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# Integrated Strategies to Improve the Quality of Wheat End-Products: Optimization of Processes and Development of Functional Food

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## PhD RESEARCH PROGRAM

**Strategie integrate per il miglioramento qualitativo di prodotti da forno a base di grano: ottimizzazione dei processi produttivi e sviluppo di alimenti funzionali.**

Sono state sviluppate tecnologie applicate ai prodotti da forno per il miglioramento dei processi produttivi e lo studio di alimenti funzionali. In particolare è stato condotto uno studio su trattamenti sanitizzanti al fine di ridurre le contaminazioni microbiche (soprattutto lieviti) sul prodotto finito. E' stata proposta una soluzione a base di perossido e argento colloidale in confronto con soluzioni fenoliche OPP. Il monitoraggio, biennale, è stato svolto nelle celle di lievitazione e raffreddamento del prodotto finito (pane di grano duro) ed è stata rilevata l'incidenza microbica percentuale. I ceppi sono stati isolati e sequenziati per l'identificazione specie/specifica. Per la riduzione degli scarti di natura plastica nel settore alimentare sono state eseguite prove sullo spessore del packaging alimentare applicato ai prodotti da forno. In particolare sono stati comparati campioni di pane confezionato con due tipologie di macchine confezionatrici e le differenze (chimico-fisiche, sensoriali e strutturali) sono state monitorate fino a 100gg di shelf-life. Sempre nella tematica miglioramento dei processi produttivi, sono stati caratterizzati gli scarti industriali della mandorlicoltura (cuticole e acque di pelatura) per sviluppare semilavorati per produrre alimenti funzionali, in particolare biscotti. Inoltre sono state svolte prove industriali per la messa a punto di alimenti funzionali. E' stato sviluppato un pane iposodico con livelli di sale secondo la normativa in vigore (REG 1924/2006) che è stato caratterizzato dal punto di vista morfologico, chimico e sensoriale. E' stata messa a punto una miscela enzimatica a base di amilasi e lipasi per la riduzione dello staling e il miglioramento della shelf life del pane industriale di grano duro. Valutazioni chimico-fisiche, della texture e della microstruttura sono state condotte per monitorare gli effetti degli enzimi sul prodotto finito. Infine è' stata eseguita la caratterizzazione chimico fisica e sensoriale della Pagnotta del Dittaino DOP e della semola utilizzata per produrla. Sono stati esaminati campioni di tipo artigianale e industriale. Le attività di ricerca del presente programma di dottorato sono state svolte presso industria alimentare di prodotti da forno sita in provincia di Enna e presso industria lavorazione mandorle sita in provincia di Siracusa.

**Integrated strategies to improve the quality of wheat end-products: optimization of processes and development of functional food.**

Innovative technologies applied to baked goods for the improvement of processes and the development of functional foods were performed. In particolar, a study was conducted on sanitizing treatments in order to reduce the microbial contamination (spoilage yeasts) on the finished product. The aim was to evaluate the effects of solution of peroxide and colloidal silver treatment, in comparison with the conventional fumigating treatment, on the incidence of chalk defects of the market baked goods. The strains have been isolated and sequenced for species/specification identification. To the reduction of the plastic waste in food industries, another line of research was carried out on the thickness of the food packaging applied to the baked good. In particular, samples of bread are compared with two types of packaging machines and the differences (chemical-physical, sensory and textural attributes) have been monitored up to 100gg of shelf-life. Same topic of process optimization, including almond byproducts, trials were made by blanced water and almond skins dried at different thermal conditions. Industrial byproducts have been characterized to develop semi-finished products for functional food (cookies). In addition, industrial tests were performed for the development of functional foods. A low-salt bread with salt content according to the fixed limits of REG (EU) 1924/2006 on nutrition and health claims was characterized. It was developed an amylase and lipase formulation for staling reduction and the improvement of the shelf life. Chemical-physical, rheological parameters and the microstructure evaluations were carried out to monitor the effects of the enzymes on the finished product. The physico-chemical properties and sensory profile of durum wheat Dittaino PDO (Protected Designation of Origin) bread and quality of re-milled semolina used for its production were studied. The research activities of this PhD program were performed to the food industry of bakery products located in Assoro (EN, Italy) and at the almond processing industry located in Floridia (SR, Italy).

# Chapter 1

## General introduction and outline of the thesis

# 1. Introduction

## 1.1 Wheat

### 1.1.1 Wheat Industry

Wheat is a cereal grain of the genus Triticum within the grass family Poaceae. Australia's first wheat was grown at the Botanic Gardens in Sydney 200 years ago (Crew, 2005). During the 19th century, wheat farms were established in all the Australian colonies. Two thirds of the world's wheat production is used for human consumption and a sixth for livestock feed. Industry (which uses small quantities to produce starch, paste, malt, dextrose, gluten, alcohol, and other products), seed requirements and post harvest losses make up the remainder. Ten to twenty per cent of the daily diet of the global population is wheat or wheat products. Botanically, there are more than 30 000 wheat varieties, categorised into six major classes according to planting and harvesting dates as well as hardness, colour and shape of the kernels: (1) hard red spring, (2) hard red winter, (3) soft red winter, (4) hard white wheat, (5) soft white wheat and (6) durum. Hard wheats contain greater levels of protein and gluten and are primarily used for leavened bread making. Common white wheats and soft red winter wheats have low protein content and are used for pastries, crackers, cookies and cakes. Blends of soft and hard wheats are combined to make all-purpose flour. Durum wheat is the hardest wheat variety known and has larger, more elongated kernels that are more golden-orange in colour. Durum wheat has a dense structure with high protein content and high gluten strength. Durum wheat is used to produce pasta, semolina, couscous and Arabic flat breads. Today wheat is one of the world's most important grains, as it covers more of the earth's surface than any other grain crop. Annual global wheat production exceeds 650 million tonnes, making the world wheat market valuable. Wheat is Italy's most important grain crop, with 4,3 million tonnes of durum wheat and 2,8 million tonnes of soft wheat. (Mipaaf, 2017)

### 1.1.2 Wheat Structure

Wheat kernels consist of three distinct layers: the endosperm, bran and germ (Figure 1). The centre of the wheat kernel is the endosperm, which is abundant in cellulose, starch and gluten,

surrounded by a single cell aleurone layer. The endosperm is about 83% of the weight of the kernel and contains 70–75% of the protein present in wheat. The bran layers surround the endosperm and are rich in vitamins and minerals as well as containing high-quality protein (19%) and large amounts of insoluble dietary fibre. The bran layer accounts for about 14.5% of the weight of the kernel. The germ is the embryo of the kernel and is only about 2.5% of the weight of the kernel. Wheat germ contains vitamins B and E, protein (8%), fat and minerals. Wheat is a nutritious food; it is an excellent source of carbohydrate, protein, vitamins, minerals and fibre. The wheat bran and germ layers removed during milling contain 75% of the phytonutrients (Slavin, 2003; Jones et al., 2004) in the wheat kernel. Two hundred and ten thousand phytochemicals present in plants have been isolated and characterised according to the Dictionary of Natural Products but a large percentage of phytochemicals remain unknown. 'Phytochemical' refers to every naturally occurring chemical substance present in plants, especially those that are biologically active (Zielinski and Kozlowska, 2000). The known phytochemicals in wholegrains and wheat include: dietary fibre, vitamins, minerals, lignans, phytoestrogens, phenolic compounds and phytic acid as well as benzoic and cinnamic acids, anthocyanidins, quinines, flavonols, chalcones, flavones, flavonones and amino phenolic compounds, flavonoids, coumarin derivatives, polyphenols, phytosterines, saponins, catechins, tocotrienls and tocopherol, tannin, cartenoids, ferulic acid and diferulates (Adom and Liu, 2002; Zielinski and Kozlowska, 2000; Slavin, 2003; Adom et al., 2003; Jones et al., 2004).

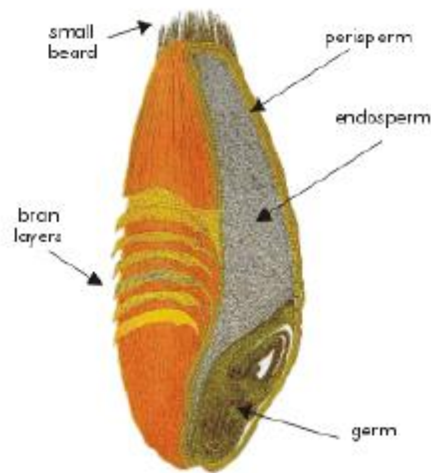


Fig. 1 Descriptive diagram of the kernel morphology

1.1.3 Wheat Processing

The main product from wheat is flour, which is produced by milling wheat kernels. There are many different techniques and processes in milling flour, depending on the types of wheat and their varying components. The endosperm is the source of white flour. Wheat bran is included in whole-wheat flour but is removed in the refining process during white flour production. Wholemeal flour is mainly white flour with wheat bran added. Germ is often separated from the flour during milling due to the fat content (approximately 10%) and essential oils, which limit the flour's shelf life. However, germ can be purchased alone and is included in wholewheat flour. In general, when wheat arrives at a mill it is assessed and graded. The wheat kernels are cleaned and soaked with water, which makes the outer bran layers easier to remove. Wheat is conveyed to the first pair of corrugated breaker rolls and this begins the separation process by removing the endosperm from the bran. The wheat endosperm is then ground and sifted to a course, granular meal called millings. Millings are further ground, sifted and purified to fine flour. Millers may then alter the flour to improve quality, by adding iron and vitamins and enhance its look by bleaching. During the milling of wheat kernels a few by-products are extracted. Gluten is concentrated in the endosperm and is a greyish white flour-like substance. When combined with water, gluten has a gluey texture

and is responsible for the elasticity in dough, enabling bread to rise by capturing the air bubbles. Gluten is also used in pet food, small goods, glues and other chemicals. Starch has many uses such as glues, fillers, confectionary, soft drinks, cordials, food thickeners, papermaking, textile sizing and mineral flocculation. Other by-products from the wheat milling process include glucose and bran. Wheat bran from a commercial milling process is likely to be a combination of pericarp, seed coat, aleurone and some remnants of the endosperm, since total separation is virtually impossible (Peyron et al., 2002).

## 1.2 Main topics

### *1.2.1 Microbial spoilage on wheat bread*

In bakeries, losses due to microbial spoilage varied between 1% and 3% depending on season, type of product and method of processing (Malkki and Rauha, 1978). Another estimate from one bakery in the US was 5% losses (Killian and Krueger, 1983). Even assuming only 1% losses, moulds could be spoiling over 23,000 tons of bread worth nearly £20 million in the UKevery year. Throughout Western Europe the annual losses could be around 225,000 tons of bread worth £242 million (Legan, 1993). Another estimate was a total loss of £200 million (Hickey, 1998). Mould growth on bread is a serious problem, especially in the UK (Saranraj and Geetha, 2012). During the years 1987–90 roughly 60% of bread spoilage was caused by moulds, 25% by bacteria, and only 15% by yeasts (Legan and Voysey, 1991). Nevertheless, yeast spoilage can be costly and inconvenient for the baker on occasions, especially on the continent. Problems due to spoilage of yeasts in bread usually result from post-baking contamination, slicing machines, bread coolers, conveyor belts and racks (Saranraj and Geetha, 2012). Many yeasts can produce visible growth on the surface of bakery products. Some of them, referred to as "chalk moulds" or "chalk yeasts" grow in low, white, spreading colonies that sometimes look like sprinkling of chalk dust on the product surface (Legan and Voysey, 1991). These authors reported that in the UK, *Hyphopichia burtonii* was the most frequently isolated chalk mould with *Saccharomycopsis fibuligera* found in one occasion. These two yeasts show mould-like structures called pseudo hyphae that are chains of budded yeast cells that did not separate after duplication (Barnett et al., 2000). Another troublesome yeast is Pichia anomala that can produce ethyl acetate in high yields from glucose or ethanol (Fredlund et al., 2004; Phaff et al.,

1978), but at aw's greater than 0.87 only (Tudor and Board, 1993). The odour of ethyl acetate is quite unexpected in bakery products and because of the “chemical” nature of the smell it generatesmore vigorous customer complaints than more obviously “fermented” odours (Legan and Voysey, 1991). The threshold of ethyl acetate is 0.87 ppm (Nagata, 2003). Several studies for modelling the effect of environmental factors on the growth of chalk yeasts were investigated. The combined effects of temperature, water activity, and solutes (Lahlali et al., 2008), temperature, water activity, NaCl, and pH (Burgain et., 2015) on the growth of P. anomala on synthetic media were modelled by second order polynomial models. The effect of water activity and pH, on the growth of *H. burtonii, P. anomala*, and *S. fibuligera* was examined on Malt Extract Agar by Deschuyffeleer et al. (2011). A good approach using efficient treatments on production areas is essential for the future development of adequate conservation strategies against chalk mould defects on bread, topic little studied in the last 15 years in the field of packed bread expecially in Italy.

### *1.2.2 Food packaging waste*

In today’s society, packaging is pervasive and essential. It surrounds, enhances and protects the goods we buy, from processing and manufacturing, through handling and storage, to the final consumer. Without packaging, materials handling would be a messy, inefficient and costly exercise and modern consumer marketing would be virtually impossible. The packaging sector represents about 2% of the gross national product (GNP) in developed countries, and about half of all packaging is used to package food. Packaging performs a series of disparate tasks: it protects its contents from contamination and spoilage, makes it easier to transport and store goods and provides uniform measuring of contents (Hine, 1995). By allowing brands to be created and standardized, it makes advertising meaningful and large-scale distribution possible. Special kinds of packages with dispensing caps, sprays and other convenience features make products easier to use. Packages serve as symbols of their contents and a way of life and, just as they can very powerfully communicate the satisfaction a product offers, they are equally potent symbols of wastefulness once the product is gone. Four primary functions of packaging have been identified: containment, protection, convenience and communication. These four functions are interconnected and all must be assessed and considered simultaneously in the package development process. The containment function of

packaging makes a huge contribution to protecting the environment from the myriad of products that are moved from one place to another on numerous occasions each day in any modern society. Faulty packaging (or under-packaging) could result in major pollution of the environment. Even today, the containment function of packaging is not always addressed satisfactorily, as evidenced by the number of packaged foods that leak their contents, especially around the closures and seals. Modern industrialized societies have brought about tremendous changes in lifestyles and the packaging industry has had to respond to those changes. Now an ever-increasing number of households are single person, many couples either delay having children or opt not to at all and a greater percentage of women are in the workforce than ever before. All these changes, as well as other factors such as the trend toward "grazing" (i.e., eating snack-type meals frequently and on the run rather than regular meals), the demand for a wide variety of food and drink at outdoor functions such as sports events, and increased leisure time, have created a demand for greater convenience in household products. Products designed to increase convenience include foods that are preprepared and can be cooked or reheated in a very short time, preferably without removing them from their primary package, and sauces, dressings and condiments that can be applied simply through aerosol or pump-action packages that minimize mess. Thus, packaging plays an important role in meeting the demands of consumers for convenience. Convenient packages promote sales. Food packaging has done enormous progress in the last decades, driven by the increasing demand for high-quality, safe food and by the growing concern towards environmental issues. The role of food packaging in the overall sustainability of food productions is controversial: the popular belief that packaging is responsible for high environmental impacts collides with scientific evidence of packaging benefits in terms of food waste reduction potential. Overall, the positive environmental role of food packaging is wellestablished for the insiders, while it should be clarified to the public opinion, whose position remains somehow hostile. Packaging reputation by consumers can be inferred from a study by Tanner and Kast (2003) who report that an environment-friendly food product is, ideally, "domestically produced rather than imported from abroad; furthermore, it is organically grown, seasonal, fresh (rather than frozen), and unwrapped". Indeed, it is unquestionable that packages are responsible for some environmental impact associated with their life cycles (Huang and Ma, 2004; Ingrao et al., 2015a), and especially with the production

of rawmaterials, processing and end-of-life phase, including recycling, incineration and landfill disposal. Recent studies on the life cycle impact of various packaging materials have increased awareness and made available useful information which can represent the basis for environmentally-responsible choices (Siracusa et al., 2014; Speck et al., 2015). According to Peelman et al. (2013), sustainability of food packaging can be achieved at three levels; 1) at the raw materials level: the use of recycled materials and of renewable resources are two strategies for reducing $CO_2$ emission and the recourse to fossil resources; 2) at the production level, through more energy-efficient processes; 3) at the waste management level, considering reuse, recycling and biodegradation. On one hand, much effort has been dedicated to decreasing the packaging impacts, by the development of novel biobased materials and through the optimization of packaging use and the improvement of materials performances which, in turn, allows the shift to lighter and thinner packages. On the other hand, packaging innovations have been developed, with the aim of increasing the packaged product quality, extending the shelf life and ultimately reducing the possibility of food to turn into waste. Plastics are the most widely used materials for packaging purposes, due to several advantages such as low cost and light weight, high versatility, flexibility, transparency, heat sealability, good mechanical and barrier performances. The high consumption of packaging is accompanied by a hugewaste generation: according to data from EU-28 referred to 2013, 156.9 kg of packaging waste was generated per inhabitant (Eurostat), plastic representing 19% of the total consumed plastic. The end of life of plastics, especially, raises environmental concern, as these materials are not biodegradable and they are difficult to recycle (Sorrentino et al., 2007). Michaud, Laura Farrant, and Jan (2010) proved that mechanical recycling is the most environmentally favourable option for waste treatment of plastics, followed by incineration and landfill. However, recycling is not always viable for food packages. Siracusa et al. (2008) observed that recycling of food packaging materials is often impracticable or non-convenient since they are contaminated by food residues, suggesting that biopolymers can be considered a solution to waste-disposal problems associated with synthetic plastics. Apart from the environmental problems caused by plastic packaging waste treatment, conventional materials contribute to the depletion of fossil resources. Another important issue in sustainable packaging is materials reduction which, unexpectedly, still represents a viable path towards the improvement of

sustainability. The concept of "overpackaging" is not new, however, the fight against overpackaging still offers good potential for sustainability improvement.

*1.2.3 By-products of food industry*

Fruit and vegetables have been recognized as important sources for a wide array of non digestible components and phytochemicals that individually, or in combination, may act synergistically to contribute to the nutritional and health benefits of these food commodities. World Health Organization (WHO) and worldwide health authorities such as United States Department of Agriculture (USDA) promote a high consumption and variety of fruit and vegetables. In addition, the source of fibre in a healthy dietary pattern such as Mediterranean Diet has been described as an important qualitative difference on health. Fruit and vegetable related dietary fibre transports a significant amount of polyphenols and carotenoids linked to the fibre matrix through the human gut. Despite these effects and recommendations, the intake of plant foods remains low and, consequently, both dietary fibre and antioxidant compounds are usually deficient in most diets around the world. On the other hand, the food industry processing plant foods produces large amounts of waste and residues (leaves, stems, wastewaters, etc.) that are good sources of dietary fibre and phytochemicals. Fruit and vegetables, have been consistently identified in epidemiological research as the key components of dietary patterns that reduce risk for the development of chronic and degenerative diseases, including atherosclerotic cardiovascular diseases, insulin resistance and type II diabetes and many cancers (Hu et al., 2000). One of the predominant mechanisms of their protective action is due to their antioxidant activity and the capacity to scavenge free radicals. There has been increasing interest in the nutritional properties of fruit and vegetables as sources of dietary fibre and other health-promoting phytochemical compounds (Kris-Etherton et al., 2004; Most, 2004). Byproducts obtained when processing cereal, algae, fruit and vegetables can be added as functional ingredients, providing advantageous dietary fibre and bioactive compounds. These byproducts serve as non-caloric bulking agents, enhance water and oil retention, and improve emulsion and oxidative stability. The literature reports addition of fibre to food products such as baked goods, beverages, confectionary, dairy, meat and pasta (Elleuch et al., 2011). Addition of byproducts in bakery products are muffin butter supplemented with peach dietary fibre (Grigelmo-Miguel et al., 1999), and cake dough

enhanced with prickly pear cladode fibre (Ayadi et al., 2009) at levels up to 5%. Nuts are oily food products traditionally associated with the Mediterranean diet. Their regular consumption, in moderate doses, reduces the blood levels of total cholesterol and LDL cholesterol and they are associated, together with other foods, with a lower incidence of cardiovascular diseases. In addition to their lipid content and profile, the beneficial effects of nuts on human health are attributed to the presence of compounds with antioxidant activity (Wijeratne et al., 2006), among others. These antioxidant compounds are found both in the seed and in various coatings of the fruits and prevent or delay the oxidation of fatty acids. Almonds (*Prunus dulcis* [Mill.] D. A. Webb) are eaten raw, roasted, fried, or caramelized. They can also be used to make snacks, desserts, salads, sauces, pates, ice creams, and some sweets such as "turr´on" and marzipans. On other occasions, the almond is processed tomake nutritional products such as almondmilk used as a substitute cow'smilk or as dietary supplements (Aranceta et al., 2006). Industrial processing of almonds starts with removal of the external coating (mesocarp or green shell cover) by natural drying or heat treatment. In a subsequent step, the brown hull is removed, obtaining the whole almond with its skin (seed coat). The almond can then be used after roasting it in an industrial oven, or the skinless almond can be obtained by separating the tegument by blanching and later peeling. The industrial process yield for the almond seed varies from 20% to 48% depending on the variety, with the skin contributing to around 6.0% to 8.4% of the seed (Soler et al., 1988). The percentage of by-products obtained from the industrial processing of almonds is, therefore, very high, which has led to the need for the industry to consider ways of treating or using these by-products. At present, they are mainly used for cattle feed (Grasser et al., 1995), although they are also used to obtain energy in gasification plants (Gonzalez et al., 2006), and active carbon can be produced from the almond shells (Ayg¨un et al., 2003). Over the past few years, several studies have focused on the phenolic composition of these by-products, and we now know that the composition of almond skins is based on hydroxybenzoic acids and aldehydes, hydroxycinnamic acids, flavan-3-ols, flavonols, dihydroflavonols, and flavanones (Brieskorn et al., 1998; Lazarus et al., 1999; Frison and Sporns 2002; Sang et al., 2002; Chen et al, 2005; Milbury et al., 2006; Wijeratne et al., 2006; Monagas et al., 2007). Recently, it was found that the flavan-3-ol composition of almond skins comprises proanthocyanidins of both B-type (linked through C4→C8 or C4→C6 bonds) and A-type (linked through C4→C8 or C4→C6 and

C2→O7 bonds) (Monagas et al., 2007). All these compounds possess antioxidant capacity as well as other properties. It is noteworthy that the flavonols and flavan-3-ols have numerous beneficial effects on the gastrointestinal tract as well as antiviral, antiinflammatory, antiallergic, antimutagenic, anticarcinogenic, and anticholesterolemic activities (Sanchez-Moreno, 2002; Brahmachari and Gorai, 2006). The antioxidant activity of almond by-products has been studied using several methodologies (Siriwardhana and Shahidi 2002; Wijeratne et al., 2006; Garrido et al., 2007; Harrison and Were, 2007). Based on these findings, almond skins could be used to obtain compounds/fractions with good antioxidant properties, which can be used as additives to control oxidative processes in the food industry or as functional ingredients in dietary supplements (Bartolome et al., 2007).

*1.2.4 Functional foods*

Many lifestyle modifications have been proved to be helpful for the prevention and control of high bloody pressure and, in turn, for reduction of cardiovascular risk, including, besides reduction of sodium chloride intake, correction of overweight, regular physical exercise, higher fruit and vegetable consumption, and avoidance of excess alcohol (Whelton et al., 2003). The beneficial effects of these measures are at least partly additive. A bulk of strong and consistent evidence indicates that excess salt intake endangers our health and that reducing salt consumption at the population level is a highly cost effective potential instrument to reduce CVD risk (WHO, 2007; Appel et al, 1997; Strazzullo et al. 2009), to the extent that its implementation has been authoritatively indicated as the second of five immediate priority actions for prevention of non-communicable diseases (Beaglehole et al., 2011). As dietary habits are developed during childhood (Beauchamp and Mennella, 2009), including the preference for salted foods (Strazzullo et al, 2012b), education to keep a low dietary salt and an adequate potassium intake during childhood is crucial (Strazzullo et al., 2012a; Donfrancesco et al., 2012). In a recent study (Campanozzi et al., 2015) about salt consumption by italian children and adolescents the data indicate that the average daily sodium consumption in Italy exceeds the official recommendations in both genders, in all age categories and, in all regions. There were several european and worldwide initiatives for reduction of salt intake. A WHO/FAO joint report in 2003 recommended a reduction of salt intake at the population level to no more than 5 g/ day (2 g or 87 mmol of sodium), while

ensuring adequate iodisation of salt (WHO, 2003); WHO released updated recommendations about the implementation of measures useful to achieve reduction of population salt intake (WHO, 2006); in 2008, an European Salt Action Network (ESAN) was established under the auspices of WHO and the support of the UK Food Standards Agency (FSA), with the aim to promote harmonisation of salt intake reduction programs in the EU member states, among which Italy; Working Group for Dietary Salt Reduction in Italy (GIRCSI), established in October 2007 has established a strategic collaboration with the Department of Prevention and Communication of the Italian Ministry of Health, and has recognised the importance of seeking the collaboration of a number of stakeholders, identified in: food industry representatives at a national and local level, governmental agencies and public institutions such as the National Institute of Health (ISS), the National Research Institute on Food and Nutrition (INRAN) and the Institute of Food Science (CNR-ISA), local administrations, communication media, academic institutions, the catering system, the food sales organisations, the public and private school system, non-governmental social and religious organisations, consumer representatives and professional organizations (Strazzullo et al., 2011). Natural foods in fact contain quite modest amounts of sodium (Eaton et al, 1985) and presently up to two thirds of the total salt content of a Western diet is not discretionary, salt having been added to the foods during their industrial preparation (Leclercq et al., 1991). It was roughly estimated that 15% of the total was added either at the table or in the cooking, approximately 5% was naturally present in the food (i.e. 0.6 g) and the remaining 80% (7.5 g) was ‘courtesy’ of the food industry. Eighty food categories were identified as major contributors to sodium chloride intake and targets were set for each category that the food industry was asked to meet within a certain time period. The advent of modern storage techniques has obviously reduced the need to use salt to this end: nevertheless, added salt still plays a multi-purpose role in many manufactured foods and drinks. While these technological issues ought to be taken into account and dealt within proper ways, the food industry may be tempted to exploit them to slow down the product reformulation process because the use of salt in foods, beyond minimal requirements, may produce economical advantages in several ways, e.g. by favouring adaptation of the customers’ taste to saltiness and making low quality food more palatable. GIRCSI and the Ministry of Health proposed The food reformulation strategy requires identification of the products making the largest contribution to the

population salt intake, transfer of information to producers as well as to consumers, development of a concerted action with the producers aiming at lowering the salt content of those particular products, regular monitoring of the salt content of foods, assistance to small local enterprises in reducing their products' salt content, encouragement to highlight the salt content of foods in the food shops. Initiatives for reduction of the salt content of bread and bakery products, a major source of sodium chloride in Italy. Recently, the Reg (EU) 1924/2006 on nutrition and health claims made on foods makes it possible to claims on a label 'low sodium/salt' (<0.12g/100g), 'very low sodium/salt' (<0.04g/100g) and 'sodium free or salt free' for foods containing low salt levels, according to the fixed limit.

*1.2.5 Enzymes on baked goods*

Bread is an unstable, elastic, solid foam, the solid part of which contains a continuous phase composed in part of an elastic network of cross-linked gluten molecules and in part of leached starch polymer molecules, primarily amylose, both uncomplexed and complexed with polar lipid molecules, and a discontinuous phase of entrapped, gelatinized, swollen, deformed (wheat) starch granules. Neither the bread system nor the staling process is understood well at the molecular level. Staling can be defined as the group of changes, other than the ones caused by spoilage microorganisms, which take place during bread storage and make the product less acceptable to consumers (Zobel et al., 1996). This process involves different physicochemical transformations. However, the firming of crumb with time is the most used parameter to follow bread staling during storage (Xu et al., 1992; Inagaki et al., 1992, Leon et al., 2002). Boyacioglu and D'Appolonia (1994) provided information about staling properties on durum wheat bread. However, bread firming and starch retrogradation are not synonymous, suggesting that other factors are involved (Ovadia et al., 1996). Despite the known facts, the staling mechanism is still a matter of debate. Several additives are utilized in bread formulas as shelf-life improvers to inhibit physical changes that lead to crumb firming. Surfactants and enzymes are the ones mostly employed for this purpose. Amylose-surfactant complexation is part of the explanation for the antifirming effect of commercial emulsifiers. These complexes may prevent amylose/amylopectin retrogradation or lead to fewer nuclei that could promote amylopectin retrogradation and the development of a crystalline structure. Among the enzymes added to bread recipes, $\alpha$-amylases are the more commonly used (Bano

et., 2011; Barrett et al., 2005). Blaszczak et al. (2004) verified the structural changes in wheat dough and flour with the addition of $\alpha$-amylases on a reduction of the starch level, whereas Martin and Hoseney (1991) assigned its effect to starch-protein or protein-protein entanglement interference by low molecular weight dextrins. Besides, lipases can be used to retard bread staling by forming monoglycerides, which act as surfactants (Van Eijk, 1996). Dextrins produced by enzymatic hydrolysis of starch interfere with amylopectin retrogradation (Leon et al., 1997; Rojas et al., 2001). Because the literature on bread staling is so extensive, any review of bread staling confined to a limited space cannot discuss all available information, hypotheses, or conclusions; nor can it give in-depth treatment to the aspects covered. It is believed, however, that most important pieces of known information, concepts, principles, hypotheses, and conclusions are presented here. Several previous reviews on staling (the process, the mechanism, its measurement, and factors that affect it) have appeared (referred to elsewhere and in the references), and 2 books (Hebeda and Zobel 1996; Chinachoti and Vodovotz 2000) are available for a more thorough treatment. To understand the mechanism of staling in breads, it is important to understand the natures of the major components that make up the system. Protein, hydrated gluten is the continuous phase of wheat flour doughs (Davies, 1986). During baking, gluten is denatured, and protein-protein crosslinking occurs via formation of disulfide bonds (Schofield, 1986). The resulting network, combined with partially gelatinized starch granules, is most certainly responsible for the semirigid structure of baked products (Blanshard, 1988; Palacios et al., 2004). Wheat flour contains 84 to 88% (db) of starch. During baking of bread dough, the starch granules are generally gelatinized, but little else other than restricted swelling followed by collapse happens to them because of the limited amount of water present in the dough system, so deformed wheat starch granules can be isolated from the crumb (Hoseney et al., 1978, Purhagen et al., 2011). Ragarding Non starch polysaccharides, arabinoxylans and arabinogalactans (arabinogalactan-proteins) are the “pentosans” (more properly pentoglycans) of wheat flour. Arabinoxylans are divided into 2 classes (“water-soluble” and “water-insoluble”) and have been much more extensively studied than have the arabinogalactans (Loosveld et al., 1997), because they are present in greater concentrations and are believed to play a more important role in both the preparation and the shelf-life of bakery products. Both classes of arabinoxylans of hard wheat flours have been investigated with

regards to structure (Izydorczyk et al., 1991; Izydorczyk and Biliaderis 2000) and to differences in structure as a function of cultivars (Cleemput et al., 1993; Izydorczyk and Biliaderis 1993; Rattan et al., 1994). Their influence on breadmaking and bread quality is still being debated.

