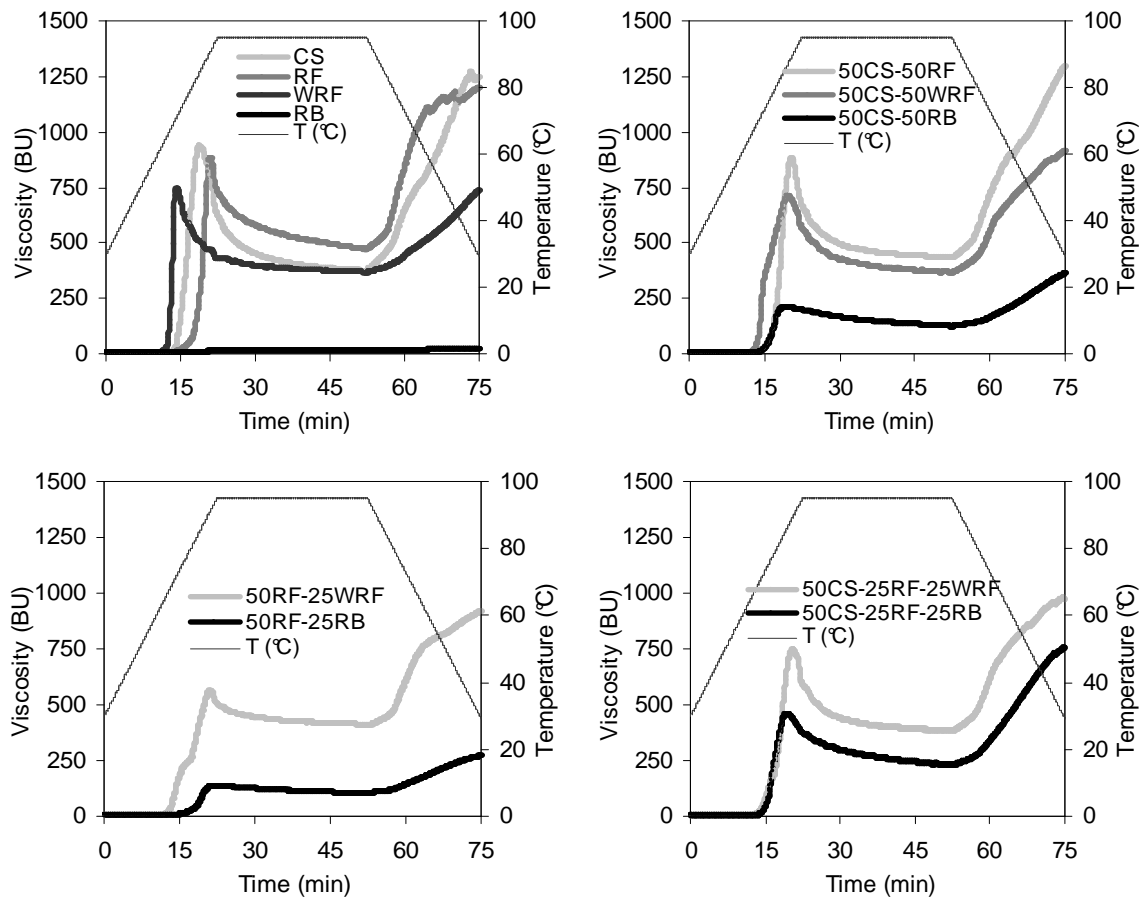


Figure 3-1. Viscoamylographic profiles of the raw materials and their mixtures.

One of the most important indices useful to understand starch tendency to retrogradation, used as a predictor of the short-term staling aptitude of flours, is the SB value, corresponding to the difference between the final viscosity and the minimum viscosity reached before the cooling period. SB values of the different raw materials were significantly different ($P < 0.05$). As mentioned before, CS showed a high tendency to retrograde, while WRF exhibited a low increase of viscosity during the cooling phase (SB, 370 ± 11 BU). Consequently, the presence of WRF in the mixtures determined a SB reduction, dependent not only on starch concentration but also on raw material type. Indeed, it can be noticed that 50CS-50WRF, 50RF-50WRF and 50CS-25RF-25RB mixtures had the same SB value (549 ± 47 BU, 509 ± 48 BU and 529 ± 22 BU, respectively), though containing different starch amounts (93.29%, 87.78% and 75.8%, respectively), while 50CS-25RF-25WRF showed a SB value slightly higher (594 ± 40 BU) even if its starch content was 97.82%. Also the final viscosity (FV) of the gels varied in a wide range, depending on the tendency of each raw material and/or mixture to arrange itself in a more organized structure, on cooling.

3.3.3 *Rheological properties of the different gels*

To study their hardening behavior, the gels obtained as reported at § 3.2.3 (with the exception of RB, as it did not form a gel during the MVA analysis) were stored at 4°C for 7 days and periodically evaluated for their mechanical properties both via compression tests (a) and dynamic oscillatory measurements (b).

a) Response to compression. Results of the compression test are reported in Tables 3-3a and 3-3b. If the different samples are compared at the same storage time, significant differences ($P < 0.05$) in terms of gels hardness (estimated both as stiffness and maximum force) were present among the samples just after 30min resting at 25°C. In particular, on the basis of their stiffness, gels could be ranked as follows: CS, 50CS-50RF, RF, 50CS-25RF-25WRF, 50CS-25RF-25RB, 50CS-50WRF, 50RF-50WRF, 50CS-50RB, 50RF-50RB, WRF. CS gel thus showed the highest hardness while WRF gel exhibited the lowest one, just after the production. A significant correlation was found between gel hardness (both in terms of stiffness and F1) and the viscoamylographic FV ($r = 0.807$, $P < 0.005$; $r = 0.771$, $P < 0.01$, respectively), indicating that the gels formed into the MVA maintained their properties even

when evaluated in different shear conditions. Another important parameter useful in describing the mechanical properties of a gel is the elasticity index (EI), *i.e.* the capability of a material to withstand to a prolonged stress: closer to 1 its value, more elastic and less viscous the sample. Also for this property, the gels resulted significantly different ($P < 0.05$). In particular, WRF gel showed a more viscous behavior (EI, 0.23 ± 0.01), while a more elastic nature characterized CS gel (EI, 0.60 ± 0.01), just after the production. It has also to be underlined how the presence of RB or WRF in the mixtures always reduced the elasticity of the resulting gels. Adhesiveness (determined only on fresh gels) was measured from the negative area of the compression curve registered at the end of the test, when the force was removed pulling the probe off the gel. Samples RF, WRF and the mixtures containing these flours presented the highest values. As regards gels ageing, it was observed that the consistency of the samples increased at different extents and rates. Data obtained from gels evaluation at each storage time can be observed in Tables 3-3a and 3-3b. ANOVA was performed both for the same sample at the different storage times, to evaluate if the observed increase in gel hardness was significant, and among the different samples at the same storage time. In order to make easier the comparison of all these data, another parameter was calculated ($\%_{7-0}$, *i.e.* the percentage increase of each index during storage) and included in Tables 3-3a and 3-3b. After 7 days of storage, CS gels were characterized by the highest consistency (a stiffness value as high as $3.34 \pm 0.11 \text{ N/mm}$), and the same was observed for 50CS-50RF gels among the binary mixtures ($3.33 \pm 0.03 \text{ N/mm}$). CS and RF coupling, actually very frequent in commercial blends for GF bread production, thus originated elastic gels but highly given to hardening. When ternary mixtures were tested (50CS-25RF-25WRF and 50CS-25RF-25RB), it came out how WRF was more effective than RB in delaying gel hardening. This is evident not only considering stiffness absolute values but, even more, when $\%_{7-0}$ indices are observed: the presence of WRF in the blend, in fact, determined lower stiffness increase during ageing in comparison to RB, although RB was more effective against gel hardening at t_0 . As regards EI, an increase during ageing was observed for the majority of the gels, due to structure strengthening induced by the reorganization of amylose chains. In particular, CS and RF samples reached EI values of 0.87 ± 0.02 and 0.71 ± 0.03 , respectively, corresponding to an increase of 45% and 97% if compared to the EI of the fresh made gels. WRF showed an opposite trend, given its constant EI values for the whole storage period.

Table 3-3a. Texture of the different gels stored at 4°C up to 7 days.

Sample	Storage (days)	Stiffness (N*mm ⁻¹)	F1 (N)	F2 (N)	Elastic Index	Ad (mJ)
CS	t0	1.20 ^{a, H}	2.07 ^{a, G}	1.24 ^{a, F}	0.6 ^{a, G}	0.354 ^A
	t1	1.20 ^{a, G}	2.09 ^{a, F}	1.51 ^{ab, G}	0.7 ^{b, I}	-
	t2	1.08 ^{a, F}	2.21 ^{a, D}	1.66 ^{b, F}	0.7 ^{b, I}	-
	t3	1.57 ^{b, I}	2.79 ^{b, E}	2.19 ^{c, G}	0.7 ^{c, H}	-
	t4	1.85 ^{c, F}	3.03 ^{b, G}	2.40 ^{c, F}	0.7 ^{c, F}	-
	t7	3.34 ^{d, H}	5.12 ^{c, G}	4.44 ^{d, F}	0.8 ^{d, I}	-
	% ₇₋₀	177.3	147.5	259.2	45.3	-
RF	t0	0.71 ^{a, F}	1.91 ^{a, F}	0.69 ^{a, E}	0.3 ^{a, E}	2.830 ^F
	t1	0.79 ^{b, F}	1.88 ^{a, E}	1.03 ^{b, E}	0.5 ^{b, F}	-
	t2	0.89 ^{c, E}	2.11 ^{a, D}	1.26 ^{bc, E}	0.6 ^{c, F}	-
	t3	1.14 ^{d, H}	2.60 ^{b, E}	1.54 ^{cd, E}	0.5 ^{c, E}	-
	t4	1.21 ^{d, E}	2.61 ^{b, F}	1.65 ^{d, E}	0.6 ^{d, E}	-
	t7	1.61 ^{e, F}	3.22 ^{c, E}	2.29 ^{e, D}	0.7 ^{e, G}	-
	% ₇₋₀	127.1	68.9	232.8	97.2	-
WRF	t0	0.08 ^{a, A}	0.22 ^{a, A}	0.05 ^{a, A}	0.2 ^{bc, B}	0.799 ^B
	t1	0.10 ^{b, A}	0.25 ^{b, A}	0.05 ^{b, A}	0.2 ^{bc, AB}	-
	t2	0.13 ^{c, A}	0.28 ^{c, A}	0.06 ^{b, A}	0.2 ^{ab, A}	-
	t3	0.08 ^{a, A}	0.23 ^{ab, A}	0.05 ^{a, A}	0.2 ^{bc, A}	-
	t4	0.16 ^{d, A}	0.31 ^{d, A}	0.06 ^{c, A}	0.2 ^{a, A}	-
	t7	0.14 ^{c, A}	0.29 ^{cd, A}	0.06 ^{d, A}	0.2 ^{b, B}	-
	% ₇₋₀	78.5	29.7	30.6	0.0	-
50CS-50RF	t0	0.93 ^{a, G}	2.35 ^{a, H}	1.17 ^{a, F}	0.5 ^{c, F}	1.354 ^C
	t1	1.58 ^{b, H}	3.55 ^{b, G}	2.34 ^{b, H}	0.6 ^{c, H}	-
	t2	1.73 ^{b, G}	3.70 ^{b, F}	2.67 ^{c, G}	0.7 ^{b, H}	-
	t3	2.20 ^{d, J}	4.15 ^{c, F}	3.03 ^{d, H}	0.7 ^{ab, G}	-
	t4	2.73 ^{e, G}	5.21 ^{d, H}	4.02 ^{e, G}	0.7 ^{b, F}	-
	t7	3.33 ^{f, H}	6.69 ^{e, H}	5.61 ^{f, G}	0.8 ^{a, H}	-
	% ₇₋₀	257.6	185.0	379.5	68.1	-
50CS-50WRF	t0	0.42 ^{a, D}	0.68 ^{a, C}	0.19 ^{a, B}	0.2 ^{a, C}	1.599 ^D
	t1	0.56 ^{b, D}	1.49 ^{b, D}	0.39 ^{b, C}	0.2 ^{a, C}	-
	t2	0.57 ^{bc, D}	1.63 ^{c, C}	0.49 ^{c, C}	0.3 ^{b, C}	-
	t3	0.55 ^{b, D}	1.45 ^{b, C}	0.53 ^{d, C}	0.3 ^{c, B}	-
	t4	0.62 ^{c, C}	1.79 ^{d, D}	0.66 ^{e, C}	0.3 ^{c, C}	-
	t7	0.78 ^{d, D}	2.36 ^{e, D}	0.92 ^{f, B}	0.3 ^{d, C}	-
	% ₇₋₀	87.5	243.9	393.0	43.2	-

Abbreviations: Ad, Adhesiveness; %₇₋₀, Percentage increase of each parameters after 7 days of storage. *Note:* For each sample, at the different storage times, values followed by the same small letter in the same column are not significantly different (P<0.05). Among the samples, at the same storage time, values followed by the same capital letter in the same column are not significantly different (P<0.05).

Table 3-3b. Texture of the different gels stored at 4°C up to 7 days.

Sample	Storage (days)	Stiffness (N*mm ⁻¹)	F1 (N)	F2 (N)	Elastic Index	Ad (mJ)
50CS-50RB	t0	0.22 ^{a, BC}	0.6 ^{a, C}	0.1 ^{a B}	0.3 ^{a, D}	1.224 ^C
	t1	0.30 ^{b, B}	0.9 ^{b, C}	0.4 ^{b C}	0.5 ^{b, E}	-
	t2	0.32 ^{c, B}	0.9 ^{b, B}	0.5 ^{c, C}	0.5 ^{c, E}	-
	t3	0.64 ^{e, E}	1.2 ^{c, C}	0.7 ^{d C}	0.5 ^{c, D}	-
	t4	0.58 ^{d, C}	1.2 ^{c, C}	0.7 ^{d C}	0.5 ^{bc, D}	-
	t7	0.90 ^{f, E}	1.8 ^{d, C}	0.9 ^{e B}	0.5 ^{b, D}	-
	% ₇₋₀	302.7	201.4	401.0	66.1	-
50RF-50WRF	t0	0.28 ^{a, C}	0.3 ^{a, B}	0.0 ^{a A}	0.2 ^{a, A}	1.346 ^C
	t1	0.38 ^{b C}	0.4 ^{c, B}	0.0 ^{b A}	0.2 ^{ab A}	-
	t2	0.44 ^{d, C}	0.5 ^{e, A}	0.1 ^{d AB}	0.2 ^{b, A}	-
	t3	0.33 ^{b, B}	0.3 ^{b, A}	0.0 ^{b AB}	0.2 ^{c, A}	-
	t4	0.45 ^{d, B}	0.4 ^{d, A}	0.1 ^{c, AB}	0.2 ^{ab A}	-
	t7	0.42 ^{c B}	0.5 ^{f, A}	0.1 ^{d A}	0.2 ^{bc, AB}	-
	% ₇₋₀	51.5	44.1	53.1	6.3	-
50RF-50RB	t0	0.17 ^{a, B}	0.3 ^{a, A}	0.0 ^{a A}	0.2 ^{bc, C}	0.952 ^B
	t1	0.37 ^{b, C}	0.8 ^{b, C}	0.2 ^{b B}	0.2 ^{bc, BC}	-
	t2	0.40 ^{c, C}	0.8 ^{b, B}	0.2 ^{c, B}	0.2 ^{c, B}	-
	t3	0.46 ^{d, C}	1.0 ^{c, B}	0.2 ^{c, B}	0.2 ^{b, A}	-
	t4	0.45 ^{d, B}	1.0 ^{c, B}	0.2 ^{c, B}	0.2 ^{b, B}	-
	t7	0.60 ^{e, C}	1.1 ^{d, B}	0.2 ^{c, A}	0.2 ^{a, A}	-
	% ₇₋₀	249.3	253.9	181.0	-20.8	-
50CS-25RF-25WRF	t0	0.72 ^{b, F}	1.2 ^{a, D}	0.3 ^{a C}	0.3 ^{a, D}	2.180 ^E
	t1	0.64 ^{a, E}	1.9 ^{c, E}	0.7 ^{b D}	0.3 ^{b, D}	-
	t2	0.62 ^{a, D}	1.6 ^{b, C}	0.8 ^{c, D}	0.5 ^{c, D}	-
	t3	0.81 ^{c, F}	2.1 ^{d, D}	1.0 ^{d D}	0.5 ^{c, C}	-
	t4	0.94 ^{d, D}	2.2 ^{d, E}	1.2 ^{e D}	0.5 ^{d, D}	-
	t7	1.63 ^{e, F}	3.2 ^{e, E}	2.0 ^{f, C}	0.6 ^{e, E}	-
	% ₇₋₀	127.7	163.7	427.1	99.8	-
50CS-25RF-25RB	t0	0.56 ^{a, E}	1.4 ^{a, E}	0.5 ^{a D}	0.3 ^{a, E}	1.261 ^C
	t1	0.72 ^{b, F}	2.0 ^{b, F}	1.2 ^{b F}	0.5 ^{b, G}	-
	t2	0.87 ^{c, E}	2.6 ^{cd E}	1.6 ^{c, F}	0.6 ^{c, G}	-
	t3	0.98 ^{d, G}	2.7 ^{d, E}	1.8 ^{d F}	0.6 ^{d, F}	-
	t4	1.25 ^{e, E}	2.5 ^{c, F}	1.6 ^{c, E}	0.6 ^{cd, E}	-
	t7	2.24 ^{f, G}	4.5 ^{e, F}	2.9 ^{e E}	0.6 ^{cd, F}	-
	% ₇₋₀	303.8	206.0	452.8	80.7	-

Abbreviations: Ad, Adhesiveness; %₇₋₀, Percentage increase of each parameters after 7 days of storage. *Note:* For each sample, at the different storage times, values followed by the same small letter in the same column are not significantly different ($P < 0.05$). Among the samples, at the same storage time, values followed by the same capital letter in the same column are not significantly different ($P < 0.05$).

b) Response to small amplitude oscillatory measurements. One of the major advantages of small amplitude oscillatory measurements is that they provide simultaneous information on the elastic (G' , storage modulus) and viscous (G'' , loss modulus) nature of the material. In this study, the effect of strain was firstly investigated (strain sweep test). For all the samples, G' and G'' remained almost constant at least up to 2% strain (*data not shown*). Beyond this limit, the induced forces became destructive and the gels structure was progressively destroyed. The limits of linear viscoelasticity of these gels were similar to those found by other authors for rice starch-galactomannan mixtures (Kim *et al.*, 2006) or rice amylose gel (Shao *et al.*, 2007), while much wider value were registered for GF doughs or batters based on rice flour, corn starch and different hydrocolloids (Lazaridou *et al.*, 2007) or on corn starch, amaranth flour and pea isolates (Mariotti *et al.*, 2009).

The heating/cooling treatments during gel formation inside the viscoamylograph has induced the networking of the gelatinized starch from the amorphous state to a crystalline form, characterized by a higher resistance to an increasing deformation. Generally, at lower level of strain, G' exceeded G'' , suggesting a solid-like behavior of the gels. At higher strains, the increase of G'' and the decrease of G' was recorded. A cross-over point between G' and G'' was observed at every storage time for samples WRF, 50RF-50WRF and 50RF-50RB, whereas for the other samples the same phenomena was evidenced only after some days of storage (*data not shown*).

The rheological behavior of gels during their ageing was studied by frequency sweep tests over the 0.1-10Hz frequency range, at 1% strain. Figures 3-2a, 3-2b, 3-3a and 3-3b show G' and $\tan \delta$ as a function of frequency for the various samples, at the different ageing times. G' was always higher than G'' in the whole range of frequencies, and its magnitude increased with frequency, with a stronger dependency for G'' , as described by Yoo (2006). The highest G' values were registered for CS and 50CS-50RF gels; on the contrary, WRF originated very weak gels. The addition of WRF both at 25% or 50% concentration strongly reduced G' values of the mixtures containing CS or RF. The same effect was observed also when RB was added to the mixture, both at 25% and 50%. These data, even if recorded under different testing conditions, are in agreement with those obtained from the compression test. The damping factor ($\tan \delta = G''/G'$) was, at every frequency considered, lower than 0.2 for CS, RF, 50CS-50RF and 50CS-25RF-25RB gels, indicating the prevalence of a solid-like behavior; in particular, CS gels were characterized by very low $\tan \delta$ values, reflecting the elasticity of this material. On the contrary,

WRF gel exhibited a more viscous behavior, especially at lower frequencies, with a strong dependence of $\tan \delta$ on the frequency. The same tendency was also evidenced by 50CS-50WRF and 50RF-50WRF gels, even if to a lower extent. WRF, thus, seems to be very effective in changing the rheological properties of gels containing CS and RF. In fact, considering the evolution of G' frequency sweep curves at the different ageing times, it is evident how CS, RF and 50CS-50RF curves shifted progressively towards higher values, while WRF curves were virtually overlapped.

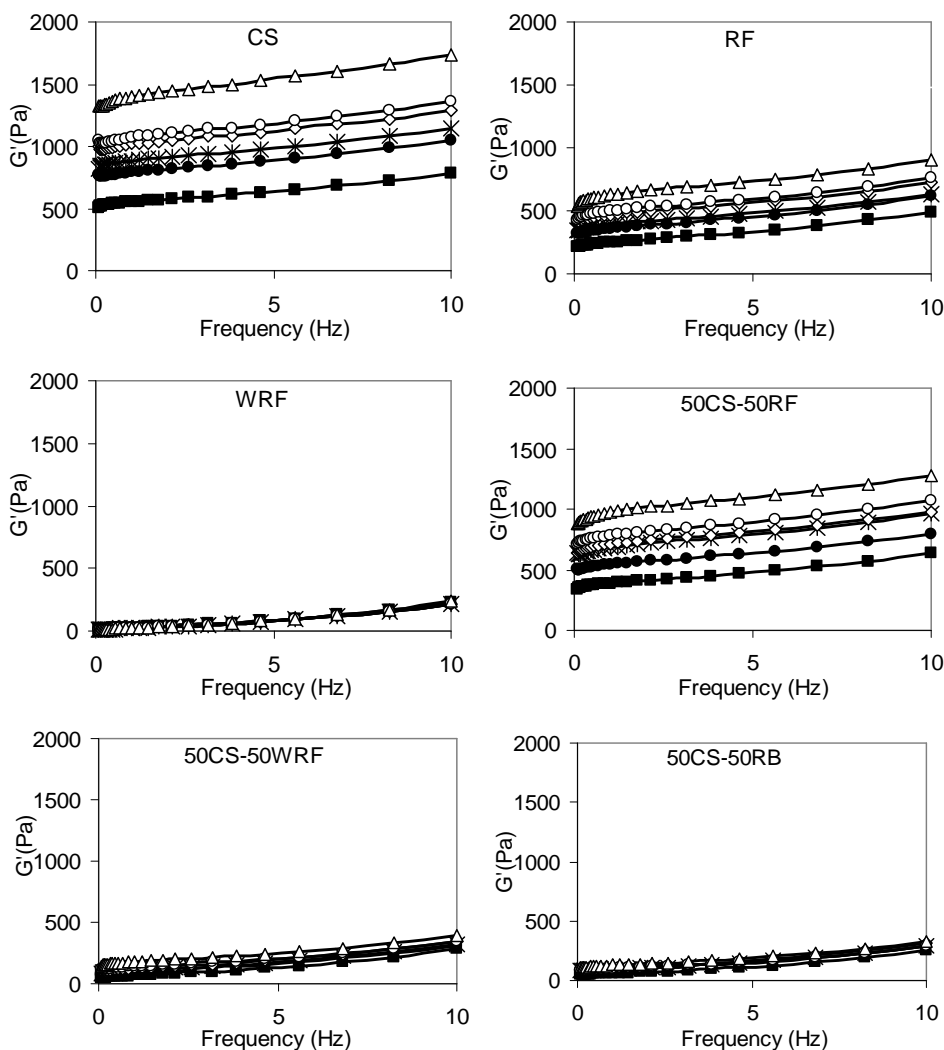


Figure 3-2a. Storage modulus (G') of gels stored at 4°C for (■) 0, (●) 1, (⋈) 2, (◇) 3, (○) 4, (△) 7 days.

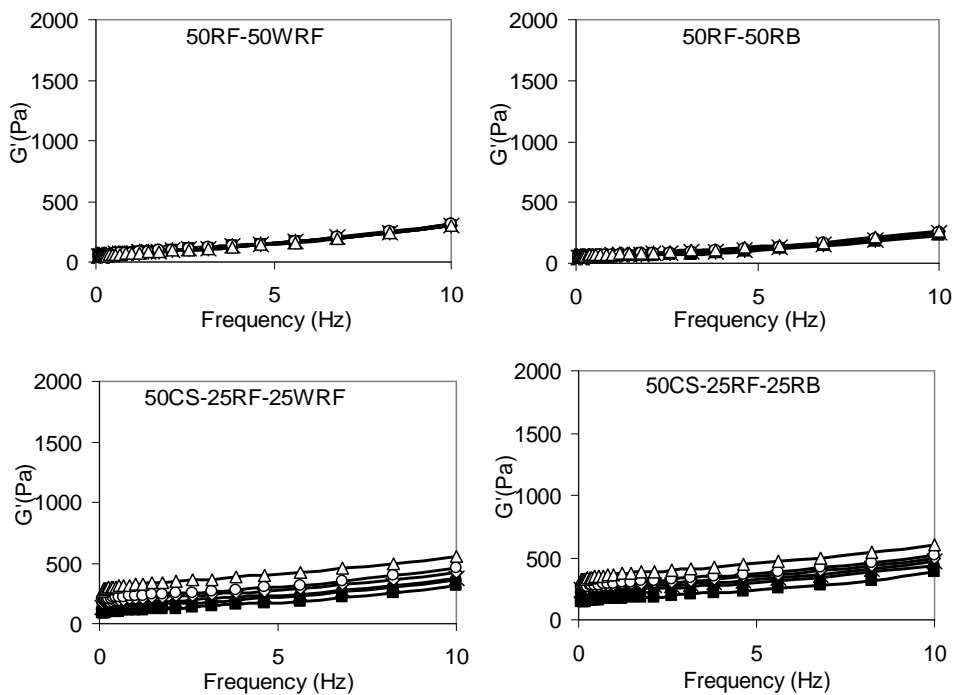


Figure 3-2b. Storage modulus (G') of gels stored at 4°C for (■) 0, (●) 1, (⌘) 2, (◊) 3, (○) 4, (Δ) 7 days.

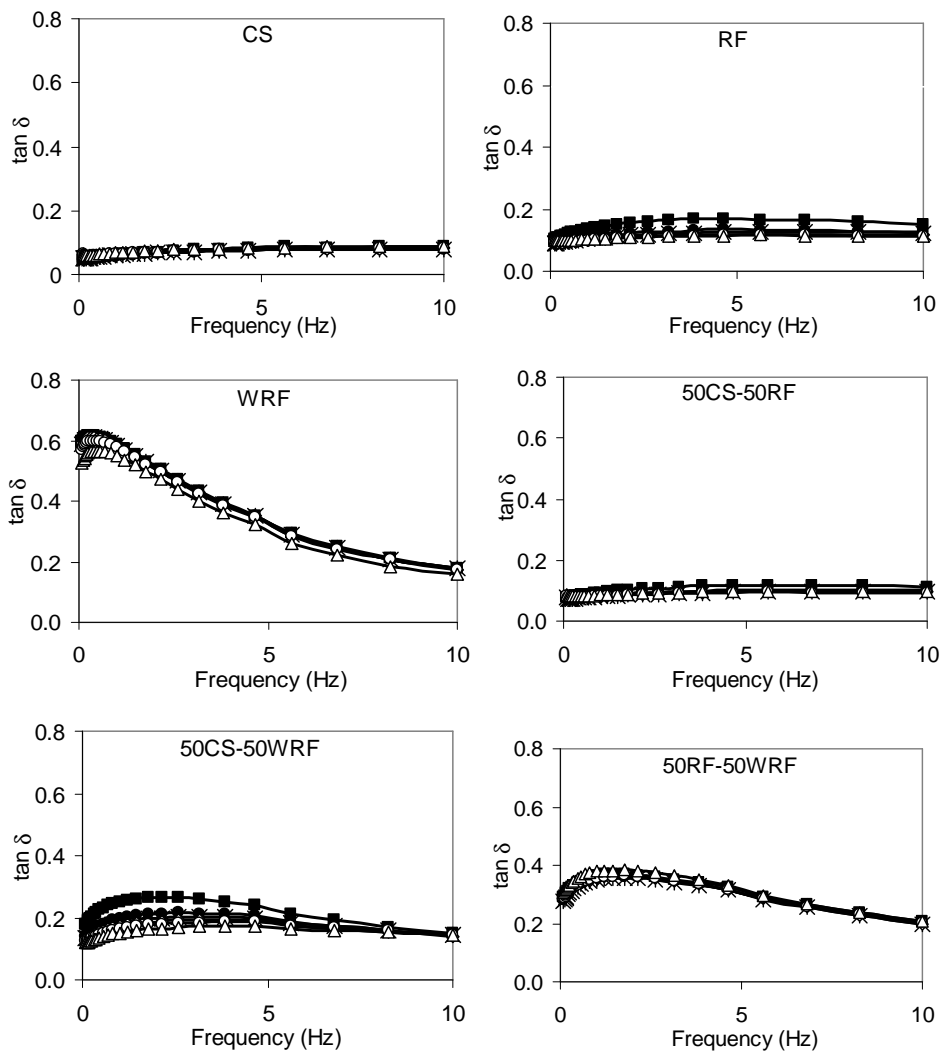


Figure 3-3a. Damping factor ($\tan \delta$) of gels stored at 4°C for (■) 0, (●) 1, (⌘) 2, (◇) 3, (○) 4, (△) 7 days.

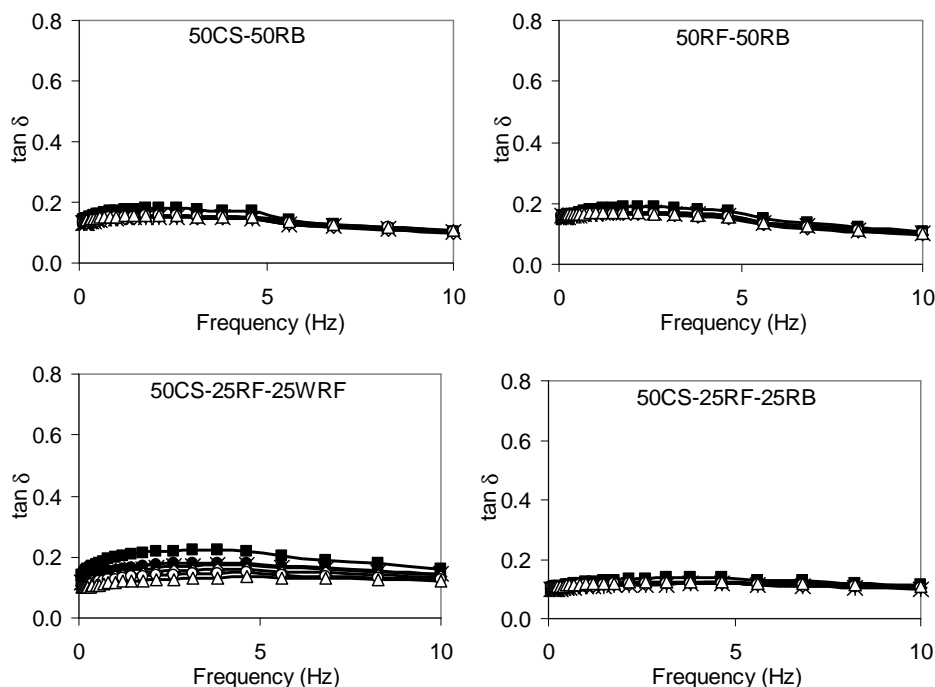
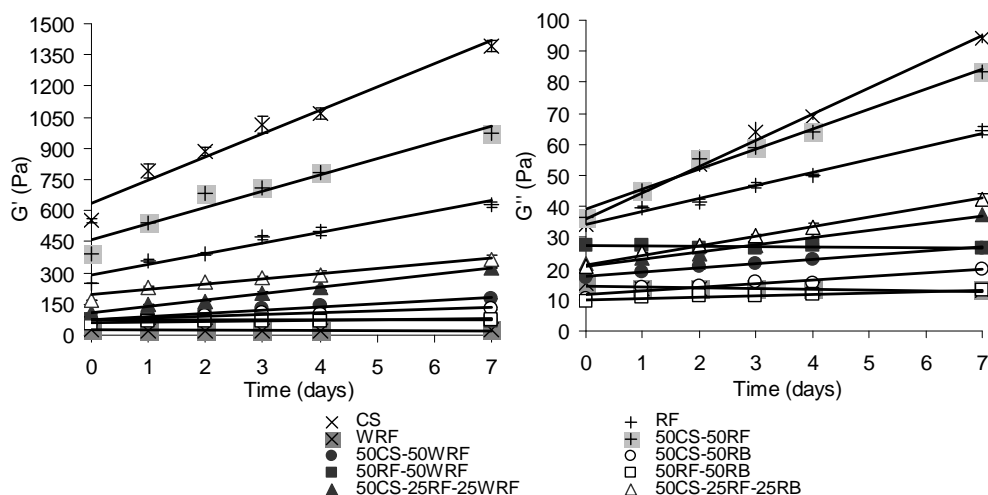


Figure 3-3b. Damping factor ($\tan \delta$) of gels stored at 4°C for (■) 0, (●) 1, (⋈) 2, (◇) 3, (○) 4, (△) 7 days.

As for all the samples the frequency sweep curves obtained at the different ageing times had a parallel trend, G' and G'' measured at 1Hz were extracted to compare the gels hardening kinetics to represent the textural modifications of the samples during storage. Moduli at 1Hz were thus plotted against ageing time and the resulting graphs together with the slope (a) and the y-intercept (b) of each curve are reported in Figure 3-4. CS gels were not only characterized by G' values at t_0 higher than those of the other samples, but also by a faster hardening kinetic. Similar results were found for RF and 50CS-50RF, while no differences were evidenced between G' values evaluated on WRF fresh gels and those obtained after 7 days of storage. The presence of WRF or RB in starchy mixtures containing CS and RF was very effective in delaying the increase of G' and G'' moduli, suggesting a sharp reduction in the reordering rate of the starchy matrix.



Sample	G'			G''		
	a	b	r	a	b	r
CS	112.4	633	0.98	8.4	36.2	1.00
RF	51.1	288	0.98	*	*	*
WRF	*	*	*	*	*	*
50CS-50RF	78.4	458.2	0.97	6.4	39.1	0.99
50CS-50WRF	15.0	77.6	0.99	*	*	*
50CS-50RB	8.2	75.0	0.91	1.2	11.6	0.96
50RF-50WRF	*	*	*	1.4	17.5	0.99
50RF-50RB	2.8	58.7	0.90	0.4	10.1	0.95
50CS-25RF-25WRF	30.1	110.7	1.00	2.3	20.8	0.99
50CS-25RF-25RB	25.5	192.8	0.98	3.0	21.4	1.00

* no changes during ageing

Figure 3-4. Linear constants relationship ($y=ax+b$) between G' or G'' and ageing time, and storage modulus (G') and loss modulus (G'') values evaluated at 1Hz.

3.4 CONCLUSIONS

Starches and/or GF flours of various origin are generally combined with different hydrocolloids in order to mimic the viscoelastic properties of gluten, aiming to improve structure, acceptability and shelf life of GF baked products. On the other hand, the high amount of starch included in these formulations is mostly responsible for the quality decay of the product, mainly due to a rapid loss of crumb softness. Actually, as underlined by this study, CS and RF coupling, very frequent in commercial blends for GF bread production,

originated elastic gels but highly subject to hardening. On the contrary, WRF (that contains a limited amount of amylose) and RB (due to its low starch content and its high level of fibre) originated very weak gels and resulted very effective in delaying gel hardening. WRF, in particular, came out to be more effective than RB. Both WRF and RB, at 25% and 50% level of substitution, strongly reduced G' values of the mixtures containing CS or RF and, for the same gels, G' curves overlapped up to seven days, indicating very slow hardening kinetics. Therefore, WRF and RB seem to be potentially effective in enhancing the shelf-life of GF baked products when included in the starchy matrix. The next ongoing step is the identification of the proper amount of WRF and/or RB to be included in GF bread formulations, in order to allow both an appropriate workability of the dough and the maintenance of the softness of the product during a prolonged storage.

3.5 ACKNOWLEDGMENTS

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CHAPTER 4

HIGH HYDROSTATIC PRESSURE (HHP) TREATMENTS ON CORN STARCH AND RICE FLOURS

This research was performed at Washington State University (Pullman, WA, USA) under the supervision of Prof. Gustavo V. Barbosa-Cánovas (Professor of Food Engineering and Director of Center for Nonthermal Processing of Food).

4.1 INTRODUCTION

High hydrostatic pressure (HHP) treatments are generally used to increase food shelf-life by inactivating microorganisms without using high temperatures that can alter the sensory and nutritional attributes of the products. Besides, depending on the conditions applied, HHP can modify functional properties of components such as proteins, and inactivate enzymes that are responsible for shortening product shelf-life (Estrada-Giron *et al.*, 2005).

During the HHP treatments the pressure is isostatically and homogeneously applied at each point of the product, due to the fact that the pressure is instantaneously and uniformly distributed within the HHP chamber (Estrada-Giron *et al.*, 2005). Therefore, the processing time is not a function of sample size. This is an advantage, if compared to the traditional thermal processes whose length depends on size and geometry of the product (San Martín *et al.*, 2002). On the contrary, the variables to be considered in a HHP process are the pressure applied, the holding time, the fluid used for transmitting pressure and the operational mode of pressure raising (continuously or cyclic); all these variables straight influence the effects of the HHP treatments (Welti-Chanes *et al.*, 2005). It is also possible to adjust the temperature of the chamber and, therefore, usefully combine HHP pressure with heat treatments.

The HHP acts on most of the foods components such as water, starch and proteins; San Martín *et al.* (2002) reported that the typically tetrahedral arrangement of water molecules is modified under high pressure to a more compact structure with distorted hydrogen-bond angles. Consequently, also the interactions between water and the other food components can be modified by the pressure applied; for this reason the effects of HHP are strongly related to the water content. Effects of hydrostatic pressure on biomembranes were investigated by Kato and Hayashi (1999) that evidenced solubilization and leakage of intracellular substances from yeast cytoplasm, leakage of metallic ions and permeation of extracellular compounds into cells and tissues. This last effect could enhance mass transfer during osmotic dehydration (Rastogi and Niranjan, 1998) or in fluidized bed drying when combined with freezing (Eshtiaghi *et al.*, 1994). Gomes *et al.* (1998) observed an increase of α - and β -amylase activities from malt barley when 10% wheat or barley flour slurries were subjected to pressures between 400MPa and 600MPa for a short time; whereas, after 20min at 600MPa the enzymatic activity decreased due to protein denaturation and modifications of the active sites. As regards cereals, Kato *et al.* (2000) observed some modifications in rice grains treated under

pressure, such as a partial destruction of the endosperm cells, solubilization and diffusion of proteins from endosperm cells into the surrounding solution. In addition, Yamazaki and Sasagawa (1998) reported that, in presence of a sufficient amount of water, a continuous increase of pressure up to 600MPa causes a portion of the starch granules to swell.

In this study the HHP was used at low temperature to induce physical and structural changes to starchy compounds. The following raw materials were tested: corn starch (CS), rice flour (RF) and waxy rice flour (WRF), that are quite commonly used in gluten-free (GF) products. Three different variables were considered: the pressure holding time (5min or 10min), the pressure applied (400MPa or 600MPa) and the temperature at which the pressure was applied (20°C or 40°C).

4.2 MATERIALS AND METHODS

4.2.1 Materials

Rice Flour (RF) and Waxy Rice Flour (WRF) were provided by Beneo-Remy NV (Leuven-Wijgmaal, Belgium); Corn Starch (CS) was kindly donated by Roquette America Inc. (Iowa, USA).

4.2.2 Chemical composition of raw materials

The moisture content of the raw materials was determined according to the Official Standard Method AACC 44-40.02 (2000). The total nitrogen content was evaluated by a thermal conductivity measurement after an organic combustion through a Leco FP-528 instrument (LECO Corporation, St. Joseph, MI, USA), using Helium as carrier gas. The protein content was calculated adopting 6.25 as conversion factor. The amounts of total starch (TS) and damaged starch (DS) were determined using the “Total Starch Assay Kit” and the “Starch Damage Assay Kit”, respectively (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland). The amylose content was assessed by the UNI ISO 6647 Method (1991) and expressed as the proportion by weight of amylose (g/100g db). All these determinations were made at least in duplicate ($n \geq 2$).

4.2.3 High hydrostatic pressure treatments

Each sample was previously hand-mixed with water for 5min at room temperature to reach a final moisture content of 40g/100g, and conditioned at 15°C for 1h to permit moisture equilibration. The samples were packaged twice in a polyethylene thermosealed bag (LayFlat Poly Tubing Rolls, 0.10mm thick from Consolidated Plastics, Twinsburg, Ohio, USA) to isolate the sample from the pressure transmitting fluid. Afterwards, the High Hydrostatic Pressure (HHP) treatment was applied through an indirect compression systems, using a 2L capacity warm isostatic press (Engineered Pressure Systems Inc. Haverhill, Maryland, USA; Figure 4-1). A synthetic cutting fluid, 10% of oil (Hydralubic 123B; Houghton International Valley Forge, Pennsylvania, USA) in water, was used as pressure transmitting fluid. The pressure (400MPa and 600MPa) was almost instantaneously (less than 50s) applied for the desired treatment time (5min and 10min) and then released. The temperature of the treatment chamber (20°C and 40°C) was reached by an electric resistance heating band and continuously monitored; both 20°C and 40°C were below the gelatinization temperature of the raw materials at atmospheric pressure.

After the HHP treatment, the samples were dried in a vacuum oven (67kPa; mod. 1410, VWR, USA) at 35°C, until a moisture content of 14g/100g was reached, and then crushed with a mortar.

From each raw material, samples mixed with water up to a final moisture content of 40g/100g of sample and subsequently dried to a moisture content of about 14g/100g before crushing (named “untreated samples”) were prepared too, in order to evaluate the potential changes due to the hydration/dehydration process.

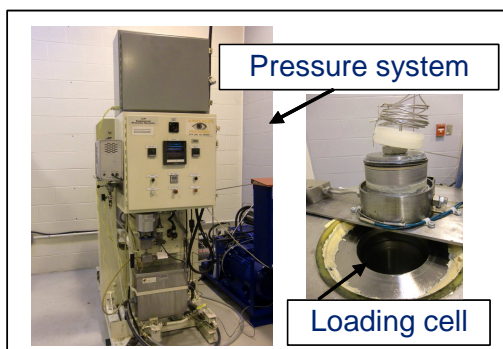


Figure 4-1. High hydrostatic pressure equipment (Engineered Pressure Systems Inc. Haverhill, Maryland, USA).

4.2.4 Evaluation of the effects of the HHP treatments

Solvent retention capacity

The solvent retention capacity (SRC) was evaluated in accordance with the Standard Method AACC 56-11 (2000), with some minor modifications: 1.5g of sample were weighed in a calibrated 15mL centrifuge tubes having a conical bottom. Then, 7.5mL of the different solvents (water; 5.0% (w/w) sodium carbonate in water; 50.0% (w/w) sucrose in water; 5.0% (w/w) lactic acid in water) were added to the powder, and the mixtures were vigorously shaken for 5s to suspend the sample. Afterwards, the mixtures were shaken every 5 minutes up to 20min, and centrifuged for 15min at 1000xg at room temperature (25°C). The solvent retention capacity (SRC) was calculated as the weight of solvent held by samples after centrifugation, supernatant separation and gel drainage for 10min. SRC was expressed as percent of sample weight, on a 14% moisture basis. The following SRC values were considered: Water Retention Capacity (WRC), Sodium Carbonate SRC (SCSRC), Sucrose SRC (SuSRC) and Lactic Acid SRC (LASRC). Generally, SCSRC is associated with starch damage, LASRC with protein properties and SuSRC with pentosan characteristics. WRC is influenced by all the constituents of the sample. The different SRC were calculated as follows (Haynes *et al.*, 2009):

$$\text{SRC}(\%) = \left(\frac{\text{gel (g)}}{\text{flour (g)}} - 1 \right) * \left(\frac{86}{100 - \text{flour moisture}(\%)} \right) * 100$$

All these measurements were made at least in duplicate ($n \geq 2$) and the coefficient of variation of the SRC values was less than 4.0%.

Pasting properties

The pasting properties of the samples, untreated and treated by means of HHP, were measured using a Brabender® Micro-Visco-Amylograph (MVA; Brabender OHG, Duisburg, Germany) that provides an accurate control of time-temperature and shear profiles. Twelve grams of sample were dispersed in 100mL of distilled water, scaling both powders and water weight on 14% sample moisture basis.

The suspensions were subjected (stirring at 250min^{-1} and using a $300\text{cm} \cdot \text{g}_f$ cartridge) to the following standard temperature profile: heating from 30°C up to 95°C, holding at 95°C for 30min, cooling from 95 °C to 30°C. A heating/cooling rate of 3°C/min was applied. The following indices were taken from the resulting curves: gelatinization temperature (GT, °C; temperature at

which an initial increase in viscosity occurs); peak viscosity (PV, Brabender Units, BU; maximum paste viscosity achieved during the heating cycle); time necessary to achieve the peak viscosity (Ptime, min); breakdown (BD, BU; index of viscosity decrease during the holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95°C); final viscosity (FV, BU; paste viscosity achieved at the end of the cooling cycle) and setback (SB, BU; index of the viscosity increase during cooling, corresponding to the difference between FV and the viscosity reached after the holding period at 95°C). Results are the average of at least two replicates ($n \geq 2$).

Differential scanning calorimetry

The thermal properties of the raw materials, as well as those of the untreated and treated samples at 600MPa for 5min at 40°C, were determined using a differential scanning calorimetry (DSC) (Pyris1, Perkin-Elmer Corp., Norwalk, CT). The instrument was calibrated with Indium and all measurements were performed under a Nitrogen atmosphere (20mL/min). Flour (10mg, dry basis) and distilled water (20 μ L) were placed in a stainless steel sample pan, and hermetically sealed, then equilibrated for at least 30min at room temperature. Each sample was heated from 20°C to 180°C at a heating rate of 10°C/min, then immediately cooled (cooling rate of 40°C/min) to 20°C, held at this temperature for 1min and finally reheated to 180°C at a heating rate of 10°C/min. A capsule with aluminum oxide and water was used as reference. For each endothermic peak, the onset temperatures, end temperatures and peak temperatures were determined using the Pyris Manager data processing software (Perkin Elmer). The transition enthalpy (J/g, db) was calculated from the peak area. The reported values are the average of at least two measurements ($n \geq 2$).

X-ray diffraction

A X-ray Diffractometer (Siemens D-500, Germany; Figure 4-2) was used to study the X-ray diffraction (XRD) patterns of the raw materials, as well as those of the untreated and treated samples at 600MPa for 5min at 40°C. The diffractometer was used with Ni-filtered CuK α radiation, operated at room temperature at 35kV and 30mA. The samples were scanned in the range of 3-30° 2 θ of scattering angle, with a 0.02° step size and 1s counting time. The samples were accurately lodged into a specific pan with a rectangular shape. The system was supported with MDI Data Scan software and MDI Jade 8

elaborate software. The diffractograms were smoothed using a Parabolic Filter (55 points). All the samples were analyzed at least in duplicate ($n \geq 2$).

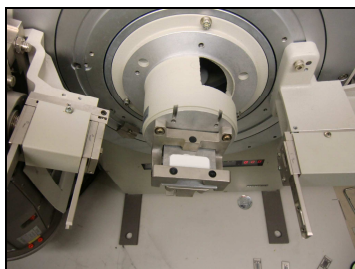


Figure 4-2. X-ray diffraction equipment (Siemens D-500, Germany).

Environmental scanning electron microscopy

A Quanta 200F Environmental Scanning Electron Microscopy (FEI, Field Emission Instruments, Hillsboro, OR, USA) using the Extended Vacuum Mode (Environmental Scanning Electron Microscopy, ESEM) was used to observe the untreated and treated samples at 600MPa for 5min at 40°C. Samples were mounted on stubs, sputter-coated with gold, and their ultrastructures were imaged at an accelerating voltage of 20kV at a pressure of $3\text{-}5 \times 10^{-4}$ Pa.

4.2.5 *Statistical analyses*

In order to assess the differences between samples, data were processed by STATGRAPHIC®*Plus* for Windows v. 5.1 (StatPoint Inc., Virginia, USA). One-way analysis of variance (ANOVA) was performed using the Least Significant Differences (LSD) test to compare the sample means; differences were considered significant at $P < 0.05$.

4.3 RESULTS AND DISCUSSION

The raw materials used in this research were chosen considering the ingredients that are actually used in GF bread production and that are mainly responsible for its quality decay, such as corn starch (CS) and rice flour (RF). Furthermore, waxy rice flour (WRF), that presents a very low amylose content (0.7g/100g db, Chapter 2 and 3), was here included as an ingredient potentially able to delay the retrogradation phenomenon occurring during the shelf-life of starchy based products.

4.3.1 Chemical composition of the raw materials

The chemical composition of the 3 raw materials is reported in Table 4-1. The moisture content was equal to $11.34 \pm 0.02 \text{g}/100\text{g}$ for CS, $11.09 \pm 0.24 \text{g}/100\text{g}$ for RF and $9.99 \pm 0.05 \text{g}/100\text{g}$ for WRF. The protein content of CS, as expected, was under the detectable level, whereas RF and WRF had a protein content of $8.06 \pm 0.04 \text{g}/100\text{g db}$ and $7.02 \pm 0.04 \text{g}/100\text{g db}$, respectively. CS showed the highest total starch content ($97.97 \pm 1.30 \text{g}/100\text{g db}$), whereas RF and WRF were characterized by not significantly different values ($P < 0.05$): $86.94 \pm 1.97 \text{g}/100\text{g}$ and $88.61 \pm 2.18 \text{g}/100\text{g}$, respectively. Starch enzymatic accessibility, assessed as damaged starch (DS), was considered as an index of starch granule organization (the highest DS the lowest the maintenance of the native structure). Both RF and particularly WRF exhibited high DS levels ($7.05 \pm 0.31 \text{g}/100\text{g}$ and $13.24 \pm 0.20 \text{g}/100\text{g}$, respectively), whereas CS presented very low DS values.

Table 4-1. Chemical composition of the raw materials.

Sample	Moisture (g/100g)	Protein (g/100g db)	TS (g/100g db)	DS (g/100g db)	DS/TS (%)	Amylose (g/100g db)
CS	11.34 ± 0.02^b	-	97.97 ± 1.30^b	0.76 ± 0.01^a	0.78	26.3
RF	11.09 ± 0.24^b	8.06 ± 0.04^b	86.94 ± 1.97^a	7.05 ± 0.31^b	8.11	26.2
WRF	9.99 ± 0.05^a	7.02 ± 0.04^a	88.61 ± 2.18^a	13.24 ± 0.20^c	14.95	0.7

Abbreviations: TS, total starch; DS, damaged starch.

Note: values followed by same letter in the same column are not significantly different ($P < 0.05$).

4.3.2 Evaluation of the HHP treatment effects

a) Starch enzymatic accessibility

The starch granule structure and organization is one of the parameters that most influence starch gelatinization-retrogradation phenomena (Mariotti *et al.*, 2005). This property was evaluated only for the samples treated for 5min at 600MPa with a temperature of 40°C, as they represented one of the more drastic HHP conditions adopted in the research. Pressurized RF and WRF DS values were not significantly different ($P < 0.05$) ($7.46 \pm 0.12 \text{g}/100\text{g}$ and $13.04 \pm 0.07 \text{g}/100\text{g}$, respectively) from those obtained for the raw materials. On the contrary, the enzymatic accessibility of CS sample was strongly modified by the HHP treatment: while this index was equal to $0.76 \pm 0.01 \text{g}/100\text{g}$ for the

raw material, it raised to $6.79 \pm 0.23 \text{g}/100 \text{g}$ for the treated sample, that is almost 9 times higher.

b) Solvent retention capacity

Solvent Retention Capacity (SRC) tests have originally been designed to predict the functionality of the North American wheat flours, but they have been recently used by Duyvejonck *et al.* (2011) to evaluate European wheat flours, too.

In the current study, the solvent retention capacity (SRC) was considered as an useful tool for evaluate the effects on sample properties of the HHP treatments performed at different conditions: 400MPa and 600MPa for 5min or 10min, at 20°C or 40°C. In order to highlight possible changes in SRC related to the sample humidification performed before HHP treatments and its drying after HHP treatments, also the untreated samples were evaluated. Results are reported in Table 4-2.

Samples hydration and drying generally determined a decrease of the SRC of all the samples (CS40, RF40 and WRF40), whereas - contrary to expectations - the raw materials and the samples treated at low pressure had similar SRC values. The 3 raw materials had different WRC values: CS showed the lowest one ($76.52 \pm 2.38\%$), when compared to the rice flours (RF, $116.46 \pm 0.17\%$; WRF, $111.99 \pm 0.26\%$); this can be attribute to their different chemical composition and to their damaged starch content (Table 4-1).

Regarding the HHP samples, few differences among the SRC values of the differently treated waxy rice flour (WRF) were evidenced, suggesting that the pressure treatments did not have important effects on the sample affinity for the different solvents used. On the contrary, RF starch and proteins seemed to be influenced by a pressure of 600MPa; in fact, an increase of SCSRC and LASRC, respectively related to the properties of starch and protein, was obtained. Furthermore, the raw RF and the untreated and treated samples at 400MPa, independently from the temperature and the pressure holding time, showed a significantly lower water retention capacity (WRC) if compared to those of the samples treated at 600MPa. Besides, at the higher pressure (600MPa), data highlighted that starch was more affected by the pressure at room temperature (higher SCSRC values) than at 40°C, whereas proteins were mostly influenced by the pressure holding time (LASRC values after an holding time of 10min were significantly higher).

Table 4-2. Solvent retention capacity of the raw materials (CS, RF and WRF) and of the samples untreated (CS40, RF40 and WRF40) and treated under different conditions.

Sample	WRC (%)	SCSRC (%)	LASRC (%)	SuSRC (%)
CS	76.52 ± 2.38 ^b	79.46 ± 0.33 ^b	n.d.	94.36 ± 1.14 ^{ab}
CS40	70.57 ± 0.34 ^a	75.13 ± 0.00 ^a	n.d.	91.22 ± 1.12 ^a
CS40_400x5	75.98 ± 0.95 ^b	79.13 ± 0.77 ^b	n.d.	94.47 ± 0.09 ^{ab}
CS40_400x10	75.84 ± 2.19 ^b	79.02 ± 1.89 ^b	n.d.	98.36 ± 0.51 ^c
CS40_400x5_T40	77.03 ± 2.21 ^b	78.78 ± 0.26 ^b	n.d.	97.57 ± 3.01 ^{bc}
CS40_400x10_T40	78.42 ± 0.32 ^b	83.72 ± 0.54 ^c	n.d.	105.15 ± 2.43 ^d
CS40_600x5	92.25 ± 1.74 ^c	105.57 ± 0.91 ^d	n.d.	120.34 ± 1.21 ^e
CS40_600x10	98.25 ± 1.30 ^d	104.14 ± 0.74 ^d	n.d.	137.66 ± 0.87 ^f
CS40_600x5_T40	110.72 ± 2.83 ^e	117.94 ± 1.10 ^e	n.d.	158.89 ± 2.10 ^h
CS40_600x10_T40	109.82 ± 2.52 ^e	122.02 ± 1.02 ^f	n.d.	152.87 ± 0.49 ^g
RF	116.46 ± 0.17 ^{de}	116.62 ± 0.04 ^e	107.93 ± 0.11 ^a	154.74 ± 1.92 ^{bc}
RF40	102.83 ± 1.16 ^{ab}	103.16 ± 0.45 ^b	107.60 ± 0.92 ^a	149.91 ± 0.10 ^{ab}
RF40_400x5	108.43 ± 2.04 ^{bc}	110.10 ± 0.60 ^c	140.41 ± 3.79 ^b	145.36 ± 0.37 ^a
RF40_400x10	110.98 ± 2.70 ^{cd}	109.08 ± 0.74 ^c	140.35 ± 2.63 ^b	164.03 ± 5.68 ^c
RF40_400x5_T40	96.92 ± 1.01 ^a	98.54 ± 2.30 ^a	142.31 ± 1.65 ^b	153.06 ± 0.58 ^{bc}
RF40_400x10_T40	121.38 ± 6.13 ^e	113.12 ± 0.39 ^d	149.77 ± 3.88 ^c	165.36 ± 4.18 ^c
RF40_600x5	133.85 ± 1.38 ^{fg}	133.68 ± 1.58 ^g	164.36 ± 2.64 ^d	162.50 ± 3.53 ^c
RF40_600x10	130.26 ± 2.35 ^{fg}	133.65 ± 1.06 ^g	181.10 ± 2.50 ^e	161.27 ± 2.40 ^c
RF40_600x5_T40	140.49 ± 1.83 ^h	127.02 ± 0.16 ^f	151.84 ± 3.23 ^c	167.17 ± 2.04 ^c
RF40_600x10_T40	138.26 ± 4.11 ^{gh}	139.44 ± 0.19 ^h	177.39 ± 2.80 ^e	163.28 ± 1.59 ^c
WRF	111.99 ± 0.26 ^{gh}	137.16 ± 0.45 ^g	114.00 ± 0.44 ^e	158.41 ± 1.86 ^e
WRF40	82.21 ± 2.17 ^a	90.83 ± 0.78 ^a	90.42 ± 2.09 ^a	132.62 ± 0.13 ^a
WRF40_400x5	90.54 ± 0.63 ^d	94.28 ± 3.30 ^{ab}	100.00 ± 0.56 ^b	136.70 ± 0.62 ^{ab}
WRF40_400x10	87.19 ± 0.02 ^{cd}	96.89 ± 2.23 ^{bc}	105.17 ± 3.64 ^{bc}	143.07 ± 2.42 ^{bcd}
WRF40_400x5_T40	119.38 ± 1.51 ^h	108.80 ± 3.38 ^e	112.49 ± 3.94 ^{de}	139.73 ± 0.19 ^{bc}
WRF40_400x10_T40	95.85 ± 1.21 ^e	99.85 ± 0.35 ^c	111.51 ± 4.19 ^{de}	150.11 ± 0.25 ^d
WRF40_600x5	83.53 ± 0.53 ^{ab}	101.31 ± 0.89 ^{cd}	104.62 ± 2.80 ^{bc}	141.91 ± 3.80 ^{bc}
WRF40_600x10	85.78 ± 0.74 ^{bc}	116.80 ± 2.38 ^f	108.06 ± 2.81 ^{cd}	143.21 ± 2.86 ^{bcd}
WRF40_600x5_T40	95.80 ± 0.42 ^e	96.40 ± 3.46 ^{bc}	120.80 ± 1.83 ^f	132.20 ± 6.27 ^a
WRF40_600x10_T40	99.40 ± 3.93 ^{fg}	106.50 ± 3.19 ^{de}	117.19 ± 0.79 ^{ef}	145.23 ± 1.45 ^{cd}

Abbreviations: Water Retention Capacity (WRC), Sodium Carbonate SRC (SCSRC), Sucrose SRC (SuSRC), Lactic Acid SRC (LASRC), n.d., not determined. *Note:* Among the same sample, values followed by same letter in the same column are not significantly different (P<0.05).

As regards CS, significantly different ($P < 0.05$) SRC values were achieved, in particular for WRC and SCSRC. In fact, the untreated sample and those treated at 400MPa were less capable to retain solvents ($WRC < 78.42 \pm 0.32\%$, $SCSRC < 83.72 \pm 0.54\%$) when compared CS samples treated at 600MPa ($WRC > 92.25 \pm 1.74\%$, $SCSRC > 104.14 \pm 0.74\%$). Furthermore, significant differences ($P < 0.05$) were underlined among the samples treated at 600MPa at room temperature or at 40°C, suggesting that process temperature had an important impact on the sample capability to retain solvent (water or 5.0% (w/w) sodium carbonate in water), whereas the holding time (5min or 10min) resulted less important.

c) Pasting properties

The Brabender Viscoamylograph has been widely used as standard analytical tool to assess the pasting characteristics of starch and flour suspensions during heating and cooling cycles (Shuey and Tipples, 1980; Mariotti *et al.*, 2008). In this research, the same procedure was applied both on raw materials and on pressurized samples, as the extent of the phenomena related to starch gelatinisation and retrogradation during the heating and cooling cycles inside the Viscoamylograph may reflect the molecular changes occurred during the HHP process. As already mentioned, during the HHP treatments 3 different variables were taken into consideration: the pressure holding time (5min or 10min), the pressure applied (400MPa or 600MPa) and the temperature at which the pressure was applied (20°C or 40°C).

The viscoamylographic indices of the raw materials (CS, RF and WRF), as well as those of the untreated (CS40, RF40 and WRF40) and pressurized samples are showed in Tables 4-3a and 4-3b. WRF gelatinized at lower temperature (GT around 65°C) in comparison with CS (70°C) and R F (73°C). These differences could be mainly related to the starch granule amount and organization in the different samples, as total starch and in particular damaged starch and amylose contents were extremely different among the samples. Sandhya Rani and Bhattacharya (1995) demonstrated that low amylose starch granules, pasted at 12% concentration, resulted weak and fragile; for that reason an easy swelling and disintegration occurred. High amylose rice starch resulted, according to the authors, relatively strong and rigid, withstanding swelling and disintegration. These results are in agreement with Kiribuchi-Otobe *et al.* (1998), too: if starch consists exclusively of amylopectin it exhibits low initial pasting temperature, high paste clarity, low syneresis, and high resistance to retrogradation.

In addition to the SRC data, also the viscoamylographic indices of the differently treated WRF were not significantly different ($P < 0.05$), suggesting that the HHP treatments did not modify WRF characteristics. On the contrary, CS and RF pasting behavior seemed to be partially altered by the treatments applied. In particular, CS treated at 600MPa - at both the considered times and temperatures - showed higher values of final viscosity (FV) and slower gelatinization kinetics (Ptime; time necessary to achieve the peak viscosity) if compared to the untreated CS and to the sample treated at 400MPa (Table 4-3a). The shift of the pasting curves of CS pressurized at 600MPa suggests the formation of a more compact structure (Figure 4-3). No curves shifts were found for the other samples (WRF and RF). However, as regards RF, a clear increase in GT and a slight decrease of peak viscosity (PV) were observed at both the applied pressures (400MPa and 600MPa). In fact, the PV values ranged from 582 to 659BU for the RF sample pressurized and from 756 to 780BU for the untreated samples, indicating that RF can be partially gelatinized at low pressures, too (400MPa). Furthermore, the highest GT values obtained suggested that the starch modified by the HHP process could present a more compact structure, thus requiring a higher temperature to gelatinize.

These results could lead to the conclusion that HHP treatments, performed as previously reported, have a predominant effect on those samples containing a high amylose amount; furthermore it was noticed that HHP time and temperature have a minor influence, if compared to the pressure applied, on the pasting properties of the samples.

Table 4-3a. Viscoamylographic indices of the raw materials (CS and RF) and of the samples untreated (CS40, RF40) and treated under different conditions.