*1.2.6 PDO of durum wheat bread*

During the last decades, European and worldwide consumers have come ever more to appreciate traditional and typical foods. Traditional is the definition used for foods that historically are part of the cultural heritage of people living in a certain geographical area (European Parliament & European Council, 2012). Typical is the attribute used for a food produced using one or more ingredients having characteristics strictly depending on the geographical area it comes from (D'amico, 2004). Mainly due to the long history of regional political division, about 200 different types of bread are manufactured throughout Italy with large differences of recipes and traditions (Atlante dei pani tipici, 2000). Various types of bread are produced from durum wheat in Italy, mainly in the southern regions and in the major islands. Some of these breads are very popular with consumers due to their desirable sensory properties and good resistance to staling (Grosch and Schieberle, 1997; Raffo et al., 2003). Since these breads are not produced at industrial levels, their production processes still exhibit artisanal characteristics, and they are therefore more expensive to produce. To ensure the survival of these products, the bakers have asked for either protected denomination of origin (PDO) or protected geographical indication (PGI) status (European Union, 2003; Istituto Poligrafico e Zecca dello Stato (2004). Some breads have already received the Protected Designation of Origin (PDO) (Pane di Altamura and Pagnotta del Dittaino) or the Protected Geographical Indication (PGI) (Pane di Matera, Pane Casareccio di Genzano, and Coppia Ferrarese). In spite of the differences, almost all traditional/typical Italian breads use sourdough as the natural starter. Among them, Altamura PDO bread and Dittaino PDO bread, although being produced using different cultivars and in different areas, are both obtained from durum wheat re-milled semolina, according to a bread-making tradition consolidated in Southern Italy (Pasqualone, 2012). Altamura PDO bread has been extensively studied (Bianchi et al., 2008; Brescia et al., 2007; Chiavaro et al., 2008; Pasqualone et al., 2007; Pasqualone et al., 2010). On the contrary, no research has been aimed until now to the quality characterization

of Dittaino PDO bread, apart the inclusion of its sourdough in an array of samples for a survey on microbiotas used for traditional/typical Italian breads (Minervini et al., 2012).

### 1.3 Outline of the thesis

This thesis investigated new strategies to improve the quality of baked goods, focused main objectives from processes to final product. In order to accomplish those general objectives, the current PhD dissertation is thus divided into six coherent chapters. Basically, the activity I investigated the opportunity to reduce microbial defects on bread using innovative chemical treatment on food industry plant. The actvity II investigated the way to reduce wastes on food company especially for packaging, several trials to compare three different system were performed. The third activity developed several protocols to extract compounds by food by-products (in particular from almonds processes) in order to obtain functional food. In the same topic we carried out some trials (activity IV) to produce low salt content bread according to the 'health claims' of REG UE 1924/2006. Activity number 5 provides knowledge about new formulation of mix enzymes to reduce staling on industrial packed bread. The final activity (VI) concerns the characterization of PDO Dittaino bread as example of agricultural system.

### 1.4 References

AACC. (2000). Approved Methods of the American Association of Cereal Chemists. 10th Ed. Vol. II. AACC Methods 74-09, 74-10A, and 74-30. American Association of Cereal Chemists. St. Paul, Minn.

Adom K. K. and Liu R. H. (2002) Antioxidant Activity of Grains. *Journal of Agricultural and Food Chemistry,* 50**:** 6182-6187.

Adom K. K., Sorrells M. E., Liu R. H. (2003) Phytochemical Profiles and Antioxidant Activity of Wheat Varieties. *Journal of Agricultural and Food Chemistry,* 51**:** 7825-7834.

Appel L.J., Moore T.J., Obarzanek E., Vollmer W.M., Svetkey L.P., Sacks F.M. (1997). A clinical trial of the effects of dietary patterns on blood pressure. *N Engl J Med*; 336 (16):1117-1124.

Aranceta J, Perez Rodrigo C., Naska A., Ruiz Vadillo V., Trichopoulou A. (2006). Nut consumption in Spain and other countries. *Brit J Nutr*, 96: S 3–11.

Ayadi M. A., Abdelmaksoud W., Ennouri M., Attia H. (2009). Cladodes from *Opuntia ficus indica* as a source of dietary fiber: Effect on dough characteristics and cake making. *Industrial Crops and Products,* Vol.30, No.1, 40-47.

Aygün A., Yenisoy-Karakaş S., Duman I. (2003). Production of granular activated carbon from fruit stones and nutshells and evaluation of their physical, chemical and adsorption properties. *Microporous and Mesoporous Materials*, 66(2-3): 189-195.

Bano S., Ul Qader S.A., Aman A., Syed M.N., Azhar A. (2011). Purification and Characterization of Novel $\alpha$-Amylase from Bacillus subtilis KIBGE HAS. *American Association of Pharmaceutical Scientists*, 255-261.

Barnett J. A., Payne R. W., Yarrow D. (2000). Yeasts: Characteristics and Identification, 3rd Edn. Cambridge: Cambridge University Press.

Barrett A. H., Marando G., Leung H., Kaletunç G. (2005). Effect of different enzymes on the textural stability of shelf-stable bread. *Cereal Chemistry*, 82: 152–157.

Bartolome B, Monagas M., Garrido I., Lebr´on-Aguilar R., Quintela J.C., De la Fuente E., Jara A., Gomez-Cordoves C. (2007). Inventors; CSIC and EXXENTIA, assignee. 2007 June 12. Almond skins phenolic extracts containing procyanidins, properlagonidins and prodelphinidins, and their procedure of obtention. Patent ES200701616.

Beaglehole R., Bonita R., Horton R., Adams C., Alleyne G., Asaria P, et al. (2011). Priority actions for the non-communicable disease crisis. *Lancet*, 377: 1438-1447.

Beauchamp G.K., Mennella J.A. (2009). Early flavour learning and its impact on later feeding behaviour. JPGN: S25–S30.

Bianchi F., Careri M., Chiavaro E., Musci M., Vittadini E. (2008). Gas chromatographic–mass spectrometric characterisation of the Italian protected designation of origin "Altamura" bread volatile profile. *Food Chemistry*, 110: 787–793.

Blanshard J.M.V. (1988). Elements of cereal product structure. In: Blanshard JMV, Mitchell JR, editors. Food Structure – Its Creation and Evaluation. London: Butterworths, 313-330.

Błaszczak W., Sadowska J., Rosell C. M., Fornal J. (2004). Structural changes in the wheat dough and bread with the addition of alpha-amylases. *European Food Research and Technology*, 219: 348–354.

Boyacioglu M. H., D'Appolonia B. L. (1994). Characterization and utilization of durum wheat for breadmaking. III. Staling properties of bread baked from bread wheat flours and durum wheat flours. *Cereal Chemistry*, *71:* 34–41.

Brachmachari G., Gorai D. (2006). Progress in the research on naturally occurring flavones and flavonols: an overview. *Curr Org Chem* 10(8):873–898.

Brescia M. A., Sacco D., Sgaramella A., Pasqualone A., Simeone R., Peri, G., Sacco A. (2007). Characterisation of different typical Italian breads by means of traditional, spectroscopic and image analyses. *Food Chemistry*, 104: 429–438.

Brieskon C.H., Betz R. (1998). Procyanidin polymers crucial to the structure of the almond seed coat. Z Lebensm Unters Forsch 187: 347–353.

Campanozzi A, Avallone S, Barbato A, Iacone R, Russo O, De Filippo G, et al. (2015) High Sodium and Low Potassium Intake among Italian Children: Relationship with Age, Body Mass and Blood Pressure. *PLoS ONE* 10(4): 0121183

Chen C.Y., Milbur P.E., Lapsley K., Blumberg J.B. (2005). Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. *J Nutr* 135: 1366–1373.

Chiavaro E., Vittadini E., Musci M., Bianchi F., Curti E. (2008). Shelf-life stability of artisanally and industrially produced durum wheat sourdough bread ("Altamura bread"). *LWT-Food Science and Technology*, 41: 58–70.

Chinachoti P., Vodovotz Y., editors. (2000). Bread Staling. Boca Raton, Fla.: CRC Press.

Cleemput G., Roels S.P., Van Oort M., Grobet P.J., Delcour J.A. (1993). Heterogeneity in the structure of water-soluble arabinoxylans in European wheat flours of variable bread-making quality. *Cereal Chem*, 70: 324.

Crew G. (2005) *Australian Primary Industries - Wheat,* from viewed 18th March, http://www.rochedalss.eq.edu.au/wheat.htm .

D'Amico A. (2004). The enhancement of the typical products value: From commodity to experience. The case of Esperya.com. *British Food Journal*, 106: 793–805.

Davies A.P. (1986). Protein functionality in bakery products. In: Blanshard JMV, Mitchell JR, editors. Chemistry and Physics of Baking. London: Butterworths, 313-330.

Deschuyffeleer N., Audeenaert K., Samapundo S., Ameye S., Eeckhout M., Devlieghere F., (2011). Identification and characterization of yeasts causing chalk mould defects on par-baked bread. *Food Microbiol.* 28: 1019–1027.

Donfrancesco C., Ippolito R., Lo Noce C., Palmieri L., Iacone R., Russo O., et al. (2013). Excess dietary sodium and inadequate potassium intake in Italy: results of the MINISAL study. *Nutr Metab Cardiovasc Dis*, 850-856.

Eaton S.B., Konner M. (1985). Paleolithic nutrition. A consideration of its nature and current implications. *N Engl J Med*, 312: 283-289.

Elleuch M., Bedigian D., Roiseux O., Besbes S., Blecker C., Attia H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry,* Vol.124, No.2, 411-421.

European Parliament and European Council (2012). Regulation (EU) no. 1151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs. Official Journal of the European Union, L343, 1–29.

European Union (2003) Official J Europ Union L181(July 19), EC Regulation 1291/2003, 46: 12–19.

Fredlund E., Blank L.M., Schnürer J., Sauer U., Passoth V., (2004). Oxygen- and glucosedependent regulation of central carbon metabolism in Pichia anomala. *Appl. Environ. Microbiol.*, 10: 5905–5911.

Frison S., Sporns P. (2002). Variation in the flavonol glycoside composition of almond seedcoats as determined by MALDI-TOF mass spectrometry. *J Agric Food Chem* 50: 6818–6822.

Garrido I., Monagas M., Gomez-Cordoves C., Bartolome B. (2007). Extraccion de antioxidantes a partir de subproductos del procesado de la almendra. Grasas y aceites. Grasas Aceites 58(2): 130–135.

Gonzalez J. F., Ganan J., Ramiro A., Gonzalez-Garcia C.M., Encinar J.M., Sabio E., Roman Suero S. (2006). Almond residues gasification plant for generation of electric power. Preliminary study. *Fuel Processing Technology* 87(2):149-155.

Grasser L.A., Fadel J.G., Garnett I., Depeters E.J. (1995). Quantity and economic importance of nine selected by-products used in California dairy rations. *Journal of Dairy Science,* 78: 962–971.

Grigelmo-Miguel N., Carreras-Boladeras E., Martín-Belloso O. (1999). Development of high-fruit-dietary-fibre muffins. *European Food Research and Technology*, Vol. 210, No. 2, 123-128.

Harrison K., Were L.M. (2007). Effect of gamma irradiation on total phenolic content yield & antioxidant capacity of Almond skin extracts. *Food Chemistry* 102(3):932-937.

Hebeda R.E., Zobel H. (1996). Baked Goods Freshness. New York: Marcel Dekker.

Hickey C.S. (1998). Sorbate spray application for protecting yeast-raised bakery products. *Baker's Digest* 54, 4–7.

Hine T. (1995). The total packaging: The secret history and hidden meanings of boxes, bottles, cans and other persuasive containers. New York: Little Brown.

Hu F. B., Rimm E. B., Stampfer M. J., Ascherio A., Spiegelman D., Willett W. C. (2000). Prospective study of major dietary patterns and risk of coronary heart disease in men. *The American Journal of Clinical Nutrition,* Vol.72, No.4, (October 2000), 912-921.

Huang C. C., Ma H.W. (2004). A multidimensional environmental evaluation of packaging materials. *Science of The Total Environment*, 324: 161-172.

Inagaki T., Seib P. A. (1992). Firming of bread crumb with cross-linked waxy barley starch substituted for wheat starch. *Cereal Chem.* 69: 321-325.

Ingrao C., Lo Giudice A., Bacenetti J., Mousavi Khanegha A., Sant'Ana A. D. S., Rana R., et al. (2015). Foamy polystyrene trays for fresh-meat packaging: Lifecycle inventory data collection and environmental impact assessment. *Food Research International*, 76: 418-426.

Istituto Nazionale di Sociologia Rurale. (2000). Atlante dei prodotti tipici: il pane, p 13. Agra-Rai Eri, Rome, Italy.

Istituto Poligrafico e Zecca dello Stato (2004) Italian Official Bulletin (Gazzetta Ufficiale della Republica Italiana) 51: 58–60.

Izydorczyk M., Biliaderis C.G., Bushuk W. (1991). Comparison of the structure and composition of water-soluble pentosans from different wheat varieties. *Cereal Chem*, 68: 139.

Izydorczyk M., Biliaderis C.G. (1995). Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydr Polym,* 28: 33.

Izydorczyk M., Biliaderis C.G. (2000). Structural and functional aspects of cereal arabinoxylans and b-glucans. *Dev. Food Sci* (Novel Macromolecules in Food Systems): 361.

Jones J. M., Reicks M., Adams J., Fulcher G., Marquart L. (2004) Becoming proactive with the whole-grains message. (Food Science). *Nutrition Today,* 39**,** 10 (18).

Killian D., Krueger J. (1983). Potassium Sorbate Spray Eliminates Returns due to Mold. *Baker Industry*, 54–55.

Kris-Etherton P. M., Lefevre M., Beecher G. R., Gross M. D., Keen C. L., Etherton T. D. (2004). Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annual Review of Nutrition,* Vol.24: 511-538.

Kulp K., Ponte J.G. (1981). Staling of white pan bread: fundamental causes. CRC Critical Rev Food *Sci Nutr* 15:1.

Lahlali R., Bajji M., Serrhini M.N., Jijakli, M.H. (2008). Modelling the effect of temperature, water activity and solute on the in vitro growth of the biocontrol yeast Pichia anomala strain K. *Biotechnol. Agron. Soc. Environ*. 12: 353–359.

Lazarus S.A., Adamson G.E., Hammerstone J.F., Schmitz H.H. (1999). High performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in food and beverages. *J Agric Food Chem* 47: 3693–3701.

Leclercq C., Ferro-Luzzi A. (1991). Total and domestic consumption of salt and their determinants in three regions of Italy. *Eur J Clin Nutr*,45: 151-159.

Legan J.D. (1993). Mould spoilage of bread: the problem and some solutions. *Int. Biodeterior. Biodegrad.* 32, 33–53.

Legan J.D., Voysey P.A. (1991). Yeast spoilage of bakery products and ingredients. *J. Appl. Bacteriol.* 70, 361–371.

Leon A., Duran E., De Baber C.B. (2002). Utilization of enzyme mixtures to retard bread crumb firming. *Journal of Agricolture and Food Chemistry, 50:* 1416-1419.

Leon A., Duran E.; Benedito de Barber C. (1997). A new approach to study starch changes occurring in the dough-baking process and during bread storage. *Z. Lebensm. Unters. Forsch., 204*: 316-320.

Loosveld A.M., Grobet P.J., Delcour J.A. (1997). Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. *J Agric Food Chem*, 45: 1998.

Martin M. L., Hoseney R. C. (1991). A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. *Cereal Chem*, 68: 503-507.

Michaud J.C., Laura Farrant L., and Jan O. (2010). Environmental Benefits of Recycling e 2010 Update. Available at: http://www.wrap.org.uk/sites/files/wrap/ Environmental_benefits_of_recycling_2010_update.3b174d59.8816.pdf accessed March 8, 2017.

Milbury P.E., Chen C.Y., Dolnikowski G.G., Blumberg J.B. (2006). Determination of flavonoids and phenolics and their distribution in almonds. *J Agric Food Chem* 54: 5027–5033.

Minervini F., Di Cagno R., Lattanzi A., De Angelis M., Antonielli L., Cardinali, G., Gobbetti, M. (2012). Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical Italian breads: Interactions between ingredients and microbial species diversity. *Applied and Environmental Microbiology*, 78: 1251–1264.

Ministero per le Politiche Agricole. (1999). Decreto ministeriale 8 Settembre 1999, no. 350. Gazzetta Officiale della Repubblica Italiana 12 Ottobre 1999, no. 240.

Monagas M., Hernandez-Ledesma B., Gomez-Cordoves C., Bartolome B. (2006). Comercial dietary ingredientes from *Vitis vinifera* L. leaves and grape skins: antioxidant and chemical characterization. *J Agric Food Chem* 54: 319–327.

Most M. M. (2004). Estimated phytochemical content of the dietary approaches to stop hypertension (DASH) diet is higher than in the control study diet. *Journal of the American Dietetic Association*, Vol.104, No.11: 1725-1727.

Nagata Y. (2003). Measurement of odor threshold by triangle odor bag method. Odor Measurement Review. *Japan Ministry of the Environment*, 118–127.

Ovadia D. Z., Walker C. E. (1996). Re-examination of the bread firming curve. *Starch*, 48, 137-144.

Palacios H. R., Schwarz P. B., D'Appolonia B. L. (2004). Effect of $\alpha$-amylases from different sources on the retrogradation and recrystallization of concentrated wheat starch gels: relationship to bread staling. *Journal of Agricultural and Food Chemistry*, *52*: 5978–5986.

Pasqualone A. (2012). Italian durum wheat breads. In M. T. Pedrosa Silva Clerici (Ed.). Bread consumption and health (pp. 57–79). Hauppage, NY: Nova Science Publisher Inc.

Pasqualone A., Alba V., Mangini G., Blanco A., Montemurro C. (2010). Durum wheat cultivar traceability in PDO Altamura bread by analysis of DNA microsatellites. *European Food Research and Technology*, 230: 723–729.

Pasqualone A., Summo C., Bilancia M. T., Caponio F. (2007). Variation of the sensory profile of durum wheat Altamura PDO (Protected Designation of Origin) bread during staling. *Journal of Food Science*, 72: S191–S196.

Peelman N., Ragaert P., De Meulenaer B., Adons D., Peeters R., Cardon L., et al. (2013). Application of bioplastics for food packaging. *Trends in Food Science & Technology*, 32(2): 128-141.

Peyron S., Chaurand M., Rouau X. and Abecassis J. (2002) Relationship between bran mechanical properties and milling behaviour of durum wheat (Triticum durum Desf.). Influence of tissue thickness and cell wall structure. *Journal of Cereal Science,* 36**:** 377-386.

Phaff H.J., Miller M.W., Mrak E.M., (1978). The Life of Yeasts. 2nd Ed. Harvard University. Cambridge, MA (154 pp.).

Purhagen J. K., Sjöö M. E., Eliasson A. C. (2011). Starch affecting anti-staling agents and their function in freestanding and pan-baked bread. *Food Hydrocolloids, 25*: 1656–1666.

Rattan O., Izydorczyk M.S., Biliaderis C.G. (1994). Structure and rheological behavior of arabinoxylans from Canadian bread wheat flours. *Food Sci Technol* (London) 27:550.

Regulation (EC) no 1924/2006 of the european parliament and of the council of 20 December 2006 on nutrition and health claims made on foods.

Rojas J. A., Rosell C. M., Benedito de Barber C. (2001). Role of maltodextrins in the staling of starch gels. *Eur. Food Res. Technol.,* 212: 364-368.

Sanchez-Moreno C. (2002). Compuestos polifenolicos: efectos fisiologicos. Actividad antioxidante. *Alimentaria* 329:29–40.

Sang S., Lapsley K., Jeong W.S., Lachance P.A., Ho C.T., Rosen R.T. (2002). Antioxidant phenolic compounds isolated from almond skins (*Prunus amygdalus* Batsch*). J Agric Food Chem* 50: 2459–2463.

Saranraj P., Geetha M. (2012). Microbial spoilage of bakery products and its control by preservatives. *Int. J. Pharm. Biol. Arch*. 3: 38–48.

Saura F., Canellas J., Soler L. (1988). La almendra. Composicion, variedades, desarrollo y maduraci´on. Madrid: INIA

Schofield J.D. (1986). Flour proteins: structure and functionality in baked products. In: Blanshard JMV, Frazier PJ, Galliard T, editors. Chemistry and Physics of Baking. London: The Royal Society of Chemistry, 14-29.

Siracusa V., Ingrao C., Lo Giudice A., Mbohwa C., Dalla Rosa M. (2014). Environmental assessment of a multilayer polymer bag for food packaging and preservation: An LCA approach. *Food Research International,* Volume 62: 151-161.

Siracusa V., Rocculi P., Romani S., Dalla Rosa M. (2008). Biodegradable polymers for food packaging: A review. *Trends in Food Science and Technology*, 19(12): 634-643.

Siriwardhana S.S.K.W., Shahidi F. (2002). Antiradical activity of extracts of almond and its by-products. *J AmOil Chem Soc* 79: 903–908.

Slade L., Oltzik R., Altomare R.E., Medcalf D.G. (1987). Accelerated staling of starch based products. U.S. Patent 4657770.

Slavin J. (2003) Why whole grains are protective: biological mechanisms. *Proceedings of the Nutrition Society,* 62**:** 129-134.

Soler L., Canellas J., Saura-Calixto F. (1988). Oil content and fatty acid composition of almond seeds. Journal of Agricultural and Food Chemistry, 36(4): 695-697.

Sorrentino A., Gorrasi G., Vittoria V. (2007). Potential perspectives of bionanocomposites for food packaging applications. *Trends in Food Science & Technology*, 18: 84-95.

Speck R., Selke S., Auras R., Fitzsimmons J. (2015). Choice of life cycle assessment software can impact packaging system decisions. *Packaging Technology and Science*, 28: 579-588.

Strazzullo P., Cairella G., Campanozzi A., Carcea M., Galeone D., Galletti F., et al. (2012). Population based strategy for dietary salt intake reduction: Italian initiatives in the European framework. *Nutr Metab Cardiovasc Dis*, 22: 161–166.

Strazzullo P., Campanozzi A., Avallone S. (2012). Does salt intake in the first two years of life affect the development of cardiovascular disorders in adulthood? *Nutr Metab Cardiovasc Dis,* 22: 787–792.

Strazzullo P., D'Elia L., Kandala N.B., Cappuccio F.P. (2009). Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. *Br Med J*, 339: 4567.

Tanner C., Kast S. W. (2003). Promoting sustainable consumption: Determinants of green purchases by Swiss consumers. *Psychology and Marketing*, 20: 883-902.

Tudor E.A., Board R.G., (1993). Food spoilage yeasts. In: Rose, A.H., Harrison, J.S. (Eds.), Yeast Technology vol. 5. Academic Press, London, UK, 436–516.

Van Eijk J. H.; Hille J. D. (1996). Nonamylolitic enzymes. In *Baked Goods Freshness*; Hebeda, R. E., Zobel, H. F., Eds.; Dekker: New York, 131-150.

Whelton P.K., He J., Gail T. (2003). Lifestyle modifications for the prevention and treatment of hypertension. Basel, New York: Marcel Dekker.

WHO (2007). Reducing salt intake in populations. Report of a WHO forum and technical meeting, 5 e 7 October 2006. Paris: WHO; 2007.

Wijeratne S.S.K, Abou-Zaid M.M., Shahidi F. (2006). Antioxidants polyphenols in almond and its coproducts. *J Agric Food Chem*, 54: 312–318.

Xu A., Chung O. K., Ponte J. G. (1992). Bread crumb amylograph studies. I. Effects of storage time, shortening, flour lipids, and surfactants. *Cereal Chem*., 69: 495-501.

Zielinsk H., Kozlowska H. (2000) Antioxidant Activity and Total Phenolics in Selected Cereal Grains and Their Different Morphological Fractions. *Journal of Agricultural and Food Chemistry,* 48**:** 2008-2016.

Zobel H.F., Young S.N., Rocca L.A. (1988). Starch gelatinization: an x-ray diffraction study. *Cereal Chem* 65: 443.

Zobel H. F., Kulp K. (1996). The staling mechanism. In *Baked Goods Freshness*; Hebeda, R. E., Zobel, H. F., Eds.; Dekker: New York, 1-64.

# Chapter 2

## Effects of innovative and conventional sanitizing treatments on the reduction of *Saccharomycopsis fibuligera* defects on industrial durum wheat bread

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**Abstract**

*Wickerhamomyces anomalus*, *Hyphopichia burtonii* and *Saccharomycopsis fibuligera* are spoilage yeasts causing chalk mold defects on sliced bread packaged under modified atmosphere. The first objective of this study, carried out in a bread-making company for two consecutive years, was to genetically identify yeasts isolated from spoiled sliced bread in Modified Atmosphere Packaging (MAP) and to determine the dominant species amongst identified strains. The second objective was to evaluate the effects of Hydrogen Peroxide and Silver solution 12% (HPS) treatment in the leavening cells and cooling chambers, in comparison with the conventional Ortho-Phenylphenol (OPP) fumigating treatment, on the incidence of chalk defects of the commercialized products. One-hundred percent of the isolated yeasts were identified as *S. fibuligera*, while *H. burtonii* and *W. anomalus* were not detected. Concerning mean water activity ($a_w$) and moisture content values, packaged bread samples were, respectively, included in the range 0.922-0.940 and 33.40-35.39%. *S. fibuligera* was able to grow in a wide range of temperature (11.5 to 28.5 °C) and relative humidity (70.00 to 80.17%) in the processing environments, and product $a_w < 0.94$. Compared to OPP, the combined treatment with hydrogen peroxide and silver solution, in association with MAP, reduced to a negligible level yeast contamination of industrial sliced bread. The identification of the spoilage organisms and a comprehensive understanding of the combined effects of $a_w$, $pO_2/pCO_2$ inside the packages, environmental conditions and sanitizing treatment on the growth behaviour is essential for future development of adequate preventive process strategies against chalk mold defects.

**Key words**: Chalk mold defects; Baked goods; 26S sequencing; MAP packaging; Water activity; Environmental biodecontamination

## 2.1 Introduction

Consumer preferences push towards alternative techniques that maintain the fresh and natural character of baked goods without the use of traditional chemical preservatives. Along with the increased interest towards health benefits, this trend is also known as green consumerism (Corbo et al., 2009). Bakery products are intermediate to high moisture products and by consequence highly susceptible to spoilage by fungi. To control this loss, manufacturers of bakery products use traditional chemical preservatives (i.e. sorbic, benzoic and propionic acid and their corresponding salts at ambient storage temperatures) or Modified Atmosphere Packaging (MAP). MAP is now commonly used as an alternative preservation technique for a specific type of bakery product namely, baked goods packed in protective atmosphere. The utilization of MAP meets consumer demands since only a gas mixture of nitrogen ($N_2$) and carbon dioxide ($CO_2$), generally applied at levels of 40 and 60% respectively (Galic et al., 2009), is used to prevent spoilage caused by aerobic microrganisms. $CO_2$ is the most important gas in the mixture since it is both fungistatic and bacteriostatic (Galic et al., 2009). In addition, the low levels of $O_2$ (<1%) ensure the inhibition of molds and other aerobic spoilage organisms. However, if under these strict conditions of MAP, baked goods are well protected against aerobic spoilage organisms, the growth of anaerobic microrganisms causing early spoilage of bread may occur. Among spoilage organisms, yeasts *Wickerhamomyces anomalus* (formerly *Pichia anomala*), *Hyphopichia burtonii* and *Saccharomycopsis fibuligera*, usually resulting from post-baking contamination, cause visually spoiled breads by growing in low, white, spreading colonies that sometimes look like sprinkling of chalk dust on the product surface (Legan and Voysey, 1991). Two of these yeast species, *H. burtonii* and *S. fibuligera*, even show structures resembling molds as they have the tendency to grow as hyphae and form mycelium. These hypha-like structures are called pseudo hypha and are actually chains of budded yeast cells that did not separate after duplication. Because of this close resemblance to molds and the white, powdery colonies they produce, *H. burtonii* and *S. fibuligera*, are also referred to as chalk molds (Legan and Voysey, 1991). With reference to the latter species, Suhr & Nielsen (2005) demonstrated that it is the least affected by the different $O_2$ residual levels, as it is not inhibited by any MAP treatments. Research on spoilage of bakery products has to date mainly focused on molds. Less attention has been paid to yeasts, probably because their public health significance is negligible, since human consumption of viable yeast cells present

in fermented foods and beverages had no adverse effects. However, the early bread spoilage caused by yeasts represents an actual problem for the bakery industry in terms of production losses. The majority of these microorganisms is carried by dust particles and water droplets suspended in the air, which constitute the so-called "bio aerosol." The formation of bio aerosol is influenced by different factors, like the use of high-pressure water for surface cleaning (Braymen, 1969; Spurlock and Zottola, 1991), air currents, relative humidity, and temperature (Stetzenbach, 2007). To contribute to the reduction of the bakery industry losses due to yeast spoilage, without affecting the additive-free character of MAP packaged bread, the main objectives of this study, carried out in an industrial bread-making company for two consecutive years, were to identify the spoilage yeasts of sliced bread and to evaluate the influence of environmental conditions, moisture content and water activity of bread, MAP packaging and two different sanitizing treatments applied to leavening and cooling chambers on the spoilage incidence.

## 2.2 Materials and methods

### *2.2.1. Study design*

The study was performed in a bread-making company located in Sicily (37°33′52.15″N 14°27′44.74″E), from August 2013 to December 2014.

### *2.2.2. Plant environmental conditions and sanitizing treatments*

The following processing areas of a bakery plant, as two of the main critical points in which microbiological contamination may occur, were considered: leavening cells and cooling cells (Fig. 1). Antimicrobial treatment, used from August to December 2013 (season 2013), consisted in 20% OrthoPhenylPhenol (Fumispore OPP, International PBI, Milano, Italy) conventionally used as fungicide of facilities and equipment used for the storage and production of foodstuffs intended for human consumption. A new generation commercial sanitizing product, based on 120mL/L hydrogen peroxide stabilized by 30 mg/Kg colloidal silver complex (Nocolyse ®, OXYPHARM, Champigny-Sur-Mame, France), manufactured according to ISO 9001 and EN 13485, was used from August to December 2014 (season 2014). Environmental temperature and relative humidity were measured by using agro-meteorological bulletin Decade of Agira

(EN), information service agro-meteorological Sicily Region. Weather parameters monitored included maximum and minimum daily air temperature, daily total precipitation, and daily relative humidity.

### 2.2.3. *Bread sample production and packaging*

A base dough formulation, consisting of durum wheat semolina, water (66% semolina basis), compressed yeast (0.47% semolina basis), salt (2.2% semolina basis), anti-staling enzyme (0.05% semolina basis) and mono and diglycerides of fatty acids (E 471, 0.22% semolina basis), was used to produce the bread. Dough was mixed for 17 min in high-speed mixer (Pietro Berto, Marano Vicentino (VI), Italy). Final dough temperature was 26±1 °C. The dough was rested in bulk for 15 min, scaled into 1160±20 g portions, placed into the proofer set and leavened at 32 ±1 °C and 66±2 % relative humidity (RH) for 150 min. The baking was carried out at 240 °C for 60 min, in industrial tunnel oven measuring 33 × 3 m (Pavailler Engineering, Galliate (NO), Italy). The baked loaves, weighting approximately 1.040 kg each, were automatically transported to the cooling chambers (S.L.C, Copit, Trecate (NO), Italy; Tecnopool, S. Giorgio in Bosco, Padova (PD), Italy) set at 20±1 °C for 120 and 75 min respectively. After cooling, the loaves were sliced by means of an automatic slicing machine (Brevetti Gasparin, Marano Vicentino (VI), Italy) to 11±1 mm thickness. About 500 g of sliced bread per loaf were packaged under modified atmosphere (70:30 $N_2$: $CO_2$). The packages were constituted by two plastic films (kindly provided by Cryovac Sealed Air, Elmwood Park, NJ, USA), one for the lid and another for the bottom. The latter was thermoformed (MIX 9000, Tecnosistem, Coccaglio (BS), Italy) into a bowl before inserting the bread slices. The characteristics of thickness, water vapor transmission rate (WVTR, g/m$^2$/24 h), and oxygen transmission rate (OTR, cm$^3$/m$^2$/24 h) of the bottom film (type T6011B, Cryovac Sealed Air, Elmwood Park, NJ, USA) were: 275 μm thickness, WVTR ≤ 10, OTR = 1; those of the lid film (type T9250B, Cryovac Sealed Air, Elmwood Park, NJ, USA) were: 125 μm thickness, WVTR < 10, OTR < 3. Nitrogen and carbon dioxide for bread packaging were supplied by SOL (Monza, Italy). Table 1 summarizes the sanitizing procedures and their frequency, the number of produced bread units per month, and the number of monitored bread units for calculating yeast spoilage incidence. Samples were stored for up to 90 days in the company warehouse at environmental temperature and RH. The determination of headspace composition of packages, moisture content and water

activity ($a_w$) of bread samples manifesting yeast spoilage was performed after 7 days of storage.

### *2.2.4. Determination of headspace gas composition of packed bread*

The internal $O_2$ and $CO_2$ composition of packages was monitored during the shelf-life by a Dansensor Checkpoint portable gas analyzer (Dansensor, Ringsted, Denmark) on three replicates during 90 days storage period, analyzing 10 mL of the package headspace (Rodriquez et al., 2003).

### *2.2.5. Determination of moisture content and water activity of bread*

Moisture content of bread crumb was determined by oven drying at 105 °C until constant weight according to AOAC method no. 945.15 (AOAC, 2000). Three bread slices (11±1 mm thickness) were used, and moisture was determined on one square crumb sample (40 mm diameter) taken from the center of each slice. $A_w$ was determined by Hygropalm 40 AW (Rotronic Instruments Ltd, Crawley, UK) according to manufactures' instructions. Three bread slices (11±1 mm thickness) were used, after the removal of the crust. For each set of determinations, separate loaves were used.

### *2.2.6. Isolation of spoilage yeasts from MAP packed bread*

For two consecutive production seasons, 2013 and 2014, once a month from August to December, about 10% of total monthly production in MAP packaging with 90 days labelled shelf life, was used to isolate spoilage yeasts. The bread samples were stored within the company warehouse and yeast isolation was performed after 7 days of storage on the ones presenting visual spoilage (so called chalk mold defects) (Fig. 2). Briefly, ten grams of each sliced bread sample, from different batches of the sliced bread loaves packed in MAP technique, were aseptically weighed and homogenized in a Stomacher (Bag Mixer®, Interscience, St Nom la Bretêche, France) for 60 s at room temperature (20±1 °C) with 90 mL of sterile Maximum Recovery Diluent (OXOID, CM0733, Basingstoke, UK). Further decimal dilutions were made with the same diluent. Rose Bengal Chloramphenicol Agar (OXOID, CM0549) supplemented with cloramphenicol selective supplement (OXOID, SR0078) and incubated at 25 °C for 48-72 h was used for yeast isolation. For the isolation and identification

of the spoilage organisms causing chalk mold defects, white and chalky colonies were picked from the plates with a sterile loop and streaked out three times on sterile YEPG agar (g/L distilled water: glucose 20, yeast extract 10, bacteriological peptone 10, agar 20) plates in order to obtain pure cultures, which were maintained at 4°C in sterile vials containing the same medium for subsequent use (Giannone et al., 2010).

#### *2.2.7. Identification of the isolated spoilage yeasts*

Yeast strains (84), isolated from sliced durum wheat bread, were grouped by PCR/RFLP of the internal transcribed spacer (ITS) regions (Esteve-Zarzoso et al., 1999). In details, the yeast strains were grown overnight in liquid YPD (g/L distilled water: yeast extract, 10; peptone, 10; dextrose, 20) at 27 °C with rotatory shaking. DNA was extracted from 3mL of this culture according to the method of Platania and co-workers (2012). The purified DNA was suspended in a solution containing MyTaq™ Mix (Bioline, London, UK). The rDNA ITS regions were amplified using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The amplification conditions were the following: 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 5 s. The digestion was performed on 5–10 μl of amplified DNA in a final volume of 20 μl with *Hae*III, *Hinf*I and *Hha*I restriction enzymes (New England BioLabs, Beverly, MA). Restriction fragments were quantified in a 2% Agarose D1 LE (Conda, Torrejón de Ardoz, Madrid, Spain) gel containing GelRed Nucleic Acid Gel Stain (Biotium Inc., Hayward, CA). The isolated yeast strains belonging to each pattern were analyzed by sequencing of the D1 and D2 domains of the 26S rRNA gene. For this purpose, PCR amplification of the D1-D2 region of 26S rRNA gene using the universal primers NL1 (50-GCATATCAATAAGCGGAGGAAAAG-30) and NL4 (50-GGTCCGTGTTTCAAGACGG-30) (Kurtzman and Robnett, 1998) was performed, and the resulting products were commercially sequenced. The corresponding sequences were finally compared with the sequences present in public data library (GenBank) using the Blast search program to determine their closest known relatives (Altschul et al., 1997; http://www.ncbi.nlm.nih.gov/BLAST/).

#### *2.2.8. Statistical analysis*

Data (at least in triplicate) of $pCO_2$, $pO_2$, $a_w$, crumb moisture, environmental temperature and relative humidity, yeast spoilage incidence (%) were subjected to one-way ANOVA, Duncan's multiple range test at $P < 0.05$, and Principal Component Analysis (PCA) using the statistical software SPSS (Statistical Package for Social Science) 8.0 for Windows. PCA analysis, using a correlation matrix, was carried out to find the effect of different sanitizing treatments on the occurrence of spoilage yeasts.

### 2.3. Results and Discussion

#### *2.3.1. Identification of spoilage yeasts*

The PCR amplification of the ITS regions yielded for all yeast isolates a fragment 680 bp long; also the subsequent digestion with *Hha* I, *Hinf*I and *Hae*III restriction endonucleases evidenced for all strains a unique restriction profile (size in bp: *Hha*I 330 + 330; *Hae*III 600 + 80; *Hinf*I 350 + 330), where restriction fragments smaller than 50 bp could not be visualized. A representative number (7) of the yeast isolates were randomly selected for the sequencing of the D1-D2 region of the 26S rRNA gene and, after the analysis of the percentage sequence identity to known isolates in the NCBI database, they were identified as *S. fibuligera* with 99-100% sequence similarity. Out of the yeast species causing chalk mold defect in bread, only *S. fibuligera*, which can secrete a large amount of $\alpha$-amylase and glucoamylase (Chi et al., 2009) is described in literature as common on continental breads (Legan and Voysey, 1991). *H. burtonii* is described as more common on British breads while *W. anomalus* was reported as dominant in Belgian par-baked breads packaged under modified atmosphere (Deschuyffeleer et al., 2011), which could explain the absence of these species in this study.

#### *2.3.2. Effects of Ortho-Phenylphenol (OPP) and Hydrogen Peroxide with colloidal Silver (HPS) treatments on inactivation of spoilage yeasts in sliced durum wheat bread*

Table 2 shows the effects of the two sanitizing treatments, as well as the environmental conditions and the quality parameters of bread samples. Average environmental conditions, in the processing areas, were very similar in terms of temperature and humidity. The temperature range for the two periods were respectively 11.60-28.30 °C and 11.43-28.20 °C, while the humidity has highlighted the range of 70.00-79.83 % and 72.00-80.17 % respectively.

The influence of water activity ($a_w$) on yeast growth has revealed that the values summarized in Table 2 (0.920 to 0.940) allowed the proliferation of spoilage yeasts on wheat bread samples. Deschuyffeleer et al. (2011) investigated the effect of $a_w$ on the growth of yeast strains belonging to the species *W. anomalus*, *H. burtonii* and *S. fibuligera* causing chalk mold defects on par-baked breads packaged under modified atmosphere; the optimum water activity for growth was different for the three strains and varied between 0.96 and 0.98 and minimum $a_w$ for *S. fibuligera* isolate growth was 0.90. These growth data were further used to develop secondary models that describe the relationship between $a_w$ and the radial growth rate of the colony (g, mm/d) or the lag phase duration. Similar trends were found by Burgain et al. (2015) who focused on the effects of temperature, T, and $a_w$ on the growth of *H. burtonii, W. anomalus, and S. fibuligera* on Sabouraud Agar Medium and on bread. The combined effects of T, $a_w$, and pH on the growth of these species were described by the gamma concept and validated on bread in the range of temperature 15–25 °C, $a_w$ 0.91–0.97 aw, and pH 4.6–6.8. The optimum growth rates on bread were 2.88, 0.259, and 1.06 mm/day for *H. burtonii*, *W. anomalus*, and *S. fibuligera*, respectively. The optimal growth rate of *S. fibuligera* on bread was about 2 fold that obtained on Sabouraud. Over the study periods, changes in moisture content matched the variations in $a_w$, with the lowest values recorded in September and December and the highest ones in August and November. Bakery products, similar to the rest of processed foods, can undergo physical, chemical, and microbiological spoilage. Moreover, classification of products based on their pH and $a_w$ can assist on recognizing the spoilage and safety potential of bakery products. Although chemical spoilage problems can considerably limit the shelf life of low- and intermediate-moisture bakery products, there is a major hazard of microbiological spoilage in intermediate- and high-moisture products. Several high-moisture, unfilled, and filled, bakery products have also been involved in outbreaks of food borne illness and, therefore, pose safety concerns (Smith et al., 2004). Microbiological spoilage is frequently the major hazard limiting the shelf life of high- and intermediate-moisture bakery products. Visible yeast growth most often occurs in high $a_w$ short shelf-life products, whereas fermentative spoilage is more frequently linked with low $a_w$ long shelf-life products. Some yeasts are resistant to low $a_w$ without causing any spoilage until they get adapted to the new conditions, or the conditions in the product are modified, thereby allowing their growth (Legan, 1993). The use of MAP packaging greatly reduced the incidence of aerobic bacteria

and molds on bread packed and improved the shelf life of baked goods. Carbon dioxide at 20%–60% has bacterio- and fungistatic properties and will prolong the lag phase and generation time of susceptible microorganisms (Coles et al., 2003; Daifas et al., 1999). In this study, the packaging material showed good barrier performances. The headspace $O_2$ concentration was less than 0.4% during the whole storage period for all sliced bread samples. Also, the internal content of $CO_2$ accounted for 30.26% at the beginning, and no significant differences ($P>0.05$) were observed among $CO_2$ values in the different samples. Similar findings were reported by Licciardello et al. (2014) and Degirmencioglu et al. (2011). The effect of the two sanitizing treatments in reducing the incidence of spoilage yeasts on sliced durum wheat bread is reported in Table 2. The percentage of incidence of yeast spoilage on sliced bread packaged in MAP revealed that the most effective treatment is HPS. In fact, conventional OPP sanitization treatment evaluated in this study during the production season 2013, promoted a spoilage incidence between 6.03 and 11.59%, while HPS treatment adopted in season 2014 reduced the incidence to values ranging from 0.31 to a maximum of 0.98%. Especially for industrial companies engaged in research and development activities, the use of alternative strategies to control growth of post-baking contaminants, which can meet the increasing demand by consumers of foods without chemical preservatives, is of primary importance. Several post-baking traditional and novel methods have been comparatively investigated with regard to reduce contaminants of high- and intermediate-moisture bakery products, including ultraviolet light (UV), infrared radiation (IR), microwave (MW) heating, low irradiation dose, pulsed light technology, and ultrahigh pressure (UHP) (Smith et al., 2004). The results of the study, however, demonstrated that preventive sanitizing technologies of industrial plant are effective alone in reducing chalk mold spoilage and may be considered as a valid approach when microbial inactivation, e.g. through thermal sterilization or deep-freezing, is not feasible.

*2.3.3. Correlations among process parameters, microbial community quality and characteristics of sliced durum wheat bread*

Principal component analysis is a statistical method that highlights the similarities and differences among bread samples. A principal component is a linear combination of the original variables among samples. Combinations are chosen to show the maximum variation

in the samples. PCA was carried out using the following variables: environmental condition in industrial plant (temperature and humidity), physical parameters of bread ($a_w$, crumb moisture), microbial spoilage (samples contaminated by yeasts) (Fig. 3). The two Principle Components (PC) of the model explained 69.4 % of the variation of the data. Selected raw data from this analysis are presented in Table 2. Overall environmental process conditions (especially temperature), $a_w$ and moisture of bread did not significantly affect the growth of yeasts on bread samples. The effect of environmental humidity was also negligible. In the first component the most important variables are samples contaminated by yeasts, bread moisture, $a_w$ and environmental temperature, while in the second component the highest weight is due to environmental humidity. Significant correlations were observed within the parameters used to evaluate quality characteristics of bread samples (Table 3). A linear relationships were observed within water-related properties of bread and environmental conditions. In particular, environmental humidity showed significant positive correlations with temperature ($r=0.699$) and $a_w$ ($r=0.705$); also moisture content was strongly correlated with $a_w$ ($r=0.577$). Figure 4 shows the scores plot of differences in inhibitory effect of the two sanitizing treatments HPS exhibited a stronger long-term inhibitory effect on spoilage yeasts, suggesting noteworthy differences between the two sanitizing treatments.

### 2.4. Conclusions

*S. fibuligera* was identified as the cause of early spoilage of sliced durum wheat breads packaged under a modified atmosphere. MAP alone was not enough to inhibit this spoilage organisms and to ensure a sufficient shelf life, with important economic losses for the bakery industry. The results of the investigation, carried out for two consecutive years in an industrial plant, confirmed that the growth occurrence of *S. fibuligera* in industrial durum wheat bread was not influenced by environmental conditions and bread parameters, but strongly affected by the sanitizing strategy applied to leavening and cooling chambers.

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## 2.5 References


Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J., 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, *25*, 3389-3402. Available at http://www.ncbi.nlm.nih.gov/BLAST/.

AOAC, 2000. Official methods of analysis of the Association of Official Analytical Chemists (17th Ed.). Gaithersburg, MD: Association of Official Analytical Chemists.

Braymen, D. T., 1969. Survival of micro-organisms in aerosols produced in cleaning and disinfecting. Public Health Rep. 84, 547-552.

Burgain, A., Bensoussan, M., Dantigny, P., 2015. Validation of a predictive model for the growth of chalk yeasts on bread. Int. J. Food Microbiol. 204, 47-54.

Chi, Z., Chi, Z., Liu, G., Wang, F., Ju, L., Zhang, T., 2009. *Saccharomycopsis fibuligera* and its applications in biotechnology. Biotechnology Adv. 27, 423-431.

Coles, R., Mcdowell, D. Kirwan, M. J., 2003. *Food Packaging Technology*. London, U.K.: Blackwell Publishing, p. 63.

Corbo, M.R., Bevilacqua, A., Campaniello, D., D'Amato, D., Speranza, B., Sinigaglia, M., 2009. Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches - a review. Int. J. Food Sci. Technol. 44, 223-241.

Daifas, D. P., Smith, J. P., Blanchfield, B., Austin, J. W., 1999. Effect of pH and CO2 on growth and toxin production by *Clostridium botulinum* in English-style crumpets packaged under modified atmospheres. J. Food Prot. 62, 1157-1161.

Degirmencioglu, N., Duygu, G., Inkaya, A. N., Aydin, E., Guldas, M., Gonenc, A., 2011. Influence of modified atmosphere packaging and potassium sorbate on microbiological characteristics of sliced bread. J. Food Sci. Technol. 48, 236-241.

Deschuyffeleer, N., Audenaert, K., Samapundo, S., Ameye, S., Eeckhout, M., Devlieghere, F., 2011. Identification and characterization of yeasts causing chalk mold defects on par-baked bread. Food Microbiol. 28, 1019-1027.

Esteve-Zarzoso, B., Belloch, C., Uruburu, F., Querol, A., 1999. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int. J. Systematic Bacteriol. 49, 329-337.

Galic, K., Curic, D., Gabric, D., 2009. Shelf life of packaged bakery goods - a review. Crit. Rev. Food Sci. 49, 405-426.

Giannone, V., Longo, C., Damigella, A., Raspagliesi, D., Spina, A., & Palumbo, M. (2010). Technological properties of bakers' yeasts in durum wheat semolina dough. J. Ind. Microbiol. Biot. 37, 371-379.

Kurtzman, C.P., Robnett, C.J., 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73, 331-371.

Legan, J.D., Voysey, P.A., 1991. Yeast spoilage of bakery products and ingredients. J. Appl. Bacteriol. 70, 361-371.

Legan, L. D., 1993. Mold spoilage of bread—The problem and some solutions. Int. Biodeter. Biodegr. 32, 33-53.

Licciardello, F., Cipri, L., Muratore, G., 2014. Influence of packaging on the quality maintenance of industrial bread by comparative shelf life testing. Food Packaging and Shelf Life 1, 19-24.

Malkki, Y., Rauha, O., 1978. Mold Inhibition by Aerosol 52. The Bakers Dig. p. 47.

Platania, C., Restuccia, C., Muccilli, S., Cirvilleri, G., 2012. Efficacy of killer yeasts in the biological control of *Penicillium digitatum* on Tarocco orange fruits (*Citrus sinensis*). Food Microbiol. 30, 219-225.

Rodriguez, M., Medina, L.M., Jordano, R., 2003. Influence of modified atmosphere packaging on the shelf-life of prebaked pizza dough with and without preservative added. Nahrung 47, 122-125.

Smith, J. P., Daifas, D. P., El-Khoury, W., Koukoutsis, J., El-Khoury, A., 2004. Shelf life and safety concerns of bakery products—A review. Crit. Rev. Food Sci. 44, 19-55.

Spurlock, A. T., Zottola, E. A., 1991. The survival of Listeria monocytogenes in aerosols. J. Food Prot. 54, 910-912.

Stetzenbach, L. D., 2007. Introduction to Aerobiology, Chapter 72. In C. J. Hurst, R. L. Crawford, J. L. Garland, D. A. Lipson, A. L. Mills, L. D. Stetzenbach (Eds.). Manual of

Environmental Microbiology, (2nd edn) (pp. 925–938). Washington: Amer Society for Microbiology.

Suhr K.I., Nielsen, P.V., (2005). Inhibition of fungal growth on wheat and rye bread by modified atmosphere packaging using volatile mustard essential oil. J. Food Sci. 70, M37-M44.

**Table 1**.
Sanitizing procedures, number of produced bread units per month and number of monitored bread units for calculating yeast spoilage incidence.

| Sanitizing treatment | Sanitizing treatment modality * | Number of 1.040 kg bread loaves /month ** | Number of monitored products (0.500 kg MAP bread)*** |
|---|---|---|---|
| **OPP** | | | |
| 2013, August | Disinfectant application: fog; Volume of use for preventive action: 350-500m$^3$; Biodegradability: > 75%; Rates of use after start of treatment: 8h; Residue measured on surfaces 0.93 mg/m$^2$. | 178560 | 17900 |
| 2013, September | | 176640 | 17720 |
| 2013, October | | 172800 | 17390 |
| 2013, November | | 161280 | 16430 |
| 2013, December | | 168000 | 16920 |
| **HPS** | | | |
| 2014, August | Disinfectant application: dry fog atomized at a speed of 80 m/s (particle size of about 5μ); Volume of use for preventive action: 350-500 m$^3$; Biodegradability: > 99%; Rates of use after start of treatment: 15 min; No presence of residues and toxic compounds | 171120 | 17420 |
| 2014, September | | 165600 | 16750 |
| 2014, October | | 167040 | 16880 |
| 2014, November | | 161280 | 16550 |
| 2014, December | | 161280 | 16610 |

* The treatments were performed on a weekly basis and according to the instructions of the supplier companies (technical data sheets)
** Data were provided by production archives of the company
*** Bread samples, not for sale, stored in the company warehouse at environmental temperature and relative humidity to monitor the occurrence of yeast spoilage

**Table 2.**
Values of environmental conditions, physical attributes and yeast spoilage incidence in durum wheat sliced breads analyzed 7 days after production. Results are expressed as average ± standard deviation of samples analyzed in triplicate. OPP, FUMISPORE OPP (20% Ortho-Phenylphenol); HPS, Hydrogen Peroxide (120 mL/L) stabilized by a colloidal Silver complex (30 mg/Kg).

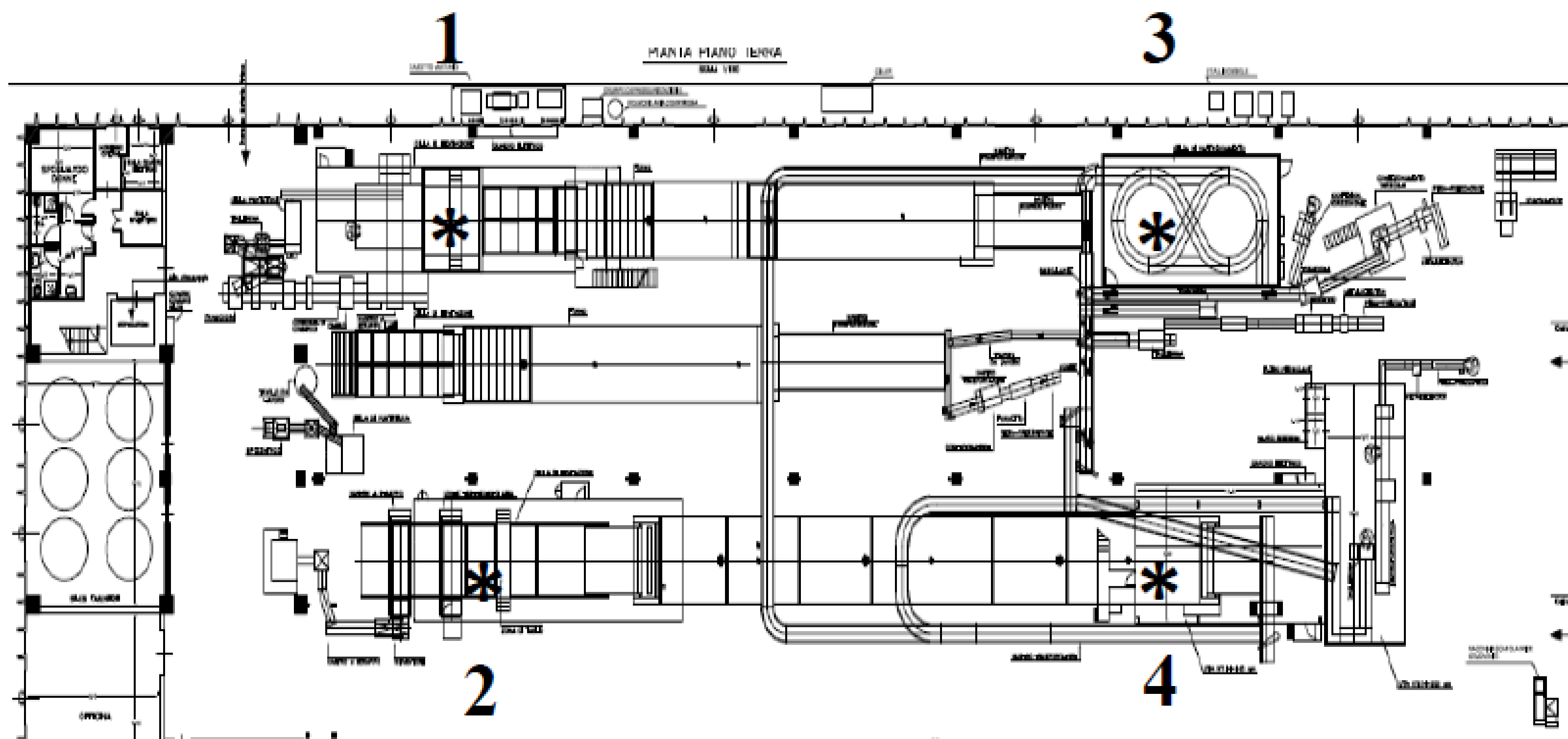
| Sampling time) | Sanitizing treatment | Environmental conditions | | Bread samples | | |
|---|---|---|---|---|---|---|
| | | Temperature (°C) | Relative humidity (RH%) | $a_w$ | Crumb moisture (%) | Yeast spoilage incidence (%) |
| 2013, August | OPP | 28.30$^{C}$ ±2.27 | 79.17$^{C}$ ±0.76 | 0.940$^{D}$ ±0.002 | 34.91$^{C}$ ±0.210 | 11.59$^{d}$ ±1.26 |
| 2013, September | OPP | 25.10$^{C}$ ±3.03 | 79.83$^{C}$ ±0.76 | 0.932$^{BC}$ ±0.001 | 33.40$^{A}$ ±0.361 | 8.64$^{c}$ ±1.07 |
| 2013, October | OPP | 19.37$^{B}$ ±1.33 | 79.83$^{C}$ ±0.76 | 0.931$^{B}$ ±0.003 | 34.18$^{B}$ ±0.329 | 10.04$^{cd}$ ±1.24 |
| 2013, November | OPP | 12.93$^{A}$ ±0.90 | 78.00$^{BC}$ ±1.73 | 0.937$^{CD}$ ±0.003 | 35.36$^{C}$ ±0.373 | 6.03$^{b}$ ±1.23 |
| 2013, December | OPP | 11.60$^{A}$ ±1.28 | 70.00$^{A}$ ±0.01 | 0.922$^{A}$ ±0.002 | 33.83$^{AB}$ ±0.153 | 8.13$^{c}$ ± 0.70 |
| 2014, August | HPS | 28.20$^{C}$ ±0.85 | 80.17$^{C}$ ±0.29 | 0.939$^{D}$ ±0.003 | 34.87$^{C}$ ±0.219 | 0.89$^{a}$ ±0.89 |
| 2014, September | HPS | 27.70$^{C}$ ±2.14 | 78.33$^{BC}$ ±1.53 | 0.933$^{BC}$ ±0.001 | 33.43$^{A}$ ±0.306 | 0.91$^{a}$ ±0.91 |
| 2014, October | HPS | 19.73$^{B}$ ±3.63 | 78.33$^{BC}$ ±1.53 | 0.932$^{BC}$ ±0.002 | 34.16$^{B}$ ±0.298 | 0.98$^{a}$ ±0.00 |
| 2014, November | HPS | 16.03$^{AB}$ ±1.70 | 76.00$^{B}$ ±1.00 | 0.936$^{BCD}$ ±0.002 | 35.39$^{C}$ ±0.335 | 0.31$^{a}$ ±0.54 |
| 2014, December | HPS | 11.43$^{A}$ ±1.05 | 72.00$^{A}$ ±1.00 | 0.922$^{A}$ ±0.002 | 33.80$^{AB}$ ±0.173 | 0.35$^{a}$ ±0.60 |

Mean values in the same column followed by different upper case letters ($P \leq 0.01$) and different lower case letters ($P \leq 0.05$) are significantly different.

**Table 3.**
Significant relationships amongst environmental conditions, microbial contamination and physicochemical attributes in durum wheat sliced breads.

| | Environmental temperature (°C) | Environmental humidity (RH%) | $a_w$ | Crumb moisture (%) | Samples contaminated by yeasts |
|---|---|---|---|---|---|
| Environmental temperature (°C) | 1 | | | | |
| Environmental humidity (RH%) | 0.699** | 1 | | | |
| $a_w$ | 0.570** | 0.705** | 1 | | |
| Crumb moisture (%) | -0.074 | 0.183 | 0.577** | 1 | |
| Samples contaminated by yeasts | 0.184 | 0.253 | 0.120 | -0.116 | 1 |

**$P$<0.01




* **Plant areas subjected to antimicrobial treatments**

**Figure 1**. Production site map and sanitizing treatment points. 1, leavening cell (30 ± 2 °C, 60± 0.5 % RH); 2, leavening cell (32 ± 2 °C, 64 ± 0.5 % RH); 3, cooling cell 320 $m^3$ (20 ± 1 °C, 75 min); 4, cooling cell 375 $m^3$ (20 ± 1 °C, 150 min).