Sample	GT (°C)	PV (BU)	Ptime (min)	FV (BU)	BD (BU)	SB (BU)
CS	70.45 ± 0.21 ^a	931 ± 11 ^a	19.67 ± 0.23 ^a	1250 ± 49 ^a	438 ± 16 ^e	740 ± 47 ^a
CS40	70.20 ± 0.17 ^a	948 ± 2 ^{ab}	20.33 ± 0.14 ^b	1322 ± 3 ^{abc}	434 ± 24 ^e	798 ± 14 ^{abc}
CS40_400x5	70.05 ± 0.21 ^a	917 ± 13 ^a	20.30 ± 0.53 ^{ab}	1288 ± 19 ^{ab}	391 ± 27 ^{cde}	737 ± 1 ^a
CS40_400x10	70.00 ± 0.28 ^a	972 ± 26 ^b	20.42 ± 0.01 ^b	1319 ± 50 ^{abc}	450 ± 43 ^e	769 ± 61 ^{abc}
CS40_400x5_T40	70.15 ± 0.07 ^a	925 ± 31 ^a	19.67 ± 0.01 ^a	1306 ± 57 ^{abc}	408 ± 44 ^{de}	765 ± 71 ^{abc}
CS40_400x10_T40	70.10 ± 0.01 ^a	939 ± 16 ^{ab}	20.13 ± 0.77 ^{ab}	1341 ± 53 ^{bc}	414 ± 41 ^{de}	789 ± 68 ^{abc}
CS40_600x5	70.65 ± 0.78 ^a	923 ± 1 ^a	21.13 ± 0.06 ^c	1388 ± 24 ^c	329 ± 24 ^{ab}	796 ± 6 ^{abc}
CS40_600x10	70.80 ± 0.71 ^a	927 ± 1 ^a	21.29 ± 0.06 ^c	1392 ± 30 ^c	300 ± 33 ^{ab}	751 ± 2 ^{ab}
CS40_600x5_T40	70.45 ± 0.07 ^a	941 ± 10 ^{ab}	21.34 ± 0.23 ^c	1483 ± 47 ^d	350 ± 28 ^{abc}	850 ± 33 ^{bc}
CS40_600x10_T40	71.00 ± 1.13 ^a	918 ± 11 ^a	21.46 ± 0.06 ^c	1484 ± 61 ^d	317 ± 49 ^a	858 ± 81 ^c
RF	73.30 ± 1.14 ^a	780 ± 13 ^f	22.08 ± 0.09 ^a	1024 ± 13 ^c	342 ± 12 ^f	584 ± 16 ^a
RF40	75.45 ± 0.35 ^b	756 ± 4 ^e	22.13 ± 0.18 ^{ab}	976 ± 8 ^{bc}	359 ± 13 ^f	575 ± 18 ^a
RF40_400x5	77.15 ± 0.21 ^{cd}	654 ± 16 ^d	22.29 ± 0.06 ^{abc}	960 ± 28 ^{ab}	279 ± 4 ^{de}	577 ± 9 ^a
RF40_400x10	76.65 ± 0.21 ^c	659 ± 4 ^d	22.33 ± 0.01 ^{abc}	966 ± 11 ^{ab}	272 ± 8 ^{cde}	573 ± 16 ^a
RF40_400x5_T40	77.30 ± 0.01 ^{cd}	655 ± 11 ^d	22.34 ± 0.23 ^{abc}	957 ± 6 ^{ab}	283 ± 9 ^e	578 ± 6 ^a
RF40_400x10_T40	77.30 ± 0.01 ^{cd}	644 ± 4 ^{cd}	22.42 ± 0.12 ^{abc}	953 ± 23 ^{ab}	267 ± 5 ^{cde}	569 ± 23 ^a
RF40_600x5	77.05 ± 0.07 ^c	632 ± 2 ^{bc}	22.50 ± 0.00 ^{bc}	951 ± 4 ^{ab}	236 ± 1 ^b	550 ± 1 ^a
RF40_600x10	77.00 ± 0.01 ^c	656 ± 13 ^d	22.42 ± 0.35 ^{abc}	971 ± 10 ^{ab}	260 ± 18 ^{dcd}	571 ± 6 ^a
RF40_600x5_T40	78.25 ± 0.35 ^d	622 ± 6 ^b	22.54 ± 0.30 ^c	943 ± 36 ^{ab}	253 ± 9 ^{bc}	566 ± 37 ^a
RF40_600x10_T40	77.45 ± 0.07 ^{cd}	582 ± 12 ^a	22.58 ± 0.01 ^c	935 ± 13 ^a	212 ± 3 ^a	560 ± 4 ^a

Abbreviations: GT, gelatinisation temperature; PV, peak viscosity; Ptime, time necessary to achieve the peak viscosity; FV, final viscosity; BD, breakdown; SB, setback. *Note:* Among the same sample, values followed by same letter in the same column are not significantly different ($P < 0.05$).

Table 4-3b. Viscoamylographic indices of the raw material (WRF) and of the sample untreated (WRF40) and treated under different conditions.

Sample	GT (°C)	PV (BU)	Ptime (min)	FV (BU)	BD (BU)	SB (BU)
WRF	65.00 ± 0.71 ^{ab}	705 ± 23 ^e	14.67 ± 0.01 ^a	621 ± 12 ^c	393 ± 18 ^e	300 ± 6 ^e
WRF40	65.15 ± 0.49 ^{ab}	662 ± 11 ^d	15.04 ± 0.06 ^{bc}	600 ± 5 ^{abc}	352 ± 6 ^d	280 ± 1 ^{abcd}
WRF40_400x5	65.30 ± 0.14 ^b	607 ± 33 ^b	15.04 ± 0.06 ^{bc}	584 ± 25 ^{ab}	312 ± 18 ^b	279 ± 9 ^{abc}
WRF40_400x10	64.85 ± 0.07 ^{ab}	640 ± 9 ^{bcd}	15.00 ± 0.01 ^b	595 ± 3 ^{abc}	337 ± 5 ^{cde}	283 ± 1 ^{abcd}
WRF40_400x5_T40	65.65 ± 0.35 ^b	623 ± 24 ^{bcd}	15.08 ± 0.01 ^{bcd}	601 ± 25 ^{bc}	318 ± 8 ^{bc}	285 ± 8 ^{bcd}
WRF40_400x10_T40	64.80 ± 0.14 ^{ab}	653 ± 3 ^{cd}	15.08 ± 0.01 ^{bcd}	617 ± 3 ^c	335 ± 4 ^{cde}	290 ± 2 ^{cde}
WRF40_600x5	64.75 ± 0.21 ^{ab}	602 ± 7 ^{ab}	15.04 ± 0.06 ^{bc}	584 ± 1 ^{ab}	305 ± 5 ^b	276 ± 1 ^{ab}
WRF40_600x10	64.15 ± 0.07 ^a	614 ± 13 ^{bc}	15.13 ± 0.06 ^{bc}	584 ± 6 ^{ab}	316 ± 10 ^{bc}	277 ± 3 ^{abc}
WRF40_600x5_T40	65.55 ± 1.06 ^a	566 ± 1 ^a	15.17 ± 0.01 ^c	569 ± 3 ^a	279 ± 4 ^a	272 ± 1 ^a
WRF40_600x10_T40	64.85 ± 0.49 ^{ab}	636 ± 13 ^{bcd}	15.13 ± 0.06 ^{bc}	617 ± 11 ^c	319 ± 11 ^{bc}	291 ± 9 ^{de}

Abbreviations: GT, gelatinisation temperature; PV, peak viscosity; Ptime, time necessary to achieve the peak viscosity; FV, final viscosity; BD, breakdown; SB, setback. *Note:* Among the same sample, values followed by same letter in the same column are not significantly different (P<0.05).

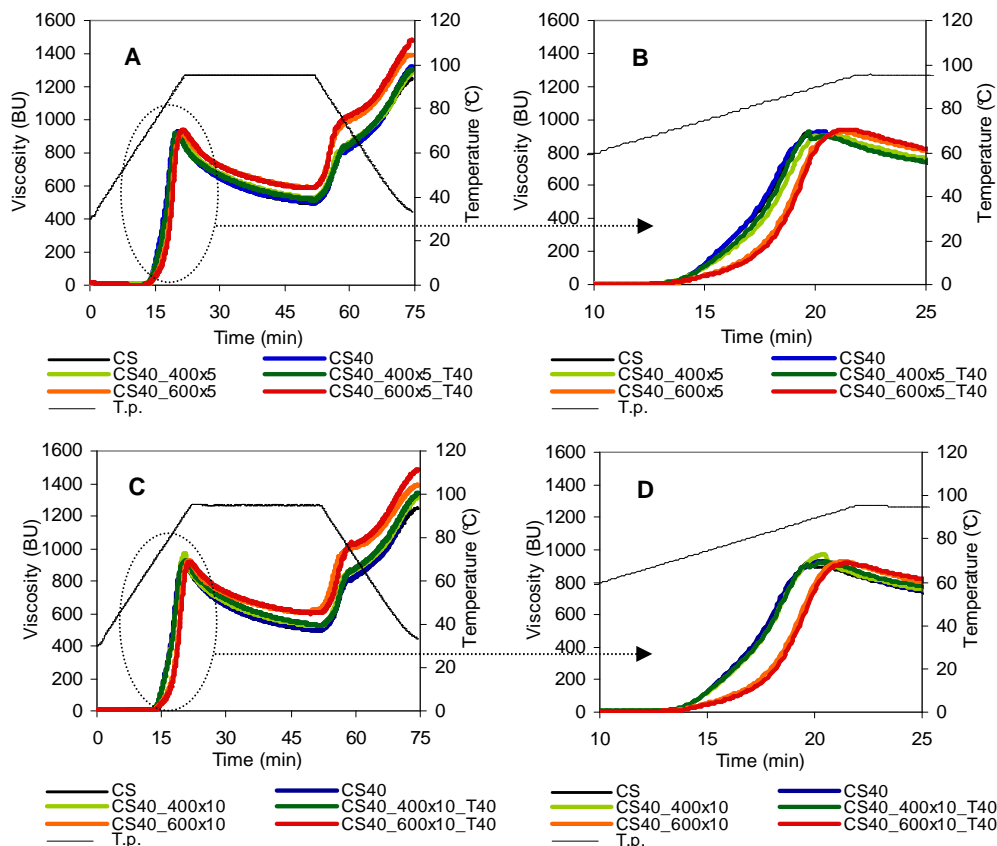


Figure 4-3. Viscoamylographic profiles of raw corn starch (CS) and untreated (CS40) and treated sample for 5min (A and B) or for 10min (C and D) at room temperature or at 40°C (T40) for 400MPa or 600MPa. Abbreviations: T.p., temperature profile.

d) Thermal properties

The thermal properties of the raw samples (CS, RF and WRF), as well as those of the untreated (CS40, RF40 and WRF40) and treated samples at 600MPa for 5min at 40°C (CS40_600x5_T40, RF40_600x5_T40 and WRF40_600x5_T40) were evaluated using a differential scanning calorimeter (Pyris1, Perkin-Elmer Corp., Norwalk, CT). The DSC scans were performed on 10mg of sample (dry basis), adding 20 μ l of water; in this condition, all water molecules were absorbed and bounded with starch and protein molecules. For this reason, in accordance with Zhong and Sun (2005), no free water was available to form ice and no ice melting was observed during the second scan. The first DSC scan of the raw samples, on the contrary, presented an endothermic peak around 70°C related to starch gelatinization (Table 4-4).

WRF started to gelatinize at lower temperatures (61°C), if compared to RF (62°C), and CS (65°C).

Even if all the samples presented a gelatinization peak located around 70°C, they could be easily distinguished for the shape of the peaks: it was thin and higher for CS, smaller and broader for WRF, and double and short for RF (first peak at 69°C; second peak at 77°C). No significant ($P<0.05$) differences in terms of peak temperatures were evidenced among the untreated and treated samples.

Using the Pyris Manager data processing software (Perkin Elmer), the transition energy (J/g db) was calculated for every endothermic peak. As reported in Table 4-4, the rice flours (RF and WRF) though having the same starch content (Table 4-1), required a different energy to gelatinize (RF, 8.11 ± 0.12 J/g db and 9.43 ± 0.30 J/g db) indicating a different organization of starch granules; the highest energy was found for CS (14.12 ± 0.56 J/g db), probably related to its high total starch content. No differences were found between the raw materials and the corresponding untreated samples (CS40, RF40, WRF40), suggesting that dehydration and drying processes did not have any effect on starch gelatinization. Instead, it is interesting to note that the HHP treatment at 600MPa for 5min at 40°C was responsible of minor changes in the transition energy (ΔH) of the rice flours (RF and WRF); whereas a 35% decrease was evidenced for CS. This can probably be related to a partial gelatinization of corn starch during the pressure treatment.

Table 4-4. Thermal indices of the raw materials (CS, RF and WRF) and of the untreated (CS40, RF40 and WRF40) and treated samples at 600MPa for 5min at 40°C.

Sample	Onset T (°C)	End Temp. (°C)	ΔH (J/g db)	1 st Peak (°C)	2 nd Peak (°C)
CS	65.38 ± 0.49^b	77.42 ± 0.65^a	14.12 ± 0.56^b	70.17 ± 0.58^a	-
CS40	65.01 ± 0.17^b	76.47 ± 0.28^a	14.41 ± 0.19^b	69.74 ± 0.26^a	-
CS40_600X5_T40	63.10 ± 0.17^a	76.64 ± 0.72^a	9.13 ± 0.14^a	69.01 ± 0.35^a	-
RF	62.16 ± 0.07^a	85.97 ± 0.33^a	8.11 ± 0.12^{ab}	68.92 ± 0.12^a	77.91 ± 0.10^a
RF40	62.24 ± 0.26^a	85.97 ± 0.24^a	8.93 ± 0.74^b	69.20 ± 0.02^a	77.48 ± 0.51^a
RF40_600X5_T40	63.82 ± 0.13^b	85.26 ± 0.11^a	7.11 ± 0.04^a	69.11 ± 0.28^a	77.44 ± 0.22^a
WRF	61.28 ± 0.13^b	79.07 ± 0.40^a	9.43 ± 0.30^a	69.51 ± 0.35^a	-
WRF40	61.06 ± 0.06^{ab}	79.27 ± 0.07^a	9.57 ± 0.14^a	69.43 ± 0.00^a	-
WRF40_600X5_T40	60.88 ± 0.07^a	79.53 ± 0.42^a	9.54 ± 0.31^a	69.68 ± 0.12^a	-

Note: Among the same sample, values followed by same letter in the same column are not significantly different ($P<0.05$).

In accordance with Zhong and Sun (2005), no endothermic transition beyond the gelatinization transition was highlighted from the second scan, indicating that no re-crystallization of starch molecules (retrogradation) occurred during cooling in the DSC measurements.

e) *X-ray diffraction*

The X-ray diffraction (XRD) patterns of the raw samples and the untreated and treated samples at 600MPa for 5min at 40°C are reported in Figure 4-4. The rice flours and CS presented several important reflections at 2θ of around 15° and 23° and an unresolved doublet at 17° and 18°. Besides, the patterns of the CS and RF raw materials showed different reflections at 2θ around 20° where broad peaks were visible, whereas WRF presented a peak of lower intensity. These patterns are in accordance with those detected from other researchers (Cheetham and Tao, 1998; Fernández-Martín *et al.*, 2008; Jiranuntakul *et al.*, 2011). As reported by Fernández-Martín *et al.* (2008) all these intensities were compatible with the A-type pattern. As reported by Cheetham and Tao (1998), the absence of peaks at 5° indicates the absence of B pattern, whereas the intensity of the peak at 15° is related to the amylose content: it decreases if the amylose content reaches 40% or higher values.

The raw materials and the corresponding untreated samples presented the same X-ray patterns, suggesting that the samples preparation process (hydration and dehydration) did not alter the starch crystalline patterns of rice and corn. On the opposite, the HHP treatment at 600MPa for 5 minutes at 40°C slightly reduced the scattering intensities of the peaks at 17-18° and 23° 2θ , indicating a lower degree of starch crystallinity in the sample.

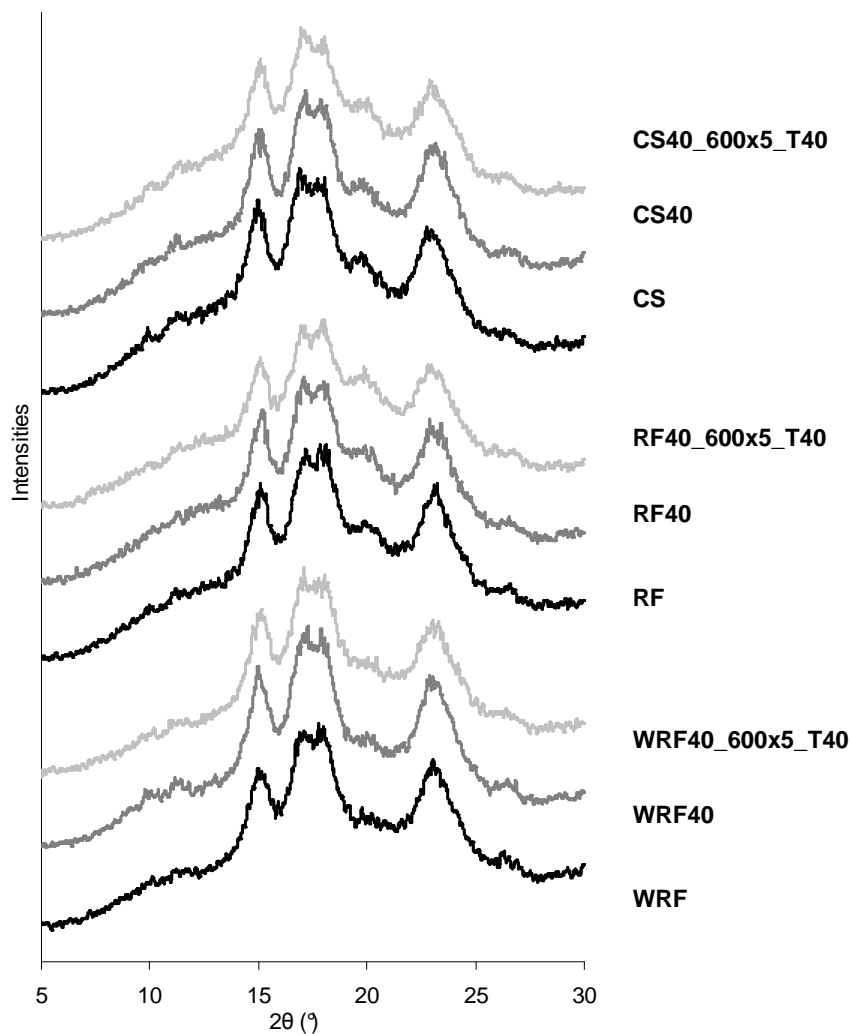


Figure 4-4. X-ray patterns of corn starch (CS), rice flour (RF) and waxy rice flour (WRF). The black lines are related to the raw materials, the grey lines to the untreated samples and the light grey lines to those samples treated at 600MPa for 5 minutes at 40°C.

f) Environmental scanning electron microscopy

An environmental scanning electron microscope was used to observe the untreated (CS40, RF40 and WRF40) and treated samples at 600MPa for 5min at 40°C (CS40_600x5_T40, RF40_600x5_T40 and WRF40_600x5_T40) (Figure 4-5).

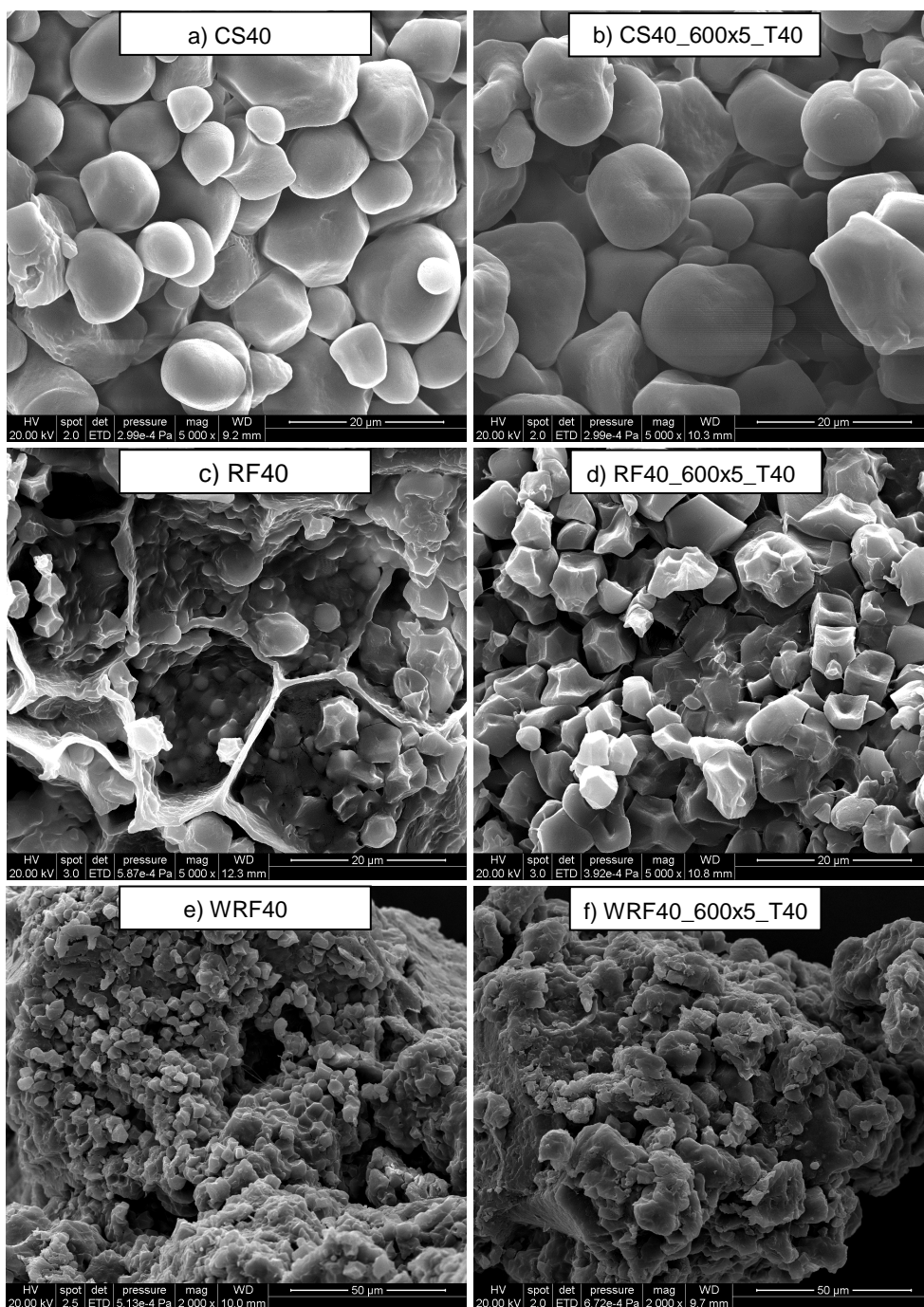


Figure 4-5. Scanning electron micrographs of the untreated (on left) and treated samples at 600MPa for 5min at 40°C (on right); (a)- (d) images: 5000x; (e) and (f): 2000x.

In accordance with Jiranuntakul *et al.* (2011), normal rice and waxy rice starch granules displayed similar sizes and shapes. As known, rice granules were small (3-8 μ m) and had angular, polyhedral shapes with smooth surfaces, whereas corn starch granules were rounded, ellipse-shaped or irregular-shaped, with a granular size of 5-20 μ m. The HHP treatment slightly modified WRF starch granules, whereas CS and RF seemed to be more intensively affected by the treatment. A partial gelatinization of corn starch occurred, as evidenced by the swelling and the aggregation of the granules. As regards RF, the cell walls were not visible after the HHP treatment and the granules appeared more compact and aggregated. RF starch granules, treated at 600MPa for 5 minutes at 40°C, presented a different shape in comparison to the native starch, indicating an effect of the pressure treatment.

4.4 CONCLUSIONS

The high hydrostatic pressure is commonly used to inactivate microorganisms as well as enzymes responsible for shortening the shelf-life of a product, but it could be potentially used for many other applications (San Martin *et al.*, 2002). In this research, the possibility of modifying the physical and structural characteristics of corn starch, rice flour and waxy rice flour (actually the basic ingredients of GF bread recipes) by means of HHP treatments was investigated.

The results evidenced that the pressure holding time and the processing temperature were not discriminating parameters, whereas a different organization of the macromolecules was obtained in relation to the pressure applied. In fact, from the viscoamylographic profiles of the untreated and treated RF, it was evidenced how starch was partially gelatinized at both the pressures adopted (400MPa or 600MPa): peak values (about 650BU and 620BU, respectively) were in fact lower when compared to those of the unpressurized sample (756BU). A higher solvent retention capacity was observed, too: RF40 had a WRC equal to 103%, whereas it was 121% for the samples treated at 400MPa and higher than 130% when a 600MPa pressure was applied. Few differences were found among WRF samples treated at the different conditions, indicating that this sample was less affected by the HHP treatments applied. On the contrary, CS samples pressurized at 600MPa presented a slower gelatinization trend compared to the untreated CS and to the sample treated at 400MPa; in fact, even if all the samples began to gelatinize at the same temperature (70°C), a clear shift of the

viscoamylographic curves was evidenced for CS samples treated at 600MPa, suggesting the formation of a more compact structure. To conclude, this research highlights that HHP can be used to modify the physical characteristics of RF and CS. In a further study the effect of these changes on the properties GF doughs and breads containing the treated samples will be evaluated.

4.5 ACKNOWLEDGMENTS

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CHAPTER 5

EFFECT OF DIFFERENT AMOUNTS OF FIBRE AND WATER ON DOUGH PROPERTIES AND BREAD QUALITY

5.1 INTRODUCTION

Celiac disease is a permanent intolerance to the storage-proteins of wheat (improperly named gluten), an affection of the small intestine influenced by genetic factors, that can appear both in children and adults. At present, the only treatment for celiac disease is a total lifelong avoidance of gluten ingestion, so celiacs must strictly follow a gluten-free (GF) diet, eating only dedicated foods.

In GF bread production, the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final product. Critical are the rheological properties of the dough that, lacking in gluten, shows limited abilities of gas expansion and retention during leavening, factors that inevitably lead to bread with a reduced volume and a low softness of the crumb (Mariotti, 2004). Nowadays, to provide for the lack of gluten and to simulate its viscoelastic behavior, the addition of hydrocolloids is quite common because these ingredients have a strategic role in making dough workable and in improving the texture of the final product (Gallagher *et al.*, 2004; Kobylański *et al.*, 2004; Lazaridou *et al.*, 2007). Recent studies have also investigated the possibility to enrich GF bread with vegetal and animal proteins and with dietary fibre (Gallagher *et al.*, 2004, Guarda *et al.*, 2004; Lazaridou *et al.*, 2007) to increase the nutritional value and the sensorial quality of GF products. Though in recent years some studies have been performed, more investigations are needed as the quality of the products available on the market does not fully meet the consumers' expectations. Besides the ingredients used, also the GF breadmaking process can influence the quality of the final product. In GF bread production the discontinuous system is largely diffused: it is characterized by different steps and by a leavening phase with compressed yeast (straight dough system; Chapter 1, Figure 1-7) that originates bread with a coarse cell structure. Compared to the traditional bread, more liquid-dough systems are preferred for GF bread production, as reported by Mariotti (2004).

The main aim of this part of the research was to produce and to evaluate experimental GF doughs containing different levels of sugar beet fibre (as fibre source; SB), Psyllium flour (as thickening agent; Psy) and different water amounts in order to: (1) evaluate the influence of the different fibres on the rheological properties of the dough; (2) study the importance of dough consistency for the quality of the final bread; and (3) produce breads with good softness and prolonged shelf-life. Keeping in mind these aims, the

characteristics of both experimental doughs and breads, fresh and stored (72h at 20°C and 60% RH), were investigated.

5.2 MATERIALS AND METHODS

5.2.1 *Materials*

The GF bread recipe here adopted included many of the raw materials introduced in Chapter 1 and the majority of those investigated in Chapter 2: corn starch (CS), rice flour (RF_b), rice starch (RS), margarine, rice protein (RP), sugar, yeast, hydroxypropyl methyl cellulose (HPMC), sugarbeet fibre (SB), Psyllium (Psy), lupin protein, locust bean and guar gum (Caremix), sorbitol, tartaric acid, enzyme, sodium chloride and distilled water. Two dough consistencies were investigated: 200 and 500 Brabender Unit (BU); the first one corresponds to a “liquid-like” dough, suitable to be poured into moulds, whereas the second one is more “solid”, to be shaped by hand or with an industrial forming machine. The two sources of fibre were added as follow:

- **A200:** 0.5% SB and 2.5% Psy; 200BU;
- **A500:** 0.5% SB and 2.5% Psy; 500BU;
- **B200:** 1.5% SB and 1.5% Psy; 200BU;
- **B500:** 1.5% SB and 1.5% Psy; 500BU.

The fibre amount is expressed as a percentage of total ingredients weight.

5.2.2 *Raw materials characterization*

As previously described in Chapter 2, the chemical-physical characteristics of the following raw materials were evaluated: corn starch (CS); Psyllium (Psy); rice flour (RF); rice protein (RP); rice starch (RS); sugar beet fibre (SB). Moisture content (AACC 44-15A, 2000), protein (N*6.25, AOAC 920.87, 1999), total starch (TS) and damaged starch (DS) (“Total/Damage Starch Assay Kit” by Megazyme International Ireland Ltd) contents were measured. The method outlined by Anderson *et al.* (1969) was used to determine the water absorption index (WAI). The pasting properties were measured using a Brabender® Micro-Visco-Amylograph (Brabender OHG, Germany) as reported in Chapter 2. All these determinations were made at least in duplicate ($n \geq 2$).

5.2.3 Dough production and evaluation

The dough mixing properties were examined with the Brabender Farinograph (Brabender OHG, Germany). Powders were put into the farinographic bowl and pre-mixed for 5min, then the test was started (1 more minute for mixing the flours) and the remaining ingredients were added into few minutes in the following order: yeast diluted in part of the water, enzyme, margarine (pre-heated to make it fluid) and NaCl dissolved in water. Afterwards, the remaining water was carefully added up to the desired consistency (200BU or 500BU) using a graduated burette. The kneading was prolonged up to 15min at a temperature of 30°C controlled by a water cooling/heating system.

The amount of water to be added during mixing was the first parameter obtained from the farinographic test, that allows to calculate the water amount to be used in breadmaking; this parameter is related to the different ingredients used in the recipe and to their affinity for water.

Dough development during leavening, and gas production and retention in the dough were investigated with the Chopin F3 Rheofermentometer (Chopin SA, Villeneuve-La-Garenne, France). A suitable method, based on a previous methodology developed by Mariotti *et al.* (2006) for the evaluation of mixtures containing flours other than wheat, was used. This procedure differs from the reference method suggested by the Chopin SA in how the dough is prepared: 300g of sample instead of 250g; water according to the farinographic water absorption index instead of a constant water concentration; the farinographic mixer rather than the alveographic one. Moreover, no weight is applied to the dough during the test (instead of 2kg weight), and the temperature at which the test is performed is 30°C instead of 28.5°C.

The current test was performed for 1h at 30°C on a portion (315g) of the dough just produced in the Farinograph mixer (as previously described). The following indices were taken from the rheofermentographic curves: Hm (mm; dough maximum development during the test), Hf (mm; dough height at the end of the test), Tx (min; time of dough porosity appearance), CO₂ TOT (mL; total gas production during the test), CO₂ RET (mL; CO₂ retained by the dough during the test), CO₂ REL (mL; CO₂ released by the dough during the test), % RET (%; percentage of the CO₂ retained by the dough).

The remaining part of the dough produced through the Brabender Farinograph (Brabender OHG, Germany) was divided and moulded in a spherical shape (10g), put into six Petri dishes and leavened in a climatic chamber up to 1h at 30°C; at the beginning of the test (t₀) and then every 10min, the images of the

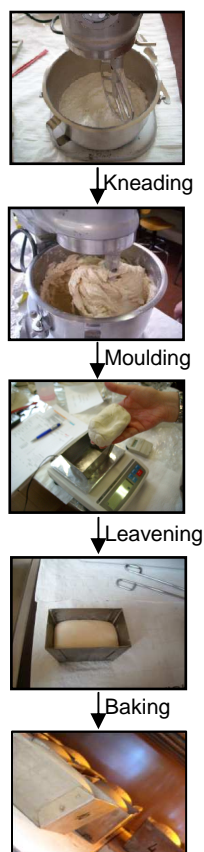
Petri dishes were acquired at a resolution of 300 dpi with a scanner HP SCANJET 8300. Using the Image Pro-Plus software (v. 4.5.1.29 Media Cybernetics, USA), images were processed and the dough percentage increases (%) in diameter and area during leavening were extrapolated.

Moreover, the surface texture of the doughs at the different leavening times was evaluated applying advanced image analysis techniques by means of the ImageJ software (1.44c National Institute of Health, USA). These techniques are able to discriminate the surface texture of foods (Kvaal *et al.*, 1998; Chen, 2007) by analysing the spatial distributions, frequency and grey level intensity values of each pixel of the image. The pixel characteristics are directly related to the physical surface texture (smoothness or roughness) of the acquired objects. Several different approaches in image texture analysis can be used, depending on the purpose of the analysis. In this research, statistical approaches such as Gray Level Co-occurrence Matrix (GLCM) method (Bharati *et al.*, 2004) and Angle Measure Technique (AMT), followed by multivariate statistical approaches based on Principal Component Analysis (PCA) were applied (Kvaal *et al.*, 1998). GLCM permits to obtain quantitative information on the homogeneity of the image by measuring different parameters: contrast (defined as a measure of the intensity contrast between a pixel and its neighbour over the whole image; contrast is 0 for a constant image), angular second moment or energy (measure of the image order; energy is 1 for a constant image) and entropy (measure of randomness, it is a measure of the image disorder). Multivariate statistical techniques were used to discriminate the doughs only on the basis of their texture surface characteristics. At least three images for every proofing time were collected and analysed ($n \geq 3$).

5.2.4 *Breadmaking process*

Breadmaking was performed as reported by Mariotti (2004), with some adjustments due to the different raw materials used in the current recipe.

To produce enough dough, rather than using the Farinograph mixer (that works with around 600g of final dough) as well as for dough evaluation, the Hobart N-50 mixer equipped with a flat beater (Hobart Corporation, Troy, Ohio, USA), that can work with more than 1.5kg of final dough (Figure 5-1), was preferred. In a complex recipe, as generally known, the order in which the ingredients are added is important for the final dough homogeneity. For this reason the same order previously used was maintained (§ 5.2.3). All the powders were pre-



mixed for 5min at the first mixing-speed, then water, yeast suspended in water and the enzyme were added. At last, margarine (pre-heated to make it fluid) and NaCl dissolved in the remaining water were added. All the ingredients were added within the first 4min mixing, and then mixed for 4 more min, at low speed (first speed=60rpm). At the end of this period, the mixing was interrupted and the dough manually recovered from the bowl, before mixing it for 2min more at the second speed (124rpm) and, finally, for other 5min at the first speed. The whole process lasted 20min (5min of pre-mixing and 15min of kneading), to simulate the conditions used during the Farinographic test.

At the end of the kneading, the dough was collected, divided into 150g aliquots and moulded by hand (A500 and B500) or by means of two spoons (A200 and B200), then placed into the baking moulds. Afterwards, the dough was leavened at 30°C and 80% RH for 35min and then baked in an oven (Lotus S.r.l., San Vendemiano, Treviso, Italy) for 30min at 230°C (bottom) and 200°C (top). At the end of baking, the samples were allowed to cool for 1h at room temperature, before being removed from the moulds. GF breads were then evaluated as described below.

Figure 5-1. Breadmaking process.

5.2.5 Bread quality evaluation

Fresh bread (after 1h cooling at room temperature) was characterized for its height (mm; using a calliper), volume (mL; AACC Method 10-05.01, replacing rapeseeds with sesame seeds) and specific volume (mL/g; dividing volume by loaf weight), weight loss (as the difference between the dough weight before leavening and the bread weight at the end of baking and cooling), and crust and crumb colour (Minolta Chroma Meter CR 210; Minolta, Osaka, Japan). Colour was expressed in the CIELAB space, as L* (lightness; from 0=black to 100=white), a* (+a=redness, -a=greenness) and b* (+b=yellowness, -b=blueness). At least 10 replicates of all these determinations were performed for each bread recipe ($n \geq 10$). Through Image Analysis techniques, also the crumb porosity, in terms of holes number (%) and alveolar area (%), was investigated. At this purpose, the images of two slices of every experimental

bread (n=20) were acquired at 300dpi with a scanner (HP SCANJET 8300) and processed by the Image Pro-Plus software (v. 4.5.1.29).

Bread crumb softness (both just after the production and during storage at 20°C, 60% RH up to 72h, into paper bags) was investigated through a compression test, using a TA-HD*plus* Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK). Bread was sliced and 3 slices for each bread (2.5cm thick) were compressed up to 40% of deformation using a 36mm diameter cylindrical probe, moving at a compression speed of 1mm/s. The following parameters were evaluated: hardness of the crumb (N, as load at 25% of deformation) and Young's modulus (N/mm², as the slope of the stress vs. strain curve calculated from the initial linear trait of the compression curve). At least six replicates were performed for each bread recipe at each storage time (n≥6).

5.3 RESULTS AND DISCUSSION

5.3.1 Dough characteristics

Table 5-1 refers the results of dough characterization, in terms of farinographic and rheofermentographic indices. The dough water absorption (WA), ranged from 57% to 85% depending on the amount of fibres in the dough and the dough consistency achieved. The presence of 2.5% of Psy determined, in particular for those doughs having a consistency of 200BU, an increase of WA from 74% to 85%. This could be related to the extremely different water absorption index (WAI) exhibited by SB and Psy: 9.48±2.13gH₂O/g db and 48.29±2.77gH₂O/g db, respectively (as reported in Chapter 2).

Table 5-1. Farinographic and Rheofermentographic properties of the doughs.

Sample	Farinograph		Rheofermentograph						
	Dough		Dough		Gas				
	Consistency (BU)	WA (%)	Hm (mm)	Hf (mm)	Tx (min)	CO ₂ TOT (mL)	CO ₂ REL (mL)	CO ₂ RET (mL)	% RET (%)
A	200	85.0	44.7	44.7	/	628	4	624	99.3
	500	58.1	14.3	13.1	25	744	38	706	95
B	200	74.0	43.1	43.1	/	614	4	609	99.3
	500	57.3	6.2	5.6	25	691	32	659	95.4

Abbreviations: A, formulation containing 0.5% SB and 2.5% Psy; B, formulation containing 1.5% SB and 1.5% Psy; WA, dough water absorption; Hm, maximum dough height; Hf, final height; Tx, time of dough porosity appearance; CO₂ TOT, total gas production; CO₂ RET, gas retained; CO₂ REL, gas released; % RET, percentage of the CO₂ retained.

The dough development during leavening and the volume of CO₂ produced from yeast activity were investigated through the Rheofermentographic test (Table 5-1 and Figure 5-2). In accordance with Mariotti (2004), that suggested that a more liquid-like dough had to be used in GF breads production, the maximum dough height (Hm) was 3 times higher for the two doughs having a 200BU consistency in comparison to the 500BU-doughs. Furthermore the 200BU-doughs did not exhibit the Tx index (the moment in which the CO₂ produced begins to escape from the dough) and the volume of CO₂ REL was only 4mL compared to more than 34mL for the 500BU-doughs.

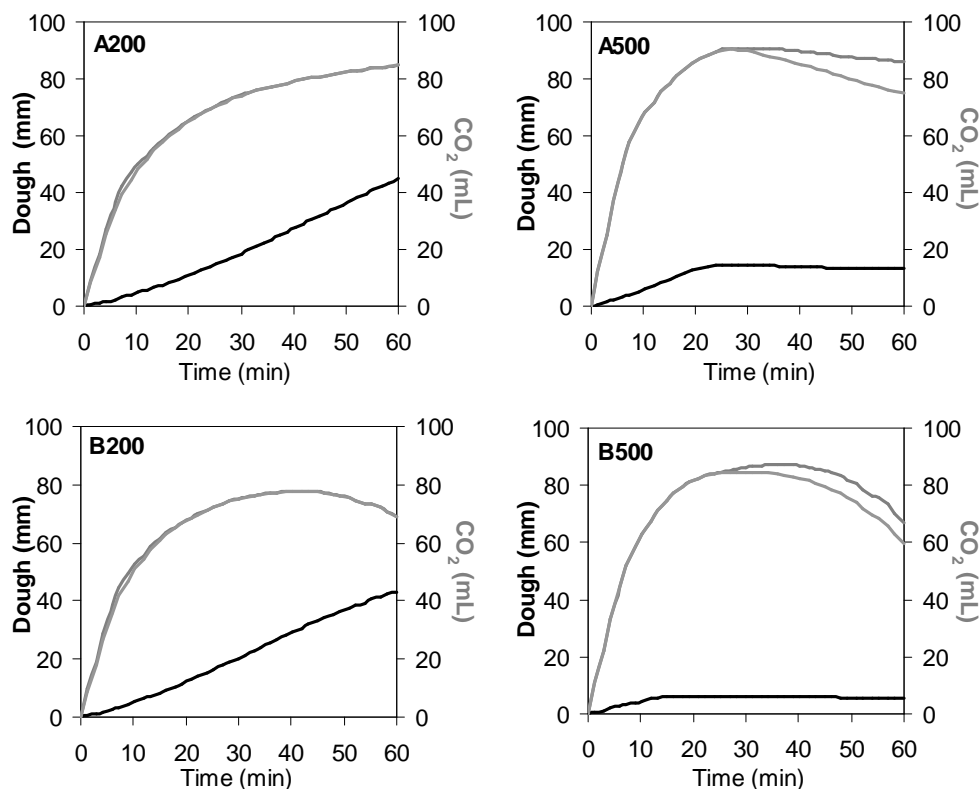


Figure 5-2. Dough height development (mm; black line) and CO₂ production and retention (mL; grey lines).

As can be appreciated in Figure 5-2, the amount of water added during kneading had an important effect of dough development. In fact, curves related to doughs having a different consistency showed a completely different trend: the 500BU-doughs showed a slight increase in height, and limited to the first 10-20 minutes of the test, whereas the 200BU-doughs exhibited a continuous increase in dough height. On the contrary, not too many differences were

observed when the percentage of Psyllium (1.5 or 2.5%) in the recipe was changed.

By means of Image Analysis techniques, accurate measurements of the dough growth, in terms of geometrical indices (diameter and area) and texture surface changes, during leavening up to 1h at 30°C into Petri dishes, were obtained. As expected, diameter and area increased with the same trends (Figure 5-3): doughs having a higher consistency (A500 and B500) increased their volume during the first 20-30min, showing then a constant increase up to the end of the leavening phase (60min); the “liquid-like” doughs (A200 and B200), on the contrary, showed a continuous increase during proofing. The evaluation of dough area increase came out to be more useful than the assessment of diameter increase in describing dough growing during leavening.

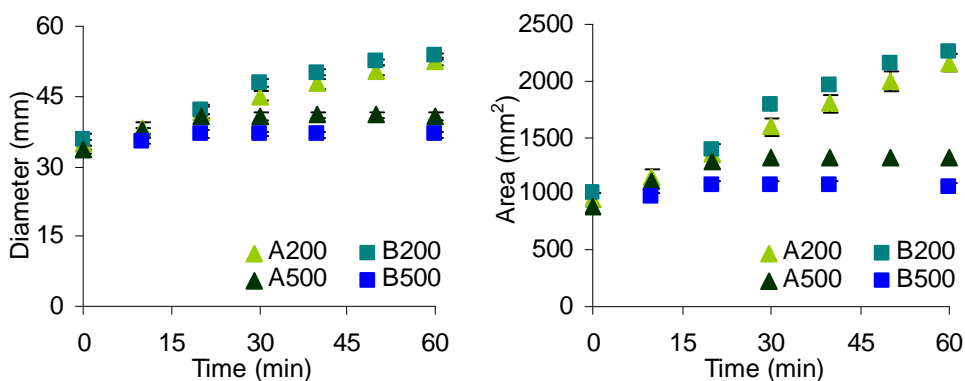


Figure 5-3. Dough diameter and area increase during leavening.

Image analysis was also used to investigate the surface texture of the dough. All the indices measured (Energy, Contrast and Entropy) resulted very useful to quantify the changing of the texture surface of the dough due to the developing and growing of air bubbles as a consequence of microbial fermentation. Here, only the Contrast values are reported, as Energy and Entropy gave the same information.

As can be appreciated in Figure 5-4 and 5-5, at the beginning of the leavening phase the Contrast measured from the images of the doughs having different formulation (A=0.5% SB and 2.5% Psy and B=1.5% SB and 1.5% Psy) and consistency (200BU or 500BU) appeared similar: no significant differences ($P < 0.05$) were observed at t_0 and very small variations were appreciated after 10 and 20min leavening. After 30min, the differences became more clear: the Contrast values of A500 and B500 were not significantly different ($P < 0.05$) and both the samples showed a low increase of the heterogeneity of the surface;

on the contrary, A200 was characterized by intermediate levels of contrast (as well as Energy and Entropy - *data not reported*) and B200 showed the highest contrast values, indicating that an extensive gas expansion occurred in this sample during proofing.

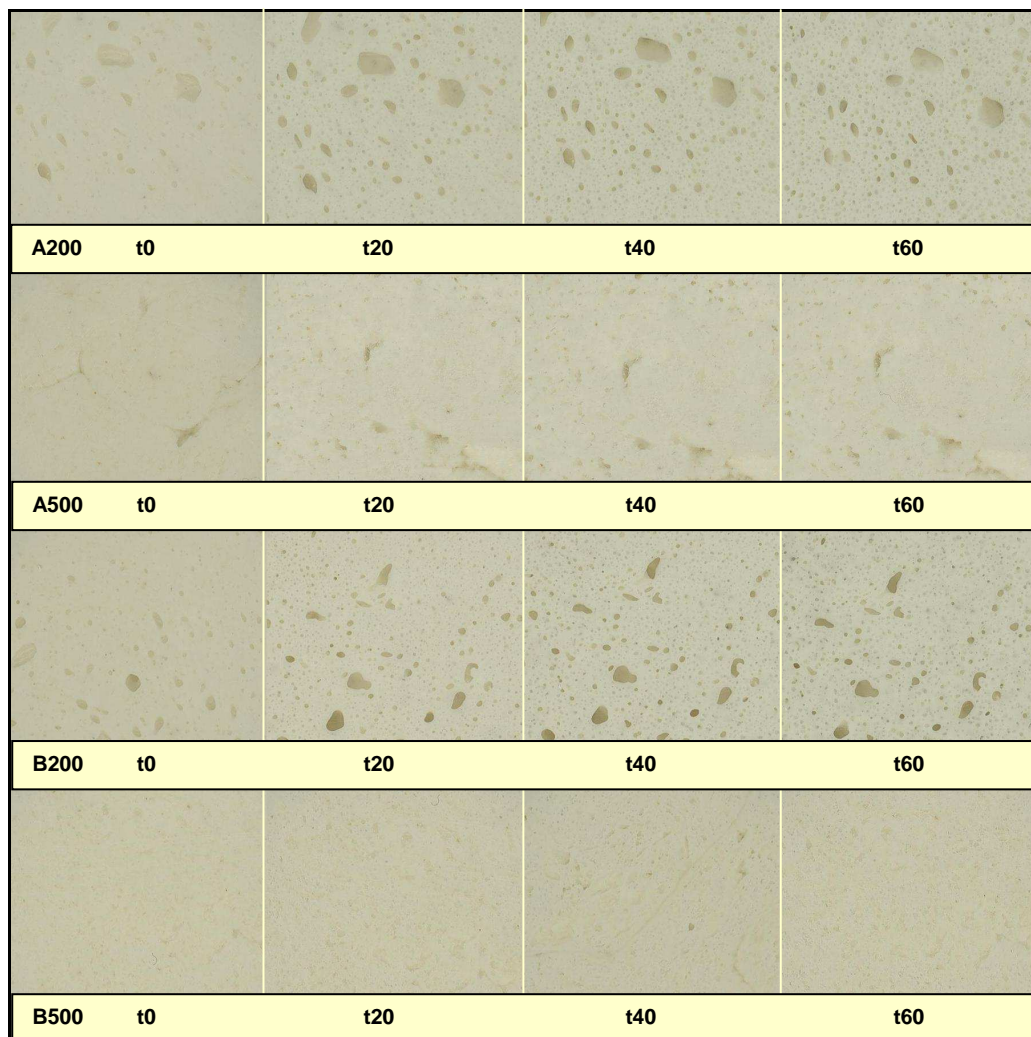


Figure 5-4. Surface images of the doughs at the different leavening times: 0, 20, 40 and 60min.

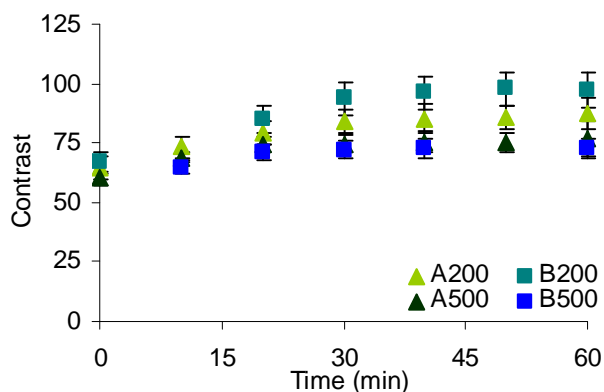


Figure 5-5. Contrast values as indices of doughs homogeneity at the different leavening times.

Combining advanced image analysis with multivariate statistical techniques doughs were discriminated only on the basis of their texture surface characteristics. As can be appreciated in Figure 5-6, the 98.73% of the explained variance was expressed by PC1; in particular, in the left square of the scores plot are located the images acquired at t0 and t10, whereas the majority of those acquired after a more prolonged leavening time are arranged on the right side of the scores plot. Furthermore, in comparison with the more liquid doughs (200BU), the 500BU doughs were less discriminated on the basis of their surface texture (mainly explained by PC1): independently from the formulation, the doughs at 500BU are not able to retain the CO₂ produced by the microorganisms and consequently their surface texture at the different leavening times change very little. The opposite happened for the 200BU doughs, that were able to retain progressively the CO₂ produced; this involved an increase of the dough heterogeneity, due to the co-presence of continuous dough and alveolate regions (Figure 5-4).

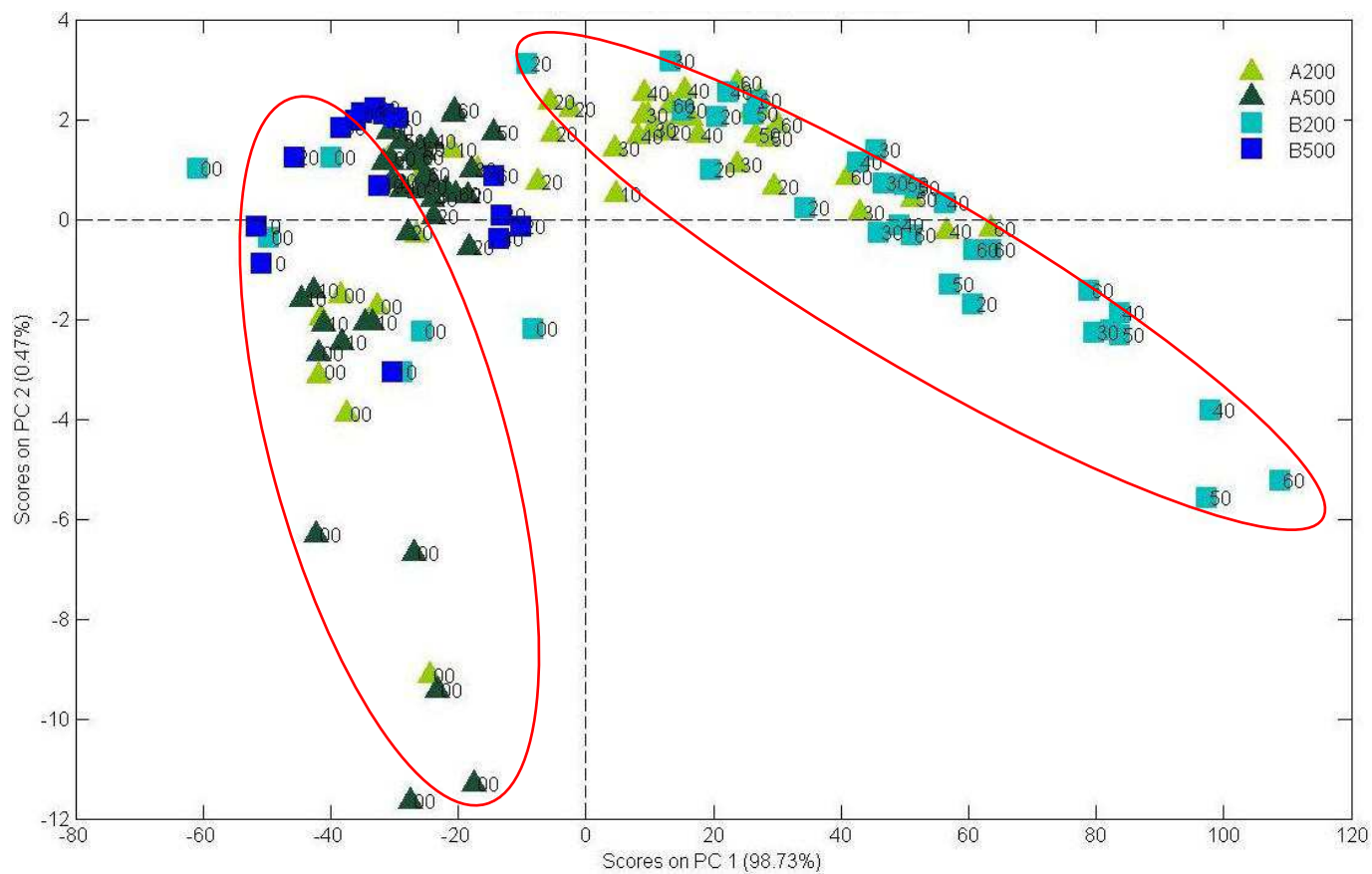


Figure 5-6. Discrimination of the doughs on the base of their texture surface characteristics at t₀ (00) and after 10, 20, 30, 40, 50, 60min leavening.

5.3.2 Bread characteristics

After these preliminary studies on the dough, the bread was produced and characterized as previously described. Breads obtained from dough having a lower consistency (A200 and B200) showed, as expected, a higher moisture content, specific volume, height and a good crumb softness (low hardness) (Table 5-2). However, in contrast with the expectations, 500BU-doughs containing 2.5% of Psy (A500), that presented a limited development during leavening (Farinographic test), showed the highest height increase during baking and the highest specific volume due to a higher amount of gas retained into the dough; this suggests that the amount of Psyllium plays a central role on bread development. On the contrary, the water content of the dough is crucial for bread crumb softness (it is worth noting that A500 bread is harder than A200). At lower Psyllium content (1.5%, B formulation) dough consistency resulted the most critical parameter for product development.

Table 5-2. Fresh bread characteristics.

Bread	Crumb moisture (g/100g)	Slice moisture (g/100g)	Height (mm)	Specific volume (mL/g)	Hardness (N)
A200	52.84±0.04 ^d	46.17±0.30 ^d	52.4±1.1 ^c	2.40±0.17 ^c	7.43±0.82 ^a
A500	44.94±0.13 ^b	37.50±0.43 ^a	61.3±2.1 ^d	2.74±0.26 ^d	12.14±1.72 ^b
B200	50.06±0.05 ^c	42.34±0.31 ^c	48.0±1.3 ^b	2.11±0.12 ^b	10.63±0.59 ^b
B500	44.77±0.13 ^a	38.48±0.21 ^b	39.4±1.5 ^a	1.79±0.16 ^a	n.d.

Note: values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Crumb porosity, as a further index of bread quality, was also investigated by Image Analysis. All breads showed a good porosity (more than 20% of the total area - *data not reported*) and, as it can be appreciated in Figure 5-7, the total slice area was characterized by a prevalence (around 50%) of intermediate size holes (area from 0.5 and 1mm²). Only the structure of sample B500 appeared more compact: a high number of fine holes (from 0.2 and 0.5mm²) was present and only the 37% of the total alveolar area was occupied by intermediate size holes.

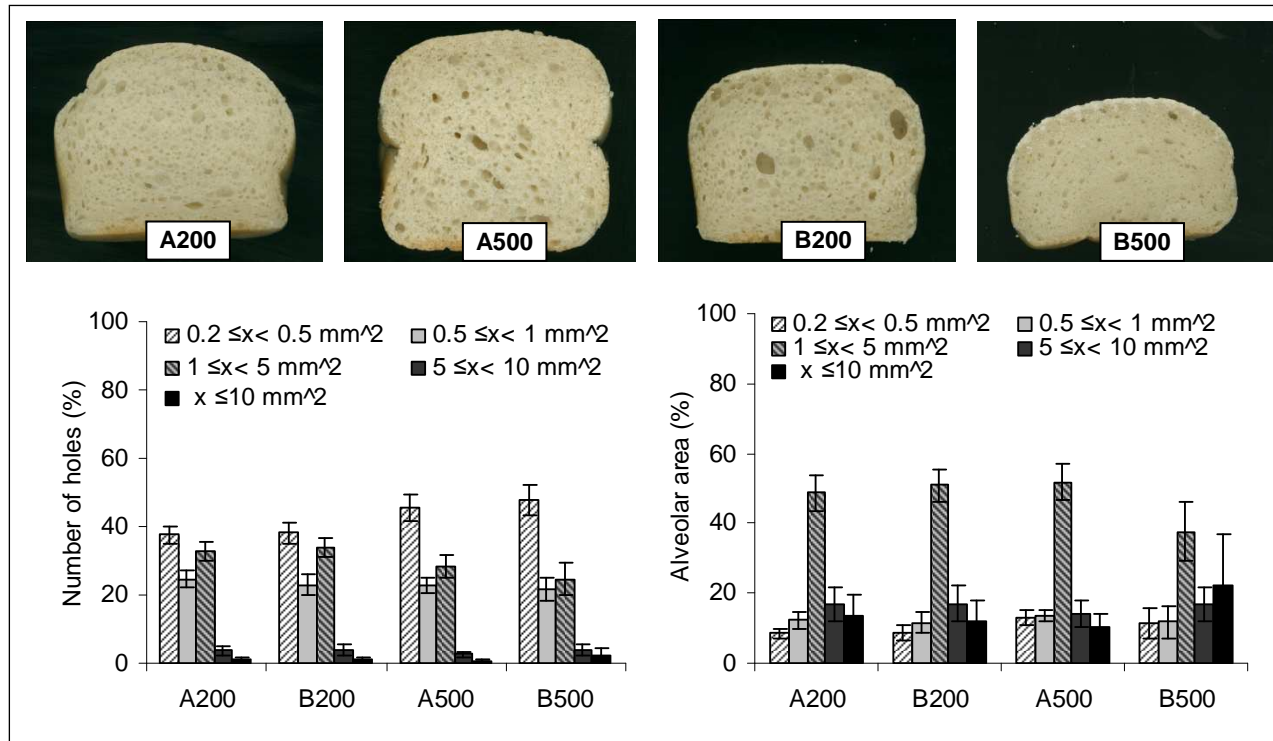


Figure 5-7. Bread crumb appearance and porosity.

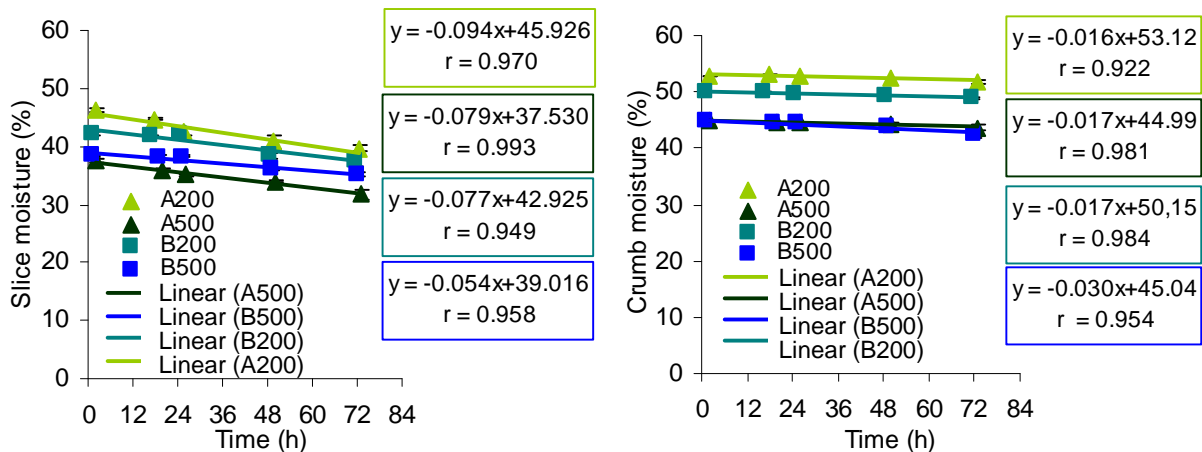
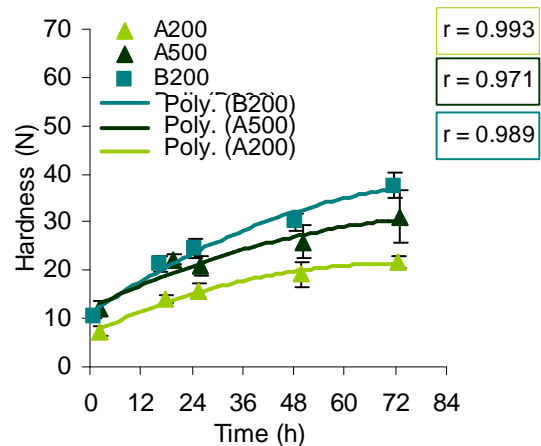


Figure 5-8. Bread characteristics during storage.

Note: crumb hardness of the B500 bread was not measured as it was not enough developed.



In Figure 5-8 bread properties during storage are showed. A limited moisture decrease (2-5%) in bread crumb was observed, whereas it was much more evident in bread slice (from 9% to 16%). Furthermore, significant differences ($P < 0.05$) in crumb softness were found between the fresh 200BU-breads ($7.43 \pm 0.82\text{N}$ for bread A200 vs. $10.63 \pm 0.59\text{N}$ for bread B200) and these differences became more evident after 72h of storage ($21.71 \pm 1.34\text{N}$ vs. $37.56 \pm 2.57\text{N}$, respectively) indicating a higher anti-staling effect of Psy in comparison to SB fibre. As regards the effect of the water amount added to the dough, both hardness and Young's modulus values highlighted that a higher water content is a prerequisite to maintain the sample softer during its shelf-life. Particularly, A200-bread had a Young's modulus of $0.038 \pm 0.01\text{N/mm}^2$ at t_0 and of $0.147 \pm 0.02\text{N/mm}^2$ at the end of storage, whereas A500 had a more compact structure from the beginning (Young's modulus: $0.067 \pm 0.01\text{N/mm}^2$ and $0.210 \pm 0.06\text{N/mm}^2$, respectively).

5.4 CONCLUSIONS

GF doughs are often characterized by limited abilities of gas expansion and retention during leavening, factors that lead to a bread with reduced volume and crumb softness (Mariotti, 2004). Furthermore, these products are more sensible to staling due to the high presence in the recipe of starches from different origins. Thus, a current challenge for scientists is to simulate the gluten properties by adding ingredients, such as hydrocolloids and fibres, able to improve the rheological properties of the dough and, consequently, to enhance the final bread quality. However, the use of new ingredients in the recipe often requires an adjustment of the breadmaking process in terms of optimal dough consistency and leavening and baking conditions.

In this study the effect of two different levels of Psyllium (Psy) and sugar beet (SB) fibres on the baking aptitude of GF doughs having two different consistencies (200 and 500BU) was evaluated. The results suggested that for GF products a lower consistency (200BU) is preferred to the traditional dough consistency (500BU) to achieve good performances during leavening, particularly if ingredients with a high water affinity (as Psyllium) are present in the recipe. In fact, 500BU-doughs were characterized by a reduced development and gas retention during proofing. However, the knowledge of dough behaviour during leavening is not always sufficient to predict the final quality of bread. Frequently, as for the 500BU-doughs of this study, it has to be supported by real baking trials.