**Figure 2**. Colonies of *Saccharomycopsis fibuligera* grown on MAP packed durum wheat bread after 7 days of storage.

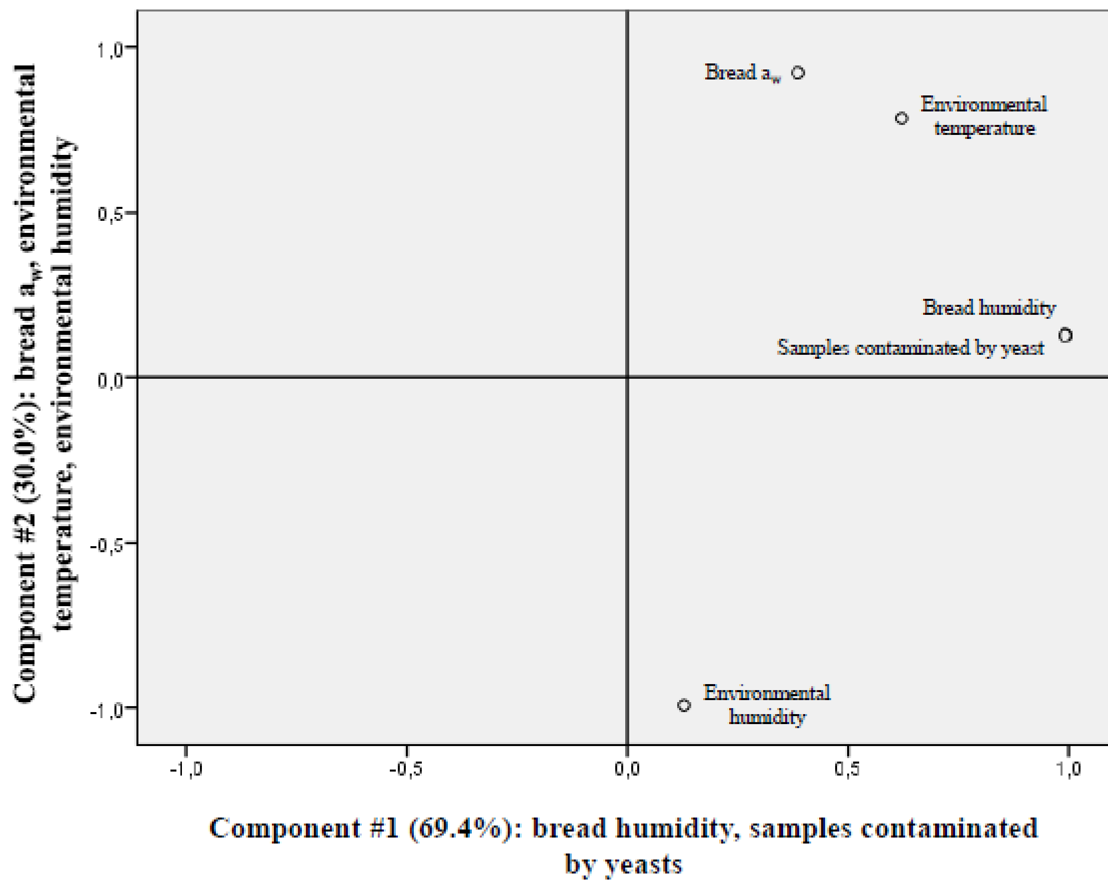



**Figure 3**. Loading plot of first and second principal components after component analysis based on data of process parameters (environmental temperature and humidity), quality parameters of bread ($a_w$, crumb moisture), microbial community (samples contaminated by yeasts).

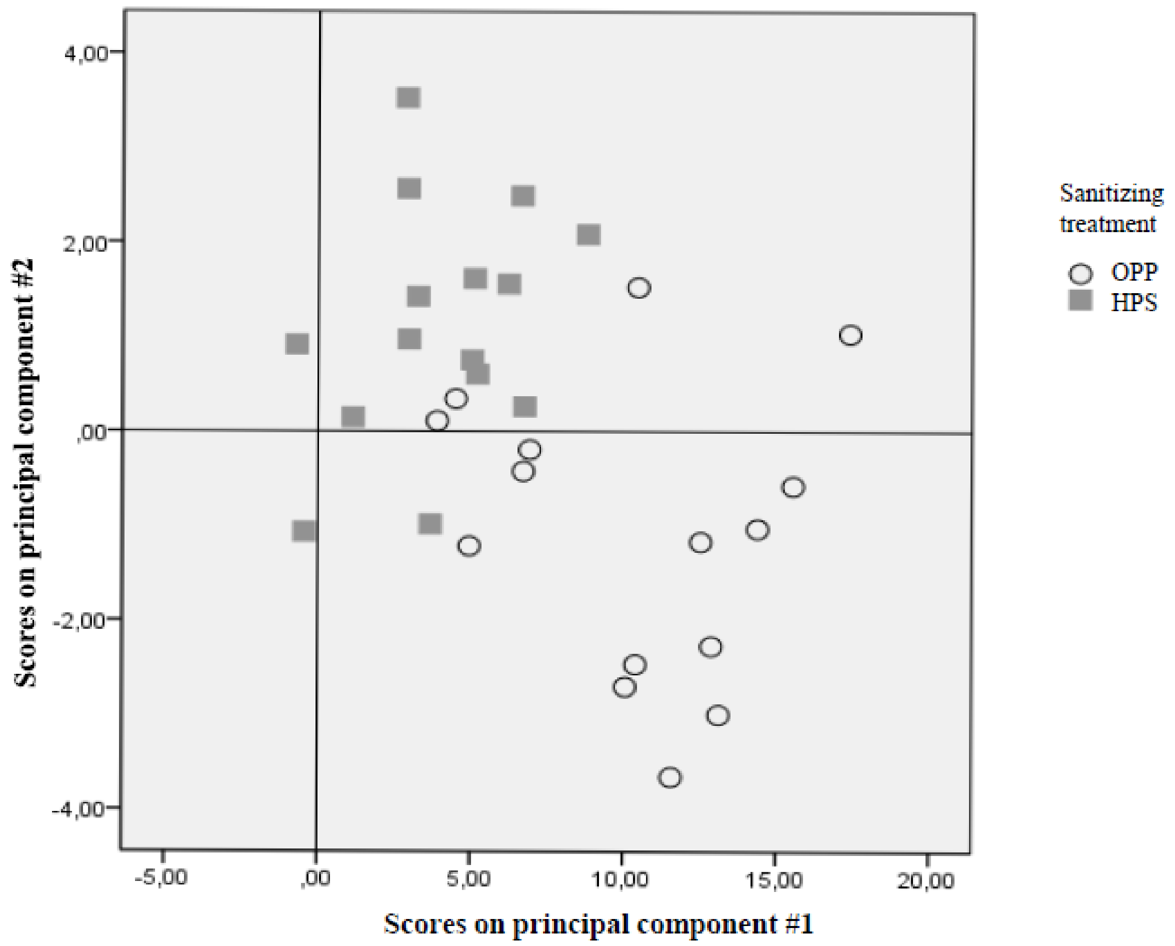



**Figure 4**. Score plot of samples between two different sanitizing treatments ( ○ OPP, OrthoPhenylPhenol; ■ HPS, hydrogen peroxide stabilized by a colloidal silver complex).

# Chapter 3

## Shelf life assessment of industrial durum wheat bread as a function of packaging system

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**Abstract**

This study compared the effect of different packaging systems on industrial durum wheat bread shelf-life, with regard to thermoformed packaging (TF) and flow-packaging (FP). Two TFs having different thickness and one FP were compared by assessing physico-chemical and sensorial properties and volatile compounds of sliced bread during 90 days of storage. Texture, $a_w$ and bread moisture varied according to a first-order kinetic model, with FP samples ageing faster than TFs. Sensorial features such as consistency, stale odor, and sour odor, increased their intensity during storage. Furans decreased, whereas hexanal increased. The Principal Component Analysis of the whole dataset pointed out that the TF system at reduced thickness could be adopted up to 60 days, without compromising the standard commercial life of industrial bread and allowing to save packaging material. The FP system would allow further saving, but it should be preferred when the expected product turnover is within 30 days.

**Key words:** durum wheat bread; shelf life; packaging system; volatile compounds; textural properties; sensorial properties

### 3.1. Introduction

The shelf life of food, defined as the period of time during which quality loss does not exceed a tolerable level, can be decisively influenced by packaging. Bread shelf life is mainly affected by staling, a complex degradative phenomenon which, in turn, depends on starch retrogradation and moisture loss (Bollaìn, Angioloni, & Collar, 2005; Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006). Staling results in chemical and physical changes such as decrease of softness and cohesiveness, as well as loss of aroma and flavor (He & Hoseney, 1990). It is well known that durum wheat bread, especially popular in the Mediterranean area due to its specific sensory and textural properties (Pasqualone, 2012; Quaglia, 1988), undergoes slower staling compared with soft wheat bread, due to high water-binding capacity of durum wheat semolina (Boyacioglou & D'Appolonia, 1994; Hareland & Puhr, 1998; Quaglia, 1988; Rinaldi et al., 2015). The addition of enzymes, such as lipase and amylase, to bread formulation (Bollaín et al., 2005; Giannone et al., 2016; Palacios, Schwarz, & D'Appolonia, 2004), or the use of sourdough (Pasqualone, Summo, Bilancia, & Caponio, 2007; Rinaldi et al., 2015), can further reduce durum wheat bread staling. Bread staling results in a decrease of consumer acceptance and in great economic losses. As bakery products are becoming a major part of the international food market, the baking industry is undergoing a period of rapid change and modernization, involving the setup of bakery plants with improved technology and new products development (Byrne, 2000). In order to achieve longer shelf lives, refrigerating conditions have been applied to dough, prebaked or not (Rask, 1989; Selomulyo & Zhou, 2007). In addition, new packaging technologies have been investigated. Packaging is the last step of production and food technologists have to select the most suitable type of packaging to ensure the longest shelf life. The success in the market is equally based on product intrinsic quality and packaging effectiveness in preserving, and communicating, this quality. The conventional packaging procedure applied in baking industry uses atmospheric air and approved lidding materials for foods. However, modern packaging is performed under modified atmosphere and with composite materials specifically formulated in order to retain the inert gases. Several studies evidenced the effectiveness of packaging in maintaining the quality characteristics of bread, slowing down moisture loss and molds growth, by using: i) suitable materials (Licciardello, Cipri, & Muratore, 2014; Pagani, Lucisano, Mariotti, & Limbo, 2006); ii) active packaging (Latou, Mexis, Badeka, & Kontominas, 2010;

Mihaly Cozmuta et al., 2015); iii) modified atmosphere (Del Nobile, Martoriello, Cavella, Giudici, & Masi, 2003; Piergiovanni & Fava, 1997). An essential issue in the present day is the selection of packaging systems which are not only effective, i.e. able to maintain quality characteristics, but also efficient, i.e. able to contain environmental impact and costs generated by packaging production and disposal. In a preliminary study, Licciardello et al. (2014) have assessed the feasibility of reducing the thickness of materials used in thermoformed packaging of durum wheat bread, finding that potential gains are possible without compromising the standard shelf life. However, no study has compared the effect of different packaging systems on bread shelf life, with special regard to thermoformed packaging and flow-packaging. Flow-packaging has the advantage of high working speed and could allow further saving of packaging material. The choice of packaging materials is often based on packaging performances, with special regards for gas barrier properties; however, in the case of thermoformed packages, the film properties in the finished product differ from those of the material as received due to thermal stretching, and need to be verified in the conditions of use. Hence, the comparison and choice cannot be made only on the basis of technical sheets available. The objective of the present study was to evaluate the influence of different packaging systems (namely, one commonly used two-piece thermoformed packaging, a two-piece thermoformed packaging at reduced thickness, and flow-packaging by a very thin material), on quality variations of industrial durum wheat bread by monitoring physico-chemical and sensorial parameters during 90 days of storage.

## 3.2. Materials and methods

### *3.2.1 Sample preparation*

Bread was prepared at a local bread-making company (Valle del Dittaino Società Cooperativa Agricola, Assoro, Italy), according to a consolidated industrial process based on the following formulation: durum wheat remilled semolina, water (66% on semolina basis), compressed yeast (0.47% on semolina basis), NaCl (2.2% on semolina basis), maltogenic $\alpha$-amylase (0.05% on semolina basis). The ingredients were mixed and kneaded for 17 min by means of a diving arms kneader. The final dough temperature was 26±1 °C. The dough was rested in bulk for 15 min, scaled into 980±20 g portions (100 loaves, repeated for three production trials), proofed

for 150 min (32±1 °C and 66±2% RH) and baked at 240 °C for 60 min, in industrial tunnel oven. The baked loaves, weighting approximately 800 g each, were automatically transported to a cooling chamber, set at 20±2 °C for 120 min. After cooling, the loaves were sliced by means of an automatic slicing machine to 11±1 mm thickness.

*3.2.2 Packaging systems*

After slicing, portions of 400 g of bread slices were packaged. Three packaging systems were compared; two of them consisted of two-piece packages made up of a thermoformed bottom and a lid. The first packaging system ('thermoformed 1' or TF1, commonly used by the baking industry were the trials were carried out) consisted of a 275 μm bottom film and a 125 μm lid; the second was similar to TF1, but with thinner films, 225 ◎m and 33 ◎m for bottom and lid, respectively (packaging system 'thermoformed 2' or TF2). The third system involved flow-packaging using a 62 ◎m coextruded film ('flow-packaging' or FP). All films were made of multilayered polyolefin materials. An automatic industrial thermoforming machine (MIX 9000, Tecnosistem snc, Coccaglio, Italy) shaped the bottom films for TF1 and TF2 before inserting the sliced bread and sealing with the corresponding lid film, whereas FP was filled and formed by a flow-packaging machine (Jaguar, Record spa, Garbagnate Monastero, Italy). All packaging systems included sprayed ethanol (1.6% on bread weight basis) and modified atmosphere composed of 30% $CO_2$ and 70% $N_2$. The packaging materials were kindly supplied by Cryovac Sealed Air S.r.l. (Passirana di Rho, Italy). Permeability properties, as from the technical sheets of the supplier, were as follows. $O_2$ transmission rate (OTR): i) TF1 lid film < 3 g/$m^2$, 24 h, bar; bottom film = 1 g/$m^2$, 24 h, bar; ii) TF2 lid film = 4 g/$m^2$, 24 h, bar; bottom film = 1 g/$m^2$, 24 h, bar; iii) FP = 4.5 g/$m^2$, 24 h, bar. Water vapor transmission rate (WVTR): i) TF1 lid film < 10 g/$m^2$ 24 h; bottom film ≤ 10 g/$m^2$, 24 h; ii) TF2 lid and bottom films = not reported; iii) FP = 4 g/$m^2$, 24 h. Packaged breads TF1, TF2, and FP were analyzed on the same day of baking ($t_0$) and after 7, 15, 30, 60, and 90 days of dark storage at 20±1 °C and 55% relative humidity. Three breads ($n$ = 3) per each of three packaging systems considered and per each of six sampling times were analyzed, for a total of 54 samples.

*3.2.3 Headspace gas composition analysis*

The internal $O_2$ and $CO_2$ composition of packages was determined by means of Dansensor

Checkpoint portable gas analyzer (Dansensor, Ringsted, Denmark). Ten mL of headspace were analyzed, with three replications.

*3.2.4 Determination of moisture, water activity, alkaline water retention capacity*

Moisture content of bread crumb and crust was determined by oven drying at 105 °C until constant weight. Two bread slices (11±1 mm thickness) for each of two repetitions were used, and moisture was determined on one square crumb sample (40 mm × 40 mm) taken from the center of each slice, and on approximately 3 g crust samples manually cut from the same slices. Crumb to crust ratio of breads was 3:1 (w/w). Water activity ($a_w$) was determined by Hygropalm 40 AW (Rotronic Instruments Ltd, Crawley, UK) according to manufacturers' instructions. Three bread slices (11±1 mm thickness) were used, after removal of the crust. For each set of determinations, separate loaves were considered. Alkaline water retention capacity (AWRC) was determined according to the method described by Yamazaki (1953), conveniently modified for the analysis of bread crumb (Licciardello et al., 2014). Briefly, 1 g of bread crumb, previously dried until constant weight and ground in a mortar, was put in 15-mL tubes (W1), added with 5 mL 0.1 N $NaHCO_3$ and vortexed for 30 s, then let at room temperature for 20 min. The slurry was centrifuged at 3000 rpm for 15 min, the supernatant was discarded and tubes were let drip for 10 min upside down inclined by 15°. Dried tubes were then weighed (W2). AWRC was calculated as [(W2 - W1)/W1] × 100, where W1 is the weight of the tube containing the dry sample and W2 is the weight of the tube containing the dripped sample. Analyses were conducted in duplicate.

Experimental data were fitted to the following first-order kinetic model:

$$C\,(t) = C^{\infty} + \left(C^{0} - C^{\infty}\right) \cdot \exp\left(-k \cdot t\right)$$

where: $C\,(t)$ is the value of the descriptor at time $t$, $C^{\infty}$ is the value of the descriptor at equilibrium (infinite time), $C^0$ is the initial value of the descriptor (time zero), $k$ is the kinetic constant, $t$ is the time.

*3.2.5 Texture Profile Analysis*

The Texture Profile Analysis (TPA) of bread was carried out by means of an Universal Testing machine (model 3344, Instron, Norwood, MA, USA), equipped with a 5.0 cm diameter cylindrical probe and a 2000 N load cell. Data were acquired through Bluehill® 2 software (Instron, Norwood, MA, USA). Cyclic compression tests (30s gap between first and second compression) were set up: trigger load and crosshead speed were 5 g and 3 mm/s respectively, the force required to compress the samples by 40% was recorded on 5-cm side square portions of 22-mm thick slices, and the average value of five replicates was taken. Three primary TPA parameters (firmness, springiness, and resilience), and one derived parameter (chewiness) were calculated: firmness (N), defined as the peak force during the first compression cycle; springiness (mm), i.e. the elastic recovery that occurs when the compressive force is removed, defined as the height to which the food recovers during the time that elapses between the end of the first and the start of the second compression; resilience, defined as the adimensional ratio between the negative force input and the positive force input during the first compression, or Area 5/Area 4; chewiness (N mm), defined as the product of firmness, resilience and springiness.

With the aim of studying gradients of firmness during aging, crumb firmness was fitted to the modified Avrami equation (Armero & Collar, 1998):

$$\theta = (F_\infty - F_t) / (F_\infty - F_0) = \exp(-kt^n)$$

where $\theta$ is the fraction of the total change in the crumb firmness still to occur. $F_0$, $F_t$ and $F_\infty$ are experimental values of fitness at times zero, t, and infinite (or limiting value), $k$ is the rate constant, and $n$ is the Avrami exponent. All parameters were obtained from the modelling process. Springiness, resilience and chewiness data were fitted to the first-order kinetic model previously described in paragraph 2.4.

*3.2.6 Color parameters*

Two slices of bread for each sample were scanned by a scanner Canoscan N650U (Canon Computer System, Inc., Costa Mesa, CA, U.S.A.). Four images (sized 2 × 2 cm) from different

points of each replicate slice were acquired at 300 dpi resolution and processed by the software Image Color Summarizer v0.5 # 2006–2011 (Martin Krzywinski, http://mkweb.bcgsc.ca/color_summarizer/) obtaining the *r, g, b* (respectively: red, green and blue indexes) and *h*, *s*, *v* (respectively: hue, saturation and lightness) color indices.

### *3.2.7 Determination of volatile compounds*

Volatile compounds of bread samples were determined by solid phase micro-extraction (SPME) coupled to gas-chromatography/mass spectrometry (GC/MS). Sample delivery from productive site to the laboratory for volatile determination accounted for about 10 h, therefore $t_0$ data of volatiles have to be intended as 10 h after baking and packing. Maintaining the crumb to crust ratio of 3:1 (w/w), an amount of 400 ± 0.05 mg of bread crust and crumb (cut in pieces of 2-3 mm, then mixed together) was added of 4 mL of a 20% NaCl (w/v) aqueous solution in a 20-mL vial. The SPME analysis was made by using an Agilent 6850 gas-chromatograph equipped with an Agilent 5975 mass-spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) as in Pasqualone et al. (2015) with the following modifications: time and temperature of fiber exposure to sample headspace = 40 min at 50 °C; desorption time = 2 min; GC injector temperature = 300 °C; flow = 2.0 mL/min. Peak identification was performed by computer matching with the reference mass spectra of National Institute of Standards and Technology (NIST) and Wiley libraries. The semi-quantitative data (peak areas expressed as total ion counts - TIC) were used to compare the samples. The analysis was carried out in triplicate.

### *3.2.8 Sensory evaluation*

As for volatiles determination, sensory determinations on fresh bread ($t_0$) were performed 10 h after baking and packing. Quantitative Descriptive Sensory Analysis of bread samples was performed by a panel consisting of 8 trained members in the conditions described in a previous work (Pasqualone et al., 2007). The list of sensory terms included descriptors of appearance (crumb color), textural characteristics (crumb cohesiveness, crumb consistency), and odor (semolina, sour, toast, stale). The descriptors were rated on an anchored line scale that provided a 0-9 score range (0 = minimum; 9 = maximum intensity). The definitions of each descriptor and the scale anchors are reported in Pasqualone et al. (2007).

*3.2.9 Statistical analyses*

The data were analysed with package IBM® SPSS® Statistics 13.0 (Armonk, NY, USA) for Windows. One-way analysis of variance (ANOVA) was performed to understand the effects of different packaging on physico-chemical attributes of durum wheat bread. Tukey HSD test ($P < 0.05$) was used for post hoc comparison of means. The Principal Component Analysis (PCA) was performed with XLStat (Addinsoft SARL, New York, NY, USA) for Windows.

## 3. 3 Results and discussion

*3.3.1 Headspace gas composition analysis*

Figure 1 shows the variations of $O_2$ and $CO_2$ level inside bread packages during 90 days of storage. The initial modified atmosphere composition, i.e. 70% $N_2$ and 30% $CO_2$, underwent significant changes during storage as a function of the packaging system. In particular, the $CO_2$ decrease can be attributed both to the dissolution of the gas into the food matrix and to permeability through the packaging material. Overall, the observed $CO_2$ permeability of the tested materials followed the order TF1<TF2<FP. Until 30 days, $CO_2$ values were not significantly different ($P < 0.05$) between TF1 and TF2, while FP scored significantly ($P < 0.05$) lower values compared to the thermoformed packages already after 15 days. A similar trend was observed for the $O_2$ level: in the TF1 samples it practically did not change during storage; slight increases were observed in TF2, not exceeding 1.0% after 90 days, and more marked increases were detected in the FP system, that allowed to reach 2.3% $O_2$ after 90 days. No significant differences in the $O_2$ level were observed between TF1 and TF2 headspaces after 60 and 90 days. These results demonstrated the real behaviour of TF1 and TF2 materials, that could not be fully foreseen by the permeability properties reported in the technical sheets due to modifications involved by stretching and thermoforming.

*3.3.2 Bread moisture, $a_w$ and AWRC*

Table 1 shows the changes in moisture content of crumb and crust, $a_w$, and AWRC of differently packed durum wheat bread samples, as well as the kinetic parameters resulting from the best-fit of the experimental data to a first-order kinetic model. The initial crumb moisture content was within the typical range of fresh durum wheat bread obtained from

semolina with high protein content (Pasqualone et al., 2007; Raffo et al., 2003), and tended to decrease during storage with significant variations ($P < 0.05$) until 30 days for TF2 and FP, and until 60 days for TF1. The latter showed significantly higher crumb moisture than FP, but without significant differences with TF2 from 60 days onwards. An opposite trend was observed for crust moisture content, whose values increased dramatically in the first 7 days, with no significant increases during the rest of the storage period. The experimental data fit well the first-order kinetic model, with $C^{\infty}$ values very similar to experimental data at 90 days. Moisture variations were faster in FP than in TF1 samples, especially for crust moisture gain, as testified by higher $k$ value for FP than for TF1. Moisture values of TF1 and TF2 moisture, instead, changed at similar rates. Water migration from crumb to crust and, then, to the ambient, is one of the main events occurring just after baking. As a consequence, crumb hardens while crust first acquires a leathery consistence, then hardens itself with detrimental effects on bread quality. One of the objectives of a packaging system is to limit water loss, and this can be achieved by materials with suitable barrier to water vapor. The observed results were therefore imputable to high WVTR value of the FP film, as reported in the technical sheet. Paralleling crumb moisture loss, also crumb $a_w$ decreased in all samples during storage. This phenomenon was more evident for FP, again in agreement with higher vapor permeability of FP film: the $a_w$ decrease followed a first-order kinetics and FP showed dramatically higher kinetic constant compared to TF1 and TF2 (Table 1). The differences between the three packaging systems were significant after 7 days, while TF1 exhibited $a_w$ values significantly ($P < 0.05$) higher than those of the other samples at 30 and 60 days of conservation. At the end of the storage period all of the samples showed similar values of $a_w$, below 0.900 and very close to the calculated $C^{\infty}$ values, irrespective of the packaging system. The AWRC values significantly ($P < 0.05$) decreased until the end of the storage period for each of the packaging solutions considered, with significantly lower values for FP than for TF2 starting from 30 days. This parameter was effective in differentiating fresh bread from aged one, in agreement with previous studies (Sidhu, Al-Saqer, & Al-Zenki, 2007; Licciardello et al., 2014). AWRC is correlated with the degree of starch crystallization, since gelatinized starch has a higher capacity to bind water, compared to retrograded starch (Indrani, Rao, Sankar, & Rao, 2000). The observed trend suggests that starch retrogradation was especially involved in bread quality loss during the initial phase of ageing (15 days), when the rate of variation was faster.

The variation of AWRC, however, could not be satisfactorily described by the first-order kinetic model.

### *3.3.3 Bread textural features*

Table 2 shows the changes in textural features of differently packed durum wheat bread samples, as well as the kinetic parameters resulting from the best-fit of the experimental data to the Avrami equation (for firmness) or to a first-order kinetic model (for springiness, resilience, and chewiness). Texture is an important characteristic in consumer's perception of food and influences the purchasing decisions. Firming of bread crumb is one of the most evident events in bread ageing and one of the most common parameters used to evaluate staling. A significant increase in crumb firmness was observed for all samples during storage. In particular, firmness increased faster in FP samples than in TF2 and TF1. This result was in agreement with the AWRC measures that evidenced a greater extent of starch retrogradation in FP samples. TF1 generally retained softer crumb than TF2 samples, but at the end of the storage period the difference with TF2 disappeared. Bread hardening was particularly fast during the first 15 days, then progressively tended to a steady state, corresponding to the maximum firming. Firmness data were modeled using Avrami equation, demonstrating that FP was associated with a higher firming rate (higher $k$) compared with TF2. The model parameter $F_{\infty}$ was very close to experimental values observed at 90 days, indicating that bread had reached the maximum firmness by that time. The $n$ term varied from 0.9 for TF2 to 1.2 for FP: other authors who have modeled bread firming kinetic data by the Avrami equation have indicated that the Avrami exponent $n$ is close to 1 (Kim & D'Appolonia, 1977). Nevertheless, other publications state that the exponent $n$ can take different values; however, the determination of the $n$ exponent is often drawn for very few data points and is questionable. Le-Bail, Boumali, Jury, Ben-Aissa, & Zuniga (2009) used a simple first order model ($n$ = 1) which fitted very well the experimental results obtained during staling of bread samples baked in a miniaturized baking system. The other textural parameters were modeled using a first-order kinetic model. Springiness significantly increased during storage, well fitting the kinetic of first order. TF1 showed significantly ($P < 0.05$) lower springiness than TF2 and FP at 7 and 15 days of storage, whereas no significant differences were observed among the three packaging systems at 30 days and 60 days. At the end of the storage period FP samples showed

the highest springiness, with $C^{\infty}$ values similar to the experimental data. Overall, the kinetic model of springiness variations highlighted two different behaviors: one, which is relative to TF1, characterized by lower kinetic constant ($k = 0.040$), the other faster, with $k = 0.153$ and $k = 0.195$ for samples TF2 and FP, respectively. Resilience, that shows how well a product 'fights to regain its original position after a stress' (Abdelghafor, Mustafa, Ibrahim, & Krishnan, 2011), decreased significantly with storage time, indicating a marked tendency of bread to become crumblier, with a less cohesive structure. Similarly to springiness, a higher kinetic constant was observed for TF2 and FP ($k = 0.093$ and 0.088, respectively), while FP1 showed $k =$ as low as 0.034. The resilience value at infinite time of TF1 and TF2 were similar to each other; FP scored the lowest value ($C^{\infty} = 0.66$). The trends of variation of the derived parameter chewiness (firmness * resilience * springiness) paralleled those of firmness and springiness, increasing significantly ($P < 0.05$) during storage. Although the estimated $k$ value was the lowest for FP, the chewiness value at infinite time was significantly higher (almost double) for this sample compared with the two thermoformed systems, which were assigned similar $C^{\infty}$ values.

#### *3.3.4 Bread volatile compounds*

Figure 2 reports the variations of the most abundant volatile compounds of bread samples during storage. Furan-derivatives and aldehydes, arising from Maillard reaction and lipid oxidation, respectively, characterized the volatile profile of breads. An overall comparison of the three packaging types points out that they had a similar effect towards the volatile compounds, apart from a few sampling points. With the only exception of hexanal, the volatiles decreased during time, but keeping quite high amounts during the first 15 days. A more evident depletion affected the volatile compounds as storage went on. More specifically, the levels of 2-furanmethanol, derived from Maillard reaction and responsible for burnt note (Chang, Seitz, & Chambers, 1995), were different in the last stages of storage, with FP samples showing lower amounts than TF1 and TF2. Furfural, typically present in bread (Makhoul et al., 2015) and contributing a 'brown' note (Chang et al., 1995), significantly decreased from 15 days, due to permeation through the films, with no differences among packaging types. Benzaldehyde, derived from aminoacid degradation, also through Strecker thermal reaction (Beleggia, Platani, Spano, Monteleone, & Cattivelli, 2009), decreased faster in TF2 than in TF1 and FP. Benzaldehyde has been already observed in durum wheat bread by other authors

(Bianchi, Careri, Chiavaro, Musci, & Vittadini, 2008). Overall, the Maillard reaction volatiles positively contribute to fresh bread aroma and their decrease during storage was detrimental. This decrease was imputable to packaging permeability, allowing these compounds to escape, as well as to the possible formation of inclusion complexes with amylose (Martínez-Anaya, 1996). The differences among packaging types, when observed, were possibly due to differences in packaging selectivity and scalping phenomena. As regards lipid oxidation volatiles, hexanal and nonanal were detected, deriving from the oxidation of linoleic and oleic acids, respectively (Frankel, 1983). Hexanal and nonanal have been already reported in bread (Chang et al., 1995; Chiavaro, Vittadini, Musci, Bianchi, & Curti, 2008), as well as in other cereal-based foods such as semolina, pasta, and biscuits (Pasqualone et al., 2014; 2015). Hexanal is responsible for a green, cut grass note, which has no obvious relationship to the typical bread flavor, although in total may have some influence (Chang et al., 1995), whereas nonanal is related to a rubbery, beany note (Chang et al., 1995). The formulation of bread samples did not include fat or oil, but the lipid fraction of semolina, although scarce, is mainly polyunsaturated (Pasqualone, Caponio, & Simeone, 2004; Pasqualone, Paradiso, Summo, Caponio, & Gomes, 2014) and very susceptible to lipoxygenase activity, leading to unstable fatty acid hydroperoxides which, in turn, decompose to carbonyl compounds. The latter can be responsible for off flavors in bread (Martínez-Anaya et al., 1996). Hexanal, being originated during processing, mainly in the kneading step (Caponio, Summo, Pasqualone, & Bilancia, 2008), was present in freshly packed bread and increased after long storage, due to further oxidative phenomena involving linoleic acid, without differences among packaging types. Therefore, packaging permeability allowed hexanal to escape, but at longer times the increase in the volatile due to oxidation overcame the loss through the films. Nonanal, instead, already originated during processing as well, did not show further increase after long storage because derived from the less oxidizable oleic acid. On the contrary, nonanal even decreased during storage. In fact, having a longer carbon chain than hexanal, nonanal is more hydrophobic (the octanol/water partition coefficients are 3.56 and 1.97 for nonanal and hexanal, respectively) and, therefore, has greater affinity towards olefins constituting the packaging materials, with a consequent higher scalping potential. The decrease of nonanal during storage was greater for TF2 samples than TF1 and FP. Nonanal showed significant differences among packaging systems also at $t_0$. This difference was imputable to the time, accounting for approximately 10

h, elapsed from production and packaging to the analytical determination of volatiles.

*3.3.5 Bread color parameters*

Among the color parameters instrumentally determined by image analysis, hue (Figure 3) significantly decreased during storage: hue values observed after 30 day were significantly lower than in freshly packed breads, irrespective of packaging system. The other parameters showed slight variations, which however could not be correlated with storage time (data not shown). Hue is a parameter derived from RGB coordinates, however it is interesting to notice that the single primary parameters are not correlated with ageing, while their derived index, hue, contains more information and is able to represent the color change which occurs during storage. Color changes during durum wheat bread ageing could be due to the oxidation of carotenoids which characterize durum wheat (Pasqualone et al., 2007), however this hypothesis needs to be investigated more in depth.

*3.3.6 Bread sensory features*

Bread sensory features were monitored during storage (Figure 3), with special regard to descriptors related to color, odor notes, and textural characteristics. Freshly packed bread were characterized by brilliant yellowish, highly cohesive and quite consistent crumb, with moderately intense pleasant odor notes of semolina, toast, and slight sour. Yellow crumb color was due to carotenoid pigments, while high consistency was imputable to tenacious gluten, both usually present in durum wheat remilled semolina (Pasqualone, Caponio, & Simeone, 2004). During storage, the intensity of the sensory descriptors decreased, with the exception of consistency, stale odor, and sour odor. Overall, but with lower statistical significance, the results of sensory evaluation confirmed the trends evidenced by instrumental measures of crumb textural properties, moisture, color hue, and volatile compounds. In particular, a progressive color decrease was observed during time, though not significant in TF1 ($P = 0.119$), with no difference among packaging types. In a previous work, carried out in unpackaged durum wheat bread, yellowish crumb shifted to a paler tone due to the increase of opacity related to starch retrogradation and moisture loss, with a significant correlation between sensory and colorimetric data (Pasqualone et al., 2007). A decrease of crumb cohesiveness, leading to a marked tendency to crumble, and an increase of consistency, were observed in all

breads. The decrease of cohesiveness was faster than the consistency increase, as already reported (Pasqualone et al., 2007). TF1 samples tended to present lower crumb consistency than FP samples, with a significant difference ($P < 0.05$) at 7 days. Irrespective of packaging system, significant decreases of semolina and toast odor were evidenced in all breads at 60 days, compared with freshly packed samples, whereas stale and sour odor increased. No statistical differences were observed at the end of storage, due to high data variability. The decrease of certain odor notes was probably imputable, besides volatilization through packaging films, to interactions between aroma components and amylose (Martínez-Anaya, 1996). The increase, instead, was due to oxidative phenomena, mainly involving carbonyl compounds and carotenoid pigments (Kulp & Ponte, 1981; Martínez-Anaya, 1996), that led to the formation of off-flavors at an extent exceeding the permeability of packaging or amylose interactions. Although perceivable, however, stale odor never reached an excessively high score: it was scored around 3 (scale 0-9) after 30 days storage, and remained on similar levels also after 60 days.

*3.3.7 Principal Component Analysis of the whole dataset*

The Principal Component Analysis (PCA) of the whole dataset pointed out that the first two principal components, PC1 and PC2, explained together about 79% of total variability. The loading plot (Figure 4 a) shows that PC1, in particular, accounted for 70% of variability and was positively correlated with all the appreciable characteristics, such as crumb moisture, color and cohesiveness, semolina odor, toast odor, water activity, crumb resilience and all the volatile compounds except hexanal, whereas it was negatively correlated with undesired stale odor, crumb consistency, crust moisture, crumb hardness, chewiness, and springiness. Therefore, PC1 allowed to discriminate bread samples in the score plot (Fig. 4 b) according to storage time: longer storage times corresponded to worse sensory and textural features. The PC2, accounting for about 9% of variability, showed a negative correlation with AWRC and sour odor, while a positive correlation with all volatile compounds except hexanal, as well as with hue, crust moisture, and springiness. This variability was mainly due, as pointed out by the score plot, to the changes occurring in breads in the initial stages of storage. As a consequence, three clearly distinct groups of breads could be observed in the score plot. The first included fresh breads ($t_0$), together with the TF1 bread stored for 7 days: this would mean

that TF1 was the only packaging system able to keep almost unaltered bread characteristics in the first week of storage. The second group includes TF2 and FP breads stored for 7 days, together with all samples stored for 15 days: these breads were involved in changes regarding loss of AWRC, and slight variations of volatile compounds and textural properties. Nevertheless, the properties of these breads remained clearly different respect to those of long-term stored breads (30-90 days, third group), which all showed the typical features of staling, although with some differentiations. In particular, at long storage times the two thermoformed packaging systems were comparable, with only a slight differentiation between them, and only the FP system was more distant. The latter, at 30 days, was similar to TF1 at 60 days. So, while in the short-term storage TF1 was by far the most effective packaging system, considering that the standard shelf-life of industrial durum wheat bread reaches 60 days, TF1 could be effectively substituted by TF2 up to this time, whereas FP could be used up to 30 days.

### 3.4. Conclusions

Based on the whole data set, and results of the PCA analysis, an overall comparison of the three packaging systems point to a significant influence on bread characteristics in the initial phase of storage, when the conventional system TF1 showed the best performance, allowing only slight changes compared to the fresh product. Data elaboration for textural parameters, crumb and crust moisture and $a_w$ changes by a first-order kinetic model allowed to highlight slower kinetic constants for TF1 and faster for TF2 and FP. However, storage times longer than 15 days, which correspond to the period when the majority of product is generally purchased, tended to smooth the differences induced by packaging. Both TF2 – thermoformed package with lower thickness – and FP could be valid alternatives to TF1: while the former would not jeopardize the standard shelf life of 60 days, the latter could be adopted when the expected product turnover is within 30 days. The adoption of TF2 or FP systems would carry a significant reduction of packaging consumption which, in turn, results in environmental and economic improvements.

### 3.5 References

Abdelghafor, R. F., Mustafa, A. I., Ibrahim, A. M. H., & Krishnan, P. G. (2011). Quality of bread from composite flour of sorghum and hard white winter wheat. *Advance Journal of Food Science and Technology*, *3*, 9–15.

Armero, E., & Collar, C. (1998). Crumb firming kinetics of wheat breads with anti-staling additives. *Journal of Cereal Science*, *28*, 165–174.

Beleggia, R., Platani, C., Spano, G., Monteleone, M., & Cattivelli, L. (2009). Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. *Journal of Cereal Science*, *49*, 301–309.

Bianchi, F., Careri, M., Chiavaro, E., Musci, M., & Vittadini, E. (2008). Gas chromatographic–mass spectrometric characterisation of the Italian protected designation of origin "Altamura" bread volatile profile. *Food Chemistry*, *110*, 787-793.

Bollaín C., Angioloni A., & Collàr C. (2005). Bread staling assessment of enzyme-supplemented pan breads by dynamic and static deformation measurements. *European Food Research and Technology 220*, 83–89.

Boyacioglu, M. H., & D'Appolonia, B. L. (1994). Characterization and utilization of durum wheat for breadmaking. III. Staling properties of bread baked from bread wheat flours and durum wheat flours. *Cereal Chemistry*, *71*, 34-41.

Byrne, M. (2000). Barilla: investing for the future. *Food Engineering and Ingredients*, *25*, 18–23.

Caponio, F., Summo, C., Pasqualone, A., & Bilancia, M. T. (2008). Effect of kneading and baking on the degradation of the lipid fraction of biscuits. *Journal of Cereal Science*, *48*, 407-412.

Chang, C. Y., Seitz, L. M., & Chambers IV, E. (1995). Volatile flavor components of breads made from hard red winter wheat and hard white winter wheat. *Cereal Chemistry*, *72*, 237-242.

Chiavaro, E., Vittadini, E., Musci, M., Bianchi, F., & Curti, E. (2008). Shelf-life stability of artisanally and industrially produced durum wheat sourdough bread ("Altamura bread"). *LWT-Food Science and Technology*, *41*, 58-70.

Del Nobile, M. A., Martoriello, T., Cavella, S., Giudici, P., & Masi, P. (2003). Shelf life extension of durum wheat bread. *Italian Journal of Food Science*, *15*, 383–393.

Frankel, E. N. (1983). Volatile lipid oxidation products. *Progress in Lipid Research*, *22*, 1-33.

Giannone V., Lauro M. R., Spina A., Pasqualone A., Auditore L., Puglisi I., & Puglisi G. (2016). A novel $\alpha$-amylase-lipase formulation as anti-staling agent in durum wheat bread. *LWT - Food Science and Technology*, *65*, 381-389.

Hareland, G. A., & Puhr, D. P. (1998). Baking performance of durum and soft wheat flour in a sponge-dough breadmaking procedure. *Cereal Chemistry*, *75*, 830-835.

Indrani, D., Rao, S. J., Sankar, K. U., & Rao, G. V. (2000). Changes in the physical-chemical and organoleptic characteristics of paratta during storage. *Food Research International*, *33*, 323–329.

Katina, K., Salmenkallio-Marttila, M., Partanen, R., Forssell, P., & Autio, K. (2006). Effects of sourdough and enzymes on staling of high-fibre wheat bread. *LWT - Food Science and Technology 39*, 479–491.

Kim, S. K., & D'Appolonia, B. L. (1977). Bread staling studies I: effect of protein content on staling rate and bread crumb pasting properties. *Cereal Chemistry*, *54*, 207-306.

Kulp, K., & Ponte, J. G. (1981). Staling of white pan bread: fundamental causes. *CRC Critical Reviews in Food Science and Nutrition 15*, 1-48.

Latou, E., Mexis, S., Badeka, A., & Kontominas, M. (2010). Shelf life extension of sliced wheat bread using either an ethanol emitter or an ethanol emitter combined with an oxygen adsorber as alternatives to chemical preservatives. *Journal of Cereal Science*, *52*, 457–465.

Le-Bail, A., Boumali, K., Jury, V., Ben-Aissa, F., & Zuniga, R. (2009). Impact of baking kinetics on staling rate and mechanical properties of bread crumb and degazed bread crumb. *Journal of Cereal Sciences*, *50*, 235-240.

Licciardello, F., Cipri, L., & Muratore, G. (2014). Influence of packaging on the quality maintenance of industrial bread by comparative shelf life testing. *Food Packaging and Shelf Life*, *1*, 19-24.

Makhoul, S., Romano, A., Capozzi, V., Spano, G., Aprea, E., Cappellin, L., Benozzi, E., Scampicchio, M., Märk, T. D., Gasperi, F., & El-Nakat, H. (2015). Volatile compound production during the bread-making process: effect of flour, yeast and their interaction. *Food and Bioprocess Technology*, *8*, 1925-1937.

Martínez-Anaya M. A. (1996). Enzymes and bread flavor. *Journal of Agricultural Food Chemistry*, *44*, 2469-2480.

Mihaly Cozmuta, A., Peter, A., Mihaly Cozmuta, L., Nicula, C., Crisan, L., Baia, L., & Turila, A. (2015). Active packaging system based on Ag/TiO2 nanocomposite used for extending the shelf life of bread. Chemical and microbiological investigations. *Packaging Technology and Science*, *28*, 271-284.

Pagani, M., Lucisano, M., Mariotti, M., & Limbo S. (2006). Influence of packaging material on bread characteristics during ageing. *Packaging Technology and Science*, *19*, 295–302.

Palacios, H. R., Schwarz, P. B., & D'Appolonia, B. L. (2004). Effect of $\alpha$-amylases from different sources on the retrogradation and recrystallization of concentrated wheat starch gels: relationship to bread staling. *Journal of Agricultural and Food Chemistry*, *52*, 5978–5986.

Pasqualone, A. (2012). Italian durum wheat breads. In M. T. Pedrosa Silva Clerici (Ed.), *Bread consumption and health* (pp. 57–79). Hauppage, NY: Nova Science Publisher Inc.

Pasqualone, A., Bianco, A. M., Paradiso, V. M., Summo, C., Gambacorta, G., Caponio, F., & Blanco, A. (2015). Production and characterization of functional biscuits obtained from purple wheat. *Food Chemistry*, *180*, 64-70.

Pasqualone, A., Caponio, F., & Simeone, R. (2004). Quality evaluation of re-milled durum wheat semolinas used for bread-making in Southern Italy. *European Food Research and Technology*, *219*, 630-634.

Pasqualone, A., Paradiso, V. M., Summo, C., Caponio, F., & Gomes, T. (2014). Influence of drying conditions on volatile compounds of pasta. *Food and Bioprocess Technology*, *7*, 719-731.

Pasqualone, A., Summo, C., Bilancia, M. T., & Caponio, F. (2007). Variations of the sensory profile of durum wheat Altamura PDO (Protected Designation of Origin) bread during staling. *Journal of Food Science*, *72*, S191-S196.

Piergiovanni L., & Fava P. (1997). Minimizing the residual oxygen in modified atmosphere packaging of bakery products. *Food Engineering and Contaminants*, *14*, 765–773.

Quaglia, G. (1988). *Other durum wheat products*. In G. Fabriani e C. Lintas, Durum wheat: chemistry and technology (pp. 263-274). St. Paul, MN: American Association of Cereal Chemistry.

Raffo, A., Pasqualone, A., Sinesio, F., Paoletti, F., Quaglia, G. & Simeone, R. (2003). Influence of durum wheat cultivar on the sensory profile and staling rate of Altamura

bread. *European Food Research and Technology*, *218*, 49-55.

Rask, C. (1989). Thermal properties of dough and bakery products: a review of published data. *Journal of Food Engineering, 9*, 167-193.

Rasmussen, P. H. & Hansen, Å. (2001). Staling of wheat bread stored in modified atmosphere. *LWT - Food Science and Technology*, *34*, 487-491.

Rinaldi, M., Paciulli, M., Caligiani, A., Sgarbi, E., Cirlini, M., Dall'Asta, C., & Chiavaro, E. (2015). Durum and soft wheat flours in sourdough and straight-dough bread-making. *Journal of Food Science and Technology, 52*, 6254-6265.

Selomulyo, V. O., & Zhou, W. (2007). Frozen bread dough: Effects of freezing storage and dough improvers. *Journal of Cereal Science, 45*, 1-17.

Sidhu J. S., Al-Saqer J., & Al-Zenki S. (1997). Comparison of methods for the assessment of the extent of staling in bread. *Food Chemistry*, *58,* 161–167.

Yamazaki, W. (1953). An alkaline water retention capacity for the evaluation of cookie baking potentialities of soft winter wheat flours. *Cereal Chemistry*, *30*, 242-246.

**Table 1.**
Changes in moisture content of crumb and crust, $a_w$, and alkaline water retention capacity (AWRC) of differently packed durum wheat bread samples (TF1 = thermoformed 1; TF2 = thermoformed 2; FP = flow-pack) during 90 days of storage, and kinetic parameters resulting from the best-fit of the experimental data to a first-order kinetic model ($k$ = kinetic constant; $C^0$ = initial value of the parameter; $C^\infty$ = value of the parameter at infinite time).

| Storage time (days) | TF1 | TF2 | FP | TF1 | TF2 | FP |
|---|---|---|---|---|---|---|
| | *$a_w$* | | | *AWRC (%)* | | |
| 0 | 0.917±0.004$^{c}$ | 0.917±0.002$^{d}$ | 0.917±0.006$^{c}$ | 315.3±2.3$^{d}$ | 315.3±2.3$^{d}$ | 315.3±2.3$^{e}$ |
| 7 | 0.916±0.003$^{cC}$ | 0.911±0.001$^{cB}$ | 0.906±0.003$^{bA}$ | 313.0±5.2$^{e}$ | 319.3±3.2$^{e}$ | 307.6±5.1$^{e}$ |
| 15 | 0.910±0.001$^{bB}$ | 0.910±0.002$^{cB}$ | 0.905±0.002$^{bA}$ | 300.0±5.6$^{cd}$ | 301.4±4.1$^{d}$ | 291.8±2.4$^{d}$ |
| 30 | 0.910±0.001$^{bB}$ | 0.905±0.002$^{bA}$ | 0.903±0.002$^{bA}$ | 292.4±0.1$^{cB}$ | 289.0±1.1$^{cB}$ | 277.0±3.7$^{cA}$ |
| 60 | 0.908±0.002$^{bB}$ | 0.903±0.002$^{abA}$ | 0.903±0.002$^{bA}$ | 265.1±3.8$^{bAB}$ | 275.3±2.0$^{bB}$ | 263.9±3.6$^{bA}$ |
| 90 | 0.899±0.004$^{a}$ | 0.899±0.002$^{a}$ | 0.897±0.003$^{a}$ | 250.6±2.1$^{aA}$ | 264.8±5.5$^{aB}$ | 239.4±3.9$^{aA}$ |
| *$k$ (×10$^{-2}$)* | 2.81±1.35 | 3.43±0.99 | 13.40±4.13 | - | - | - |
| *$C^0$* | 0.917±0.001 | 0.915±0.001 | 0.916±0.002 | - | - | - |
| *$C^\infty$* | 0.903±0.003 | 0.899±0.002 | 0.900±0.001 | - | - | - |
| | *Crumb moisture (g/100 g)* | | | *Crust moisture (g/100 g)* | | |
| 0 | 45.4±0.2$^{e}$ | 45.4±0.2$^{c}$ | 45.4±0.2$^{c}$ | 22.3±4.0$^{a}$ | 22.3±4.0$^{a}$ | 22.3±4.0$^{a}$ |
| 7 | 43.8±0.7$^{d}$ | 42.9±1.0$^{b}$ | 40.6±2.0$^{b}$ | 30.2±0.3$^{b}$ | 29.6±1.0$^{b}$ | 31.5±0.1$^{b}$ |
| 15 | 40.5±0.5$^{cA}$ | 42.3±0.1$^{bB}$ | 40.7±0.1$^{bA}$ | 30.4±0.7$^{b}$ | 30.7±0.3$^{b}$ | 33.4±2.2$^{b}$ |
| 30 | 39.1±0.4$^{bB}$ | 36.8±0.7$^{aAB}$ | 35.5±1.5$^{aA}$ | 33.2±2.2$^{b}$ | 30.7±1.3$^{b}$ | 32.8±0.6$^{b}$ |
| 60 | 38.1±0.4$^{abB}$ | 37.3±0.5$^{aB}$ | 36.0±0.3$^{aA}$ | 34.9±0.9$^{b}$ | 31.9±1.6$^{b}$ | 33.3±0.1$^{b}$ |
| 90 | 37.6±1.0$^{a}$ | 37.7±1.4$^{a}$ | 36.2±0.1$^{a}$ | 34.5±1.4$^{b}$ | 32.3±0.9$^{b}$ | 33.4±0.2$^{b}$ |
| *$k$ (×10$^{-2}$)* | 5.68±0.98 | 5.64±1.76 | 7.26±2.09 | 8.52±3.30 | 18.29±9.37 | 24.79±11.81 |
| *$C^0$* | 45.7±0.4 | 45.8±0.8 | 45.4±0.9 | 24.0±1.4 | 23.6±1.3 | 23.6±1.3 |
| *$C^\infty$* | 37.6±0.4 | 37.0±0.7 | 35.7±0.7 | 34.4±1.0 | 31.6±0.7 | 33.3±0.7 |

$^{a,A}$ Different lower case letters in column, for each parameter, indicate significant differences due to the effect of storage time (at $P < 0.05$, based on Tukey HSD test); different upper case letters in row, for each parameter, indicate significant differences due to the effect of packaging type (at $P < 0.05$, based on Tukey HSD test). Absence of letters indicates absence of significant differences.

**Table 2.**
Changes in textural parameters of differently packed durum wheat bread samples (TF1 = thermoformed 1; TF2 = thermoformed 2; FP = flow-pack) during 90 days of storage. The table also reports the parameters (*k* = kinetic constant; *n* = Avrami exponent; $F_\infty$ = limiting value of firmness at infinite time) resulting from the best-fit of Avrami equation to firmness data, as well as the parameters (*k* = kinetic constant; $C^0$ = initial value of the descriptor; $C^\infty$ = value of the descriptor at infinite time) resulting from the best fit of a first-order kinetic model to resilience, springiness and chewiness data.

| Storage time (days) | TF1 | TF2 | FP | TF1 | TF2 | FP |
|---|---|---|---|---|---|---|
| | *Firmness (N)* | | | *Resilience* | | |
| 0 | 22.2±1.3$^{a}$ | 22.2±1.3$^{a}$ | 22.2±1.3$^{a}$ | 0.91±0.02$^{c}$ | 0.91±0.04$^{c}$ | 0.91±0.03$^{c}$ |
| 7 | 31.5±2.3$^{bA}$ | 46.5±3.0$^{bB}$ | 49.1±3.1$^{bB}$ | 0.89±0.01$^{cB}$ | 0.80±0.02$^{bA}$ | 0.78±0.06$^{bA}$ |
| 15 | 59.0±4.7$^{c}$ | 60.5±2.8$^{c}$ | 63.6±0.2$^{c}$ | 0.79±0.02$^{b}$ | 0.75±0.06$^{ab}$ | 0.74±0.02$^{b}$ |
| 30 | 74.9±4.7$^{dA}$ | 83.0±2.0$^{dB}$ | 90.7±3.5$^{dC}$ | 0.77±0.04$^{bB}$ | 0.75±0.01$^{abAB}$ | 0.70±0.04$^{abA}$ |
| 60 | 79.3±3.2$^{dA}$ | 86.0±0.6$^{dB}$ | 113.0±4.3$^{eC}$ | 0.77±0.04$^{b}$ | 0.72±0.06$^{ab}$ | 0.68±0.05$^{ab}$ |
| 90 | 97.1±4.2$^{eA}$ | 99.9±4.0$^{eA}$ | 114.7±4.1$^{eB}$ | 0.68±0.03$^{a}$ | 0.68±0.01$^{a}$ | 0.64±0.05$^{a}$ |
| $k$ ($\times 10^{-2}$) | 3.4±1.9 | 6.4±2.0 | 2.3±0.6 | 3.40±1.22 | 9.35±2.47 | 8.82±2.18 |
| $n$ or $C^0$ | 1.1±0.2 | 0.9±0.1 | 1.2±0.1 | 0.91±0.02 | 0.90±0.02 | 0.90±0.02 |
| $F_\infty$ or $C^\infty$ | 92.3±4.3 | 99.4±4.2 | 117.2±2.2 | 0.70±0.03 | 0.71±0.01 | 0.66±0.01 |
| | *Springiness (mm)* | | | *Chewiness (N mm)* | | |
| 0 | 4.53±0.90$^{a}$ | 4.53±0.91$^{a}$ | 4.53±0.20$^{a}$ | 91±5$^{a}$ | 91±5$^{a}$ | 91±5$^{a}$ |
| 7 | 4.68±0.54$^{aA}$ | 6.46±0.22$^{bB}$ | 6.77±0.80$^{bB}$ | 99±3$^{aA}$ | 178±6$^{bB}$ | 256±8$^{bC}$ |
| 15 | 5.73±0.52$^{abA}$ | 6.80±0.17$^{bB}$ | 7.28±0.12$^{bB}$ | 208±8$^{bA}$ | 231±4$^{cB}$ | 257±5$^{bC}$ |
| 30 | 6.43±0.18$^{b}$ | 6.98±0.92$^{b}$ | 7.40±0.61$^{b}$ | 276±6$^{cA}$ | 354±8$^{dB}$ | 467±6$^{cC}$ |
| 60 | 6.73±0.74$^{b}$ | 7.23±0.10$^{b}$ | 7.47±0.32$^{b}$ | 305±6$^{dA}$ | 334±6$^{eB}$ | 575±7$^{dC}$ |
| 90 | 7.05±0.40$^{bA}$ | 7.55±0.36$^{bAB}$ | 7.64±0.21$^{bB}$ | 353±6$^{eA}$ | 384±2$^{fB}$ | 621±2$^{eC}$ |
| $k$ ($\times 10^{-2}$) | 4.01±1.48 | 15.30±4.50 | 19.50±5.60 | 4±1 | 6±1 | 3±1 |
| $C^0$ | 4.37±0.26 | 4.56±0.28 | 4.53±0.27 | 75±10 | 87±11 | 101±15 |
| $C^\infty$ | 7.10±0.32 | 7.24±0.16 | 7.49±0.15 | 357±14 | 373±9 | 656±24 |

$^{a,A}$ Different lower case letters in column, for each parameter, indicate significant differences due to the effect of storage time (at $P < 0.05$, based on Tukey HSD test); different upper case letters in row, for each parameter, indicate significant differences due to the effect of packaging type (at $P < 0.05$, based on Tukey HSD test). Absence of letters indicates absence of significant differences.

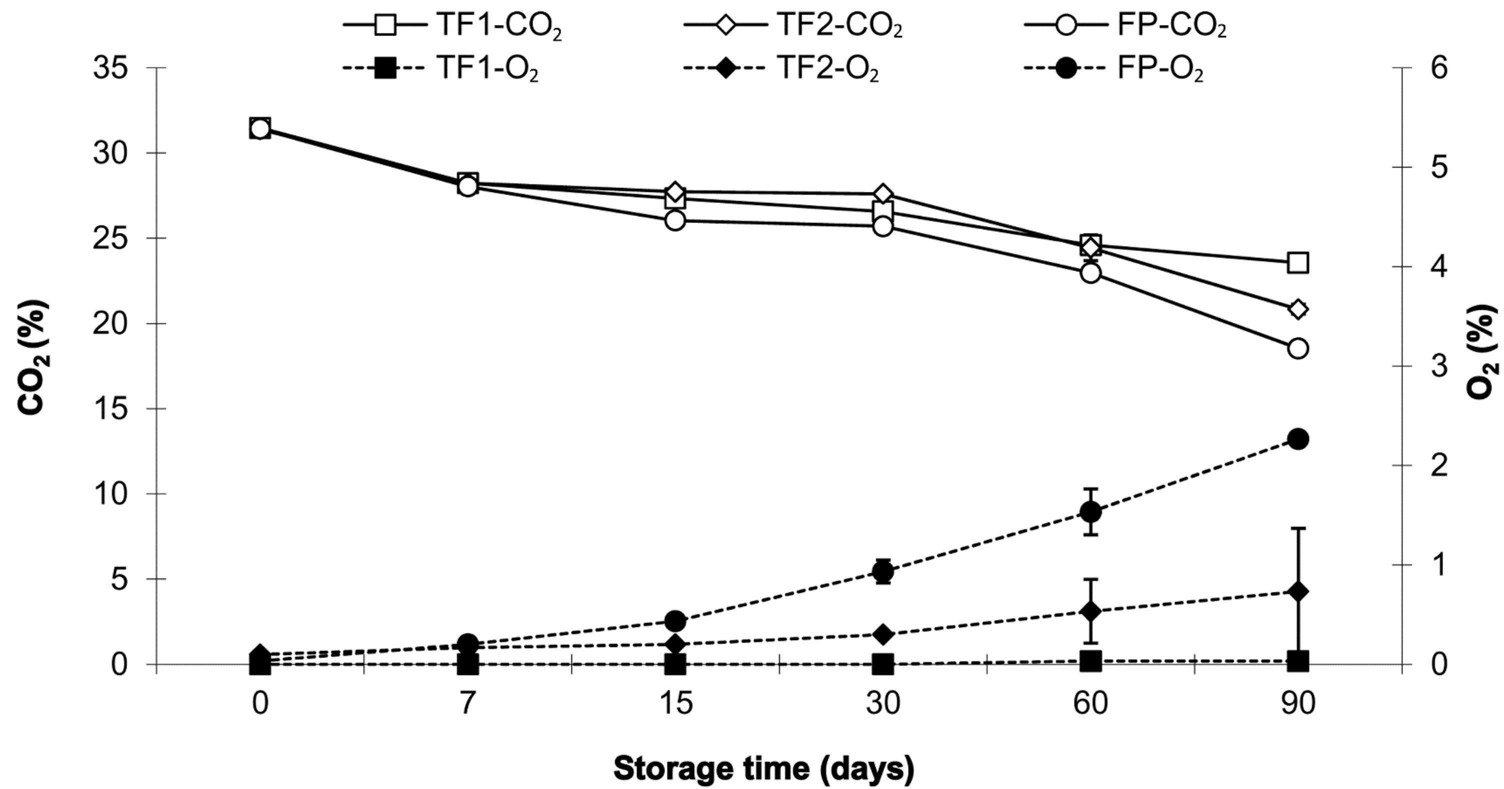


**Figure 1.** Variations of headspace $CO_2$ and $O_2$ composition (mean ± standard deviation) of durum wheat bread package during 90 days of storage, as a function of packaging system (TF1 = thermoformed 1; TF2 = thermoformed 2; FP = flow-pack).

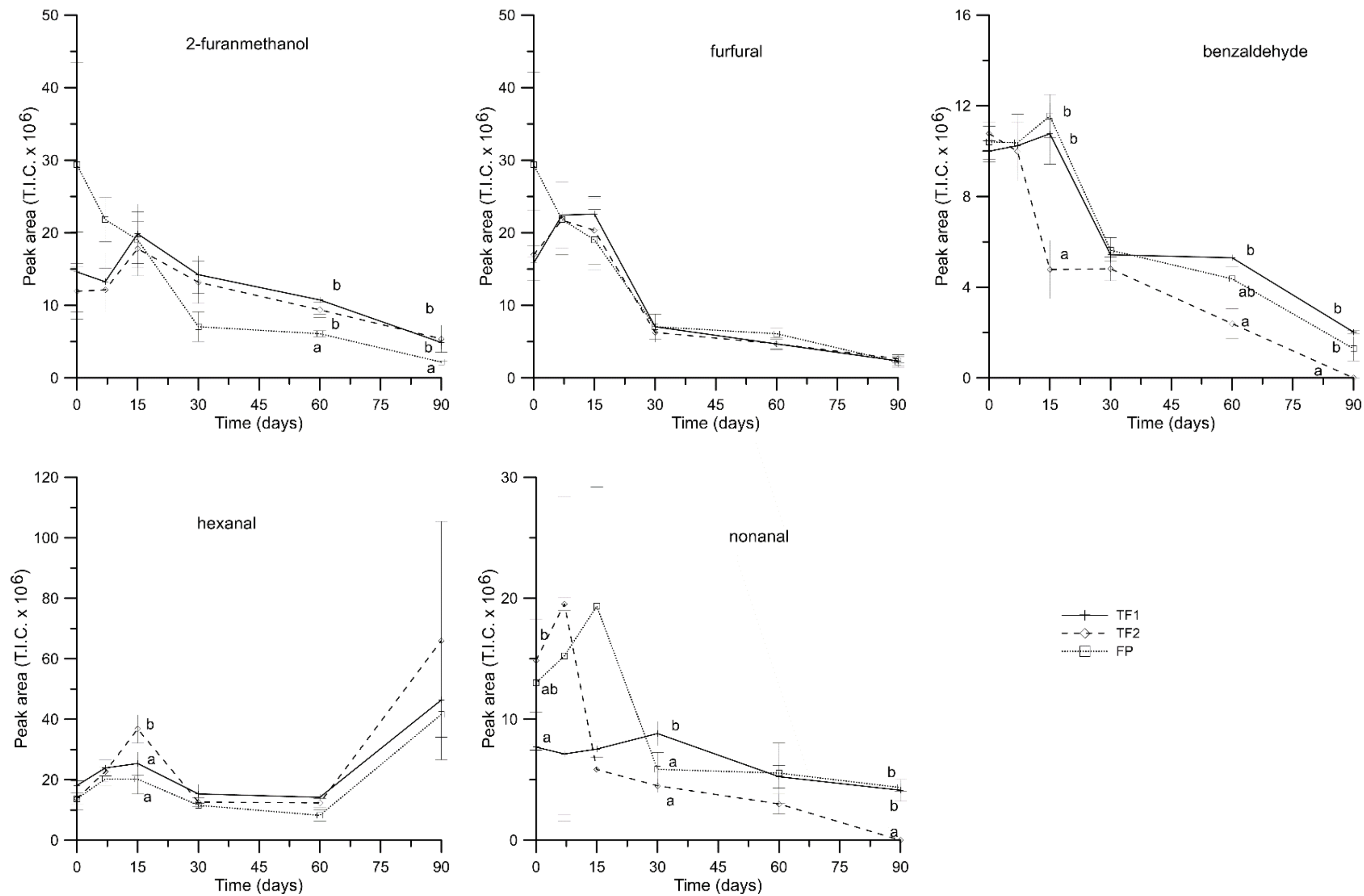


**Figure 2.** Variations of volatile compounds of durum wheat bread during 90 days of storage, as a function of packaging system (TF1 = thermoformed 1; TF2 = thermoformed 2; FP = flow-pack). Different letters indicate significant differences due to the effect of packaging type (at $P < 0.05$, based on Tukey HSD test).