As regards this part of the research, it can be concluded that hydrocolloids and fibres play a strategic role in improving the workability of GF dough, enhancing at the same time their water binding capacity and the shelf-life of the final product. In addition, a more effective anti-staling effect of Psy in comparison to SB after 3 days of storage was evidenced.

5.5 REFERENCES

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CHAPTER 6

GLUTEN-FREE BREADMAKING TRIALS: COMPRESSED YEAST *VERSUS* SOURDOUGH

This research was performed in collaboration with
Prof. Roberto Foschino and Dr. Claudia Picozzi
(DiSTAM-Microbiologia Agraria Alimentare ed Ecologica)

6.1 INTRODUCTION

Gluten-free (GF) breads have a texture and a crumb grain quite different from the wheat counterparts. In GF bread production, the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final product. To simulate gluten properties agents that increase the viscosity of the system and emulsifiers able to enhance a starch-continuous system are frequently added to the recipe (Gallagher *et al.*, 2004; Lazaridou *et al.*, 2007). Nevertheless, critical are the rheological properties of the dough that, lacking in gluten, shows limited abilities of gas expansion and retention during leavening, factors that lead to a bread with reduced volume and crumb softness (Mariotti, 2004). Moreover, the high presence in the formulation of starches from different origins and of flours containing high amounts of starch makes the product more sensible to staling and reduces its shelf-life (Arendt *et al.*, 2008).

Generally, GF breads are produced by a straight-dough process, using compressed yeast as leavening agent. However, as in wheat-based baked products it is well known that an improvement of bread quality and shelf-life can be obtained by using sourdough, this procedure could become an interesting alternative in GF bread production too.

Sourdough is a spontaneous fermented dough, frequently inoculated with a wild microbial starter called “mother”, which is constantly renewed in a cycling way, using strict conditions of recipe and ripening. The microflora of sourdough generally consists of yeasts and homofermentative and heterofermentative lactobacilli. As reported by Foschino *et al.* (1995), *Lactobacillus sanfranciscensis* is the typical and predominant bacterium in the sourdough and it can form a mutualistic association with maltose-negative yeast species (*Candida holmii* or *Candida humilis*).

In the last years, some researchers have demonstrated an improvement in bread volume and crumb structure (Corsetti *et al.*, 2000 and Clarke *et al.*, 2002), flavour (Thiele *et al.*, 2002) and mold-free shelf-life (Lavermicocca *et al.*, 2000 and Dal Bello *et al.*, 2006) when sourdough was used as a leavening agent. The flavour obtained is mainly due to the starter cultures used, and to the organic acids and aminoacids released during fermentation. Besides, the gas-holding of the dough seems to be positively affected by the acidification of the flour. Incorporation of 20% sourdough seems to have remarkable effects on the final bread quality, delaying the bread staling and the growth of spoilage organisms (Moore *et al.*, 2008). Even if the use of sourdough makes the

breadmaking process longer and the research in this field is still at its beginning, data available up to now indicate that sourdough could represent an attractive tool to improve the quality (e.g. flavour and shelf-life) of GF bread.

In the current research, the first step was the set up of a GF sourdough (SD), starting from selected bacteria and yeasts isolated from a wheat-based sourdough conventionally used for Panettone production. This starter was constantly and continuously propagated and its properties were regularly monitored. In a second phase, breadmaking trials were performed to compare the quality of GF breads obtained using a traditional compressed yeast (*Saccharomyces cerevisiae*; CY) or the developed GF-sourdough (SD) as leavening agents, as well as their combination (CY/SD).

6.2 MATERIALS AND METHODS

6.2.1 Microbial counts

Lactobacillus sanfranciscensis and *Candida humilis* were isolated from a wheat-based sourdough conventionally used for Panettone production. The microbial counts were conducted on the traditional sourdough, and before and after each refreshment of the GF sourdough here developed. For this purpose, approximately 10g of dough was diluted in 90mL of sterile peptone water, and homogenized in a Colworth Stomacher 400 (Seward, London, UK) for 2min at 230rpm. Lactobacilli were counted by plating on Sanfrancisco medium (SFM) modified by Foschino *et al.* (2001), after an incubation under anaerobic conditions (GasPak System, Merck KGaA, Germany) at 30°C for 3-5 days. Yeasts were plated on yeast glucose chloramphenicol agar (YGC) after an incubation at 25°C for 5 days in aerobic conditions. Every count was conducted in duplicate (n=2).

6.2.2 Dried yeast extract and microbial liquid inoculum production

The GF dried yeast extract was produced by suspending 80g of dried yeast (Springaline, BA95/0-PW; Bio Springer, France, Maisons-Alfort Cedex) into 1L of distilled water, followed by sterilization, centrifugation (4500rpm for 30min at 10°C) and filtration to recover the cytoplasmatic material. Aliquots of the extract were then poured into plastic container (90mL each) and stored at -20°C. For the preparation of the microbial *inoculum* the pure strains stored frozen (-20°C for the bacteria; -80°C for the yeasts) into 20% v/v glycerol, were suspended into 10mL of SFM broth (*Lactobacillus sanfranciscensis*) or 10mL of Yeast

Extract Peptone Dextrose (YEPD) broth (*Candida humilis*). These suspensions were then incubated at 30°C for 48h (lactobacilli) and at 25°C for at least 12h (yeast). Then, 6mL of the corresponding broth was centrifuged at 15000rpm for 10min and the microbial cells were suspended into 10mL of GF dry yeast extract, in order to have a final microbial concentration equal to 10⁹CFU/mL for *Lactobacillus sanfranciscensis* and 10⁷CFU/mL for *Candida humilis*.

6.2.3 Sourdough development and refreshments

The GF recipe was defined by studying the related literature, considering some commercial recipes and through the knowledge acquired from preliminary studies carried out at DiSTAM-Food Technology Section, that included also the evaluation of the foaming stability of different pea proteins (Cream Tester CT II, Gerber Instruments; Chapter 2). The final recipe for the GF dough set up in this research consisted of: 89.4% corn starch (CS, Roquette Italia SpA, Alessandria, Italy) and rice flour (RF, Beneo-Remy NV, Leuven-Wijgmaal, Belgium); 7% isolated pea protein (IPP-F9, Cosucra, Warcoing, Belgium); 3.6% hydroxypropylmethylcellulose (HPMC, Food Grade Modified Cellulose, F4M; The Dow Company, Midland, Michingan, Usa) and *Psyllium* fiber (Psy, Roeper GmbH, Hamburg, Germany) (Tedesco, 2010). Maltose (Merck KGaA, Darmstadt, Germany; 2.8% w/w of the dough) was used as microbial substrate.

All the powders (300g wet basis) were put into the Brabender Farinograph chamber (Brabender OHG, Germany), thermostated at 25°C, and pre-mixed for 5min; then the remaining ingredients were added, within few minutes, as follows: 70mL of GF dry yeast extract, 10mL of the inoculum containing *Lb. sanfranciscensis*, 10mL of the inoculum containing *Candida humilis*, and distilled water to reach a final consistency of the dough equal to 230 Brabender Unit (BU) (water absorption - WA, %). The mixing step was prolonged for 15min. Then part of the dough (300g) was maintained at 25°C for 22h into a Chopin F3 Rheofermentometer (Chopin SA, Villeneuve-La-Garenne, France) both to allow lactobacilli and yeasts growing and to monitor the leavening process. The GF sourdough thus developed was stored at 4°C in a plastic bag until the following refreshment.

In order to carry on the refreshments, the GF recipe previously described was pre-mixed for 5min with a part of the fermented GF dough (10% GF-mix) in the Brabender Farinograph (Brabender OHG, Germany) thermostated at 25°C.

Then 90mL of the GF dry yeast extract were added to the mix, followed by water addition up to achieve a final consistency of 230BU. Mixing was prolonged up to 15min. The dough thus obtained (300g) was fermented at 25°C using a Chopin F3 Rheofermentometer (Chopin SA, Villeneuve-La-Garenne, France) for the time necessary to reach the pH 3.8-4.0. The refreshed and leavened GF sourdough was then stored at 4°C in a plastic bag, until the next refreshment.

6.2.4 Sourdough quality assessment

The developed GF sourdough was refreshed and monitored constantly in terms of number and type of microorganisms (§ 6.2.1), capability to produce/retain CO₂ and pH variations. According to a previous methodology developed by Mariotti *et al.* (2006) for the evaluation of the leavening performances of mixtures containing flours other than wheat, the leavening properties of the GF sourdough were investigated by means of the Chopin F3 Rheofermentometer (Chopin SA, Villeneuve-La-Garenne, France). The following indices were taken from the rheofermentographic curves: Hm (mm; maximum dough development), T1 (h:min; time of maximum dough development), CO₂ TOT (mL; total gas produced), CO₂ REL (mL; CO₂ released), CO₂ RET (mL; CO₂ retained), % RET (%; percentage of CO₂ retention) and Tx (h:min; time of dough porosity appearance).

During fermentation, also the pH of the sourdough (50g) was constantly checked at 25°C using a pH-meter PHM 220 (Radiometer; A. De Mori Strumenti SpA, Milano, Italy). The pH values were continuously recorded in real time along with the fermentation process; only when pH values were equal to 3.80-4.0 the fermentation process was considered completed.

6.2.5 Bread production

When a stable association between microorganisms was reached, as well as a quite constant technological quality of the GF sourdough, some breadmaking trials were performed.

The GF bread recipe included: 77.8% CS and RF, 6.2% IPP-F9, 3% HPMC and Psy, 0.6% emulsifier (DIMODAN PH100 and PANODAN-DATEM 517; Danisco A/S, Copenhagen, Denmark), 6.2% extra-virgin oil, 2.1% sodium chloride and 4.1% maltose.

Depending on the leavening agent used, three GF breadmaking trials were carried out:

- CY BREAD: containing only compressed yeast as leavening agent (2% of the GF recipe);
- SD BREAD: with only sourdough as leavening agent (20% of the GF recipe);
- CY/SD BREAD: using both compressed yeast (2% of the GF recipe) and sourdough (20% of the GF recipe) as leavening agents.

Breadmaking was performed as reported by Mariotti (2004), adopting some adjustments due to the different raw materials used in the current recipe. All the powders (and sourdough, when present) were pre-mixed with a Hobart N-50 mixer (Hobart Corporation, Troy, Ohio, USA) for 5min at 60rpm. Those ingredients dispersed in water (maltose, sodium and - when present - compressed yeast) were then added, followed by the addition of the remaining water (being the total water amount previously determined by the farinografic test, aiming to reach a final consistency of 180BU). Oil was included in the mix at the end. All these ingredients were added within the first 2 minutes, mixing and kneading was prolonged up to 15min.

The dough was divided, placed into baking moulds (150g in each one) and leavened in a climatic chamber (Heraeus Votsch) at 25°C and 80% RH for different times, depending on the leavening agent used (CY, 1h 30min; SD, 3h and CY/SD, 1h). The optimal fermentation time was determined for each type of dough by means of rheofermentographic tests. The leavened dough was baked in an oven (Lotus S.r.l., San Vendemiano, Treviso, Italy) for 30min at 230°C (bottom) - 200°C (top), then cooled at room temperature for 1h and finally removed from the moulds. The quality of these GF experimental breads was then evaluated as follows.

6.2.6 Bread quality evaluation

a) Fresh bread

After baking, the loaves were removed from the moulds and cooled for 60min at room temperature before being characterized for weight (g), height (cm; calliper) and volume (mL; AACC Method 10-05.01, replacing rapeseeds with sesame seeds). The specific volume (mL/g) was calculated. Weight loss (%; difference between the weight of the dough before leavening and bread weight after baking, divided by the dough weight) was evaluated, too. Fresh breads were also characterized as following indicated.

b) Bread quality evaluation during storage

The GF loaves were evaluated in accelerated storage conditions (69h, paper bags, 25°C and 60% RH). At each sampling time (1h- t_0 , fresh bread; 23h- t_1 ; 46h- t_2 ; 69h- t_3), 2 breads for each formulation were weighted, then sliced and characterized as follows.

Crust and crumb colour were measured using a Minolta Chroma Meter CR 210 (Minolta, Osaka, Japan); results were expressed in the CIELAB space, as L^* (lightness; 0=black, 100=white), a^* (+a=redness, -a=greenness) and b^* (+b=yellowness, -b=blueness). At least 8 replicates were performed for each bread recipe ($n \geq 8$). The two back ends of each loaf were then removed and used for Images Analysis, whereas the 3 slices (20mm thick) obtained from the central part of each loaf were used for the evaluation of bread moisture and crumb consistency. The moisture of the central slice and of the crumb was determined according to the AACC Official Standard Method 44-15A (2000). Bread crumb softness was investigated through a TA-HDplus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) using a Texture Profile Analysis test (500N load cell). From each slice a cylindrical crumb sample (CCS, 25mm diameter, 20mm height) was obtained. Each CCS was compressed twice up to 25% deformation, using a 100mm diameter cylindrical probe moving at a compression speed of 2mm/s. From the resulting curve, the following parameters were considered: crumb hardness (N; load at 25% deformation) and Young's modulus (N/mm^2 ; slope of the stress vs. strain curve obtained from the initial linear trait of the compression curve). At least 6 replicates were performed for each bread recipe at each storage time ($n \geq 6$). Through Image Analysis also crumb porosity was investigated. The images of the 2 back ends of each bread loaf ($n=16$) were acquired at 600dpi with a HP SCANJET 8300, and processed by means of the software Image Pro-Plus (v. 4.5.1.29). A portion ($721.12mm^2$) of each slice was selected and analysed. The objects (holes) were counted and classified into 3 groups on the basis of their size: $0.1 \leq x \leq 1mm^2$; $1 \leq x \leq 3mm^2$ and $x > 3mm^2$. The following parameters were considered for each hole class: hole distribution (%; percentage of the total mean number of counted holes), hole area (%; percentage of the total hole area) and hole mean diameter (mm); furthermore, the total mean alveolar area (%; total hole area in the portion of the crumb analysed) was quantified.

6.3 RESULTS AND DISCUSSION

6.3.1 Sourdough characterization

The microorganisms of interest (*Lactobacillus sanfranciscensis* and *Candida humilis*) were isolated from a traditional sourdough used in Panettone production having a population of 2.3×10^7 CFU/g yeasts and 1.3×10^8 CFU/g bacteria. The pure strain isolated were added into a GF-matrix to produce a GF dough-inoculum, also named “mother”, that was fermented at 25°C for 22h and 30min. Part of this fermented dough (30g) was then used to produce the GF sourdough that was constantly refreshed with new ingredients (300g of GF-mix).

Table 6-1. GF sourdough characterization.

	WA (%)	Starting pH	Ending pH	Leavening time (h:min)	Y (CFU/g)	LB (CFU/g)
<i>inoculum</i>	96.87	5.99	4.73	22:30	5.20×10^6	2.15×10^9
1_R	88.73	5.83	4.18	22:15	1.02×10^8	1.70×10^9
2_R	87.93	5.71	4.16	22:00	9.82×10^7	1.42×10^9
3_R	86.93	5.73	4.07	20:15	4.18×10^7	7.82×10^8
4_R	87.87	5.63	3.98	19:10	6.64×10^7	2.69×10^9
5_R	88.00	5.65	3.96	15:55	9.18×10^7	nd
6_R	88.00	5.66	3.94	15:45	1.03×10^8	1.57×10^9
7_R	87.63	5.68	3.96	16:15	1.59×10^8	4.18×10^9
8_R	88.23	5.67	3.98	16:00	1.70×10^8	3.09×10^9
9_R	89.00	5.68	3.99	17:01	1.47×10^8	4.31×10^9
10_R	87.83	5.65	3.91	16:20	2.07×10^8	4.72×10^9
11_R	87.40	5.65	4.00	16:08	2.07×10^8	1.89×10^9
12_R	88.87	5.69	3.97	16:30	1.65×10^8	3.59×10^9
13_R	87.20	5.70	3.98	15:50	1.98×10^8	2.15×10^9
14_R	87.87	5.72	3.96	16:22	1.25×10^8	1.65×10^9
15_R	88.60	5.69	3.95	15:17	1.54×10^8	2.35×10^9
16_R	87.60	5.75	3.99	15:10	nd	nd
17_R	88.43	5.73	3.95	17:35	nd	nd
18_R	87.83	5.76	3.96	15:27	nd	nd
19_R	86.00	5.72	3.97	14:50	nd	nd
20_R	87.17	5.71	3.95	16:55	nd	nd
21_R	87.87	5.67	3.95	16:12	nd	nd
22_R	88.23	5.76	3.97	16:43	nd	nd
23_R	88.83	5.70	3.97	16:12	nd	nd
24_R	88.90	5.68	3.83	16:00	nd	nd
25_R	89.03	5.67	3.97	15:50	2.34×10^8	2.25×10^9

Abbreviations: R, refreshment; WA, dough water absorption; Y, yeasts growth; LB, lactobacilli growth; nd, not determined.

As it can be appreciated in Table 6-1, after 22h and 30min of fermentation at 25°C, the inoculum was composed by $5.20 \cdot 10^6$ CFU/g yeasts and $2.15 \cdot 10^9$ CFU/g bacteria. Nevertheless, the rheofermentographic indices (Table 6-2) evidenced a very low dough height increase (Hm: 10.2mm) and gas production (CO₂TOT: 225mL) during fermentation. For that reason during the first refreshment (1_R), beyond the addition of the GF dough previously fermented, further liquid yeast inoculum (10mL) containing *Candida humilis* strains was added in order to achieve the desired final microbial concentration of $1.02 \cdot 10^8$ CFU/g yeasts and $1.70 \cdot 10^9$ CFU/g bacteria as well as a better leavening performance of the dough (Hm; 18mm; CO₂TOT: 1377mL). A stable association between microorganisms, measured both in terms of microbiological population and technological properties, was obtained after few refreshments (3-4); furthermore, the leavening time necessary to reach pH values ranging between 3.8 and 4.0 decreased to 15-17h and the amount of water necessary to obtain a 180BU dough consistency stabilized around 88% (WA: 97% for the inoculum).

Refreshments were not carried on at fixed times (Table 6.2): at the beginning the sourdough (SD) was refreshed every 2-4 days, while longer periods of storage at 4°C between subsequent refreshments were then tested. It can be observed that SD leavening performances were quite constant up to 7-8 days. Longer storage times (up to 12 days) between one refreshment and the other determined lower dough developments (Hm: 21.3mm vs. Hm: 25mm, calculated as the mean Hm calculate from the 3_R to the 25_R, excluding 20_R and longer time to observe dough porosity appearance (Tx, 8h and 12min vs. 6h and 25min). These results suggest that refreshments should be performed at least every week.

Table 6-2. Rheofermentographic behaviour of the GF sourdough.

	Dough			CO ₂ production and retention				
	Days from previous R	Hm (mm)	T1 (h:min)	CO ₂ TOT (mL)	CO ₂ REL (mL)	CO ₂ RET (mL)	% RET (%)	Tx (h:min)
<i>inoculum</i>	-	10.2	3:47	225	5	221	98.0	-
1_R	2	18.0	10:16	1377	183	1195	86.7	8:57
2_R	4	22.1	8:06	1382	146	1236	89.4	6:46
3_R	1	24.2	7:01	1293	135	1159	89.6	6:18
4_R	1	24.8	7:00	1226	122	1104	90.0	5:18
5_R	1	24.8	6:55	1225	126	1099	89.7	6:16
6_R	4	25.0	7:34	1226	116	1110	90.5	6:24
7_R	3	25.4	7:07	1267	120	1148	90.6	6:21
8_R	4	26.8	7:18	1273	132	1140	89.6	6:34
9_R	3	25.0	7:09	1309	136	1173	89.6	5:45
10_R	4	24.2	7:39	1269	132	1137	89.6	6:39
11_R	7	24.4	8:51	1230	125	1104	89.8	7:42
12_R	2	26.3	6:39	1287	134	1153	89.6	5:54
13_R	5	23.4	7:34	1260	138	1122	89.1	6:43
14_R	2	24.3	7:34	1257	128	1129	89.8	6:10
15_R	5	25.4	7:03	1246	135	1111	89.2	6:30
16_R	2	23.5	6:49	1247	138	1109	88.9	6:00
17_R	5	24.9	5:51	1251	130	1121	89.6	6:18
18_R	2	25.9	6:45	1248	133	1115	89.3	6:18
19_R	8	25.3	5:58	1306	142	1164	89.1	6:33
20_R	12	21.3	9:15	1246	130	1117	89.6	8:12
21_R	8	25.1	6:48	1257	113	1144	91.0	6:46
22_R	6	24.7	8:24	1259	122	1137	90.3	7:15
23_R	6	25.6	7:43	1222	115	1108	90.6	6:19
24_R	7	24.5	7:30	1192	121	1071	89.8	6:43
25_R	7	25.7	7:22	1163	104	1059	91.1	6:34
mean after 2 nd _R		24.7±1	7:19±0:47	1256±42	128±10	1086±210	89.8±1	6:30±0:35

Abbreviations: R, refreshment; Hm, maximum height; T1, time of maximum dough height; CO₂ TOT, total gas production; CO₂ REL, gas released; CO₂ RET, gas retained; % RET, percentage of the CO₂ retained; Tx, time of dough porosity appearance.

6.3.2 Gluten-free breadmaking trials

a) Dough and fresh bread properties

When the GF sourdough microbial population was constant (*Candida humilis* and *Lactobacillus sanfranciscensis* around 10⁸CFU/g and 10⁹CFU/g, respectively) and its leavening performance judged satisfactory, both in terms

of dough development and CO₂ production/retention, some breadmaking trials were performed. The quality of GF breads leavened by means of compressed yeast (*Saccaromyces cerevisiae*; CY), the developed GF-sourdough (SD) or a combination of the two (CY/SD) were compared.

Table 6-3. Dough and fresh GF bread properties.

	CY GF BREAD	SD GF BREAD	CY/SD GF BREAD
Dough water absorption (WA, %)	82	64.5	64.5
Dough weight (g)	150.6±0.21 ^a	150.5± 0.28 ^a	150.5±0.16 ^a
Bread weight (g)	134.9±1.21 ^b	135.0± 1.03 ^b	123.1±1.33 ^a
Weight loss (%)	10.4±0.8 ^a	10.3± 0.7 ^a	18.2±0.9 ^b
Maximum height (cm)	5.61±0.13 ^b	4.69± 0.19 ^a	6.02±0.15 ^c
Minimum height (cm)	3.96±0.25 ^b	2.74± 0.13 ^a	4.30±0.28 ^c
Specific volume (mL/g)	2.57 ± 0.08 ^b	1.89 ± 0.05 ^a	3.07±0.09 ^c
L*-crust	81.3±1.5 ^b	82.2± 2.3 ^b	77.3±2.4 ^a
a*-crust	-1.9±0.2 ^a	-1.5± 0.2 ^b	1.1±0.7 ^c
b*-crust	17.0±2.4 ^a	16.9± 2.8 ^a	20.4±1.4 ^b
L*-crumb	72.6±1.1 ^a	75.1± 1.1 ^b	74.3±0.9 ^b
a*-crumb	-2.2±0.2 ^a	-2.0± 0.2 ^b	-2.3±0.2 ^a
b*-crumb	12.6±0.9 ^a	14.9± 0.3 ^b	12.8±0.6 ^a
Slice moisture (%)	51.05±0.42 ^a	50.40± 0.04 ^a	53.01±0.36 ^b
Crumb moisture (%)	46.85±0.14 ^c	46.06± 0.02 ^b	43.99±0.25 ^a
Young's modulus (N/mm ²)	0.009±0.001 ^a	0.038± 0.003 ^b	0.007±0.002 ^a
Hardness (N)	1.06±0.05 ^a	4.52± 0.28 ^b	0.84±0.12 ^a

Note: values followed by same letter, in the same row, are not significantly different (P<0.05).

In Table 6-3 the main results concerning the properties of GF doughs and fresh breads are summarized. To achieve the 180BU dough consistency, CY water absorption was equal to 82%, whereas this value was lower for the other samples due to the presence of sourdough. As regards weight losses during baking, only CY/SD bread showed a significant (P<0.05) higher value (18.2%) when compared to the others, probably due to a more intense water evaporation caused by the slightly higher oven temperature at the beginning of baking (270°C the bottom; 240°C the top). For the same reason, CY/SD had the lowest crumb moisture (43.99% vs. 46.06% and 46.85% for SD and CY, respectively); nevertheless, its slice moisture was the highest, while those of the other two formulations were not statistically different (P<0.05).

Also the colour of the crust can be influenced by baking conditions, as can be appreciated from Table 6-3 and Figure 6-1. CY/SD has the highest redness values (a*) and lowest lightness (L*) if compared to the other samples. On the

contrary, the crumb colour is more influenced by the ingredients used in the recipe that do not change among the 3 breads: it varied, in fact, within a narrow range (L^* , between 72.6 and 75.1; a^* , from -2.0 to -2.3 and b^* , from 12.6 to 14.9).

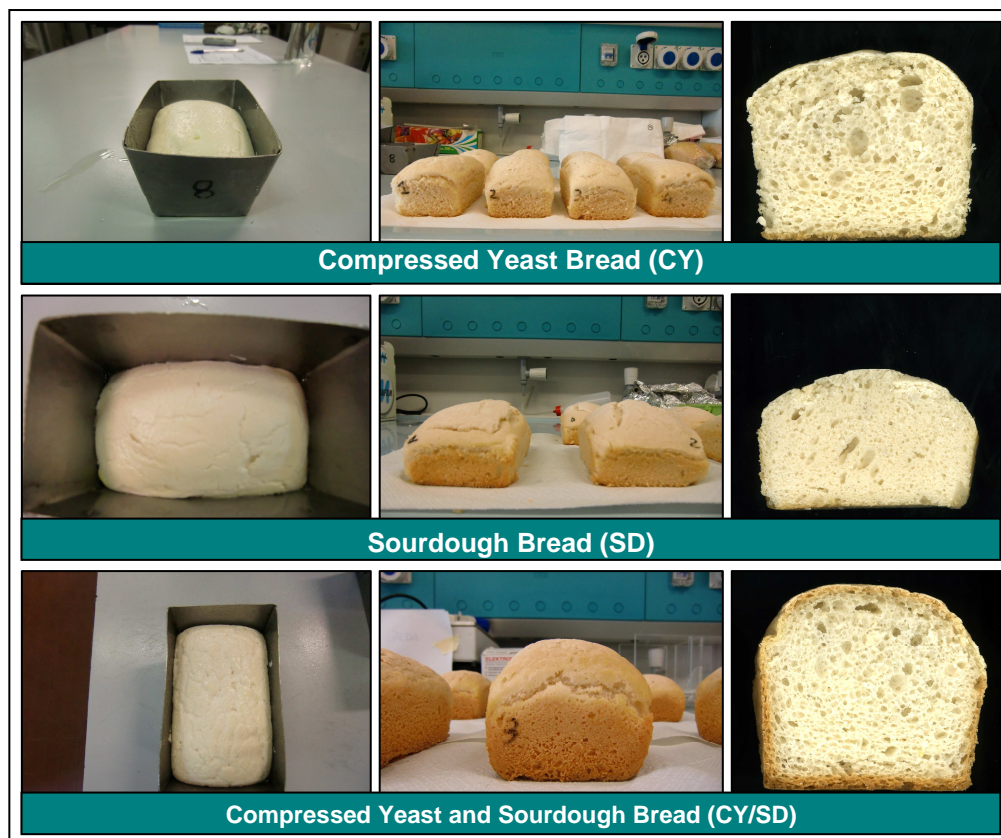


Figure 6-1. Dough development during proofing and baking and images of GF bread slices.

The most significant differences among the 3 baking trials are related to dough development during proofing and baking (Table 6-3 and Figure 6-1). SD increased very few its height during leavening and baking, reaching the lowest height and specific volume (H_{max} : 4.69cm; specific volume: 1.89mL/g), whereas CY developed during leavening but no more during baking (H_{max} : 5.61cm; specific volume: 2.57mL/g). The best performances, both during proofing and baking, were evidenced when sourdough and compressed yeasts were used together as leavening agents (H_{max} : 6.02cm; specific volume: 3.07mL/g); this association had a positive impact also on the softness of fresh bread, as both CY/SD and CY BREAD were much softer than breads leavened

using only sourdough (SD). By means of Image Analysis (Table 6-4), it was evidenced that SD was characterized by a high number of holes (94%) having a small size (between 0.1mm^2 and 1mm^2) and only 0.73% of the total holes were bigger than 3mm^2 . The other two GF breads showed a more heterogeneous hole size distribution (about 80% and 17% holes having a small and an intermediate size, respectively). In addition, CY/SD had the highest percentage of total alveolar area (25%) - expressed on the basis of the crumb area analysed (721.12mm^2) - if compared to CY and SD (21% and 16%, respectively).

Table 6-4. Crumb porosity of GF breads leavened using sourdough (SD) and/or compressed yeast (CY).

	Holes			
	Size (mm^2)	Distribution (%)	Area (%)	Diameter (mm)
CY	$0.1 \leq x \leq 1$	80.72 ± 3.59^a	44.51 ± 6.59^a	0.65 ± 0.02^b
	$1 \leq x \leq 3$	16.79 ± 2.98^b	37.99 ± 5.90^b	1.42 ± 0.04^b
	$x > 3$	2.49 ± 1.06^b	17.50 ± 8.61^a	2.33 ± 0.23^a
SD	$0.1 \leq x \leq 1$	94.04 ± 1.52^b	67.89 ± 8.57^b	0.57 ± 0.02^a
	$1 \leq x \leq 3$	5.29 ± 1.53^a	20.17 ± 5.11^a	1.41 ± 0.07^{ab}
	$x > 3$	0.73 ± 0.32^a	13.03 ± 10.39^a	2.71 ± 0.68^b
CY/SD	$0.1 \leq x \leq 1$	80.82 ± 3.48^a	46.96 ± 6.35^a	0.66 ± 0.02^b
	$1 \leq x \leq 3$	17.07 ± 3.34^b	37.79 ± 6.12^b	1.38 ± 0.02^a
	$x > 3$	2.11 ± 0.83^b	15.25 ± 7.71^a	2.34 ± 0.24^a

Abbreviations: hole distribution (%; percentage of the total mean number of counted holes), hole area (%; percentage of the total hole area) and hole mean diameter (mm) into each hole class. *Note:* within the same hole size classes, values followed by same letter, in the same column, are not significantly different ($P < 0.05$).

b) Bread quality evaluation during storage

Loaves were stored, into paper bags, at 25°C and 60% RH up to 69h. Two breads for each recipe were weighted and characterized after 1h (t_0 , fresh bread), 23h (t_1), 46h (t_2), 69h (t_3). In Figure 6-2 bread properties during storage are reported. A limited moisture decrease (1-6%, of the initial moisture) in bread crumb was observed, whereas it was much more evident in bread slice (from 18% to 27%). In particular, CY/SD that was baked at a slightly higher temperature showed the lower slice moisture and an intermediate weight loss, at each storage time. As for fresh breads (Table 6-3), significant differences ($P < 0.05$) in crumb softness were found during storage (Figure 6-2); in particular, SD was characterized by the highest Young's modulus (more

compact structure), probably due to its low development during breadmaking, and by a hardening kinetic different from the other breads, starting from 23h. On the opposite, CY exhibited a low Young's modulus during the all storage period. However, these positive results have to be carefully evaluated, as CY after 30h of storage was characterized by a crumbly behavior; this is also attested by its weight loss trend (y-intercept: 0.32 vs. 0.29 and 0.25 for CY/SD and SD, respectively). On the contrary, when the two leavening agents were combined (CY/SD), the resulting bread exhibited an intermediate weight loss and did not show a crumbly behaviour, indicating that the lower Young's modulus recorded during the storage period can be effectively attributed to a more softness texture and a lower tendency to staling. Similar results were obtained evaluating crumb hardness as the load at 25% of deformation (*data not reported*).