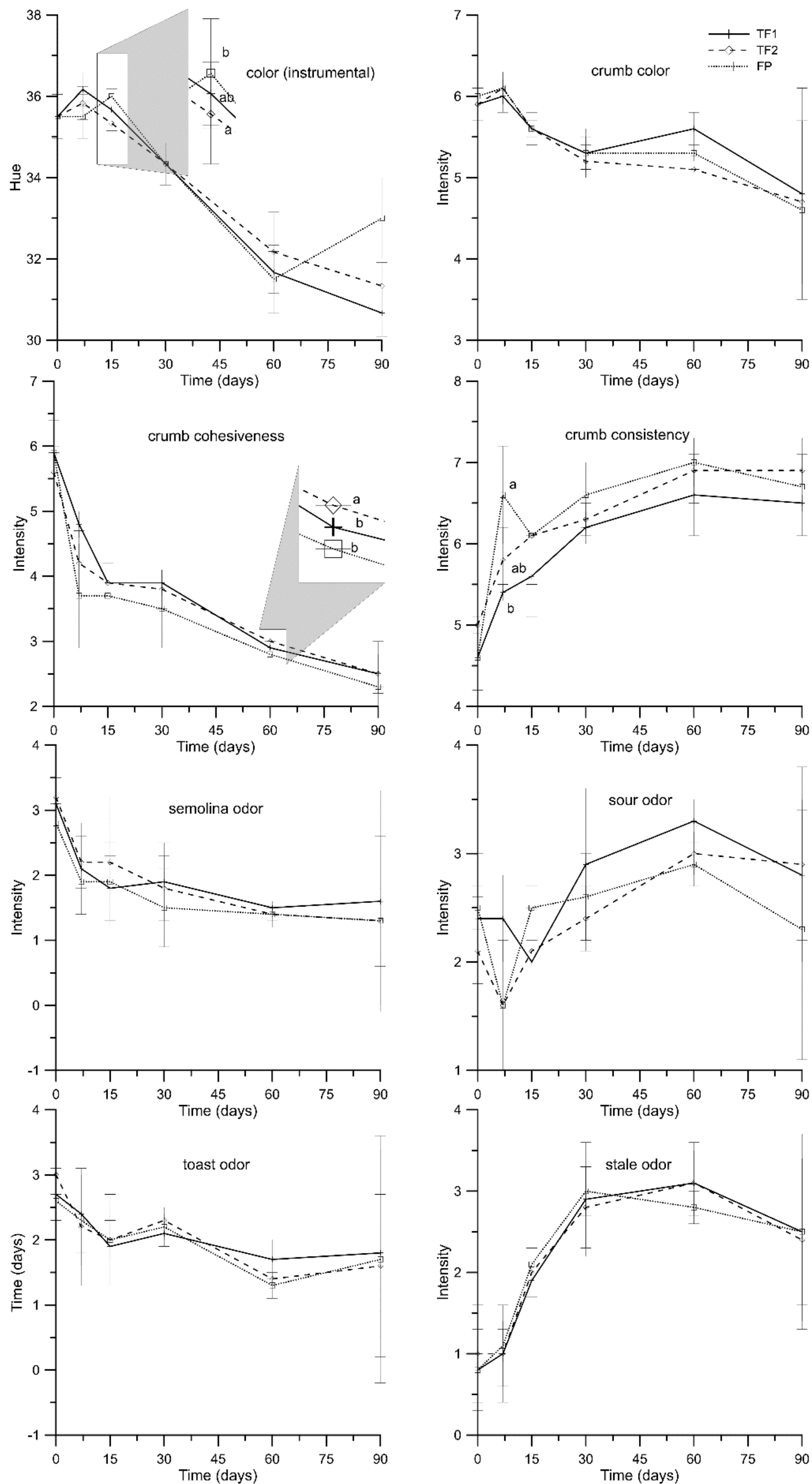


**Figure 3.** Variations of color (instrumentally determined) and sensory properties of durum wheat bread during 90 days of storage, as a function of packaging system (TF1 = thermoformed 1; TF2 = thermoformed 2; FP = flow-pack). Different letters indicate significant differences due to the effect of packaging type (at $P < 0.05$, based on Tukey HSD test).

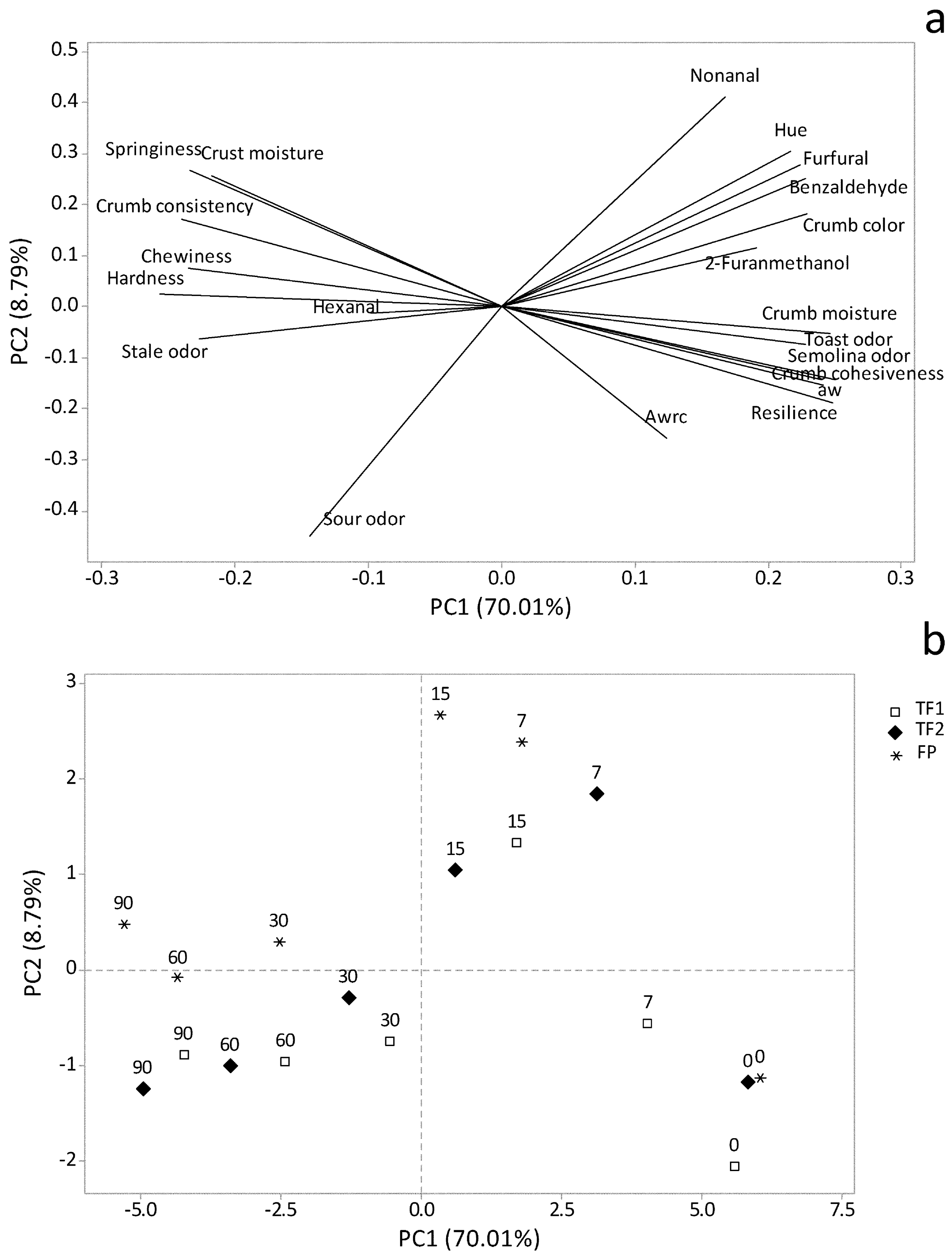


**Figure 4.** Loading plot (a) and score plot (b) of the principal components analysis carried out on the analytical data of durum wheat bread during storage, under three different packaging systems (TF1 = thermoformed 1; TF2 = thermoformed 2; FP = flow-pack). Data labels in the score plot indicate the days of storage.

# Chapter 4

## Almond by-products: extraction and characterization of phenolic compounds and evaluation of their potential use in composite dough with wheat flour

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**Abstract**

Blanched skins and blanching water, by-products of almond processing, were evaluated as potential ingredients of bakery products. The research included three phases: i) optimization of skin drying; ii) optimization of quali-quantitative determination of phenolic compounds, by comparing three extracting protocols; iii) assessment of the impact of by-products on the rheology of composite doughs with wheat flour. The least time-consuming drying mode (at 60 °C for 30 min) retained better odor notes, higher content of phenolics (814 μg/g d.m. by HPLC, with the most effective extracting method) and greater antioxidant activity than sun-drying. Blanching water showed 917 μg/mL phenolics. Dried almond skins altered alveograph and farinograph indices of dough at doses higher than 30 and 50 g/kg, respectively, whereas blanching water did not cause significant changes. Therefore, almond skins could be used in products tolerating weak gluten network, such as cookies, whereas blanching water could be added to any bakery good.

**Keywords:** almond skins; almond blanching water; bioactive compounds; antioxidant activity; rheological properties.

## 4.1. Introduction

Almonds (*Prunus dulcis* (Mill.) D.A. Webb or *Amygdalus communis* L.) are largely used in the preparation of several traditional bakery and confectionery products including almond cookies, marzipan and almond milk (Bennet, 2016). The first processing step in the production of these delicacies is the removal of the brown skin from almonds, by means of blanching in hot water and subsequent mechanical peeling. Skins account for 6-8% of the seed (Garrido, Monagas, Gómez-Cordovés & Bartolomé, 2008) and are mainly destined to cattle feeding (Grasser, Fadel, Garnett & DePeters, 1995). Blanching water represents merely a waste, and the producers have to face costs for its disposal. However, blanched skins and blanching water contain several antioxidant bioactive phenolic compounds, mainly composed of flavonoids, hydrolysable and condensed tannins and phenolic acids (Bolling, 2017; Chen, Milbury, Lapsley & Blumberg, 2005; Garrido et al., 2008; Mandalari et al., 2010), that act synergistically to protect LDL from oxidation (Amarowicz, 2016; Chen, Milbury, Chung & Blumberg, 2007). To increase the value of almond by-products, so as to reduce economic and environmental issues, the bioactive compounds could be extracted or, alternatively, the by-products themselves could be used as food ingredients. The addition of phenolic-rich by-products improves the health-promoting value of cereal-based end products, even if baking may result in the modification of phenolic content and composition (Laddomada, Caretto & Mita, 2015; Pasqualone et al., 2014; 2015). The direct use of by-products would encompass the costs of extraction and, in case of blanched skins, would also contribute dietary fiber, mostly insoluble (Mandalari et al., 2010). Bakery products such as cookies and bread, are considered a suitable carrier for functional ingredients (Laddomada et al., 2015; Rahaie, Gharibzahedi, Razavi & Jafari 2014). Functional food production, however, may involve quality drawbacks in terms of possible alterations of physico-chemical and sensory features. Fiber-rich functional ingredients, in fact, could interfere with gluten formation. These drawbacks would be minimal in case of using concentrated bioactive extracts from almond by-products, but could be more evident in case of direct addition of blanching water and, above all, of blanched skins to wheat flour. Therefore, after evaluating the physico-chemical properties, odor notes, antioxidant activity, and content of phenolic compounds of blanching water and blanched almond skins - the latter not dried and dried in different conditions- the aim of this work has been to evaluate the potential of these by-products as food ingredients in view of obtaining cereal-based bakery

products. The research followed a multi-step approach and was developed in three main phases: i) optimization of the thermal conditions for drying blanched almond skins; ii) optimization of the extraction and quali-quantitative determination of phenolic compounds of blanched skins and blanching water, by comparing three different protocols; iii) determination of the impact of dried blanched skins (opportunely milled) and blanching water on the rheological properties and color of composite dough with wheat flour, so as to choose the best practical applications in bakery.

## 4.2. Materials and Methods

### *4.2.1. Sampling of almond by-products*

Blanched almond skins (detached by soaking almonds in water at 95 ± 2 °C for 4 ± 1 min, followed by mechanical peeling) and blanching water were collected at an almond processing industry (Calafiore S.r.l., Floridia, Siracusa, Italy). Two different samplings were carried out to take into account any possible variability of the raw materials and the two samples were combined to generate one sample for analyses and comparisons. The Calafiore S.r.l. industrial plant has a daily processing capacity of 30 tons almonds (coming from California, Spain, and Southern Italy, shipped in 800-1000 kg big bags and stored at room temperature), leading to 100-150 kg blanched almond skins and 10.000-12.000 L blanching water. Blanched almond skins were dried in different conditions, i.e. by rotary air drier (Scirocco, Società Italiana Essiccatoi, Milano, Italy) at: i) 60 °C for 30 min; ii) 45 °C for 90 min; iii) 32 °C for 150 min, or iv) by natural sun-drying at 38-40 °C for 6-8 h. Time/temperature combination of the drying conditions were selected on the basis of preliminary trials.

### *4.2.2 Almond skin preparation for analyses*

Dried and not dried blanched almond skins were directly submitted to the analysis of moisture content, $a_w$, color and odor notes without any treatment (i.e. entire), whereas the same skins were powdered for the analyses of antioxidant activity, phenolic compounds, and water retention capacity. In particular, dried skins were powdered by mechanical milling (Cutting Mill SM 100, Retsch, Haan, Germany), while not dried skins were hand milled in a mortar under liquid nitrogen. Lipid content was analyzed on mechanically milled dried almond skins,

while not dried skins were first submitted to lyophilization (Analitica De Mori, Milano, Italy) and then to mechanical milling.

*4.2.3. Preparation of wheat flour composite meals containing almond by-products*

Refined wheat flour type 0 (*Triticum aestivum* L.) (Molini Spigadoro, Bastia Umbra, Italy), having 104 g/kg protein and 137 g/kg moisture, was used to prepare composite meals containing 30 g/kg, 50 g/kg, 70 g/kg and 100 g/kg of almond skins dried at 60 °C for 30 min. The skins were opportunely milled (Cutting Mill SM 100, Retsch, Haan, Germany) to a particle size in the range 450-500 μm so as to allow easy blending with flour and for obtaining a homogeneous dough.

*4.2.4. Determination of physico-chemical properties of almond by-products*

Moisture content of blanched almond skins was determined by drying (Eurotherm, Gibertini, Novate Milanese, Italy) at 105 °C until constant weight; $a_w$ was determined by AquaLab Vapor Sorption Analyzer (Decagon Devices, Pullman, WA, USA) according to manufacturers' instructions. The lipid fraction was extracted from blanched almond skins by Soxhlet apparatus using diethyl ether. Water retention capacity (WRC) was determined on 1 g of blanched almond skins, put in 15-mL tubes, added with 5 mL distilled water and vortexed for 30 s, then left at room temperature for 20 min. The slurry was centrifuged at 3000 × *g* for 15 min, the supernatant was discarded and tubes were let drip for 10 min upside down inclined by 15°. Dried tubes were then weighed. WRC was calculated as [(W2 - W1)/W1] × 100, where W1 is the weight of the tube containing the dry sample and W2 is the weight of the tube containing the dripped sample. Color parameters ($L^*$, $a^*$, $b^*$) of blanched almond skins were determined by Chromameter CR-300 (Minolta, Osaka, Japan), under the illuminant D65. Blanching water was shaken and poured into a Petri dish placed on a white paper sheet and the color of a 1-cm thick layer of sample was determined. Brown index was calculated as 100 – $L^*$.

*4.2.5. Sensory determination of main odor notes of almond by-products*

The intensity of the main odor notes of almond blanched skins and blanching water was determined by 8 trained panelists (four males, four females, aged between 24 and 49 years),

selected for their reliability, consistency and discriminating ability as in Pasqualone et al. (2011). All the samples, identified by alphanumerical codes, were randomly served to panelists into plastic cups covered by an aluminum foil. The odor notes "leafy", "rancid" and "sour" were rated on an anchored line scale that provided a 0-9 score range, 0 being absence and 9 very intense, in the conditions reported in Pasqualone et al. (2011).

*4.2.6. Extraction of phenolic compounds from blanched almond skins*

The phenolic compounds were extracted from 1 g of blanched almond skins either not dried or dried in different conditions (60°C for 30 min; 45°C for 90 min; 32°C for 150 min; 38-40°C for 6-8 h) according to three different protocols: "*A*", "*B*" and "*C*" (Figure 1). The *A* and *C* protocols were modifications of extracting methods proposed by Garrido et al. (2008) and Mandalari et al. (2010), respectively. Modification consisted of substituting the extracting solvent (methanol acidified with 1 mL/L HCl 12 mol/L) with a solution of acetone diluted with 200 mL/L distilled water, based on a preliminary comparison of the extracting efficiency of these two different solvent combinations. Extraction efficiency was determined by comparing the quantitative results obtained using the two solvent combinations on the same amount of almond skins. The *B* method consisted of sample delipidation, followed by alkaline hydrolysis, acidification and double ethyl acetate extraction as described in details in Laddomada et al. (2017). At the end of each protocol, the dried extracts were dissolved in 2 mL of a solution of methanol diluted with 200 mL/L distilled water and used for analyses.

*4.2.7. Extraction of phenolic compounds from almond blanching water*

An aliquot (20 mL) of blanching water was centrifuged at 5000 *g* for 5 min, then the supernatant was lyophilized (Analitica De Mori, Milano, Italy). The dry residue was extracted two times with 20 mL of a solution of acetone diluted with 200 mL/L distilled water, under mixing and sonication, at sonic power 200 W, for 10 min at room temperature (LBS1-10 sonicator, Tecno Lab, Brescia, Italia), followed by centrifugation (10 min at 5000 *g*, 4°C). The aqueous acetone extracts were combined and dried by rotary evaporator. The same procedure was repeated without sonication to point out the effect of ultrasound. The dried extracts were dissolved in 2 mL of a solution of methanol diluted with 200 mL/L distilled water and used for analyses.

*4.2.8. HPLC analysis of phenolic compounds*

An aliquot of 50 μL of phenolic extract obtained according to the above reported procedures was filtered on 0.45 μm polytetrafluoroethylene (PTFE) filters (Teknokroma, Barcelona, Spain) and quali-quantitatively analyzed using an Agilent 1100 Series HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed phase C18(2) Luna column (Phenomenex, Torrance, CA, USA) (5 μm, 250 × 4.6 mm). The chromatographic separation was carried out using a gradient made up of three solvents: A (water/glacial acetic acid, 98/2, v/v), B (water/acetonitrile/glacial acetic acid, 73/25/2, v/v/v) and C (acetonitrile) as mobile phase (Mandalari et al., 2010). The gradient program started with pure A solvent to reach a solution of A (200 mL/L) and B (800 mL/L) at 55 min, followed by a solution of A (100 mL/L) and B (900 mL/L) at 57 min; then pure B at 90 min and finally pure C at 105 min. The flow rate was 1 mL/min, and the column was thermostatically controlled at 25 °C (Mandalari et al., 2010). Peaks were identified by comparing their retention times and UV-Vis spectra to those of authentic phenolic standards. Phenolic acids were quantified via a ratio to 3,5-dichloro-4-hydroxybenzoic acid used as internal standard and calibration curves of phenolic acid standards. Other phenolic compounds (flavan-3-ols, flavonol and flavonone glycosides and aglycones) were quantified using calibration curves according to the external standard method (Mandalari et al., 2010). Authentic standards of phenolic acids were obtained from Sigma-Aldrich (Gillingham, UK) and included protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, chlorogenic acid and vanillic acid. Authentic standards for flavan-3-ols (as(+)-catechin and (–)-epicatechin); flavonols (quercetin-3-*O*-glucopyranoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, quercetin dehydrate, kaempferol, isorhamnetin); and flavonones (naringenin-7-*O*-glucoside and eriodictyol and naringenin) were purchased from Extrasynthèse (Genay, France).

*4.2.9. Determination of in vitro antioxidant activity*

An amount of 1 g blanched almond skin powder was mixed with 10 mL of methanol, purged with a stream of nitrogen and kept on orbital shaker at 250 rpm for 2 h in the dark. After centrifugation at 5000 *g* for 5 min, the supernatant was utilized for the determination of the *in*

*vitro* antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay in the conditions reported in Pasqualone et al. (2015). Blanching water was directly used, after a centrifugation step at 5000 *g* for 10 min and recovery of the surnatant. Antioxidant activity was expressed as μmol of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox).

*4.2.10. Rheological and color analyses of composite dough containing almond by-products*

The farinograph (Brabender instrument, Duisburg, Germany) and alveograph (Tripette et Renaud, Chopin Technologies, Villeneuve-la-Garenne, France) analyses of wheat flour and composite meals with almond by-products were carried out according to the methods AACC 54-21 (AACC International, 2000) and UNI 10453 (UNI, 1995), respectively. When almond blanching water was tested, it totally replaced the amount of liquid (water or 25 g/L NaCl water solution for farinograph and alveograph analysis, respectively) added to control wheat flour. For color analyses, performed by Chromameter CR-300 (Minolta, Osaka, Japan) under the illuminant D65, dough was prepared by farinograph and was successively compressed between two sterile Petri plates to obtain a uniform and smooth surface. Color parameters $L^*$, $a^*$, $b^*$ were determined, and brown index was calculated as $100 – L^*$.

*4.2.11. Statistical analyses*

Two different samplings were carried out, which were pooled, and all analytical tests were carried out in triplicate. Data were analyzed using SigmaStat version 11.0 software (Systat Software Inc., Chicago, IL, USA). One-way ANOVA followed by Bonferroni's post-hoc comparisons tests was performed to establish significant differences between means.

## 4.3. Results and discussion

*4.3.1. Moisture content, odor notes, antioxidant activity and color of almond by-products*

All drying treatments effectively reduced the moisture content and $a_w$ of blanched almond skins in comparison with not dried blanched almond skins (Table 1). Skins dried at 60 °C for 30 min showed the lowest moisture content and $a_w$ among the four thermal conditions considered, whereas those dried at 32 °C for 150 min were the moistest ($p < 0.05$). Considering

that blanched and peeled almond nuts are oven-dried at 70 °C to allow their successive milling into flour, the skins could be dried immediately after turning off the oven, so as to recover the residual heat and save energy. Sun-drying (at 38-40 °C for 6-8 h) could be equally inexpensive, but depends on weather conditions and makes difficult keeping high hygienic standards. The low values of moisture content and $a_w$ achieved by drying allow to expect a relatively long shelf life, with good resistance to microbiological spoilage. However, drying does not prevent oxidative degradations, which could be important in raw materials containing polyunsaturated lipids, such as almond skins. The lipid content, indeed, accounted for approximately 220 g/kg d.m., in agreement with other researches (Mandalari et al., 2010). As a consequence, it was not surprising that the sensory panelists detected an intense rancid odor note (score 6.3 ± 0.2) in sun-dried blanched skins, due to both exposure to light and longer duration compared to the other drying conditions considered. The other dried samples were characterized by an intense leafy odor (score from 5.8 to 6.4), with negligible rancid odor. No statistically significant differences were observed among the three oven-based drying conditions for all the odor notes considered ($p < 0.001$). Not dried skins exhibited a sour note, probably coming from fermentation processes occurred during shipping to the processing company and successive storage at room temperature. This odor note was substantially lost with drying, irrespective of the conditions adopted. Blanching water, which contains only traces of lipids (Mandalari et al., 2010), was characterized by moderate leafy odor and slight sour odor, with almost no rancid note. WRC of almond skins was high, probably due to water-retaining compounds such as fiber, known to be abundant in this by-product (Mandalari et al., 2010). No significant differences were observed among samples dried in different conditions. WRC of not dried skins was significantly lower than after drying, due to higher starting moisture content which lowered the rehydration capacity. All by-products were brown colored, with red tones. The intensity of this color was the result of the occurrence of Maillard reaction during heat treatment and of the enzymatic oxidation of phenolics to brown quinones due to air exposure (Taranto et al., 2017), although polyphenol oxidase activity is partly inactivated during blanching. Not dried skins showed the lowest brownness, whereas, among thermal drying treatments, sun-dried skins showed the highest brown and red indices, and the lowest yellow index. Blanching water was markedly reddish-brown, indicating that colored compounds were water soluble. The *in vitro* antioxidant activity of not dried almond

skins, determined by the DPPH radical scavenging capacity assay, accounted for 40.37 μmol Trolox/g (d.m.). A significant increase was observed after drying, irrespective of the conditions adopted, due to the antioxidant activity of Maillard-related products (Garrido et al., 2008; Piga, Del Caro & Corda, 2003). Significant differences of antioxidant activity were assessed among the different thermal treatments, with the highest value in skins dried at 45 °C for 90 min. This combination of time and temperature, in fact, was the most intense and caused a more developed Maillard reaction. The lowest antioxidant activity was observed in sun-dried skins, in agreement with the intense rancid odor perceived by sensory evaluation. The antioxidant activity of blanching water was similar to that reported for herbal medicinal products such as sage tea (aqueous infusions of *Salvia officinalis* L.), which displays an antioxidant activity in the range 4-18 μmol Trolox/mL (Walch et al., 2011).

*4.3.2. Phenolic compounds of almond by-products*

To optimize the extraction of phenolic compounds for future use, three different methods were compared (Fig. 1). The *B* method, proposed by Laddomada et al. (2017), involved an alkaline hydrolysis, whereas the *C* method, i.e. a modified protocol based on Mandalari et al. (2010), included a sonication step. Both these treatments were aimed at freeing the insoluble phenolic compounds linked to polymers of the plant cell wall. In particular, the effectiveness of alkaline hydrolysis in enhancing the extracting yield of phenolic compounds was previously evidenced in wheat bran (Laddomada et al., 2017; Pasqualone et al., 2017). The *A* protocol, based on a modification of Garrido et al. (2008), neither required these treatments nor involved the preliminary lipid removal included in *B* and *C* methods. Overall, the extracting conditions had a significant effect ($p < 0.05$) on the quali-quantitative pattern of phenolic compounds (Table 2), as pointed out by a recent review (Bolling, 2017). The lowest amount of total phenolic compounds was measured by means of A method, which was less effective probably due to the absence of sonication or hydrolysis treatments. The *C* method extracted the highest amounts of (+)-catechin, whereas the *B* method detected the highest amounts of (-)-epicatechin. B method also allowed the best extraction of phenolic acids. These findings, observed both in not dried and dried skins – in the latter case irrespective of the thermal treatment – resulted from the complex combination of chemical and physical phenomena varying from one extracting method to another: hydrolysis of glycosides, decomposition of

aglycones, partition between solvents having different polarity, and isomerization/epimerization reactions. For example, epimerization of (+)-catechin to (-)-epicatechin occurs easily in hot water or diluted alkaline solution (Kiatgrajai, Wellons, Gollob & White, 1982). Moreover, (-)-epicatechin is more hydrophobic than its stereoisomer (+)-catechin (Verstraeten, Fraga & Oteiza, 2009). Blanching treatment with hot water probably caused the formation of (-)-epicatechin, which could have been affected more than (+)-catechin by a lipid removal step included in the *B* and *C* protocols. The *B* method, which involved an alkaline hydrolysis after the lipid removal step, probably caused further conversion of (+)-catechin to (-)-epicatechin, explaining why the latter prevailed on (+)-catechin in *B* extracts. Overall, blanched almond skins showed total amounts of phenolic compounds ranging from 253.6 μg/g d.m. (in sun-dried skins extracted by *A* method) to 1773 μg/g d.m. (in not dried skins extracted by *B* method). The former result was similar to previous literature findings, whereas the latter was about seven-fold higher (Mandalari et al., 2010; Garrido et al., 2008). The most abundant compounds in both not dried and dried skins were flavan-3-ols, irrespective of the extracting method. Among phenolic acids, the most abundant was procatechuic acid, whereas among flavonols, isorhamnetin-3-*O*-rutinoside prevailed in all the extracts. Among flavanons, naringenin-7-*O*-glucoside was ascertained in moderate amounts, together with very little amounts of naringenin, whereas eriodictyol was not detected. The total amounts of phenolic compounds extracted from thermally dried skins were always lower than those extracted from not dried skins, indicating that oxidative and other degradative phenomena occurred during thermal treatment. In particular, the most evident decrease affected (+)-catechin. Moreover, significant differences were observed also between different thermal conditions ($p < 0.05$). The *C* extracts magnified these differences: the total phenolic compounds ranged from 317 μg/g d.m. (in sun-dried skins) to 857 μg/g d.m. (in skins dried at 45 °C for 90 min). Oven drying, irrespective of the temperature/time combination chosen, affected phenolic compounds less than sun-drying. Among the thermal treatments considered, drying at 45 °C for 90 min or at 60 °C for 30 min allowed to retain the highest levels of total phenolic compounds, without significant differences among them. In addition, drying at 45 °C for 90 min caused a more intense Maillard reaction yielding to higher antioxidant activity, whereas the treatment at 60 °C for 30 min led to the lowest moisture content (Table 1). For these reasons, these two treatments were the best among the thermal conditions

considered. However, the fastest drying combination, i.e. at 60 °C for 30 min, could be more easily transferred at industrial level, where speed and productive capacity are essential factors. Table 3 reports the content of phenolic compounds of almond blanching water, where (+)-catechin was the most abundant. Overall, the phenolic profile of blanching water was similar to that of blanched skins, with the exception of aglycones, that were all not detectable or almost absent. Among flavonol aglycones, in particular, isorhamnetin was markedly lower than in skins, probably due to the low hydrophilicity of aglycones compared to conjugates. The presence of a conjugate moiety of any form, in fact, aids hydrophilicity and solubility (Rothwell, Day & Morgan, 2005), and the precipitation of polyphenol aglycones from blanching water due to their nonpolarity has been reported (Hughey et al., 2012). Therefore, blanching water tended to solubilize and retain compounds having higher hydrophilicity. To point out the effect of ultrasound, the extraction of phenolics from blanching water was carried out with and without sonication. The latter condition gave worst results, in fact (+)-catechin was quantified in half amounts. The ultrasound treatment probably promoted the liberation of the catechin monomers from tannins (Ferraretto & Celotti, 2016).