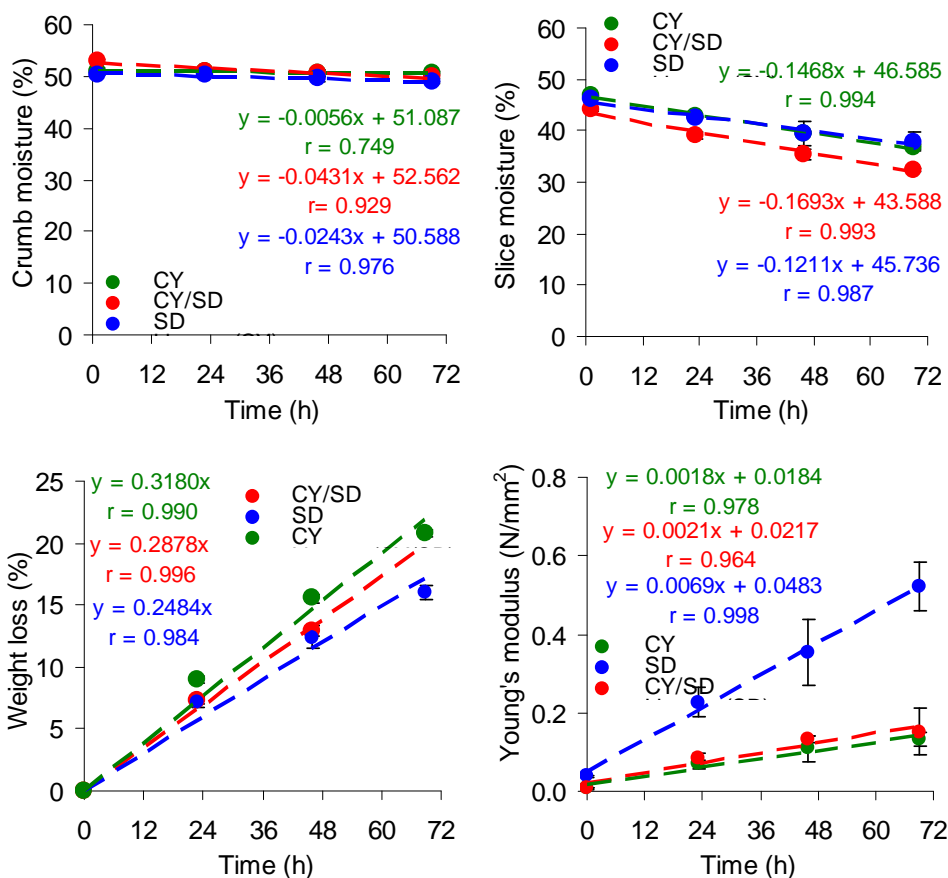


Figure 6-2. Bread properties during accelerated storage (69h into paper bags at 25°C and 60% RH). *Note:* the experimental points were interpolate by a linear equation.

6.4 CONCLUSIONS

In GF bread production, the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final product. Moreover, the high presence in the recipe of flours containing high amounts of starch makes the product more sensible to staling and reduces its shelf-life. At present, the use of sourdough to increase the shelf-life and the sensorial and nutritional quality of the final GF product has been studied only by few researchers (Di Cagno *et al.*, 2008; Moore *et al.*, 2008). Therefore, more studies concerning the production of traditional GF sourdough are necessary.

In the present study a GF sourdough (Type I), characterized by a stable interaction between bacteria and yeasts, was set up and its microbial and technological quality was assessed. The microorganisms of interest (*Lactobacillus sanfranciscensis* and *Candida humilis*) were isolated from a traditional sourdough used in Panettone production ($1.3 \cdot 10^8$ CFU/g bacteria; $2.3 \cdot 10^7$ CFU/g yeasts). The pure strains were added into a GF-matrix to produce a GF starter ($5.2 \cdot 10^6$ CFU/g yeasts and $2.2 \cdot 10^9$ CFU/g bacteria after 22h and 30min of fermentation at 25°C). The developed GF sourdough was refreshed and monitored constantly in terms of number and type of microorganisms, capability to produce/retain CO₂ and pH variations. A stable association between microorganisms, measured both in terms of dough microbial population and technological properties, was obtained after few refreshments (3rd-4th). During the whole observation time (3months), the microbial population (*Lb. Sanfranciscensis*: 10^9 CFU/g; *Candida humilis*: 10^7 - 10^8 CFU/g) and the leavening performance (Hm: 24.7 ± 1 mm; CO₂TOT: 1255.9 ± 42 mL) of the developed GF sourdough were constant and satisfactory, thus attesting that a GF sourdough has been effectively developed.

Breadmaking trials, carried out using compressed yeast (CY), sourdough (SD) or a combination of compressed yeast and sourdough (CY/SD) as leavening agents, evidenced that SD could be used as an alternative leavening agent; in particular, when used in combination with compressed yeast a synergic effect was highlighted and an improvement of GF bread quality (both in terms of bread development and crumb softness) and shelf-life was achieved.

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CHAPTER 7

**GENERAL CONCLUSIONS AND FUTURE
PERSPECTIVES**

The incidence of celiac disease (CD) is continuously growing: actually, an estimated 0.5 to 2.0% of the population in most European countries and the United States have CD. Even if several alternative treatments are now being studied, at present the only acceptable treatment for CD is a total lifelong avoidance of gluten ingestion, so celiacs must strictly follow a gluten-free (GF) diet, eating only dedicated foods. A GF diet means avoiding all those products that contain wheat, rye and barley or any of their derivatives. This means that many typical Italian foods, like bread and pasta, cannot be consumed by celiac people.

In this contest, the investigations carried out during this PhD research resulted very useful to define the optimal GF bread-dough consistency, in relation to the raw materials used, and the leavening agents that could be used to develop and improve the quality of the final GF product; furthermore, some of the mechanisms involved in GF bread storage, such as the retrogradation process, were highlighted.

As many raw materials can be used in GF production, each one having different chemical-physical properties, the first step of the research was focused on the characterization of most of these materials, by means of different techniques (Chapter 2). Among the others, the Brabender Micro-Visco-Amylograph (MVA) (Brabender OHG, Duisburg, Germany), that permits to fingerprint starch and flour *via* their pasting profiles and to select them on the basis of their susceptibility to retrogradation, came out to be very useful in defining the staling tendency of the different systems investigated. The water absorption index (WAI) and the water binding capacity (WBC) showed to be very important tools to study the water affinity of different raw materials, such as hydrocolloids and fibres. As regards the protein fraction, the foaming properties of proteins and foam stability were evaluated through the Cream Tester CT II (Gerber Instruments, Effretikon, Switzerland); this simple and quick test permitted to clearly evidence which pea protein isolates was more suitable for GF breadmaking process in comparison to the other proteins investigated.

As regards the starchy fraction, the evaluation of the rheological properties of gels made of corn starch (CS), rice flour (RF), waxy rice flour (WRF), rice bran (RB) and their mixtures (Chapter 3) highlighted how both WRF and RB were very effective in reducing gels stiffness and storage modulus (G'). Both WRF and RB, at 25% and 50% level of substitution, strongly reduced G' values of the mixtures containing CS or RF and, for the same gels, G' curves overlapped up to seven days, indicating very slow hardening kinetics. Therefore, WRF and

RB seem to be potentially effective in enhancing the shelf-life of GF baked products when included in the starchy matrix. The next ongoing step is the identification of the proper amount of WRF and/or RB to be included in GF bread formulations, in order to allow both an appropriate workability of the dough and the maintenance of the softness of the product during a prolonged storage.

The high hydrostatic pressure (HHP) was used to modify the technological aptitude of RF and CS, thanks to the partial gelatinization of starchy compounds at low temperature. In this contest further investigations should be carried out, in order to highlight the effect of HHP treatments on raw materials technological aptitude and on bread staling.

A current challenge for food scientists is to simulate gluten properties by adding different ingredients, such as hydrocolloids and fibres, in order to improve the rheological properties of the GF dough and, consequently, of the bread. However, the introduction of new ingredients into the recipe frequently requires an adjustment of the GF breadmaking process (*i.e.* the optimal dough consistency and the time of leavening and baking). As regards the formulation, the effect of Psyllium (Psy) and sugar beet (SB) fibres and water amounts on GF dough properties and bread quality (Chapter 5) was investigated. Results highlighted that, for GF products, a lower dough consistency (200 Brabender Unit, BU) is preferred compared to traditional doughs (500BU) to achieve good performances during the leavening phase, particularly if the mixture contains ingredients having high water affinity, such as Psyllium. Doughs having 500BU consistency, in fact, were characterized by reduced developments and gas retention during proofing. Hydrocolloids and fibres played a strategic role in improving the workability of GF dough, enhancing - at the same time - the water binding capacity of the GF mixture and bread shelf-life. As regards the two fibres investigated, a higher anti-staling effect was found for Psy fibre in comparison to SB fibre after 3 days of bread storage in paper bags at 20°C and 60% RH.

As it is generally known that the sourdough can be used to improve the quality and to extend the shelf-life of traditional bread, a GF sourdough was developed in our laboratories (DiSTAM). A stable interaction between microorganisms was achieved (up to the 25th refreshment), suggesting that this GF-sourdough could be potentially used in GF industries. The breadmaking trials also evidenced that the GF sourdough (SD) could be a valid leavening agent, able to improve GF bread quality and shelf-life, if used in combination with

compressed yeast (CY): in fact, a synergistic effect of SD and CY was highlighted.

The future perspectives of this research are therefore focused on GF breadmaking trials using both GF-sourdough and compressed yeast as leavening agents, in formulations containing CS and RF treated with HHP and a small amount of WRF (25% on flour base); furthermore GF breads will be packaged in propylene bags to investigate their shelf-life up to 3 months of storage.

APPENDIX

PhD PUBLICATIONS

PAPERS ON NATIONAL AND INTERNATIONAL JOURNALS

- A1) **Cappa, C.**, Lucisano, M., Mariotti, M. (2011). Rheological properties of gels obtained from gluten-free raw materials during a short term ageing. *Journal of Cereal Science* (under review).
- A2) Mariotti, M., **Cappa, C.**, Iametti, S., Lucisano, M. (2011). Valutazione degli spaghetti gluten-free presenti sul mercato italiano: proprietà del prodotto crudo. *Tecnica Molitoria*, 62(5):442-449.
- A3) Mariotti, M., Iametti, S., **Cappa, C.**, Rasmussen, P., Lucisano, M. (2011). Characterisation of gluten-free pasta through conventional and innovative methods: evaluation of the uncooked products. *Journal of Cereal Science*, 53:319-327.
- A4) Lucisano, M., **Cappa, C.**, Fongaro, L., Mariotti, M. (2010). Methods for the characterization of breadcrumb, an important ingredient of stuffed pasta. *Journal of Cereal Science*, 51(3):381-387.
- A5) Mariotti, M., Fongaro, L., **Cappa, C.**, Lucisano, M. (2010). Identificazione e monitoraggio delle proprietà del pangrattato quale ingrediente per la pasta ripiena. *Tecnica Molitoria*, 61(8):836-841.

POSTERS AND PROCEEDINGS OF NATIONAL AND INTERNATIONAL CONGRESSES

- B1) **Cappa, C.**, Lucisano, M., Mariotti, M. (2011). Rheological properties of gels obtained from gluten-free raw materials during a short term ageing. *2011 EFFoST Annual Meeting - Process - Structure - Function Relationships*, 9-11 November, Technical University Berlin, Germany (Poster).
- B2) **Cappa, C.** (2011). Gluten-free baked products: optimization of formulation and process conditions. *Proceedings of the 16th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology*, September 21-23, Lodi, Italy (Oral presentation).

- B3) Cappa, C.** (2011). Valutazione delle proprietà di spaghetti gluten-free presenti sul mercato italiano. *8° Convegno AISTEC*, 11-13 Maggio, Aci Castello (CT), Italia (Poster).
- B4) Cappa, C.** (2010). Influence of the presence of different fibers on gluten-free dough and bread properties. *Proceedings of 15th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology*, September 15-17, Portici, Italy, pp. 221-222 (Poster).
- B5) Mariotti, M., Cappa, C., Lucisano, M.** (2010). Gluten-free pasta: technology and quality evaluation. *Proceedings 2nd International Symposium on Gluten-free Cereal Products and Beverages*, June 8-11, Tampere, Finland, pp. 159-160.
- B6) Lucisano, M., Mariotti, M., Cappa, C., Luoto, S.** (2009). Evaluation of rice pasta properties through new and conventional methods. *AACC International Annual Meeting*, September 13-16, Baltimore, Maryland, USA (Poster).
- B7) Cappa, C.** (2009). Gluten-free baked products: optimization of formulation and process conditions. *Proceedings of the 14th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology*, September 16-18, Oristano, Italy, pp. 391-392 (Poster).
- B8) Mariotti, M., Fongaro, L., Cappa, C., Lucisano, M.** (2009). Identificazione e monitoraggio delle proprietà chimico-fisiche del pangrattato, un ingrediente di primaria importanza per la pasta ripiena. *Congresso di Scienze e Tecnologia degli Alimenti (9° Ciseta)*, 11-12 Giugno, Milano, Italia (Poster).

Rheological properties of gels obtained from gluten-free raw materials during a short term ageing

Carola Cappa, Mara Lucisano, Manuela Mariotti

Abstract

The high amount of starch contained in gluten-free baked products is largely responsible for their quality decay during storage. Starch retrogradation, in fact, is the major phenomenon involved in bread staling. The aim of this research was to evaluate the rheological properties of gels obtained from corn starch (CS), rice flour (RF) and their mixtures, and the capability of waxy rice flour (WRF) and rice bran (RB) to reduce starch retrogradation, when added at different ratios to the starchy matrix. Eleven gels were prepared with a Brabender® Micro-Visco-Amylograph and their rheological properties during a 7 days storage period at 4° C were evaluated through compression tests and dynamic oscillatory measurements. During ageing, gels consistency increased at different extents and rates. Samples containing CS showed the highest values. WRF originated weak gels and resulted more effective than RB in delaying gel hardening. However, both WRF and RB, at 25% and 50% level, strongly reduced G' values of the mixtures containing CS or RF. For the same gels, G' curves overlapped up to seven days, indicating very slow hardening kinetics. Therefore, WRF and RB seem to be effective in enhancing the shelf-life of GF baked products when included in the starchy matrix.

Keywords: gel; rheology; starch; waxy rice

A2

PASTA

VALUTAZIONE di SPAGHETTI GLUTEN-FREE presenti sul mercato italiano: proprietà del prodotto crudo

*Gluten-free pasta characterization: properties
of the uncooked product*

Parole chiave: pasta senza glutine, proprietà meccaniche, amido, proteine

Key words: gluten-free pasta, mechanical properties, starch characteristics, protein aggregation

MANUELA MARIOTTI^{1*} - CAROLA CAPPA¹ - STEFANIA IAMETTI² - MARA LUCISANO¹

¹DISTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche
Università degli Studi di Milano - Via G. Celoria 2 - 20133 Milano - Italia

²DISMA, Dipartimento di Scienze Molecolari Agroalimentari - Università degli Studi di Milano
Via G. Celoria 2 - 20133 Milano - Italia

*corresponding author: manuela.mariotti@unimi.it

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SOMMARIO

La formazione di un reticolo glutinico avente proprietà viscoelastiche è fondamentale per la texture del prodotto crudo ed il comportamento in cottura della pasta. L'assenza di tale struttura nella pasta senza glutine rende necessari studi di formulazione e processo finalizzati alla creazione, durante il processo produttivo, di una "impalcatura alternativa". Si tratta di una sfida tecnologica avvincente, come dimostra la continua attività di ricerca in questo settore.

Lo scopo di questo studio è stato quello di effettuare un'indagine ad ampio raggio sulle paste senza glutine attualmente presenti sul mercato italiano, cercando di individuare come alcune proprietà molecolari influenzino la qualità finale della pasta. I fenomeni legati alla retrogradazione dell'amido si sono mostrati fondamentali per la texture finale del prodotto e le interazioni proteina-proteina sono risultate governate dall'origine delle diverse proteine vegetali impiegate nelle varie formulazioni.

ABSTRACT

Formation of a gluten protein network is fundamental for the texture and the overall quality of pasta. Replacement of the gluten network in gluten-free (GF) pasta is a major technological challenge, and the conventional technological processes have to be adapted to non-gluten formulations. The wide variety of raw materials and technologies used in the production of commercial GF pasta stems from the still on-going search for solutions to these problems.

The aim of this study was to evaluate the characteristics of different commercial GF spaghetti currently available on the Italian market, focusing on how some molecular properties relate to the final structure and quality of GF pasta. Phenomena related to starch retrogradation played a central role for the final texture of the products. At the same time, the origin of proteins included in the formulation governed the protein-protein interactions, especially in those samples including proteins from different sources.



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Characterisation of gluten-free pasta through conventional and innovative methods: Evaluation of the uncooked products

Manuela Mariotti^a, Stefania Iametti^b, Carola Cappa^a, Patrizia Rasmussen^b, Mara Lucisano^{a,*}

^a *Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DiSTAM), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy*

^b *Dipartimento di Scienze Molecolari Agroalimentari (DiSMA), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy*

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ABSTRACT

Formation of a gluten protein network is fundamental for the texture and the overall quality of pasta. Replacement of the gluten network in gluten-free pasta is a major technological challenge, and the conventional technological processes have to be adapted to non-gluten formulations. The wide variety of raw materials and technologies used in the production of commercial gluten-free pasta stems from the – still on-going – search for solutions to these problems. The aim of this study was to evaluate the characteristics of different commercial gluten-free spaghetti currently available on the market, focusing on starch and protein organisation. Taking into account the chemical and biochemical properties of the samples, and their relationships to the physical characteristics of these products we looked at how some molecular properties relate to the final structure and quality of gluten-free pasta. Phenomena related to starch retrogradation were found to play a central role for the final texture of the products. At the same time, the origin of proteins included in the formulation was found to govern the protein–protein interactions, especially in those samples including proteins from different vegetable sources.

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Methods for the characterisation of breadcrumb, an important ingredient of stuffed pasta

Mara Lucisano, Carola Cappa, Lorenzo Fongaro, Manuela Mariotti*

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DiSTAM), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy

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ABSTRACT

Very few methods have been proposed to evaluate the technological characteristics of breadcrumb, an ingredient of stuffed pasta. Nevertheless, the physical properties of this raw material can play a relevant role in modifying the filling texture, which is important both for the filling workability and product consistency after cooking. The aim of this study was to determine the most suitable methodologies for describing breadcrumb characteristics, with particular attention to those features that can potentially influence the performance of this ingredient. Three lots of two different types of commercial breadcrumb were analysed for their chemical composition, particle size distributions, pasting properties, and water binding capacity. The texture of breadcrumb/water dispersion was also evaluated using different methods. Chemical analyses did not prove to give a satisfactory differentiation between samples. On the contrary, the different rheological tests adopted (Bostwick, Farinographic, and compression test) turned out to be very effective in describing the thickening properties of breadcrumb. In particular, a statistically significant differentiation ($P < 0.05$) between the samples was obtained by means of the Bostwick consistometer, an easy to use, rapid and cheap instrument that turned out to be suitable for defining the technological characteristics of breadcrumb.

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PASTA FRESCA

A5

Identificazione e monitoraggio delle PROPRIETÀ del PANGRATTATO quale ingrediente per la PASTA ripiena

*Methods for the characterization of breadcrumb,
an important ingredient of stuffed pasta*

Parole chiave: pangrattato, consistenza, lavorabilità, proprietà reologiche, pasta ripiena
Key words: breadcrumb, consistency, workability, rheological properties, stuffed pasta

MANUELA MARIOTTI* - LORENZO FONGARO - CAROLA CAPPÀ - MARA LUCISANO

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM)
Università degli Studi di Milano - Via G. Celoria 2 - 20133 Milano - Italia

*corresponding author: manuela.mariotti@unimi.it

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SOMMARIO

Nelle operazioni di formatura della pasta ripiena viene spesso riscontrata una grande variabilità nella quantità di ripieno erogata dalle macchine dosatrici, le cui cause non sono sempre di facile individuazione. In particolare, viene spesso trascurato il ruolo tecnologico del pangrattato (nonostante sia uno degli ingredienti frequentemente presente in grande quantità nel ripieno), tanto che pochi sono i parametri qualitativi richiesti nei capitolati d'acquisto aziendali. Per poter descrivere e, se possibile, prevedere l'attitudine tecnologica del pangrattato sono stati considerati due differenti campioni commerciali. Oltre a determinarne la composizione chimica e la distribuzione granulometrica, mediante Micro-Visco-Amilografo Brabender sono state condotte misure di viscosità di dispersioni diluite di pangrattato, durante cicli di riscaldamento e raffreddamento, per monitorare il comportamento della componente amido. Miscele concentrate di pangrattato e acqua sono state valutate mediante consistometro Bostwick e Farinografo Brabender, per ottenere informazioni legate alla consistenza. La caratterizzazione di queste miscele è stata infine completata con prove di compressione multipla.

Dall'insieme dei risultati è emerso che il pangrattato rappresenta un ingrediente critico per la standardizzazione delle caratteristiche del prodotto finito. Anche piccole variazioni nelle sue caratteristiche chimico-fisiche, infatti, possono produrre cambiamenti rilevanti nelle proprietà reologiche dei ripieni.

ABSTRACT

Very few methods have been proposed to evaluate the technological characteristics of breadcrumb, an ingredient of stuffed pasta. Nevertheless, the physical properties of this raw material can play a relevant role in modifying the filling texture, an important property both for the filling workability and the product consistency after cooking. The aim of this study was to determine the methodologies more suitable in describing breadcrumb characteristics, with particular attention to those features that can potentially influence the performance of this ingredient. Three lots of two different commercial breadcrumbs were analyzed for their chemical composition, particle size distribution, and pasting properties. The texture of breadcrumb/water dispersions was also evaluated setting up different methods. Chemical analyses did not prove to give a satisfactory differentiation between samples. On the contrary, the different rheological tests adopted (Bostwick, Farinographic, and compression test) came out to be very effective in describing the thickening properties of the breadcrumb. In particular, a statistically significant differentiation ($P < 0,05$) between the samples was obtained by means of the Bostwick consistometer, an easy to use, rapid and cheap instrument, that turned out to be suitable for defining the technological characteristics of breadcrumb.

Rheological properties of gels obtained from gluten-free raw materials during a short term ageing

B1



C. Cappa*, M. Lucisano, M. Mariotti

Università degli Studi di Milano.
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DISTAM),
Via G. Celoria 2, Milan, Italy; carola.cappa@unimi.it



Introduction

Many gluten-free (GF) foods, and baked goods in particular, contain a large amount of starch, whose behavior during process and storage greatly influences the final products quality and shelf-life. As reported by Lii *et al.* (1995), the formation of a gel or a paste is one of the principal events that controls the texture and quality of starch-containing foods. Gel structure depends on many factors, such as starch source and concentration, amounts and types of amylose and amylopectin leached out from starch granules, interactions among amylose, amylopectin and granules, besides heating and cooling conditions in terms of treatment temperature, length and rate (Mariotti *et al.*, 2005). In particular, starch retrogradation is the major phenomenon involved in bread staling. The rheological properties provided by the starchy matrix to the final product appear to be of great importance in GF breadmaking. Up to now, instruments such as the Rapid Visco Analyzer and the Brabender Viscoamylograph have been used as standard analytical tools to assess the pasting characteristics of starch and flour suspensions during heating and cooling cycles (Shuey and Tipples, 1980; Mariotti *et al.*, 2008); whereas dynamic oscillatory rheometry has been frequently applied to study the viscoelastic properties of starch gels (Tsai *et al.*, 1997; Hsu *et al.*, 2000).

Aim

Evaluation of the rheological properties of gels obtained from **corn starch (CS)**, **rice flour (RF)** and their mixtures, and of the capability of **waxy rice flour (WRF)** and **rice bran (RB)** to reduce **starch retrogradation**, when added at different ratios to the starchy matrix.

Materials and Methods

Eleven gels were prepared with a **Brabender® Micro-Visco-Amylograph** (Brabender OHG, Duisburg, Germany): 12g sample (14% H_2O)/100mL H_2O ; speed: 250min⁻¹; measuring range: 300cm²g; temperature increase/decrease: 3°C/min; temperature profile: heating from 30°C up to 95°C, holding at 95°C for 30min, cooling from 95°C to 30°C.

Samples investigated (% w/w):

- | | | |
|--------------------------|---------------|---------------------|
| 1) Corn Starch (CS) | 5) 50CS-50RF | 8) 50RF-50WRF |
| 2) Rice Flour (RF) | 6) 50CS-50WRF | 9) 50RF-50RB |
| 3) Waxy Rice Flour (WRF) | 7) 50CS-50RB | 10) 50CS-25RF-25WRF |
| 4) Rice Bran (RB) | | 11) 50CS-25RF-25RB |



The rheological properties of the forming gels were evaluated after 30min at 25°C (**t0**) and after 1 (**t1**), 2 (**t2**), 3 (**t3**), 4 (**t4**), and 7 (**t7**) days of storage at 4°C. Before each trial, gels were conditioned at 25°C for 30 min.



Empirical rheological measurements
(TA-HDplus Texture Analyzer; n \geq 5)

18g of each gels were submitted to compression with a cylindrical probe (diameter: 3.5cm) at a crosshead speed of 1mm/s (10N load cell), up to 30% deformation, and maintained at this deformation for 60s, before releasing the force.

Fundamental rheological measurements
(Physica RCR 300 Rheometer; n \geq 3)

After loading the sample, the excess was trimmed off and the sample was allowed to rest for 5min, before starting the test:
- *Strain sweep test* (1Hz, 0.01-300%, 25°C);
- *Frequency sweep test* (0.1-10Hz, 1%, 25°C).
(Corrugated parallel plate system, 1mm gap).

Results and Discussion

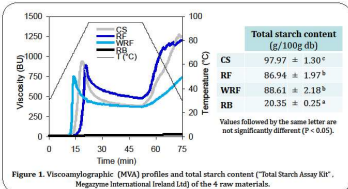


Figure 1. Viscoamylographic (MVA) profiles and total starch content ("Total Starch Assay Kit", Megazyme International Ireland Ltd) of the 4 raw materials.

CS and RF presented the highest MVA values, indicating their ability to form strong gels but a high tendency to retrograde. On the opposite, RB - characterized by the lowest total starch content and the highest level of fiber - did not form a gel in the experimental conditions adopted during the MVA test. WRF, too, exhibited a low increase of viscosity during cooling.

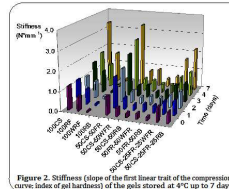


Figure 2. Stiffness (slope of the first linear part of the compression curves) of gels stored at 4°C up to 7 days.

CS gel showed the highest consistency and WRF the lowest one, just after the production. A significant correlation was found between gel stiffness and viscoamylographic final viscosity ($r=0.771$, $P<0.01$), indicating that the gels formed into the MVA maintained their properties even when evaluated in different shear conditions.

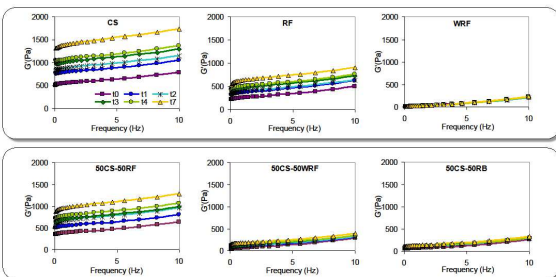


Figure 3. Storage modulus (G') of gels stored at 4°C for (a) 0, (b) 1, (c) 2, (d) 3, (e) 4 and (f) 7 days.



The highest G' values were registered for CS and 50CS-50RF gels; on the contrary, WRF (that contains a limited amount of amylose) and RB (due to its low starch content and its high level of fiber) originated very weak gels and resulted very useful in delaying gel hardening. WRF, in particular, came out to be more effective than RB. Both WRF and RB, at 25% and 50% level of substitution, strongly reduced G' values of the mixtures containing CS or RF and, for the same gels, G' curves overlapped up to seven days, indicating very slow hardening kinetics.

Therefore, WRF and RB seem to be **potentially effective** in enhancing the shelf-life of GF baked products when included in the starchy matrix.

Conclusions

CS and RF coupling, very frequent in commercial blends for GF bread production, originated elastic gels but characterized by a fast hardening kinetic. On the contrary, WRF (that contains a limited amount of amylose) and RB (due to its low starch content and its high level of fiber) originated very weak gels and resulted **very effective in delaying starchy gel hardening**, highlighting the possibility to enhance the shelf-life of GF baked products.

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**16th Workshop on the Developments in the Italian PhD Research on
Food Science, Technology and Biotechnology**

B2

Lodi (Italy), 12 - 23 September 2011

**GLUTEN-FREE BAKED PRODUCTS: OPTIMIZATION
OF FORMULATION AND PROCESS CONDITIONS**

Carola Cappa (carola.cappa@unimi.it)

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di
Milano, Italia

Tutor: Prof. Mara Lucisano; Co-tutor: Dr. Manuela Mariotti

This PhD research project has the purpose to identify the formulation and to define the process conditions that most influence the quality and shelf-life of gluten-free (GF) bread. As reported by Cappa 2009 and 2010, during the first two years of research, different activities related to the characterization of GF raw materials (starches, flours, hydrocolloids, proteins, etc.) and to the optimization of the various steps of bread production (mixing, leavening and baking conditions) have been carried out. Whereas, during the third year physical studies on starch gelatinization/retrogradation of different ingredients and the development of a GF sourdough were performed.

**PRODOTTI DA FORNO GLUTEN-FREE:
OTTIMIZZAZIONE DELLA FORMULAZIONE E DELLE
CONDIZIONI DI PROCESSO**

Questa ricerca ha lo scopo di individuare le materie prime e di definire le variabili di processo che maggiormente influenzano la qualità e la shelf-life del pane gluten-free (GF). Come riportato da Cappa 2009 e 2010, durante i primi due anni del dottorato di ricerca sono state svolte differenti attività legate alla caratterizzazione delle materie prime GF e all'ottimizzazione del processo produttivo. Durante il terzo anno, invece, sono stati condotti studi sulla gelatinizzazione/retrogradazione dell'amido ed è stato sviluppato un impasto acido GF da utilizzare come agente lievitante in alternativa o unitamente al lievito convenzionale.

Key words: starch retrogradation, high hydrostatic pressure, sourdough, bread-making.

1. State of the Art

Celiac disease is a permanent intolerance to “gluten”, an affection of the small intestine influenced by genetic factors, that can appear both in children and adults (incidence of 1:100-150 people). Those who suffer from celiac disease present anomalies of the intestinal mucosa with partial or total atrophy of the villi following the assumption of

storage proteins found in many common cereals such as wheat, rye or barley and hybrids of these grains (e.g. triticale), that are so considered harmful for the sensitive consumers. This pathology is often correlated with a malabsorption of several important nutrients including iron, folic acid, calcium and vitamins. At present, the only treatment for celiac disease is a total lifelong avoidance of “gluten” ingestion, strictly following a gluten-free diet. It is evident that many typical Italian foods like bread and pasta can not be consumed by celiac people.

In GF bread production, the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final product. Critical are the rheological properties of the dough that, lacking in gluten, shows limited abilities of gas expansion and retention during leavening, factors that lead to a bread with reduced volume and crumb softness (Mariotti, 2004). Moreover, the high presence in the formulation of starches from different origins (mainly corn, rice and potato) and of flours containing high amounts of starch (rice and corn flour) makes the product more sensible to staling and reduces its shelf-life.

At present, to provide for the lack of gluten and to simulate its viscoelastic behaviour, the addition of hydrocolloids is quite common because these ingredients have a strategic role in making dough workable and in improving the texture of the final product (Gallagher *et al.*, 2004; Kobylański *et al.*, 2004; Lazaridou *et al.*, 2007). Recent studies have also investigated the possibility to enrich GF bread with proteins and dietary fibre (Gallagher *et al.*, 2004) and to use sourdough to increase the shelf-life and the sensorial and nutritional quality of the final product (Di Cagno *et al.*, 2008; Moore *et al.*, 2008). Nevertheless more studies are necessary on the production of traditional GF sourdough (Type I) with a stable interaction between bacteria and yeasts. Also unconventional treatments on raw materials, such as High Hydrostatic Pressure, have been recently studied (Barcenas *et al.*, 2010; Vallons *et al.*, 2010) as potential treatments to reduce the retrogradation rate and to increase product shelf-life, but the optimal process conditions have not been achieved yet.

2. Objective and Experimental Plan

During the third year of this PhD the following activities have been carried out:

Step 1: Retrogradation study. The high amount of starch contained in GF baked products is mostly responsible for the quality decay due to the starch retrogradation during product staling, that involves a rapid loss of softness. To understand and delay retrogradation, a study on the mechanical and rheological properties of GF gels during a short term ageing was performed.

Step 2: Non-conventional treatments on GF raw materials. The effect of a physical treatment such as High Hydrostatic Pressure (HHP) on water/solvent affinity of GF raw materials and on their aptitude to gelatinization/retrogradation was studied. This step was performed at the Washington State University (Pullman, WA, USA) under the supervision of Prof. Gustavo V. Barbosa-Cánovas (Professor of Food Engineering and director of Center for Nonthermal Processing of Food).

Step 3: Activities aimed at improving bread quality and shelf-life. To achieve these goals, after preliminary steps to identify the best formulation, a GF sourdough (Type I) with selected bacteria and yeasts to produce a GF sourdough bread was set up.

3. Materials and Methods

3.1 Mechanical and rheological properties of GF gels during a short term ageing (Step 1)

GF gels were prepared with the Brabender® Micro-Visco-Amylograph (Brabender OHG, Duisburg, Germany) from Corn Starch (CS), Rice Flour (RF), Waxy Rice Flour (WRF) and Rice Bran (RB) and from their mixtures (50CS-50RF; 50CS-50WRF; 50CS-50RB; 50RF-50WRF; 50RF-50RB; 50CS-25RF-25WRF; 50CS-25RF-25RB, as percentage w/w sample based) in order to mimic the starchy component of a GF bread formulation. The mechanical properties of the gels, previously conditioned at 25°C for 30min, were evaluated after 30min at 25°C (t₀) and after 1 (t₁), 2 (t₂), 3 (t₃), 4 (t₄), 7 (t₇) days of storage at 4°C by a compression test using a TA-HDplus Texture Analyzer (Stable Micro Systems, Surrey, UK). The rheological behaviour of the gels was measured by performing frequency sweep tests with a MCR 300 Rheometer (Modular Compact Rheometer, Physica, Ostfildern, Germany). These measurements were carried out at 25°C, using a corrugated parallel plate system at a gap of 1mm and after 5min of equilibration at test temperature. Dynamic shear data were obtained from frequency sweeps over the range of 0.1–10Hz at 1% strain. The strain value was selected from preliminary amplitude sweep tests (from 0.01 to 300% at 1Hz). For the trials, storage modulus (G', Pa), loss modulus (G'', Pa) and tan δ (ratio between G'' and G') were computed from raw oscillatory data using the Universal Software US200 (version 2.5) (Anton Paar, Ostfildern, Germany). All measurements were performed at least in triplicate.

3.2 HHP treatments on GF raw materials (Step 2)

To improve the technological properties of GF raw materials, CS, RF and WRF were subjected to HHP using a isostatic press (Engineered Pressure Systems Inc. Haverhill, USA). The following processing variables were considered: pressure holding time (5min or 10min), pressure applied (400MPa or 600MPa) and temperature (20°C or 40°C). Before the HHP treatments flour or starch was directly mixed for 5min at room temperature with distilled water until reaching 40% of moisture (w/w). The pressurized samples were dried with a low vacuum oven (~67.727kPa; mod. 1410, VWR, USA) at 35°C and crushed with a mortar. The pasting properties of the samples were evaluated through the Brabender® Micro-Visco-Amylograph (Brabender OHG, Duisburg, Germany), whereas the Solvent Retention Capacity (SRC) was assessed in accordance to AACC International Method 56-11.02. Selected samples were also evaluated by differential scanning calorimetry (DSC; Pyris1, Perkin-Elmer Corp., Norwalk, CT), X-ray diffraction (XRD; Siemens D-500, Germany) and Scanning Electron Microscopy (SEM; Field Emission Instruments, Hillsboro, OR). All measurements were performed at least in duplicate.

3.3 Bread quality and shelf-life improvement through sourdough fermentation (Step 3)

The GF bread formulation was defined on the base of data reported in the literature, by considering commercial recipes and through knowledge acquired from preliminary studies carried out at DiSTAM-Food Technology Section, that included also the evaluation of the foaming properties of some vegetable proteins (Cream Tester CT II, Gerber Instruments) and of the technological aptitude of some hydrocolloids (bread-making trials). All measurements were performed at least in duplicate.

The microorganisms of interest (*Lactobacillus sanfranciscensis* and *Candida humilis*) were isolated and identified from a traditional sourdough for Panettone production. The pure strains were then added in a GF-matrix containing corn starch, rice flour, pea protein, hydroxypropyl methylcellulose (HPMC), psyllium and substrate for strain grow, to produce the GF sourdough. To obtain a stable interaction between bacteria and yeast strains, the GF sourdough was constantly refreshed and monitored for pH, capability to produce/retain CO₂ (Rheofermentometer F3 Chopin, Villeneuve-La-Garenne, France) and number and type of microorganisms. As the microbiological population and technological properties of GF sourdough resulted constant, bread-making trials were performed. The GF bread formulation, containing the materials previously mentioned and an emulsifier, oil, sugar and salt, was used in comparison with a traditional yeast (*Saccaromyces cerevisiae*) leavened bread. The bread-making was performed as reported by Mariotti (2004). Data related to GF bread are not reported in this paper as the study is still in progress.

3.4 Statistical analysis

All the analytical results were processed by STATGRAPHIC®Plus for Windows 5.1. Analysis of variance (ANOVA) was performed using the Least Significant Differences (LSD) test to compare sample means; differences were considered significant at $P < 0.05$.

4. Results and Discussion

4.1 Mechanical and rheological properties of GF gels during a short term ageing (Step 1)

Comparing the mechanical characteristics of gels, studied using a TA-HDplus Texture Analyzer (Stable Micro Systems, Surrey, UK), it was possible to rank them, on the basis of their stiffness, as follows: CS, 50CS-50RF, RF, 50CS-25RF-25WRF, 50CS-25RF-25RB, 50CS-50WRF, 50RF-50WRF, 50CS-50RB, 50RF-50RB, WRF. An important parameter that describes the mechanical properties of a gel is the elasticity index (EI), i.e. the capability of a material to withstand to a prolonged stress: closer to 1 the value, more elastic and less viscous the sample. WRF fresh gel had a viscous behaviour (EI, 0.23 ± 0.01), while a much more elastic nature characterized CS (EI, 0.60 ± 0.01). The presence of RB or WRF generally reduced gels elasticity. During ageing, gels consistency increased at different extents and rates: after 7 days of storage, samples containing corn starch had the highest consistency, in particular CS and 50CS-

50RB were characterized by a stiffness value of $3.34 \pm 0.11 \text{ N/mm}$. Also 50CS-25RF-25RB resulted rather hard, having a stiffness value of $2.24 \pm 0.03 \text{ N/mm}$. WRF resulted more effective than RB in delaying gel hardening; in fact, WRF, 50RF-50WRF and 50CS-25RF-25WRF remained rather soft at the end of the storage period, with a stiffness equal to $0.14 \pm 0.02 \text{ N/mm}$, $0.78 \pm 0.02 \text{ N/mm}$ and $1.63 \pm 0.02 \text{ N/mm}$, respectively. The EI of the majority of the samples increased during gel ageing due to the structure strengthening induced by the reorganization of the amylose chains. In particular CS and RF reached values of 0.87 ± 0.02 and 0.71 ± 0.03 respectively, with an increase of 45% and 97% compared to the fresh made gels. WRF showed an opposite trend, showing a rather constant EI value and a small hardening (from $0.08 \pm 0.01 \text{ N/mm}$ to $0.14 \pm 0.02 \text{ N/mm}$) during the whole storage period.

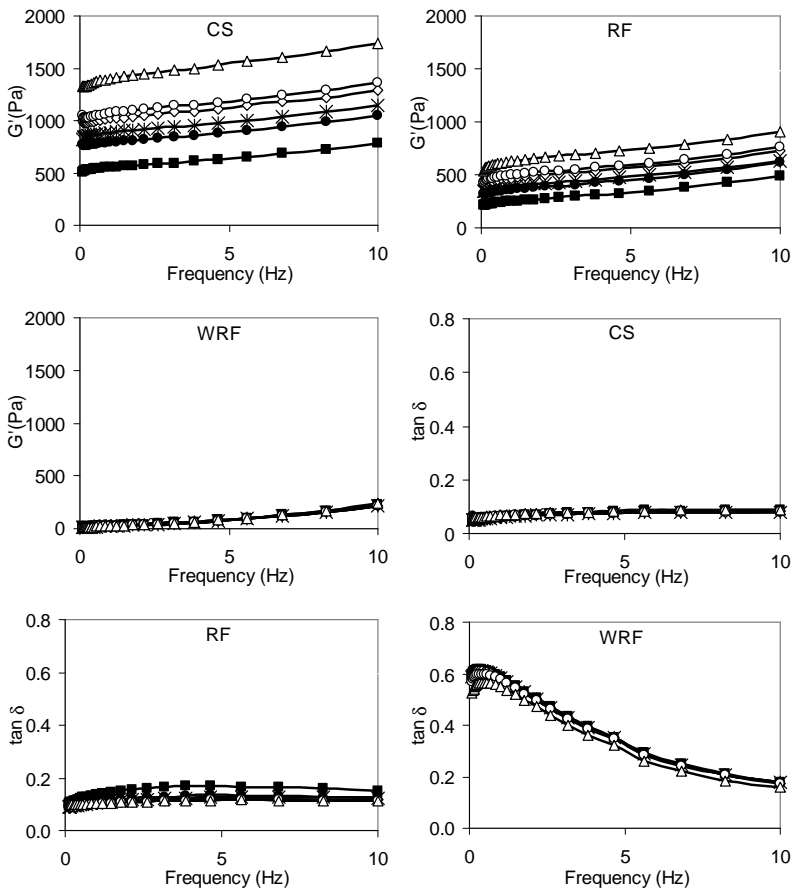


Figure 1 Storage modulus (G') and damping factor ($\tan \delta$) of gels stored at 4°C for different times: (■) t_0 , (●) t_1 , (∗) t_2 , (◊) t_3 , (○) t_4 , (Δ) t_7 ; data related to mixtures are not showed.

The viscous-elastic properties of gels, both fresh and during ageing, was studied by a MCR 300 Rheometer using frequency sweep tests over the frequency range of 0.1-10Hz at 1% strain (Fig.1). Storage modulus (G') was always higher than loss modulus (G'') in the whole range of frequencies, and its magnitude increased with frequency with a stronger dependency for G'' . The highest G' values were registered for CS and CS-RF gels; on the contrary WRF originated very weak gels. The addition of WRF both at 25% or 50% concentration strongly reduced the G' value of the mixtures containing CS or RF. The same -or even greater- effect was ascribable to the addition of RB for both the concentrations considered. The damping factor ($\tan \delta = G''/G'$) was, at every frequency considered, lower than 0.2 for CS, RF, 50CS-50RF and 50CS-25RF-25RB gels, to indicate a prevalent solid-like behaviour; in particular CS gels were characterized by very low $\tan \delta$ values, reflecting the elasticity of this material. On the contrary, WRF gel had a more viscous character especially at low frequency values, with a strong dependence of $\tan \delta$ on the frequency. This behaviour can also be found in 50CS-50WRF and 50RF-50WRF gels, even if at a minor extent. Considering the G' values of CS, RF gels and of their blend evaluated at the different ageing times, it is evident how the curve shifted progressively towards higher values, while WRF curves were virtually overlapped. CS sample, was not only characterized by a G' value at t_0 higher than the other samples, but also by a much faster hardening kinetic. Also the mechanical spectra (in particular G') of RF and 50CS-50RF increased with time, while for WRF no differences were evidenced between G' evaluated on fresh gels and after 7 days of hardening. The addition of WRF or RB to CS or RF determined a sharp reduction of starch reordering rate that resulted very pronounced in the case of the 50RF-50WRF mixture. Also the addition of 25% WRF or 25% RB to the CS-RF mixtures (50CS-25RF-25WRF or 50CS-25RF-25RB) was very effective in delaying the increase of G' and G'' .

4.2 HHP treatments on GF raw materials (Step 2)

In this study the high hydrostatic pressure was used to induce physical and structural changes of starchy compounds at low temperature. Due to the pressure applied also proteins can be partially denatured changing their aptitude to form a protein network during further processing.

The results obtained showed that pressure holding time (5min or 10min) and processing temperature (20°C or 40°C) were not discriminating parameters, whereas the pressure applied (400MPa or 600MPa) determined a different organization of the macromolecular compounds.

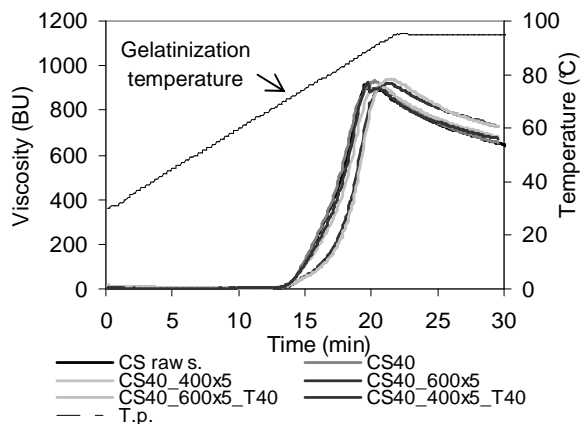


Figure 2 Pasting properties of CS treated for 5 minutes at different pressures

Observing the viscoamylographic profiles of RF untreated and treated in different conditions it was evident that starch was partially gelatinized at both the pressures used (400MPa or 600MPa): lower peak values (about 650BU -Brabender Unit- and 620BU, respectively) were measured in comparison to the unpressurized sample (756±4BU). Also a higher solvent retention capacity (SRC) in almost all the media used was observed: RF had a water SRC around 97%, whereas it ranged between 103-121% for samples treated at 400MPa and more than 130% for those at 600MPa. Few differences were found for WRF, to indicate that this sample was less influenced by pressure. On the contrary CS (Fig. 2), pressurized at 600MPa, presented a slower gelatinization trend compared to the untreated CS and to the sample treated at 400MPa; in fact, even if they started to gelatinize at the same temperature (70°C), a clear shift of the viscoamylographic curves appeared suggesting the formation of a more compact structure. The lower enthalpy required to gelatinize the 600MPa treated sample (9±0.14J/g vs. 14±0.19J/g of untreated CS), measured through differential scanning calorimetry, may be related to a partial gelatinization/retrogradation of starch.

4.3 Bread formulation and sourdough development (Step 3)

Three different brands of pea proteins were compared for Overrun Properties (OP, %) and Foaming Stability (FS, %) up to 60 min of rest. Pea protein (IPP) considered appropriate for the bread-making process presented a high protein content (90% vs. 88% and 82%), a pH of 7.6 (vs. 6.6 and 6.3), an OP value of 103% (vs. 67% and 0.77%) and a FS after 60min of 68.5% (vs. 58.2% and 0%). To define the amount of proteins and hydrocolloids to be used in the recipe, Farinographic tests aimed at determining the amount of water required to yield a dough consistency of 200BU and baking trials (Fig.3) were performed. The formulations tested contained: flours (CS and RF), IPP, psyllium, HPMC, emulsifier, oil, sugar, yeast and salt. Water absorption ranged between 79.5-84.8% depending on the ingredients used. For this preliminary study, appearance, baking weight loss and bread height, were considered. As can be appreciated in Fig. 3 and Table 1, the formulation containing 6% IPP and both psyllium and HPMC presented the better bakery aptitude, in particular when the emulsifier was added.



- | |
|--|
| 1) 76CS_RF+6IPP+3PSY (%total weight) |
| 2) 82CS_RF+0IPP+3PSY (%total weight) |
| 3) 76CS_RF+6IPP+1.5PSY+1.5HPMC (%total weight) |
| 4) 76CS_RF+6IPP+1.5PSY+1.5HPMC (%total weight)+emulsifier 0.43% of flours weight |

	Baking Loss (%)	Max Height (cm)	Min Height (cm)
1)	10.16 ^a	4.37 ^a	2.77 ^a
2)	13.05 ^{bc}	4.20 ^a	2.73 ^a
3)	10.51 ^{ab}	4.33 ^a	3.70 ^b
4)	13.50 ^c	5.07 ^b	3.97 ^b

Figure 3 and Table 1 Baking trials to define the GF recipe. Note: values followed by the same letter in the same column are not significantly different ($P < 0.05$).

At the same time a GF sourdough was developed. *Lactobacillus sanfranciscensis* and *Candida humilis* were isolated from a commercial traditional sourdough (characterized by a population of $1.5 \cdot 10^9$ UFC/g bacteria and $2.8 \cdot 10^6$ UFC/g yeast). Strains isolated, purified and identified were mixed with GF ingredients to generate the “sourdough starter” having a population of $5.90 \cdot 10^5$ UFC/g bacteria and $1.50 \cdot 10^5$ UFC/g yeast, a consistency of 230BU (Farinographic Brabender Unit) and a pH of 5.94. The resulting dough was then maintained at 25°C for 24h to permit the microorganisms growth (fermented dough had $1.05 \cdot 10^8$ UFC/g bacteria and $3.00 \cdot 10^7$ UFC/g yeast). During fermentation pH, continuously monitored, reached 4.70 and CO₂ production/retention resulted equal to 1174mL and 1076mL, respectively. A portion of the fermented dough (30g), maintained at 4°C for few days, was refreshed, fermented at 25°C until pH values of 3.80-4.00 were achieved, and constantly monitored for microbial growth and technological performance. *Lb. sanfranciscensis* and *Candida humilis* ranged around 10^8 - 10^9 UFC/g and 10^7 UFC/g respectively, during a period of one month (ten sourdough refreshments) to indicate that a stable interaction was achieved. The dough development, express as dough height after fermentation improved from 18.2mm to 31.6mm at the 10th refreshment and the CO₂ production/retention reached maximum values of 1668mL and 1380mL respectively.

5. General Conclusions and Future Perspectives

The study of the rheological properties of CS, RF, WRF and RB and their mixtures (4.1) highlighted that both WRF and RB were very effective in reducing the gels stiffness and storage modulus. In particular, the addition of both WRF or RB greatly delayed the gel networking if present at 25% level, while very limited stiffness increase was evidenced in CS and RF mixtures containing 50% WRF and RB. Furthermore using the HHP (4.2) the technological aptitude of the raw materials, particularly RF and CS, changed due to a partial gelatinization of starchy compounds that can be related to a slower retrogradation rate in the final product. As reported in sections 4.3, a GF sourdough was successfully developed and the encouraging results obtained -stable interaction between microorganisms- suggest that this GF sourdough could be used as alternative leavening agent to improve GF bread quality and shelf-life.

Future perspectives are therefore focused on bread-making trials using the GF sourdough in a formulation containing CS and RF treated at high pressures. In addition, the acquired knowledge will be used to develop a formulation for the production of new GF baked products such as a breakfast cake.

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Valutazione delle proprietà di spaghetti gluten-free presenti sul mercato italiano



9° Convegno AISTE
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Manuela Mariotti¹, Carola Cappa¹, Stefania Iametti², Mara Lucisano¹

¹Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche; ²Dipartimento di Scienze Molecolari Agroalimentari
Università degli Studi di Milano, Via G. Celoria, 2 - 20133 Milano; carola.cappa@unimi.it

Introduzione La Celiachia è una enteropatia glutina sensibile, ampiamente diffusa in Italia; attualmente l'unica alternativa per i soggetti Celiaci è una dieta priva di glutine. Come noto, il fumento possiede frazioni proteiche che, in opportune condizioni di processo, danno origine ad un reticolo, avente proprietà viscoelastiche tali da consentire una strutturazione ottimale dell'impatto durante le fasi di impastamento ed estrusione, responsabile della qualità e del comportamento in cottura della pasta. Nelle paste GF l'assenza di glutine rende necessari studi di formulazione e di processo finalizzati alla creazione, durante il processo produttivo, di una "impacatura alternativa" in grado di conferire rigidità al prodotto e limitare la perdita di solidi nell'acqua di cottura.

SCOPO: indagare ad ampio raggio sulle paste gluten-free attualmente presenti sul mercato italiano, mediante valutazioni di tipo chimico, biochimico e fisico. Vengono qui presentati i risultati relativi alla caratterizzazione del prodotto crudo.

SOMMARIO

Valutazione delle alternative gluten-free (GF) ad un prodotto tipicamente italiano: la **pasta alimentare**. Particolare attenzione è stata dedicata allo studio dell'organizzazione della matrice amidacea e proteica e della loro influenza sulla qualità finale della pasta GF. I risultati qui riportati, relativi alla caratterizzazione del prodotto crudo, hanno evidenziato una discreta variabilità tra i 14 campioni considerati, imputabile sia agli ingredienti utilizzati, diversi per tipologia e caratteristiche, che al processo tecnologico adottato dalle aziende produttrici.

Materiali e Metodi

Tabella 1. Ingredienti dei diversi campioni commerciali di spaghetti gluten-free.

Spaghetti di riso (R)		Spaghetti a base di amido di mais (CS)	
R1	100% farina di riso	CS1*	amido di mais, amido di patata, inulina, E-471, aroma cardamomo
R2	farina di riso, E-471	CS2	amido di mais, amido di patata, farina di lupino, proteine di lupino, E-471
R3	100% farina di riso	CS3	amido di mais, farina di mais, farina di riso, isolato proteico di pisello, E-471
R4	farina di riso, germe di riso, E-471	CS4	amido di mais, amido di patata, farina di lupino, proteine di lupino, E-471
Spaghetti di mais (C)		Spaghetti misti (M)	
C1	100% farina di mais	M1	70% farina di riso, 29% farina di mais, E-471
C2	100% farina di mais	M2	farina di mais, riso e grano saraceno, lievito, farina di tapioca, farina di girasole
C3	100% farina di mais	M3	farina di mais, farina di riso, isolato proteico di pisello, E-471

* Campione venduto con l'indicazione "a ridotto contenuto proteico".

CARATTERIZZAZIONE CHIMICO-FISICA (n=2)

Unità (AACC 44-15A, 2009).

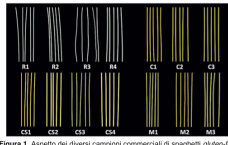
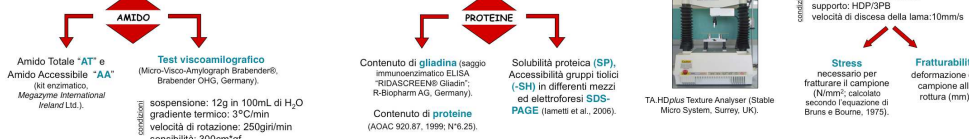


Figure 1. Aspetto dei diversi campioni commerciali di spaghetti gluten-free.

ASPETTO (n=3)

Colore (colorimetro tristimolo Minolta)
Diametro (Image Analysis; acquisizione a 600dpi, elaborazione con Image Pro-plus)

TEXTURE - Bending Test (n=7)

lunghezza spaghetti: 12cm
supporto: HD/SPB
velocità di discesa della lama: 10mm/s

Stress necessario per fratturare il campione (N/mm²), calcolato secondo l'equazione di Bruns e Bourne, 1979).
Fratturabilità deformazione del campione alla rottura (mm).

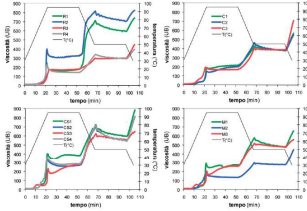
Valutazione frazione AMIDO

Tabella 2. Amido totale (AT) e amido accessibile (AA).

	AT (g/100g ss)	AA (g/100g ss)	AA/AT (%)
R1	88,54 [±]	15,07 [±]	12,33
R2	85,89 [±]	14,16 [±]	16,49
R3	87,38 [±]	10,24 [±]	11,72
R4	85,86 [±]	8,14 [±]	9,25
C1	86,25 [±]	9,49 [±]	11,01
C2	84,03 [±]	12,45 [±]	14,81
C3	82,60 [±]	15,39 [±]	18,63
CS1	88,01 [±]	18,89 [±]	22,08
CS2	82,17 [±]	11,96 [±]	14,55
CS3	82,71 [±]	15,49 [±]	18,72
CS4	85,69 [±]	15,59 [±]	18,66
M1	83,10 [±]	13,63 [±]	16,40
M2	90,41 [±]	8,07 [±]	9,02
M3	78,63 [±]	14,15 [±]	18,00

Nella stessa colonna, a stessa diversa composizione campioni significativamente (P<0,05) differenti.

Figura 2. Organizzazione della matrice amido: profili viscoamilografici.



AA	VISCOSITA'	STRUTTURAZIONE AMIDO	CONDIZIONE DI PROCESSO
R2 e CS1	●●●	●●●	poco drastiche
R3	●●●	●●●	drastiche (tecnologia orientale)
R4	●●●	●●●	forma di riso pregelatinizzata

Valutazione frazione PROTEICA

I 14 campioni considerati sono risultati essere conformi ai limiti di legge (glutine<20mg/kg di pasta).

Tabella 3. Valutazione dell'organizzazione della componente proteica.

Proteine (g/100g ss)	Solubilità proteica (mg/g pasta)		Accessibilità -SH (μmol/g proteina)	
	temperone	+ urea	+ urea/DTT	temperone
R1	7,71 [±]	2,96 [±]	21,69 [±]	3,16 [±]
R2	7,84 [±]	3,47 [±]	25,47 [±]	7,94 [±]
R3	5,95 [±]	1,80 [±]	4,38 [±]	17,21 [±]
R4	8,04 [±]	0,31 [±]	9,35 [±]	41,26 [±]
C1	8,20 [±]	3,07 [±]	4,23 [±]	18,47 [±]
C2	8,51 [±]	0,29 [±]	12,22 [±]	31,19 [±]
C3	8,18 [±]	0,08 [±]	12,93 [±]	26,28 [±]
CS1	6,36 [±]	0,36 [±]	1,80 [±]	5,98 [±]
CS2	6,03 [±]	0,83 [±]	38,90 [±]	45,95 [±]
CS3	8,72 [±]	1,45 [±]	22,56 [±]	70,89 [±]
CS4	4,65 [±]	0,50 [±]	31,72 [±]	41,09 [±]
M1	8,66 [±]	1,50 [±]	19,19 [±]	44,45 [±]
M2	8,83 [±]	1,29 [±]	8,34 [±]	14,66 [±]
M3	10,79 [±]	1,41 [±]	25,12 [±]	56,76 [±]

Nella stessa colonna, a stessa diversa composizione campioni significativamente (P<0,05) differenti.

La bassa solubilità in tampone e l'incremento aggiunto di urea e DTT stabilizzano il network proteico idrofobico e covalente.

Risultati e Discussione

AA e profili viscoamilografici come indicatori dell'organizzazione strutturale dell'amido.

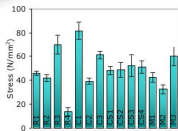


Figure 3. Stress necessario per fratturare il campione.

TEXTURE

L'organizzazione molecolare a carico dell'amido e delle proteine, in funzione del processo tecnologico adottato, esplica un ruolo chiave per la texture del prodotto crudo.

	STRESS	REFORMABILITA'	ROSSIBILI PROTAGONISTI
R3	●●●	●●●	strutturazione elevata di AMIDO e di PROTEINE
R4	●●●	●●●	presenza di GERME
C1	●●●	●●●	strutturazione elevata di amido e di PROTEINE
M2	●●●	●●●	presenza di FBRA

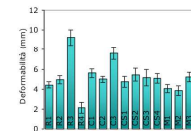


Figure 4. Deformazione alla rottura.

CONCLUSIONI

La caratterizzazione del prodotto crudo ha evidenziato una discreta variabilità tra i diversi campioni di pasta gluten-free. Fattori critici risultano sia gli ingredienti utilizzati nella formulazione che il processo tecnologico, responsabile di una diversa strutturazione dell'amido e della componente proteica. Inoltre la presenza di alcuni ingredienti, quali germe e fibra, determina un indebolimento della struttura del materiale che risulta più fragile e meno deformabile.



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INFLUENCE OF THE PRESENCE OF DIFFERENT FIBERS ON GLUTEN-FREE DOUGH AND BREAD PROPERTIES



DiSTAM
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano
via G. Celoria 2, 20133 Milano - Phone: 02-50319175, Fax: 02-50319190, e-mail: carola.cappa@unimi.it

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Technology and Biotechnology

Introduction

In gluten-free (GF) bread production, the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final product. Moreover, the high presence of starch makes the product more sensitive to staling and reduces its shelf-life. To provide for the lack of gluten and to simulate its viscoelastic behaviour, hydrocolloids, proteins and fibres are frequently used. Even if in the last years many studies have been carried out on this topic, the gluten-free products present on the market are often characterized by low nutritional value and unsatisfactory sensorial quality, in particular regarding the maintenance of their crumb softness during shelf-life.

Aim
This research was focused on the effects of 2 different combinations of Psyllium and Sugar Beet fibers on the mixing and leavening behaviour of GF doughs, which are suitable to be poured into moulds (200BU) or to be shaped by hand or with an industrial forming machine (500BU). The quality of the resulting GF breads was evaluated too.

Materials and Methods

1. Raw Materials

- Rice flour (RF)
- Rice protein (RP)
- Rice starch (RS)
- Sugar Beet fiber (SB)
- Psyllium fiber (Psy)
- Corn starch (CS)

Raw Materials Characterization (n≥2)

- ✓ Moisture (AACC 44-15A, 2000)
- ✓ Protein (AOAC 920.87, 1999; N*6.25)
- ✓ Total starch (TS) and damaged starch (DS) (Megazyme International Ireland Ltd. Kits)
- ✓ Water absorption index (WAI) (Anderson et al., 1969)
- ✓ Pasting properties (Brabender® Micro-Visco-Amylograph; Brabender OHG, Germany).

15g sample (14%Hu)/100mL H₂O, speed: 250min⁻¹; measuring range: 300cm²gf.

2. Bread-baking Trials

(Mariotti, 2004)

Mixing of the raw materials and addition of margarine, sugar, yeast, HPMC and salt (20 min, room temperature). Water was added in order to obtain two consistencies (200 and 500BU) according to the following recipes:

A200: 2.5%Psy, 0.5%SB (200BU)
A500: 2.5%Psy, 0.5%SB (500BU)
B200: 1.5%Psy, 1.5%SB (200BU)
B500: 1.5%Psy, 1.5%SB (500BU)
 (% on flour basis)

Leavening (35min, 30°C, 80% RH)

Baking (30min, 230°C bottom, 200°C top).

Dough and Bread Quality Evaluation (n≥4)

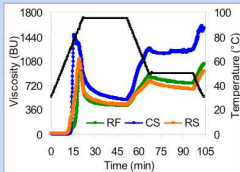
- ✓ Dough mixing properties (15min, 30°C; Brabender Farinograph; Brabender OHG, Germany)
- ✓ Dough development during leavening and CO₂ production and retention (1h, 30°C; Chopin F3 Rheofermentometer; Chopin SA, France)
- ✓ Bread crumb softness on fresh and stored bread in paper bags at 20°C, 60% RH for 72h (compression test; TA-HDplus Texture Analyzer; Stable Micro Systems, UK).

1. Properties of the Raw Materials

Tab. 1 Chemical composition (n.d., not detectable).

Raw Materials	Moisture (g/100g)	Protein (g/100g db)	TS (g/100g db)	DS (g/100g db)
RF	11.47±0.28	7.86±0.03	85.16±1.91	5.80±0.28
RP	6.90±0.30	87.44±2.34	4.58±0.19	n.d.
RS	10.35±0.13	n.d.	90.73±1.53	12.01±0.37
CS	11.05±0.03	n.d.	97.97±1.30	1.08±0.03
Psy	8.46±0.05	3.69±0.11	n.d.	n.d.
SB	9.58±0.25	9.30±0.32	1.32±0.04	n.d.