#### *4.3.3. Rheological properties and color of composite dough containing almond by-products*

Based on moisture content, antioxidant activity and phenolic quali-quantitative profile of blanched almond skins dried in different ways, only those dried at 60 °C for 30 min were considered (after being opportunely milled) for preparing composite meals with refined wheat flour at levels ranging from 30 g/kg to 100 g/kg. Besides, also blanching water was used to prepare dough. In order to choose the best practical applications of these by-products in bakery, the rheological properties and color data of dough, compared with control dough without supplementation, were considered (Table 4). Among the mixing properties of dough, determined by farinograph, dough stability (i.e. the time needed to reach the dough consistency of 500 Brabender Units) did not change significantly by adding skin powder up to 50 g/kg, followed by marked increases at higher levels. Accordingly, the softening index (i.e. the loss of dough consistency after 12 min), significantly decreased only from 70 g/kg onwards. On the contrary, dough development time (i.e., the time needed from the first addition of water to reach the maximum consistency) did not show statistically significant differences among samples ($p < 0.05$). Water absorption capacity (i.e., the percentage of water required to yield a

dough consistency of 500 Brabender Units) of the composite meals ranged from 592 g/kg to 612 g/kg. Due to their fiber content, the almond skins influenced water absorption of dough, making it significantly ($p < 0.05$) and progressively increase, compared to control, as the amount added increased. These findings agreed with the high WRC values of skins. If the effect of almond skins on the main farinograph indices was evident, on the contrary the addition of blanching water did not cause significant changes ($p < 0.05$). The alveograph indices were negatively influenced by the addition of skin powder: the deformation energy (i.e. the dough "strength") significantly decreased and the tenacity/extensibility ratio (P/L) increased, both at levels as low as 30 g/kg skins. In particular, with the addition of skins the P/L values were always above the 0.5-1.0 range suggested for bakery leavened products, and even exceeded the typical values of durum wheat re-milled semolina (Pasqualone, Caponio & Simeone, 2004). Again, the effect of blanching water was negligible also on alveograph indices. Apart the interactions between phenolic antioxidants and wheat proteins during dough preparation (Labat, Morel & Rouau, 2000; Wang and Zhou, 2004), the effect of which on rheological properties could not be excluded, these findings were mainly due to the hygroscopic effect of fiber, known to be abundant in almond skins (Mandalari et al., 2010). Furthermore, fiber interferes with the formation of a complete and strong gluten network. However, in certain bakery products, such as cookies, additional ingredients such as fats and/or emulsifiers are usually included. During mixing, lipids act as a lubricant, competing with water to coat the surface of flour, and preventing a complete formation of the gluten network (Manohar and Rao, 1999). In cookies, therefore, the values observed for farinograph and especially for alveograph indices, even at the highest level of supplementation, would be well tolerated since the friability typically required in these products takes advantage from weak gluten network and the addition of fats and/or emulsifiers could mitigate the excessively high P/L value (Addo and Pomeranz, 1992; Agyare, Addo, Xiong & Akoh, 2005). Blanching water, instead, did not affect the rheological properties of dough and could therefore be added to any kind of bakery end product, including bread. Color data show that the addition of skin powder or blanching water significantly affected the ordinary color of dough, which from pale cream turned to markedly brown. In particular, the variation of both brown and red indices, already significant at levels of supplementation as low as 30 g/kg, was progressively more evident with the increase of skin amount added. The addition of blanching water had an effect

similar to the addition of 50 g/kg dried skins. Color is an essential attribute that strongly influences consumer choice, so these alterations could be negatively considered. However, the consumer behavior has been changing. Therefore, cereal-based food products from whole wheat or containing pseudocereals and/or legumes, which show unusual sensory features but are perceived as healthier than the conventional products, are now well accepted. In this frame, also bakery products containing almond by-products should have good chances to be marketed.

### 4.4. Conclusions

This study has ascertained the great potential of almond processing by-products as food ingredients, with the aim of improving the health value of cereal-based bakery products. Blanched skins show the advantage of being, after proper drying, relatively stable to oxidation and easy to store and manage. Blanching water, instead, should be necessarily used fresh, soon after production, so that an ideal layout would see an almond-processing industry joined, on place, with a second plant exploiting the liquid by-product. Blanching water did not affect the rheological properties of dough and could therefore be added to any kind of bakery end product. On the contrary, blanched almond skins dried by the least time-consuming drying mode (i.e. at 60 °C for 30 min) could be added to cookies, in which formulation usually other ingredients are included, able to mitigate the negative effect of skins on dough rheology. From the sensory point of view, oven-dried blanched almond skins and blanching water were devoid of odor defects, but their marked brown color affected the usual appearance of dough. Further research will be carried out to evaluate key elements in food marketing such as the sensory characteristics and consumer acceptability of bakery products, either cookies or bread, containing dried almond skins or blanching water. The use of almond by-products for food specialties with functional properties could represent an important approach for a better qualification of this crop, in view of fulfilling consumer expectations for healthy niche products.

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### 4.5 References

AACC (2000). *Approved methods of analysis* (11th ed.). St Paul, MN, USA: The American Association of Cereal Chemists.

Addo, K., & Pomeranz, Y. (1992). Effects of lipids and emulsifiers on alveograph characteristics. *Cereal Chemistry*, *69,* 6–12.

Agyare, K. K., Addo, K., Xiong, Y. L., & Akoh, C. C. (2005). Effect of structured lipid on alveograph characteristics, baking and textural qualities of soft wheat flour. *Journal of Cereal Science*, *42*, 309–316.

Amarowicz R. (2016). Natural phenolic compounds protect LDL against oxidation. *European Journal of Lipid Science and Technology*, 118, 677–679.

Bennett, B. L. (2016). *Almond flour: the high-protein, gluten-free choice for baking and cooking*. Book Publishing Company: Summertown, TN, USA, pp. 1–160.

Bolling B. W. (2017). Almond polyphenols: methods of analysis, contribution to food quality, and health promotion. *Comprehensive Reviews in Food Science and Food Safety*, *16,* 346–368.

Chen, C. Y., Milbury, P. E., Lapsley, K., & Blumberg, J. B. (2005). Flavonoids from almond skins are bioavailable and act synergistically wi<th vitamins C and E to enhance hamster and human LDL resistance to oxidation. *Journal of Nutrition*, *135*, 1366–1373.

Chen, C. Y., Milbury, P. E., Chung, S. K., & Blumberg, J. (2007). Effect of almond skin polyphenolics and quercetin on human LDL and apolipoprotein B-100 oxidation and conformation. *Journal of Nutritional Biochemistry*, *18*, 785–794.

Ferraretto, P., & Celotti, E. (2016). Preliminary study of the effects of ultrasound on red wine polyphenols. *CyTA - Journal of Food*, *14*, 529–535.

Garrido, I., Monagas, M., Gómez-Cordovés, C., & Bartolomé, B. (2008). Polyphenols and antioxidant properties of almond skins: influence of industrial processing. *Journal of Food Science*, *73*, C106–C115.

Grasser, L. A., Fadel, J. O., Garnett, I., & DePeters, E. J. (1995). Quantity and economic importance of nine selected by-products. *Journal of Dairy Science*, *78*, 962–971.

Hughey, C. A., Janusziewicz, R., Minardi, C. S., Phung, J., Huffman, B. A., Reyes, L., … Prakash, A. (2012). Distribution of almond polyphenols in blanch water and skins as a function of blanching time and temperature. *Food Chemistry*, *131*, 1165-1173.

Kiatgrajai, P., Wellons, J. D., Gollob, L., & White, J. D. (1982). Kinetics of epimerization of (+)-catechin and its rearrangement to catechinic acid. *The Journal of Organic Chemistry*, *47*, 2910-2912.

Labat, E., Morel, M. H., & Rouau, X. (2000). Wheat gluten phenolic acids: occurrence and fate upon mixing. *Journal of Agricultural and Food Chemistry*, *48*, 6280-6283.

Laddomada, B., Caretto, S., & Mita, G. (2015). Wheat bran phenolic acids: bioavailability and stability in whole wheat-based foods. *Molecules*, *20,* 15666-15685. doi:10.3390/molecules200915666.

Laddomada, B., Durante, M., Mangini, G., D'Amico, L., Lenucci, M. S., Simeone, R., … Blanco, A. (2017). Genetic variation for phenolic acids concentration and composition in a tetraploid wheat (*Triticum turgidum* L.) collection. *Genetic Resources and Crop Evolution*, *64*, 587–597.

Mandalari, G., Tomaino, A., Arcoraci, T., Martorana, M., Lo Turco, V., Cacciola, F., … Wickham, M. S. J. (2010). Characterization of polyphenols, lipids and dietary fibre from skins of almonds (*Amygdalus communis* L.). *Journal of Food Composition and Analysis*, *23*, 166–174.

Manohar, R. S., & Rao, P. H. (1999). Effect of emulsifiers, fat level and type on the rheological characteristics of biscuit dough and quality of biscuits. *Journal of the Science of Food and Agriculture, 79*, 1223–1231.

Pasqualone, A., Bianco, A. M., Paradiso, V. M., Summo, C., Gambacorta, G., & Caponio, F. (2014). Physico-chemical, sensory and volatile profiles of biscuits enriched with grape marc extract. *Food Research International*, *65*, 385–393.

Pasqualone, A., Bianco, A. M., Paradiso, V. M., Summo, C., Gambacorta, G., Caponio, F., & Blanco, A. (2015). Production and characterization of functional biscuits obtained from purple wheat. *Food Chemistry*, *180*, 64-70.

Pasqualone, A., Caponio, F., & Simeone, R. (2004). Quality evaluation of re-milled durum wheat semolinas used for bread-making in Southern Italy. *European Food Research and Technology*, *219*, 630-634.

Pasqualone, A., Laddomada, B., Centomani, I., Paradiso, V. M., Minervini, D., Caponio, F., & Summo, C. (2017). Bread making aptitude of mixtures of re-milled semolina and selected durum wheat milling by-products. *LWT-Food Science and Technology*, *78*, 151–159.

Pasqualone, A., Piergiovanni, A. R., Caponio, F., Paradiso, V. M., Summo, C., & Simeone, R. (2011). Evaluation of the technological characteristics and bread-making quality of alternative wheat cereals in comparison with common and durum wheat. *Food Science and Technology International*, *17*, 135–142.

Piga, A., Del Caro, A., & Corda, G. (2003). From plums to prunes: influence of drying parameters on polyphenols and antioxidant activity. *Journal of Agricultural and Food Chemistry*, *51*, 3675–3681.

Rahaie, S., Gharibzahedi, S. M. T., Razavi, S. H., & Jafari, S. M. (2014). Recent developments on new formulations based on nutrient-dense ingredients for the production of healthy-functional bread: a review. *Journal of Food Science and Technology*, *51*, 2896–2906.

Rothwell, J. A., Day, A. J., & Morgan, M. R. (2005). Experimental determination of octanol−water partition coefficients of quercetin and related flavonoids. *Journal of Agricultural and Food Chemistry*, *53*, 4355–4360.

Taranto, F., Pasqualone, A., Mangini, G., Tripodi, P., Miazzi, M. M., Pavan, S., & Montemurro, C. (2017). Polyphenol oxidases in crops: Biochemical, physiological and genetic aspects. *International Journal of Molecular Science*, *18*, 377.

UNI. *UNI method no. 10453. Durum wheat and semolina. Determination of rheological properties using an alveograph*; Ente Nazionale Italiano di Unificazione: Milan, Italy, 1995; pp. 1–11.

Verstraeten, S. V., Fraga, C. G., & Oteiza, P. I. (2009). Flavonoid-membrane interactions: consequences for biological actions. In Fraga, C.G. (Ed.) *Plant phenolics and human health: biochemistry, nutrition and pharmacology* Vol. 1. (pp. 107-135). Hoboken, NJ: John Wiley & Sons, Inc.

Walch, S. G., Ngaba Tinzoh, L., Zimmermann, B. F., Stühlinger, W., & Lachenmeier, D. W. (2011). Antioxidant capacity and polyphenolic composition as quality indicators for aqueous infusions of *Salvia officinalis* L. (sage tea). *Frontiers in Pharmacology*, *2*, 1-6, article 79.

Wang, R., & Zhou, W. (2004). Stability of tea catechins in the breadmaking process. *Journal of Agricultural and Food Chemistry*, *52*, 8224–8229.

Wijeratne, S. S. K., Abou-zaid, M. M., & Shahidi, F. (2006). Antioxidant polyphenols in almond and its coproducts. *Journal of Agricultural and Food Chemistry*, *54*, 312–318.

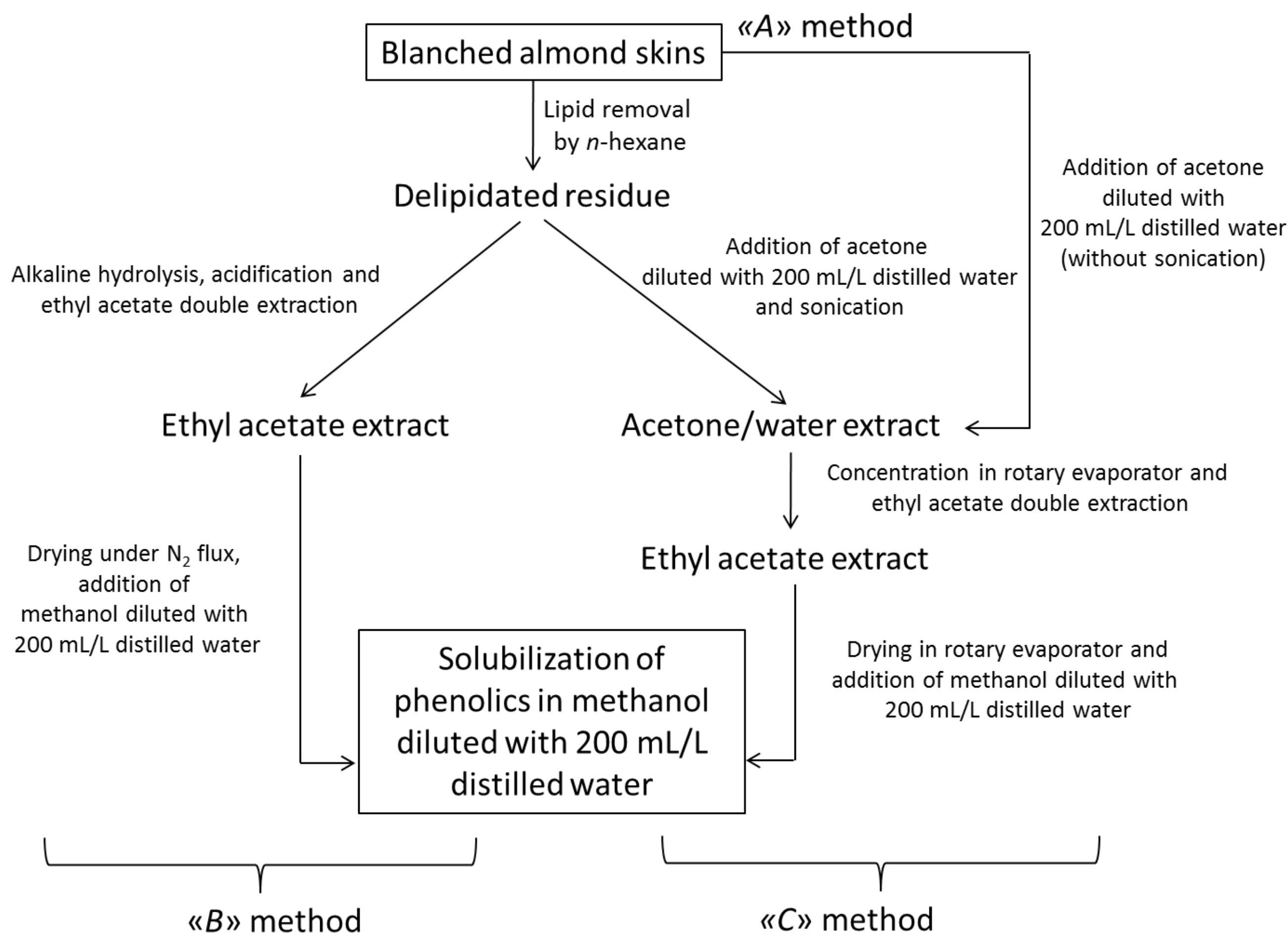


**Figure 1.** Flow chart of the main steps of the "*A*", "*B*", and "*C*" methods used to extract phenolic compounds from blanched almond skins. *A* and *C* protocols were modifications of extracting methods proposed by Garrido et al. (2008) and Mandalari et al. (2010), respectively. Modification consisted of substituting the extracting solvent (methanol acidified with 1 mL/L HCl 12 N) with a solution of acetone diluted with 200 mL/L distilled water. *B* method was as in Laddomada et al. (2017).

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**Table 1.**
Main physico-chemical properties and odor notes of blanched almond skins either not dried or dried in different conditions and of blanching water ($n = 3$).

| | Blanched almond skins | | | | | Blanching water |
|---|---|---|---|---|---|---|
| | Not dried | 60 °C for 30 min | 45 °C for 90 min | 32 °C for 150 min | 38-40 °C for 6-8 h | |
| $a_w$ | 0.98 ± 0.01 [a] | 0.41 ± 0.01 [e] | 0.44 ± 0.01 [d] | 0.54 ± 0.01 [b] | 0.48 ± 0.01 [c] | n.d. |
| Moisture content (g/kg) | 585 ± 5 [a] | 78.6 ± 0.6 [e] | 85.5 ± 0.2 [c] | 95.9 ± 0.6 [b] | 80.9 ± 0.4 [d] | 993 ± 11 |
| Water binding capacity (WBC) | 174 ± 3 [b] | 384 ± 5 [a] | 376 ± 5 [a] | 374 ± 5 [a] | 380 ± 5 [a] | n.d. |
| Lipid content (g/kg d.m.) | 219 ± 2 [§] | 220 ± 2 | 221 ± 4 | 216 ± 3 | 219 ± 3 | n.d. |
| Antioxidant activity (μmol Trolox/g d.m.) | 40.4 ± 0.2 [e] | 81.6 ± 0.5 [b] | 90.4 ± 0.9 [a] | 72.2 ± 0.7 [c] | 59.2 ± 1.5 [d] | 17.4 ± 0.1 [§§] |
| *Color* | | | | | | |
| Brown index (100 – *L**) | 43.7 ± 0.1 [e] | 52.0 ± 0.3 [b] | 50.6 ± 0.3 [c] | 49.4 ± 0.1 [d] | 58.7 ± 0.1 [a] | 63.8 ± 0.2 |
| Red index (*a**) | 10.1 ± 0.1 [d] | 11.2 ± 0.4 [abc] | 11.4 ± 0.1 [b] | 10.8 ± 0.1 [c] | 11.7 ± 0.1 [a] | 14.2 ± 0.8 |
| Yellow index (*b**) | 27.9 ± 0.1 [a] | 25.6 ± 0.1 [b] | 25.6 ± 0.1 [b] | 25.1 ± 0.1 [c] | 18.2 ± 0.3 [d] | 20.1 ± 0.3 |
| *Odor notes* | | | | | | |
| Leafy odor | 4.5 ± 1.1 [b] | 6.1 ± 0.5 [a] | 5.8 ± 0.2 [ab] | 6.4 ± 0.2 [a] | 1.7 ± 0.5 [c] | 3.3 ± 0.5 |
| Rancid odor | 0.1 ± 0.1 [b] | 0.2 ± 0.1 [b] | 0.3 ± 0.1 [b] | 0.4 ± 0.2 [b] | 6.3 ± 0.2 [a] | 0.1 ± 0.2 |
| Sour odor | 4.9 ± 1.0 [a] | 0.2 ± 0.1 [b] | 0.1 ± 0.1 [b] | 0.1 ± 0.1 [b] | 0.1 ± 0.1 [b] | 0.5 ± 0.3 |

[a-e]Values followed by the same letter, within each row, are not significantly different at $p < 0.05$ except for the odor notes, where $p < 0.001$. Blanching water was not included in statistical comparison being a totally different by-product; n.d. = not determined; d.m. = dry matter; [§]determined on lyophilized sample; [§§]expressed as mmol/L Trolox.

**Table 2.**
Content of identified phenolic compounds (μg/g d.m.) determined according to three different protocols of extraction (*A*, *B*, and *C*, schematized in Fig. 1) in blanched almond skins either not dried or dried in different conditions ($n = 3$).

| | Without drying | | | Drying at 60 °C for 30 min | | | Drying at 45 °C for 90 min | | | Drying at 32 °C for 150 min | | | Sun-drying at 38-40 °C for 6-8 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *A* | *B* | *C* | *A* | *B* | *C* | *A* | *B* | *C* | *A* | *B* | *C* |
| *Phenolic acids* | | | | | | | | | | | | | | | |
| Protocatechuic acid | 3.2±0.4$^{cC}$ | 92.4±2.8$^{cA}$ | 47.2±4.1$^{aB}$ | 2.9±0.3$^{cB}$ | 162±8$^{bA}$ | 4.1±0.9$^{bB}$ | 4.1±0.3$^{bB}$ | 163±12$^{abA}$ | 3.8±0.8$^{bB}$ | 4.4±0.8$^{bB}$ | 150±11$^{bA}$ | 5.1±0.9$^{bB}$ | 9.6±2.1$^{aB}$ | 201±15$^{aA}$ | 3.1±0.1$^{cC}$ |
| *p*-Hydroxybenzoic acid | 4.3±0.4$^{aB}$ | 32.8±0.1$^{cA}$ | 3.6±0.5$^{dB}$ | 3.9±0.9$^{aC}$ | 65.9±4.3$^{aA}$ | 14.4±0.2$^{bB}$ | 3.5±0.8$^{aC}$ | 61.1±3.9$^{abA}$ | 10.4±0.2$^{cB}$ | 3.8±0.7$^{aC}$ | 55.8±3.7$^{bA}$ | 15.3±0.1$^{aB}$ | 1.5±0.3$^{bC}$ | 57.1±4.1$^{abA}$ | 14.1±0.4$^{bB}$ |
| *p*-Coumaric acid | n.d. | n.d. | n.d. | n.d. | 7.3±0.9$^{ab}$ | n.d. | n.d. | 8.1±0.7$^{a}$ | n.d. | n.d. | 5.8±0.7$^{b}$ | n.d. | n.d. | 7.8±0.9$^{a}$ | n.d. |
| Chlorogenic acid | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Vanillic acid | 10.8±0.8$^{aB}$ | 19.9±0.1$^{bA}$ | n.d. | 3.1±0.1$^{cB}$ | 27.8±2.1$^{aA}$ | n.d. | 3.1±0.4$^{cB}$ | 30.7±2.3$^{aA}$ | n.d. | 6.4±0.3$^{bB}$ | 26.7±2.4$^{aA}$ | n.d. | 1.5±0.1$^{dB}$ | 14.9±2.1$^{cA}$ | n.d. |
| *Flavan-3-ols* | | | | | | | | | | | | | | | |
| (+)-Catechin | 126±6$^{aC}$ | 201±8$^{aB}$ | 1085±28$^{aA}$ | 29.9±1.1$^{bC}$ | 84.2±3.6$^{cB}$ | 572±12$^{cA}$ | 18.3±0.9$^{dC}$ | 1216±4$^{bB}$ | 621.2±13.6$^{bA}$ | 15.6±0.5$^{eC}$ | 118±4$^{bB}$ | 411±10$^{dA}$ | 21.1±1.1$^{cC}$ | 50.5±9.2$^{dB}$ | 111±12$^{eA}$ |
| (-)-Epicatechin | 724±24$^{aB}$ | 1393±25$^{aA}$ | 20.6±1.9$^{dC}$ | 234±11$^{cA}$ | 117±10$^{dB}$ | 51.6±2.7$^{aC}$ | 294±11$^{bA}$ | 178±12$^{bB}$ | 46.3±2.2$^{abC}$ | 279±13$^{bA}$ | 128±8$^{cdB}$ | 45.5±2.2$^{bC}$ | 161±9$^{dA}$ | 136±8$^{cB}$ | 29.4±1.5$^{cC}$ |
| *Flavonol glycosides and aglycones* | | | | | | | | | | | | | | | |
| Quercetin-3-*O*-glucopyranoside | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Kaempferol-3-*O*-rutinoside | 69.1±1.6$^{aA}$ | n.d. | 31.6±3.6$^{bB}$ | 10.3±0.1$^{eC}$ | 23.8± 2.8$^{aB}$ | 48.3±2.6$^{aA}$ | 11.5±0.2$^{dB}$ | 10.1±1.1$^{bB}$ | 47.8±2.3$^{aA}$ | 12.5±0.2$^{cC}$ | 7.8±0.9$^{bA}$ | 49.2±3.1$^{aB}$ | 13.5±0.5$^{bB}$ | 8.6±1.1$^{bC}$ | 49.7±4.4$^{aA}$ |
| Kaempferol-3-*O*-glucoside | n.d. | n.d. | 4.4±0.7$^{a}$ | 19.6±0.8$^{aB}$ | 30.2±1.5$^{aA}$ | 3.3±0.1$^{cC}$ | 17.8±0.6$^{aB}$ | 24.1±1.4$^{bA}$ | 4.3±0.1$^{aC}$ | 5.1± 0.3$^{bB}$ | 17.5±0.9$^{cA}$ | 3.5±0.1$^{bC}$ | 0.7±0.1$^{cB}$ | 33.3±1.1$^{aA}$ | n.d. |
| Isorhamnetin-3-*O*-rutinoside | 110±6$^{aA}$ | n.d. | 48.3±2.4$^{cB}$ | 26.5±1.2$^{cC}$ | 67.1±5.3$^{aB}$ | 93.2±2.1$^{bA}$ | 20.4±1.6$^{dC}$ | 45.2±2.6$^{bcB}$ | 94.4±3.1$^{bA}$ | 30.7±1.2$^{bC}$ | 47.6±2.9$^{bB}$ | 105±3$^{aA}$ | 24.1±1.1$^{cC}$ | 30.9±1.9$^{cB}$ | 92.5±2.7$^{bA}$ |
| Isorhamnetin-3-*O*-glucoside | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Quercetin dehydrate | n.d. | n.d. | n.d. | 0.9±0.1$^{bB}$ | 2.8±0.2$^{aA}$ | n.d. | 0.8± 0.1$^{cB}$ | 1.6±0.1$^{bA}$ | n.d. | 0.7±0.2$^{dB}$ | 1.9±0.8$^{aA}$ | n.d. | 1.7±0.1$^{a}$ | 1.6±0.1$^{b}$ | n.d. |
| Kaempferol | 9.1±3.2$^{aB}$ | 14.9±1.3$^{aA}$ | n.d. | 0.9±0.1$^{cB}$ | 7.5± 0.6$^{aA}$ | 0.3±1.9$^{bcB}$ | 0.8±0.1$^{cdB}$ | 5.9±0.6$^{bA}$ | 0.8±1.6$^{aB}$ | 0.8±0.1$^{dB}$ | 6.1±0.8$^{abA}$ | 0.3±0.1$^{aC}$ | 1.5±0.1$^{bB}$ | 7.2±0.7$^{abA}$ | 0.3±0.1$^{aC}$ |
| Isorhamnetin | 9.7±0.3$^{aC}$ | 38.6±2.9$^{aA}$ | 25.3±1.8$^{aB}$ | 7.8 ± 0.2$^{bC}$ | 36.1± 3.1$^{aA}$ | 12.2±0.8$^{bB}$ | 6.9±0.1$^{cdC}$ | 23.1±2.2$^{bA}$ | 12.2±1.2$^{bB}$ | 6.7±0.2$^{dC}$ | 24.2±1.2$^{bA}$ | 12.9±0.4$^{bB}$ | 7.1±0.1$^{cC}$ | 26.2±1.1$^{bA}$ | 9.6±0.4$^{cB}$ |
| *Flavanone glycosides and aglycones* | | | | | | | | | | | | | | | |
| Naringenin-7-*O*-glucoside | 14.2±1.3$^{bcAB}$ | 10.5±1.9$^{bB}$ | 17.4±0.8$^{aA}$ | 12.8±0.2$^{cB}$ | 15.7±1.1$^{bA}$ | 12.4±0.2$^{cB}$ | 14.1±0.2$^{bA}$ | 7.5±1.2$^{bcB}$ | 13.5±0.3$^{bA}$ | 20.8±0.3$^{aA}$ | 5.9±1.1$^{cC}$ | 16.1±0.5$^{aB}$ | 10.6±0.1$^{dA}$ | 2.8±0.2$^{dB}$ | 2.6±0.1$^{dB}$ |
| Eriodictyol | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Naringenin | n.d. | n.d. | 2.9±0.2$^{a}$ | n.d. | n.d. | 1.7±0.1$^{c}$ | n.d. | n.d. | 2.1±0.1$^{b}$ | n.d. | n.d. | 2.1±0.1$^{b}$ | n.d. | n.d. | 2.9±0.1$^{a}$ |
| *Total* | 1110±51$^{aC}$ | 1773±82$^{aA}$ | 1286±79$^{aB}$ | 353±19$^{bC}$ | 648±19$^{bB}$ | 814±32$^{bA}$ | 396±23$^{bC}$ | 679±21$^{bB}$ | 857±39$^{bA}$ | 387±21$^{bC}$ | 595±14$^{cB}$ | 666±18$^{cA}$ | 254±11$^{cC}$ | 577±23$^{cA}$ | 317±13$^{dB}$ |

$^{a\text{-}d}$ *In the same row and within the same extracting method, means with different small letters are significantly different ($p < 0.05$).*
$^{A\text{-}C}$ *In the same row and within the same drying treatment, means with different capital letters are significantly different ($p < 0.05$).*
*d.m. = dry matter; n.d. = not detected*

**Table 3.**
Content of identified phenolic compounds (mg/L) of almond blanching water (solid content = 7.4 $10^{-3}$ g/L) extracted according to method C (schematized in Fig. 1) with and without sonication ($n = 3$).