Different pasting properties were observed between RS and CS. In particular a lower peak viscosity and a lower tendency to retrogradation were evidenced for RS.



WAI extremely different values

The WAI of the different fibers greatly influences the water amount that has been added during the bread-making process.

SB	Psy
9.48±2.13 gH ₂ O/g db	48.29±2.77 gH ₂ O/g db

Conclusions

This research confirms that hydrocolloids and fibers play a strategic role in improving the workability of GF dough, enhancing at the same time the water binding capacity of bread and its shelf-life. A higher anti-staling effect of Psy in comparison to SB was evidenced.



Results and discussion

2. Properties of GF Dough

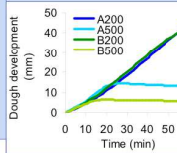


Fig. 2, Tab. 2 Dough properties during mixing and leavening.

Dough	Water absorption (%)	CO ₂ production (mL)	CO ₂ retention (%)
A200	85.0	628	99.3
A500	58.1	744	95.0
B200	74.0	614	99.3
B500	57.3	691	95.4

The 200BU doughs, that evidenced a higher farinographic water absorption during mixing, showed the highest dough development and CO₂ production during the leavening phase.

The opposite was evidenced for the 500BU doughs.

3. Properties of GF Bread

Despite the not promising results obtained for the 500BU doughs, A500 exhibited a considerable volume increase during baking, and thus the highest specific volume of the bread.

Fig. 3 Bread appearance.

Bread	Crumb moisture (g/100g)	Slice moisture (g/100g)	Height (mm)	Specific volume (mL/g)	Crumb hardness (N)
A200	52.84±0.04 ^a	46.17±0.30 ^b	52.4±1.1 ^c	2.40±0.17 ^c	7.43±0.82 ^a
A500	44.94±0.13 ^b	37.50±0.43 ^b	61.3±2.1 ^d	2.74±0.26 ^b	12.14±1.72 ^b
B200	50.06±0.05 ^c	42.34±0.31 ^b	48.0±1.3 ^b	2.11±0.12 ^b	10.63±0.59 ^b
B500	44.77±0.13 ^b	38.48±0.21 ^b	39.4±1.5 ^b	1.79±0.16 ^c	n.d.

Tab. 3 Fresh bread properties (n.d., not detectable). Note: values followed by the same letter in the same column are not significantly different (p<0.05).

Bread Shelf-life

A limited decrease (2-5%) in crumb moisture was observed after 3 days of storage at 20°C and 60% RH, whereas the moisture of the slice changed more evidently (9-16%).

Significant differences (p<0.05) in crumb softness between the two 200BU breads were found (Fig. 4). These differences were more evident after 3 days of storage indicating a higher anti-staling effect of Psy in comparison to SB.

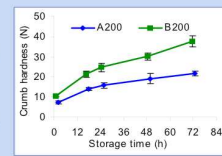


Fig. 4 Evaluation of crumb hardness during storage.

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...The next PhD year

will be focused on the setting up of different technological procedures (e.g. high pressure treatments) to improve the technological properties of the raw materials, and on the optimization of the GF bread recipe and baking process by using GF sourdough. Particular attention will be paid to the evaluation of GF bread quality during its shelf-life.

Gluten-free Pasta: Technology and Quality Evaluation

B5

Manuela Mariotti, Carola Cappa, Mara Lucisano

Università degli Studi di Milano, DiSTAM (Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche), Via G. Celoria 2, Milan, Italy

**corresponding email: mara.lucisano@unimi.it*

Introduction. Gluten forming proteins are fundamental for the production of a great variety of food, including pasta, most appropriately made from durum wheat. The replacement of gluten network, in order to produce gluten-free pasta (GFP), is a major technological challenge, and ingredients that imitate the viscoelastic properties of gluten are always required. The first attempts in the search for substances able to imitate the viscoelastic properties of gluten exploited starch gelatinization and retrogradation phenomena, modifications that can be obtained during the technological process or using pre-gelatinized starches or starchy flours as raw materials. Later on, other ingredients have been considered in GFP formulation: rice and corn flours, flours from pseudocereals, starches from different sources, vegetable proteins, emulsifiers, hydrocolloids. The technological process also plays an important role on the final quality of GFP. The traditional process for making rice noodles involves the presence of many heating and cooling phases aimed at reorganizing the starchy matrix; the batch process can be switched to a continuous one with the use of the extrusion technology performed at high temperature; the same process and the same equipment employed in the production of durum wheat pasta can be used if pregelatinized materials are used.

In consideration of such a large variety of formulations and technologies, the aim of this study was to evaluate the characteristics of as much as possible commercial GFP (spaghetti shape), in order to get a wide view of what is actually available on the Italian market. The study regarded the chemical, biochemical and physical characterization of the samples, focusing the attention on starch and protein organization. Cooking behaviour and textural characteristics of cooked pasta (at different cooking times) were also evaluated.

Methods. Fourteen commercial brands of GF spaghetti (GFS) were collected. The uncooked GFS were characterized for gluten content, color, chemical composition, protein solubility and thiol accessibility, starch accessibility, starch pasting properties (Brabender Micro-Visco-Amylograph, MVA), spaghetti fracture properties (TAHD_{plus} Texture Analyser); the cooked GFS were evaluated for water absorption, cooking loss, dimensional changes (Image Analysis), and textural properties (compression test, creep test) at different cooking times, in order to determine the kinetics of all these phenomena.

Results. Only some of the results obtained are here reported. On the basis of their ingredients the 14 GFS were identified as: rice spaghetti (coded: R1, R2, R3, R4), corn spaghetti (C1, C2, C3), corn starch based spaghetti (CS1, CS2, CS3, CS4; some of them containing also potato flour, rice flour, corn flour, pea protein isolate, lupin flour, lupin proteins), and spaghetti (M1, M2, M3) obtained from a mixture of rice flour, corn flour and other ingredients (tapioca flour, yeast, buckwheat flour, sunflower flour). These products were characterized not only by a different chemical composition (protein: 5-11% d.b.; starch: 79-90% d.b.) but also by a different protein and starch organization (starch accessibility: 8.1-16.0% d.b.; MVA peak viscosity: 167-404 BU). All these factors influenced the cooking quality of the different GFS. Some quality parameters (at the optimum cooking time, OCT), are reported in Table 1. R3,

obtained according to the oriental technology, exhibited high water absorption, low cooking loss and adhesiveness, differently from the others “R” samples. Various behaviours were also observed in legume-based GFS, due not only to dissimilar properties of proteins in botanically related species but also to different organizations of the protein network in these samples.

Table 1. Quality parameters of the GFS, at their OCT (*, *compression test*)

Sample	Water absorption (%)	Diameter increase (%)	Cooking loss (g/100g d.b.)	Young Modulus* (N/mm ²)	Adhesiveness* ($\cdot 10^{-3}$ J)
R1	100.0	44.2± 8.2	12.4± 0.1	0.39± 0.02	5.21± 0.72
R2	112.5	45.3± 7.9	8.5± 0.1	0.37± 0.01	2.47± 0.48
R3	162.5	41.0± 7.6	3.2± 0.2	0.26± 0.01	0.67± 0.05
R4	112.2	41.5± 4.5	5.6± 0.3	0.36± 0.01	2.65± 0.60
C1	125.0	52.6± 4.5	6.2± 0.1	0.36± 0.01	0.90± 0.10
C2	117.1	39.8± 2.9	5.3± 0.2	0.34± 0.01	1.48± 0.13
C3	117.1	51.2± 4.7	5.0± 0.1	0.34± 0.01	1.01± 0.16
CS1	122.0	47.0± 5.2	5.1± 0.1	0.28± 0.01	1.99± 0.69
CS2	107.3	42.8± 4.4	6.8± 0.1	0.33± 0.01	1.09± 0.17
CS3	142.5	54.3± 5.3	3.9± 0.1	0.27± 0.01	3.82± 0.18
CS4	125.0	47.7± 5.1	6.7± 0.1	0.31± 0.01	1.38± 0.26
M1	134.1	61.5± 4.7	5.1± 0.1	0.31± 0.01	1.89± 0.25
M2	122.0	39.2± 3.2	7.5± 0.1	0.36± 0.001	1.34± 0.07
M4	120.0	53.0± 4.6	2.5± 0.1	0.30± 0.01	1.87± 0.48

A creep test applied to the GFS cooked at their OCT (Figure 1) proved to be very useful in describing the viscoelastic characteristics of the cooked products: very different patterns were observed, even in the same product category (*e.g.* C1 *vs.* C3, R1 *vs.* R3), underlying the importance of the technological process adopted besides the role of the formulation.

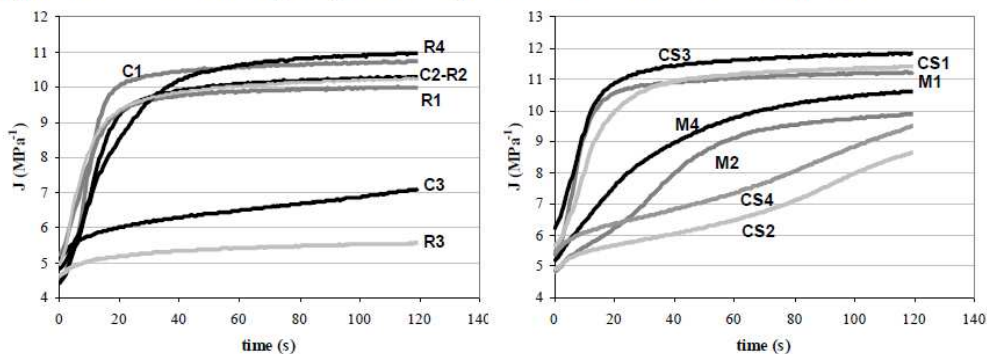


Figure 1. Viscoelastic behaviour (*creep test*) of the GFS, at their OCT.

Conclusions. The overview on GFS samples presented in this study highlighted the wide variety of raw materials and technologies adopted in this sector, indicating the on-going research of solutions providing for the gluten-network absence. At the same time, it came out how all these factors can directly influence the final structure and quality of the products. Phenomena related to starch retrogradation certainly have a central role on the final texture of the products, but also the origin of the protein included in the formulation plays an important role in the definition of the protein-protein interactions, especially in those samples including proteins from different vegetable sources.

Evaluation of rice pasta properties through new and conventional methods

Mara Luciano^{1*}, Manuela Mariotti¹, Carola Cappa¹, Sanna Luoto²

⁽¹⁾ Department of Food Science and Microbiology, University of Milan, Italy, ⁽²⁾ Department of Food Technology, University of Helsinki, Finland.

* mara.luciano@unimi.it (+39) 02 603 19185, manuela.mariotti@unimi.it (+39) 02 603 19186, carola.cappa@unimi.it (+39) 02 603 19175, sanna.luoto@helsinki.fi (+356) 6503703066



INTRODUCTION

Pasta is a traditional Italian food, but it is also frequently used in many other countries. It is one of the most common cereal based products in the world. Traditionally pasta is obtained from wheat, but there are many other non-conventional types of pasta, such as "special pasta" or "dietetic pasta", that are combined with or made of other cereals. Rice pasta, apart from being largely diffused in the oriental countries, is particularly important for people suffering from coeliac disease, who must strictly follow a gluten-free diet. In the traditional pasta-making process, semolina was kneaded with water to obtain a uniformly humidified (about 30% moisture) dough; the moistened mass is extruded to facilitate the interaction of the hydrated proteins and the formation of the gluten network; at the end of this step, the dough is formed through a die having different holes according to the desired shape of the final product, and it is finally dried. In the absence of gluten, as in rice pasta production, the traditional process has to be modified: in the oriental countries wet-milled flour is used, and many different heating-cooling steps are performed to induce the formation of a three-dimensional structure of gelatinized and retrograded starch; in western countries, the same result is obtained by pregelatinizing rice flour during the first step of the pasta-making process or by using pregelatinized rice flour as raw material.

AIM
The aim of this study was the evaluation of two commercial rice pasta (spaghetti), produce by adopting different technologies, through new and conventional methods.

MATERIALS
Two commercial samples of rice pasta (spaghetti, Table 1) prepared according to two different technological processes were considered. Sample A was obtained adopting the technology used in the oriental countries, whereas sample B was obtained using the conventional process used for wheat pasta production, which was applied to a pregelatinized rice flour.

Table 1. Composition of the two spaghetti samples

Sample	Protein (%)	Starch (%)	Water (%)
A	10.5	75.0	14.5
B	10.5	75.0	14.5

CHEMICAL CHARACTERIZATION
(Moisture (AACC 44-15A, 2000) - Protein (AOAC 920.07, 1999) - Total Starch (ST) and Damaged Starch (SD) analyzed by Megazyme International, Ireland, UK.)

MICRO-VISCO-ANALYGRAPH BRABENDER (MVA)
(Brabender GmbH, Duisburg, Germany)
Twelve grams of milled sample were dispersed into 100mL of distilled water, stirred at 200rpm, and using a 100mm³ cartridge, and subjected to the following temperature profile:
Indic.: Gelatinization Temperature (GT, °C)
Peak Viscosity (PV, Brabender Units, BU)
Breakdown (BD, BU)
Breakdown (BD, BU)
corresponding to the PV minus the viscosity after the holding period at 95°C; setback (SB, BU): index of viscosity increase during the cooling cycle; Final Viscosity (FV, BU).

GEOMETRICAL INDICES
(Gleadow)
The diameter (mm) was obtained sorting the sections of 10 spaghetti on the surface of a flatbed scanner.

Three Point Bending Test
The spaghetti cut to a length of 12cm were put on the appropriate support (ICP-216) and broken by a blade moving at 10mm/s speed.
Indic.: Force (N, corresponding to the force necessary to break the sample), Elasticity (N/mm): calculated by the following equation: Bruts, 1973; Fracturability (mm): distance covered by the blade before sample breaking; Elongity (J/10³): energy needed to break the sample;
Stiffness = F * L / (δ * A)
δ = distance between supports (mm)

IMAGE ANALYSIS
Images were acquired by a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan) at 600 dpi resolution, saved in TIF format and processed using a dedicated software (ImageJ, NIH, 4.8.1.20 (NP, Media Cybernetics Inc., 14819-2003, US).

TEXTURE ANALYSIS
by means of a Texture Analyser (Stable Micro System, Surrey, UK), interfaced with a personal computer through a specific software (Texture Equipment TEX2 3.2.4.0, Stable Micro System, UK).
Seven spaghetti were cooked for the fixed times, cooled for 20min at room temperature in an airtight container and placed in the "HDP/PPS Pasta Firmness Stickiness System".
1. Compression test
The samples were compressed (probe speed = 1mm/s) to a constant force of 50N, and maintained in this condition for 10s before removing the probe.
Indic.: Young's modulus (N/mm²): minimum of product hardness and adhesiveness (J x 10³).

COOKING CONDITIONS
Samples were cooked for 4, 7, 8, 9 and 12 min; the ratio of pasta to water was 1:15.

WATER ABSORPTION
Water absorption (%) of spaghetti, after the fixed cooking times, was determined as: (weight of cooked pasta - weight of raw pasta)/weight of raw pasta*100.
COOKING LOSS
Cooking water collected from each cooking time was evaporated until constant weight in an oven, at 105°C. The residue (%) was weighed and reported as percentage of the original spaghetti weight.

*The experimental data were obtained at least in duplicate and were processed by a statistical software (StatgraphPlus v3.11); for the same parameter, values followed by the same letter are not significantly different (P<0.05).

CHEMICAL AND PHYSICAL PROPERTIES OF DRY PASTA

A statistically significant (P<0.05) different composition was observed for the two rice spaghetti samples (Table 1). This could be due to the presence of the germ in sample "B", and to the use of wet milled rice flour in sample "A". The higher starch content of sample "A" was related to more drastic conditions adopted during the technological process and thus to a lower accessibility to amylase action.

Table 2. Chemical composition of the two rice spaghetti samples

Sample	Protein (%)	Starch (%)	Water (%)
A	10.5	75.0	14.5
B	10.5	75.0	14.5

Important information on starch organization was also obtained by the viscoamylographic test (Figure 1), a procedure which is conventionally performed on raw materials such as cerea flour or starch.



Figure 1. Viscoamylographic profile of the two rice spaghetti samples.

RESULTS AND DISCUSSION

GEOMETRICAL INDICES: Due to the water absorption and starch gelatinization, the structure of the product became more deformable and the diameter and the length of spaghetti changed. In particular, during the first 4 cooking minutes a quick increase of the diameter and a reduced change in the length was observed for both the samples (Figure 2).

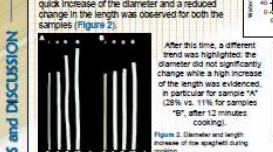


Figure 2. Geometrical and Three Point Bending indices of the two rice spaghetti samples.

Another important deformation that sample "A", though having a structure more resistant to breaking, was more deformable than sample "B": its fracturability index, in fact, was very high (Table 4). As a consequence, the energy required to break sample "A" was more than 15 times higher than the one required to reach the rupture of sample "B".

RICE PASTA COOKING QUALITY

WATER ABSORPTION: during cooking, the water absorption increased almost linearly for both the rice spaghetti samples but with a different kinetic: after 12 minutes cooking, in fact, the weight increase was equal to 200% for sample "A" and to 129% for sample "B" (Figure 3).

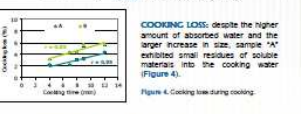


Figure 3. Water absorption during cooking.

COOKING LOSS: despite the higher amount of absorbed water and the larger increase in size, sample "A" exhibited small residues of soluble material into the cooking water (Figure 4).

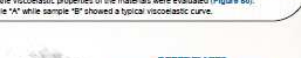


Figure 4. Cooking loss during cooking.

TEXTURE ANALYSES

The rheological tests performed came out to be useful for the description of the textural changes of the samples during cooking.



Figure 5. Young's modulus (E) and adhesiveness (A) of the two rice spaghetti during cooking, and elasticity (δ) curves at their optimum cooking time (min).

Sample "A" was characterized by a lower consistency (Young's modulus) (Figure 5a) in comparison to sample "B", and its adhesiveness (Figure 5b) remained very low and almost constant even after a prolonged cooking time. These results are in line with the high amount of absorbed water and the reduced cooking losses of this sample. In addition, the viscoelastic properties of the materials were evaluated (Figure 6): an elastic behavior was associated to sample "A" while sample "B" showed a typical viscoelastic one.

CONCLUSIONS

The traditional and the non-conventional methods used in this study were found to be suitable in describing the characteristics of dry and cooked gluten-free spaghetti. In particular, the viscoamylographic test combined with the enzymatic evaluation of starch accessibility (DS) was very useful to evidence the starch organization in the two samples. Moreover, by studying the mechanical behaviour of the materials both in their dry and cooked states, the textural analyses highlighted the different structural networking in the two rice pasta samples due to the different formulation and the technological pasta-making processes adopted.

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GLUTEN-FREE BAKED PRODUCTS: OPTIMIZATION OF FORMULATION AND PROCESS CONDITIONS

Carola Cappa; Tutor: Prof. Mara Lucisano

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano
via G. Celoria 2, 20133 Milano - Phone: 02-50319175, Fax: 02-50319190, e-mail: carola.cappa@unimi.it

1. State of the Art Celiac disease is a permanent intolerance to "gluten", a small intestine affection influenced by genetic factors, that can appear both in children and adults. In gluten-free baked products (e.g. bread), the absence of the viscoelastic gluten network makes the whole process problematic and penalizes the sensorial quality of the final products. Critical are the rheological properties of the gluten-free dough, that exhibits a limited expansion and gas retention, thus leading to bread with reduced volume and decreased softness of the crumb (Mariotti, 2003). Moreover, the high presence of starch from different origin makes the product more susceptible to staling and reduces its shelf-life (Arendt *et al.*, 2008). To provide for the lack of gluten and to simulate its viscoelastic behaviour, hydrocolloids, proteins of vegetal and animal origin and dietary fibres are frequently included in the recipe (Gallagher *et al.*, 2004; Guarda *et al.*, 2004; Lazaridou *et al.*, 2007). Although in the last years many studies have been carried out on this topic, the gluten-free products present on the market are often characterized by low nutritional value and unsatisfactory sensorial quality, in particular regarding the maintenance of their softness during shelf-life.

2. PhD Thesis Objective and Milestones

The aim of this PhD research project is to **identify the formulations** and to **define the process conditions** that most influence the quality and shelf-life of gluten-free baked products. Particularly, it will focus on **gluten-free bread**, in order to get an innovative and nutritionally balanced product able to satisfy the consumer demands and whose technology could be adapted to others gluten-free baked products.

Table 1. Gantt diagram

ACTIVITIES	MONTHS																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1. RAW MATERIAL CHARACTERIZATION																									
2. TECHNOLOGICAL TREATMENTS OF RAW MATERIALS																									
3. OPTIMIZATION OF FORMULATION AND PROCESS CONDITIONS																									
4. EVALUATION OF PRODUCT QUALITY AND SHELF-LIFE																									
5. CHARACTERIZATION OF GLUTEN-FREE BREAD ON THE MARKET																									
6. THESIS AND PAPER PREPARATION																									

The PhD project can be scheduled into the following activities (also reported in Table 1)

1. Selection and characterization of raw materials.

Corn and rice flours having different amylose contents (critical for the shelf-life of the final product), flours from pseudocereals (e.g. amaranth and buckwheat), and proteins from soy, pea, and lupine (to increase the protein fraction and to improve bread texture) will be considered.



Throughout the whole planned activities, literature will be constantly updated.

2. Assessment of treatments able to improve the technological and nutritional properties of the raw materials.

Specific physical treatments (i.e. puffing, partial dehulling) able to modify or improve the structuring properties of raw materials will be considered. The composition and the baking aptitude of these raw materials will be tested.



3. Optimization of formulation and process conditions.

The use of various hydrocolloids (in terms of types and concentration), water amount, fats with different properties, and enzymes will be considered. Moreover, the number and the length of the leavening phases and the other baking conditions will be optimized. The use of sourdough to improve gluten-free bread quality will be tested, too.

3. Selected References

4. Beside the conventional chemical analyses, empirical and fundamental rheology, Image Analysis, microscopy and FT-NIR spectroscopy will be used throughout the project. Moreover, due to the high number of variables involved (related to both formulation and process conditions), statistical multivariate approaches will be adopted.

4. Evaluation of product quality and shelf-life.

The quality profiles of both fresh and packaged gluten-free bread will be monitored by applying different techniques.

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B8

Identificazione e Monitoraggio delle Proprietà Chimico-Fisiche del Pangrattato, un Ingrediente di Primaria Importanza per la Pasta Ripiena



M. Mariotti, L. Fongaro, C. Cappa, M. Lucisano

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Italy



manuela.mariotti@unimi.it +39.0250319186, loreno.fongaro@unimi.it +39.0250319177, carola.cappa@unimi.it +39.0250319175, mara.lucisano@unimi.it +39.0250319185

Introduzione Il pangrattato, ottenuto dalla macinazione di un impasto (farina, acqua, lievito e sale) appositamente lievitato e cotto (umidità residua inferiore al 10%), è un ingrediente comunemente utilizzato, nell'industria alimentare sia per panature che per la produzione di ripieni per paste alimentari. Questo ultimo, a livello industriale, è sicuramente l'impiego di maggiore importanza. Il quantitativo di pangrattato presente all'interno di un ripieno può superare infatti anche il 40% del ripieno stesso (comunicazione personale). Da un lato l'utilizzo del pangrattato consente di ottenere un buon volume finale del ripieno, riducendo la quantità di altri ingredienti più costosi (carne, formaggio, etc.), dall'altro rende il ripieno maggiormente lavorabile facilitando le operazioni delle macchine formatrici. Nonostante il suo ruolo tecnologico sia indubbiamente importante, spesso le caratteristiche chimico-fisiche del pangrattato vengono erroneamente trascurate. Nei capitoli d'acquisto aziendali, ad esempio, frequentemente vengono considerati solo alcuni indici qualitativi. Anche nella letteratura scientifica sono pochi i parametri finora proposti per definire le caratteristiche tecnologiche di questo prodotto; tra questi, il colore, la granulometria e la capacità di legare acqua (Tireki et al., 2006)

Scopo

Determinare le tecniche e i parametri chimico-fisici che maggiormente discriminano e influenzano l'attitudine tecnologica del pangrattato come ingrediente per la produzione di paste ripiene.

Materiali

Campioni di pangrattato di due diversi marchi commerciali (A e B) utilizzati indifferentemente, da uno stesso produttore, come ingredienti per il ripieno di paste alimentari. Sono stati considerati tre lotti diversi per ciascun campione.

Metodi, Risultati e Discussione

Le analisi sono state tutte eseguite almeno in doppio ed i dati ottenuti sono stati sottoposti ad elaborazione statistica mediante il software Statgraphics®Plus v.5.1 (nota: per ogni parametro, a lettere diverse compaiono valori significativamente diversi P<0.05)

PROPRIETÀ CHIMICHE

UMIDITÀ (AACC 44-15A; 2000)
Proteine (AOAC 920.37; 1999)
Grassi (AOAC 99.25; 2000)
Amido Totale "AT" e Amido Accessibile "AA" (kit enzimatico distribuito da Megazyme International Ireland Ltd.)
Ceneri (AACC 8-1; 2000)

COMPOSIZIONE CHIMICA	CAMPIONI	
	A	B
Umidità	6.15 ± 0.43*	8.50 ± 0.04*
Proteine (DM ⁸⁷)	11.87 ± 0.33*	12.01 ± 0.13*
Grassi	2.51 ± 0.09*	2.55 ± 0.18*
AT	73.24 ± 1.02*	73.37 ± 4.11*
AA	33.86 ± 1.31*	45.28 ± 0.31*
Ceneri	1.89 ± 0.02*	0.95 ± 0.08*

I campioni A e B presentano una composizione chimica significativamente diversa (P<0.05) ad eccezione del quantitativo in grassi e AT. La minore umidità di A indica inoltre che questo campione è stato probabilmente sottoposto ad un trattamento termico più drastico.

DISTRIBUZIONE GRANULOMETRICA

Setoatore automatico a movimento vibratorio regolabile (Octagon Digital, Endeocott Ltd.).

Condizioni:
 - 50 g di campione
 - 5 minuti di vibrazione continua
 - 4 setacci con differente luce delle maglie (µm)

La DISTRIBUZIONE GRANULOMETRICA è un parametro fortemente discriminante, in quanto comporta l'esposizione di una diversa superficie, specifica e quindi una differente rapidità dei fenomeni di assorbimento superficiali da parte del materiale.

Il campione B presenta una maggiore quantità di particelle fini, oltre il 52% < 500µm - ca. il 10% < 125µm

Il campione A - oltre il 40% > 500µm

ANALISI VISCOMETROGRAFICA

Micro-Visco-Amilografo Brabender® (MVA)

Condizioni:
 - 15g di pangrattato scossi in 100mL H₂O distillata
 - gradiente di temperatura = 3°C/min
 - velocità di rotazione: 250 giri/min
 - sensibilità: 300 cmG
 - profilo termico: 40°C → 100°C → 100°C → 50°C

	TC	PV	VF	SB	SI
CAMPIONE A	68.9 ± 1.2*	529 ± 14*	1310 ± 29*	571 ± 52*	300 ± 9*
CAMPIONE B	92.9 ± 1.1*	887 ± 44*	1737 ± 45*	570 ± 9*	-

Indici considerati:
 Temperatura di gelatinizzazione (TG: °C), picco di viscosità (PV: UB), viscosità finale (VF: UB), setback (SB: UB), differenza tra la viscosità alla fine e all'inizio del raffreddamento a 50°C (SI: UB)

Poiché i campioni hanno lo stesso quantitativo di AT, le differenze possono attribuirsi al diverso grado di GELATINIZZAZIONE DELL'AMIDO, in particolare PV, VF e SB più contenuti indicano che il campione A è stato sottoposto a condizioni termiche di cottura più severe.

VALUTAZIONE DELLA CONSISTENZA

di miscela pangrattato/acqua, mediante Consistometro Bostwick (LS100 -Labo-Scientifica s.r.l., PR. IT).

Condizioni:
 - 60g di campione in 180 mL H₂O distillata
 - 1 minuto di miscelazione, 5 minuti di riposo, 1 minuto di miscelazione

Indici considerati:
 scorrimento (cm) dopo 30s e 60s

Il consistometro, generalmente utilizzato dalle aziende per valutare la consistenza di sospensioni alimentari, ha evidenziato ulteriori differenze tra i due campioni commerciali di pangrattato: il campione A, infatti, è risultato molto più scorrevole (4.63±0.40 cm/60 s) del campione B (1.86±0.59 cm/60s).

PROPRIETÀ REOLOGICHE

di miscela pangrattato/acqua (200g campione + 350mL H₂O distillata) valutato con un metodo non-convenzionale (Fanninagro Brabender, Quidburg, Germany).

Condizioni:
 - 1 minuto di miscelazione
 - aggiunta di acqua entro 3 minuti
 - miscelazione a velocità costante (83 rpm)
 - miscelazione continua per 20 minuti a 30°C

Indici considerati:
 Consistenza finale dopo 20 minuti di miscelazione (= resistenza alla miscelazione, UB)

I traccati faninografici mostrano come dopo i primi minuti di miscelazione ed idratazione i campioni sono caratterizzati un diverso comportamento reologico. A conferma di quanto evidenziato precedentemente, il campione B presenta un incremento di consistenza molto più rapido e raggiunge una consistenza finale superiore rispetto al campione A.

CAMPIONE	Hardness (N)	Compressibility (N/mm)	PLSI
A	63.7 ± 3.1*	8.9 ± 0.9*	0.88 ± 0.02*
B	48.4 ± 4.1*	26.4 ± 4.2*	0.71 ± 0.01*

Il campione B è risultato più consistente (F1) del campione A, ma possiede una minor capacità di mantenere la propria struttura (FS-F1) in condizioni di stress meccanici ripetuti.

PROPRIETÀ FISICHE

di miscela pangrattato/acqua, mediante test di compressione multipla con dinamometro TA-HDplus (Texture Analyzer, Stable Micro Systems, Surrey, UK).

Condizioni:
 - 17 g di miscela ottenuta con il Fanninagro nelle condizioni sopra indicate, condizionata per 45 minuti a 20°C
 - compressione del campione posizionato in un contenitore cilindrico (diametro = 6 cm, altezza = 1 cm) con un pistone avente diametro pari a 4.5 cm (velocità di compressione pari a 1 mm/s, frequenza pari a 0.5Hz)

Indici considerati:
 - Hardness: massima forza registrata al 1° ciclo di compressione (F1: N)
 - Compressibility: energia necessaria per comprimere il campione durante la prima compressione (N*mm)
 - PLSI: rapporto tra la forza massima registrata al 1° ciclo di compressione (FS) e quella al 1° ciclo (F1)

Conclusioni

Dalla presente sperimentazione è emerso che le analisi chimiche non sono in grado di rilevare differenze importanti tra i due campioni commerciali di pangrattato. Tali campioni, utilizzati indifferentemente dal produttore per la produzione del ripieno per pasta, causavano tuttavia problematiche impiantistiche, a livello delle macchine formatrici.

Un parametro maggiormente discriminante è risultato essere la granulometria del prodotto, caratteristica che tuttavia non fornisce alcuna indicazione sulla capacità ispessente del materiale.

Al contrario i differenti test reologici messi a punto nel corso della sperimentazione si sono dimostrati idonei nel discriminare due materiali apparentemente simili ed in grado di definire la diversa attitudine tecnologica delle due tipologie di pangrattato. In particolare, l'impiego di un consistometro di Bostwick, strumento di basso costo, facile uso ed in grado di fornire una risposta in tempi brevi, potrebbe rappresentare un mezzo per giungere ad una standardizzazione delle caratteristiche reologiche di un ripieno.

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