| | With sonication | Without sonication |
|---|---|---|
| *Phenolic acids* | | |
| Protocatechuic acid | 5.68 ± 0.81 | 5.89 ± 0.78 |
| *p*-Hydroxybenzoic acid | 2.94 ± 0.23 [b] | 4.91 ± 0.54 [a] |
| *p*-Coumaric acid | n.d. | n.d. |
| Chlorogenic acid | n.d. | n.d. |
| Vanillic acid | n.d. | n.d. |
| *Flavan-3-ols* | | |
| (+)-Catechin | 847 ± 11 [a] | 408 ± 7 [b] |
| (-)-Epicatechin | 30.9 ± 1.3 [b] | 63.9 ± 2.1 [a] |
| *Flavonol glycosides and aglycones* | | |
| Quercetin-3-*O*-glucopyranoside | n.d. | n.d. |
| Kaempferol-3-*O*-rutinoside | 11.5 ± 1.2 [a] | 8.1 ± 1.3 [b] |
| Kaempferol-3-*O*-glucoside | n.d. | 1.46 ± 0.05 |
| Isorhamnetin-3-*O*-rutinoside | 18.6 ± 1.9 [a] | 13.3 ± 1.4 [b] |
| Isorhamnetin-3-*O*-glucoside | n.d. | 3.64 ± 0.09 |
| Quercetin dehydrate | n.d. | n.d. |
| Kaempferol | n.d. | n.d. |
| Isorhamnetin | 0.04 ± 0.01 [b] | 0.27 ± 0.03 [a] |
| *Flavanone glycosides and aglycones* | | |
| Naringenin-7-*O*-glucoside | 0.49 ± 0.03 [a] | 0.31 ± 0.02 [b] |
| Eriodictyol | n.d. | n.d. |
| Naringenin | n.d. | 0.12 ± 0.01 |
| *Total* | 917 ± 41 [a] | 510 ± 23 [b] |

[a-b] *Values followed by the same letter, within each row, are not significantly different ($p < 0.05$); n.d. = not detected.*

**Table 4.**
Rheological properties and color of composite dough containing refined wheat flour and increasing amounts of blanched almond skins (dried at 60 °C for 30 min and milled) or blanching water ($n$ = 3).

| Parameter | Almond skins (g/kg wheat flour) | | | | | Blanching water* |
|---|---|---|---|---|---|---|
| | 0 (CTRL) | 30 | 50 | 70 | 100 | |
| *Farinograph data* | | | | | | |
| Water absorption (g/kg) | 575 ± 4 [d] | 592 ± 4 [c] | 602 ± 5 [bc] | 612 ± 1 [a] | 612 ± 9 [ab] | 564 ± 5 [d] |
| Dough development time (s) | 93 ± 4 | 93 ± 4 | 87 ± 12 | 87 ± 4 | 90 ± 5 | 94 ± 4 |
| Dough stability (s) | 144 ± 8 [c] | 126 ± 9 [c] | 138 ± 8 [c] | 264 ± 16 [b] | 630 ± 51 [a] | 140 ± 7 [c] |
| Softening index (B.U.) | 60 ± 5 [a] | 54 ± 5 [ab] | 45 ± 9 [ab] | 43 ± 6 [b] | 27 ± 2 [c] | 58 ± 4 [a] |
| *Alveograph data* | | | | | | |
| Deformation energy × $10^{-4}$ (J) | 176 ± 3 [a] | 170 ± 2 [b] | 163 ± 5 [b] | 133 ± 2 [c] | 117 ± 8 [d] | 173 ± 3 [a] |
| Tenacity/Extensibility ratio | 0.8 ± 0.1 [e] | 1.6 ± 0.3 [d] | 2.2 ± 0.2 [c] | 4.8 ± 0.4 [b] | 6.0 ± 0.2 [a] | 0.8 ± 0.2 [e] |
| *Color of dough* | | | | | | |
| Brown index (100 – $L^*$) | 28.2 ± 0.4 [e] | 35.4 ± 0.3 [d] | 39.3 ± 0.2 [c] | 42.3 ± 0.3 [b] | 45.2 ± 0.5 [a] | 39.7 ± 0.3 [c] |
| Red index ($a^*$) | 5.7 ± 0.1 [e] | 9.6 ± 0.1 [d] | 10.1 ± 0.1 [c] | 11.2 ± 0.1 [b] | 12.3 ± 0.1 [a] | 10.4 ± 0.2 [c] |
| Yellow index ($b^*$) | 27.2 ± 0.3 [a] | 27.3 ± 0.2 [a] | 24.7 ± 0.1 [b] | 23.8 ± 0.2 [c] | 22.3 ± 0.2 [d] | 24.6 ± 0.1 [b] |

** The amount of blanching water accounted for 56.4 mL per 100 g wheat flour for farinograph and 53.2 mL for alveograph, according to the corresponding analytical protocols. The amount added for color data determination was the same as farinograph absorption, i.e. 53.2 mL per 100 g wheat flour. [a-e] Values followed by the same letter, within each row, are not significantly different ($p < 0.05$). B.U. = Brabender Units.*

# Chapter 5

## Effect of natural sea salt on physicochemical and textural properties of low sodium durum wheat bread.

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**Abstract**

The aim of this study was the development of functional durum wheat bread, with the purpose of decreasing its sodium content (Russell, 1989; Salovaara, 1982). A natural Na-K sea salt (Atacama, Chile), with 65% of NaCl, 30% KCl, 1.5% $SO_4$ and traces of other elements was used in order to obtain functional durum wheat bread with low in sodium (<0.12g/100g) and very low sodium claim (< 0.04/100 g), according to the fixed limits of Reg (EU) 1924/2006 on nutrition and health claims. Three formulations of bread were prepared at various concentrations: 1.70%, 0.35% and 0.15% sea salt. Bread samples prepared with 1.70%, 0.35% and 0.15% commercial NaCl were used as control. The physicochemical and textural attributes of bread were evaluated. Results showed significant differences ($p<0.01$) in terms of loaf volume, height and weight and crust thickness. Significant ($p<0.05$) differences in loaf base diameter were also observed. Textural attributes (hardness, resilience and chewiness) of 0.15% sea salt-supplemented bread were significantly ($p<0.01$) higher compared to bread containing 1.70 % and 0.35% sea salt. 1.70% Na-K supplemented bread showed the greatest improvement in terms of increased softness and crumb moisture. 0.35% of sea salt supplementation allowed obtaining functional bread with low sodium content without affecting the overall of physicochemical and textural properties of the product.

**Keywords:** Durum wheat bread, Salt replacement, Physicochemical and textural attributes, functional food.

## 5.1 Introduction

In Sicily, approximately 50% of the durum wheat is used in bread-making, and the characteristic flavour of durum wheat bread is enhanced by adding a high percentage (2.0-2.4) of sodium chloride (NaCl). NaCl is an essential ingredient to control the functional properties of wheat dough (McCann and Day 2013). The traditional use of NaCl fulfils various important rheological, technological and sensory properties in manufacturing of baked goods. Today, the dietary salt intake is considerably higher than the recommendations of the EFSA (2006) for good health (3-4 g salt/day). The major adverse effect of excess dietary sodium intake is etiologically related to hypertension and cardiovascular disease (He and MacGregor, 2007; Beck et *al.*, 2010). Several initiatives have been undertaken for a reduction in salt intake in daily diet in the last years. There is a need to implement a systematic strategy for the reduction of sodium intake, especially the content of salt on bread and bakery products.

## 5.2 Materials and methods

### *5.2.1 Samples*

Bread was prepared at a local bread-making factory (Valle del Dittaino Società Cooperativa Agricola, Assoro, Enna, Italy) according to a consolidated industrial process based on the following formulation: durum wheat remilled semolina (50 kg), compressed yeast (0.5% semolina basis), water (62% semolina basis) and NaCl. Three formulations of bread were prepared at various sea salt (Saltwell ™, Salinity Sweden, Halmstad Sweden) concentrations: 1.70, 0.35 and 0.15 (% semolina basis) sea salt. Bread samples with commercial NaCl at the same contents as sea salt were used as control (Spina et *al*., 2015).

### *5.2.2 Sodium determination on experimental bread*

Sodium content, after wet digestion of samples with concentrated hydrochloric and nitric acid, was determined by atomic absorption spectrophotometry (AAS) using an instrument VARIAN SpectrAA-10 (AACC, 2000).

### *5.2.3 Determination of the physicochemical and textural attributes of breads*

Loaf volume was determined according to the rapeseed displacement in a loaf volume meter. The internal structure was visually estimated by eight evaluator, and the crumb porosity was estimated using the Mohs scale, improved by Dallmann (1958), based on the visual analysis of 8 photos representing different cross sections of loaves with diverse porosity. Loaf height and top crust thickness was measured using a digital caliper (Digi-Max™, Scienceware®, NJ, U.S.A.). Crumb color was measured with Colorimeter Minolta CR 300. Moisture content of bread crumb was determined according to AOAC method no. 945.15 (AOAC, 2000). Three bread slices (11±1 mm thickness) were used, one from center and two from extremity of the loaf. Water activity ($a_w$) was determined on the crumb of three slices using Hygropalm 40 AW (Rotronic Instruments Ltd, Crawley, UK) according to manufacturers' instructions. The Texture Profile Analysis (TPA) of bread was carried out by means of a Universal Testing machine (model 3344, Instron, Norwood, MA, USA), equipped with a 5.0 cm diameter cylindrical probe and a 250 N load cell. Data were acquired through Bluehill® 2 software (Instron, Norwood, MA, USA). Cyclic compression tests (30s interval between first and second compression) were set up: the crosshead speed was 3.3 mm/s, the force required to compress the samples by 40% was recorded on 5-cm side square portions of 24-mm thick slices, and the average value of five replicates was taken.

## 5.3 Results and discussion

Table 1 summarized the content of sodium on the experimental breads. on 0.35 and 0.15 (% semolina basis) samples with Saltwell supplementation, the content of sodium were in agreement to the fixed limits of the Reg (EU) 1924/2006 on nutrition and health claims made on foods in order to obtain claims on a label 'low sodium/salt', 'very low sodium/salt' and 'sodium free or salt free'. Table 2 shows significant differences for loaf height ($p<0.01$), internal structure ($p<0.01$), base diameter ($p<0.05$) and crumb moisture ($p<0.05$), especially between bread with 1.7% NaCl and the sample containing 1.7% sea salt. Regarding crumb porosity and $a_w$ no significant differences were measured among the bread sample, independently from the level of quantity and type of salts. As regards loaves volume only the sample with 0.15% sea salt showed a lower bread volume compared to the sample containing NaCl. The highest loaf height was obtained from the thesis 1.7% of both salts, while the lowest one in the case 0.15%

supplementation, regardless the type of salt. The three samples with NaCl showed a higher but not significance bread volume. Regarding the internal structure, only the control bread with 1.7% NaCl showed an irregular structure (value 2). The sample with 1.7% sea salt supplementation exhibited the lowest crust thickness, measured at the top loaf (3 mm), while the sample loaves with 0.15% sea salt presented the highest crust thickness (4 mm). In addition, bread with 1.7% NaCl produced loaf with a lower diameter and chroma (C) compared to other samples; however, no significant differences were found according to the type of salt at the same percentage of addition. No significant changes in moisture values were observed, even if bread with 0.15% of both salts showed a slightly higher values (>39%) compared to the other samples. 0.35% of sea salt supplementation has allowed to obtain a functional bread with low sodium content without affecting the overall of physicochemical and textural properties of the product. Textural measurement showed that 0.15% Na-K supplemented bread presented the highest ($p<0.01$) hardness, resilience and chewiness among the samples (Fig. 1). 1.7% sea salt supplementation loaf showed greater improvement in terms of softness and crumb moisture (Fig. 1). The results of this study showed that the decrease in NaCl from 1.7 to 0.15% and its replacement with Na-K sea salt supplementation in durum wheat bread is a possible strategy for the reduction of salt and $Na^+$ intake. Durum wheat bread at low salt level had generally similar properties to bread with the highest NaCl content. The values of the main physicochemical and bread quality parameters did not showed differences attributable to the different levels of salts, while the thesis with 0.15% sea salt supplementation gave bread with low volume, height and high samples value in crust thickness and hardness, gumminess and chewiness. Considering the increasing interest on durum wheat bread from consumers and the guidelines for a healthy diet, these results showed the possibility of producing durum wheat bread with low salt content (Fig. 2).

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## 5.4 References

AOAC 2000. *Official methods of analysis of the Association of Official Analytical Chemists* 17th ed. Methods 945.15. Gaithersburg, MD (USA).

Beck M., Jekle M., Becker T. 2010. Risks of sodium chloride and salt substitutes and their impact on baked goods. In: *5th World Congress of Food Science and Technology*, S. Tietze, Ed. Cape Town (South Africa), p. 66.

Dallmann H. 1958. Porentabelle. Verlag Moritz Schäfer, Detmold, Germany.

EFSA 2006. Tolerable upper intake levels for vitamins and minerals. *Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies:* 429–446.

He F. J., MacGregor G. A. 2007. Dietary salt, high blood pressure and other harmful effects on health. In *Reducing salt in foods: practical strategies* Ch. 2, D. Kilcast and F. Angus, Eds. Woodhead Publishing Limited, Cambridge (England), pp. 18–54.

McCann T. H., Day L. 2013. Effect of sodium chloride on gluten network formation, dough microstructure and rheology in relation to breadmaking. *Journal of Cereal Science*, 57(3), 444–452.

Mohs K. 1924. The size of the pores in baked bread. Cereal Chemistry 1: 149-151.

Regulation (EC) no 1924/2006 of the european parliament and of the council of 20 December 2006 on nutrition and health claims made on foods.

Russell P.L.1989. The effect of pH and NaCl Content on starch gel ageing. A study by differential scanning calorimetry and rheology. *Journal of Cereal Science,* 10(2): 123-138.

Salovaara H. 1982. Effect of partial sodium chloride replacement by other salts on wheat dough rheology and breadmaking. *Cereal Chemistry*, 59(5): 422-426.

Spina A., Brighina S., Muccilli S., Mazzaglia A., Rapisarda P., Fallico B. Arena E. 2015. Partial replacement of NaCl in bread from durum wheat (Triticum turgidum L subsp. durum Desf.) with KCl and yeast extract: evaluation of quality parameters during long storage. *Food and Bioprocess Technology* 8(5): 1089-1101.

**Table 1.**
Theoretical determination of the cation content of various bread formulation

| Sample code | Salt (%) on experimental bread | Nutritional information/100g bread (sodium g) | Regulation (EU) No 1924/2006 - nutrition claim |
|---|---|---|---|
| Control A | 1.22% commercial sea salt | 0.4894 | - |
| Control B | 1.22% Saltwell ™ | 0.3181 | - |
| 1A | 0.25% commercial sea salt | 0.1016 | 0.12 g of sodium - low sodium/salt |
| 1B | 0.25% Saltwell ™ | 0.0660 | 0.12 g of sodium - low sodium/salt |
| 2A | 0.11% commercial sea salt | 0.0436 | |
| 2B | 0.11% Saltwell ™ | 0.0283 | 0.04 g of sodium - very low sodium/salt |

**Table 2.**
Physicochemical characteristics and quality properties of samples (n = 3). Different superscript letters following numbers indicate significant differences between values within each row, according to the Tukey test (capital letter $p<0.01$; lower case letter $p<0.05$; n.s. not significant).

| | Bread samples | | | | | |
|---|---|---|---|---|---|---|
| | NaCl | | | Saltwell | | |
| | 1.7 % | 0.35 % | 0.15% | 1.7 % | 0.35 % | 0.15% |
| Na (g/100g) | 0.43% | 0.087% | 0.048% | 0.24% | 0.064% | 0.035% |
| Loaf volume ($cm^3$) | 1470 ±95$^{A}$ | 1498 ±65$^{A}$ | 1276 ±15$^{AB}$ | 1443 ±86$^{A}$ | 1420 ±70$^{A}$ | 1160 ±0.1$^{B}$ |
| Loaf height (mm) | 97±2$^{A}$ | 82±2$^{BC}$ | 76±1$^{CD}$ | 87±2$^{B}$ | 83±3$^{BC}$ | 74±1$^{D}$ |
| Loaf weight (g)[1] | 484.73 ±19.24$^{AB}$ | 489.96 ±28.09$^{A}$ | 413.04 ±6.18$^{BC}$ | 460.65 ±23.30$^{BC}$ | 459.27 ±26.69$^{ABC}$ | 391.73 ±17.27$^{C}$ |
| Crumb porosity (1:8)[2] | 6±0.0$^{n.s}$ | 6±0.0$^{n.s}$ | 6±0.0$^{n.s}$ | 6±0.0$^{n.s}$ | 6±0.0$^{n.s}$ | 7±0.0$^{n.s}$ |
| Internal structure (1: 2)[3] | 2±0.0$^{A}$ | 1±0.0$^{B}$ | 1.3±0.6$^{AB}$ | 1±0.0$^{B}$ | 1±0.0$^{B}$ | 1±0.0$^{B}$ |
| Top crust thickness (mm) | 3.5±0.0$^{ABC}$ | 3.2±0.3$^{BC}$ | 3.8±0.3$^{AB}$ | 3.0±0.0$^{C}$ | 3.8±0.3$^{AB}$ | 4.0±0.0$^{A}$ |
| Bread base diameter (cm) | 21.9±0.7$^{b}$ | 23.0±0.5$^{ab}$ | 23.6±0.6$^{a}$ | 23.3±0.5$^{ab}$ | 23.3±0.5$^{ab}$ | 23.8±0.4$^{a}$ |
| Crumb color (C) | 17.48 ±0.44$^{B}$ | 19.67 ±0.90$^{A}$ | 19.03±0.35$^{AB}$ | 19.28 ±0.72$^{AB}$ | 20.46 ±0.56$^{A}$ | 19.24 ±0.67$^{AB}$ |
| Crumb moisture (g/100g) | 38.1±0.8$^{ab}$ | 38.0±0.5$^{ab}$ | 39.4±0.6$^{a}$ | 38.3±0.7$^{ab}$ | 37.8±0.4$^{b}$ | 39.1±0.5$^{ab}$ |
| $a_w$ | 0.922±0.0$^{n.s}$ | 0.924±0.0$^{n.s}$ | 0.925±0.0$^{n.s}$ | 0.925±0.0$^{n.s}$ | 0.923±0.0$^{n.s}$ | 0.925±0.0$^{n.s}$ |

Data expressed as the mean±standard deviation; [1] referred on weight of durum wheat semolina bread; [2]1: most porous; 8: least porous; [3] 1: regular; 2: irregular.

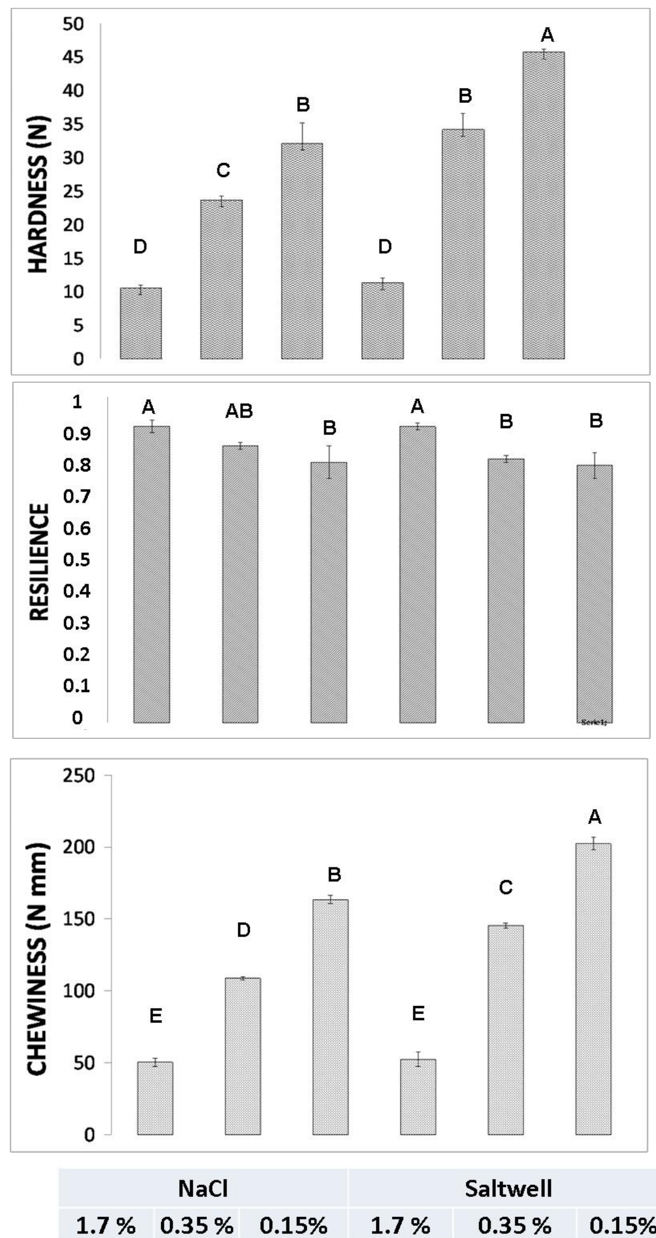


**Figure 1.** TPA parameters of breads at different salt content (n = 3). Different capital letter indicate significant differences (p<0.01).

**Figure 2.** Commercial label of low sodium content bread (PANLIFE®)

# Chapter 6

## A novel $\alpha$-amylase-lipase formulation as anti-staling agent in durum wheat bread

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**Abstract**

The aim of this work has been to evaluate the anti-staling effect exerted by a novel $\alpha$-amylase-lipase enzyme formulation on durum wheat bread, in comparison with four different commercial amylase preparations and with control without added enzymes. Bread-making trials were carried out at industrial level. Sliced bread, packed under modified atmosphere, was analyzed for texture profile, moisture content, and water activity during 90 days. Crumb sections were submitted to environmental scanning electron microscopy at the end of the storage period. The $\alpha$-amylase-lipase enzyme preparation showed synergistic interactions in preventing staling. In particular, bread added of these two enzymes in mixture was always softer and more chewable than either control or samples added of other enzymes. Moreover, $\alpha$-amylase-lipase exhibited the most marked effect in slowing down both hardening and chewiness changes during time. Starting from 7 days of storage, both water activity and moisture content of bread added of $\alpha$-amylase-lipase were higher than in control. Starting from 68 days the moisture content of $\alpha$-amylase-lipase-added bread became lower than that of other enzyme-added breads, and at the end of storage also water activity was significantly lower. Pore morphology of $\alpha$-amylase-lipase-added bread appeared markedly different from that of control bread.

**Keywords:** enzymes, durum wheat semolina, TPA, $a_w$, bread staling

## 6.1 Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) is usually the raw material for pasta. In addition, it is also used in the production of various types of bread all over the Mediterranean area (Quaglia, 1988). This ancient habit is still particularly diffused in the Southern regions of Italy and in its main islands (Sicily and Sardinia), where the pedoclimatic conditions are more favorable to durum than soft wheat cultivation (Fadda, Santos, Piga, & Collar, 2010; Pasqualone, 2012). Bread is a worldwide essential food but quickly loses its desirable qualities such as texture, flavor, and freshness. In fact, when stored at room temperature, most types of breads or bakery products with a spongy crumb undergo a progressive and often rapid deterioration of quality, commonly known as 'staling'. Some authors (Boyacioglu & D'Appolonia, 1994a) have investigated the staling behavior of bread made of durum wheat semolina or blends of durum and soft wheat flour. Durum wheat bread has been reported to have longer shelf-life than soft wheat bread, due to high water-binding capacity of semolina (Boyacioglu & D'Appolonia, 1994b). The mechanism of bread staling involves the re-crystallization (retrogradation) of gelatinized starch, responsible for the texture changes that take place during bread storage (Gray & Bemiller, 2003). During baking, amylose and amylopectin tend to separate and to accumulate within the starch granules and in the intergranular space in the form of double helices (Conde-Petit, Nuessli, Handschin, & Escher, 1998; Hug-Iten, Escher, & Conde-Petit, 1999). After baking, amylose retrogrades very quickly, stabilizing the initial structure and forming a more rigid, insoluble network. The subsequent increase in bread firmness is due to further physic-chemical changes affecting the starch components, especially the amylopectin fraction. To prevent these phenomena, anti-staling agents such as enzymes and emulsifiers are used in bread-making, as they interfere with the re-association of amylose, amylopectin, or both (Purhagen, Sjöö, & Eliasson, 2011). In particular, the enzymes most frequently used are $\alpha$-amylases of fungal, microbial, or cereal origin (Rosell, Haros, Escrivà, & Benedito de Barber, 2001). These enzymes positively act on bread texture by producing low-molecular-weight dextrins that, in turn, interfere with the amylopectin retrogradation and with the protein-starch interactions occurring during bread storage (Martinez-Anaya, Devesa, Andreu, Escrivà, & Collar, 1999). Their use optimizes the amylase activity of flour and slows down bread staling (Goesaert et al., 2005). Moreover, the $\alpha$-amylases reduce the firming rate (Rosell et al., 2001), increase bread volume, improve crumb

grain, crust and crumb color, and contribute to flavor development (Martinez-Anaya et al., 1999). A few studies, carried out mainly by micro baking tests at laboratory level, have been conducted on the lipases (Castello, Jollet, Potus, Baret, & Nicolas, 1998; Castello, Potus, Baret, & Nicolas, 2000; Schaffarczyk, Østdal, & Koehler, 2014). These enzymes have been reported to positively influence bread quality characteristics. In fact, they act on the lipid fraction by increasing the amount of molecules with emulsifying properties (monoacylglycerols and diacylglycerols) (Castello et al., 1998) which, in turn, positively influence bread volume (Castello et al., 2000). Increases of bread volume of 56-58% have been reported, depending on the type and concentration of the added lipase (Schaffarczyk et al., 2014). Moreover, exogenous lipases improve the rheological properties of the dough, and delay bread staling (Castello et al., 2000; Olesen, Si, & Donelyan, 2000), also when used in combination with $\alpha$-amylase (Siswoyo, Tanaka, & Morita, 1999; León, Durán, & Benedito de Barber, 2002). Hence, the aim of the present work has been to evaluate the anti-staling effect exerted by a novel $\alpha$-amylase-lipase enzyme formulation in comparison with four different commercial $\alpha$-amylase preparations, and with control without added enzymes. The enzymes were tested in durum wheat bread-making trials carried out at industrial level. Sliced bread, packed under modified atmosphere, was analyzed for texture profile, moisture content, and water activity during 90 days. The environmental scanning electron microscopic analysis of crumb sections was also carried out at the end of storage to point out morphological differences of crumb pores.

## 6.2. Materials and methods

### *6.2.1 Enzymes*

Four commercial enzyme preparations and a novel formulation were used: (i) the maltogenic amylase NM 15 new (Ri.fra, Marsala, Italy) (NM 15); (ii) the maltogenic amylase MAX LIFE P15 (Danisco, Cernusco sul Naviglio, Italy), (ML P15); (iii) the maltogenic amylase VERON x Tender (AB enzymes, Darmstad, Germany) (VxT); (iv) the maltogenic amylase VERON MAC (AB enzymes, Darmstad, Germany) (VMAC); (v) the novel $\alpha$-amylase-lipase enzyme formulation Prozyme's 3010 fresh (Bontà Infinite, Terme Vigliatore, Messina, Italy) (3010). All the preparations, whose details regarding activity, dosage, and composition are reported in Table 1, were used according to the recommended manufacturer's dosage.

*6.2.2 Sample preparation*

Commercial durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) re-milled semolina was provided by Molino S. Paolo of Mario Paolo Gallo & C. S.p.A. (Noto, Italy). Compressed yeast (AB Mauri, Casteggio, Italy) and NaCl employed in bread-making process were purchased at a local retailer. Bread was prepared at a local bread-making factory (Valle del Dittaino Società Cooperativa Agricola, Assoro, Italy) according to a consolidated industrial process based on the following formulation, with no added fat: durum wheat semolina, water (66% semolina basis), compressed yeast (0.47% semolina basis), NaCl (2.2% semolina basis). Six types of bread were produced: the control (without the addition of enzymes), and five $\alpha$-amylase-added types (NM 15, ML P15, VxT, VMAC, and 3010), each containing one of the five tested enzyme preparations added – at the manufacturer's recommended dosage – to the above formulation without modifications. The ingredients were mixed for 17 min by means of a diving arms mixer (Pietro Berto, Marano Vicentino, Italy). The final dough temperature was 26±1 °C. The dough was rested in bulk for 15 min, then was scaled by the automatic volumetric divider Omega (Pietro Berto, Marano Vicentino, Italy) into 145 portions weighting 1160±20 g, that were automatically shaped into round loaves by the conical rounder CO 3000 (Turri, Costa di Rovigo, Italy), and were placed for 150 min into a proofer (Pavailler Engineering, Galliate, Italy) set at 32 °C and 66% RH. The subsequent baking was carried out at 240 °C for 60 min, in an industrial tunnel oven (Pavailler Engineering, Galliate, Italy). The baked loaves, weighting approximately 1 kg each, were automatically transported to a cooling chamber (S.L.C, Copit, Trecate, Italy) set at 20±2 °C, where were kept for 120 min. After cooling, the loaves were sliced by means of an automatic slicing machine (Brevetti Gasparin, Marano Vicentino, Italy) to 11±1 mm thickness.

*6.2.3 Packaging and storage conditions*

About 500 g of sliced bread per loaf were packaged under modified atmosphere (70:30 $N_2$:$CO_2$). The packages were constituted by two plastic films (kindly provided by Cryovac Sealed Air, Elmwood Park, NJ, USA), one for the lid and another for the bottom. The latter was thermoformed (MIX 9000, Tecnosistem, Coccaglio, Italy) into a bowl before inserting the bread slices. The characteristics of thickness, water vapor transmission rate (WVTR, $g/m^2/24$

h), and oxygen transmission rate (OTR, $cm^3/m^2/24$ h) of the bottom film (type T6011B, Cryovac Sealed Air, Elmwood Park, NJ, USA) were: 275 μm thickness, WVTR ≤ 10, OTR = 1; those of the lid film (type T9250B, Cryovac Sealed Air, Elmwood Park, NJ, USA) were: 125 μm thickness, WVTR < 10, OTR < 3. As conventionally done by the producer, food-grade ethanol was sprayed on the slices into the packages, whose internal atmosphere was then replaced by a 70:30 $N_2$:$CO_2$ gas mixture (MAP MIX 9001 ME, Dansensor Italia, Segrate, Italy). Nitrogen and carbon dioxide for bread packaging and ethanol were respectively supplied by SOL (Monza, Italy) and Distillerie F.lli Russo (Santa Venerina, Italy). The packaged samples were stored up to 90 days at 20±2°C and 60±2% RH. Sample withdrawals were carried out, for the subsequent analyses, at 0, 7, 14, 21, 28, 48, 68, and 90 days.

*6.2.4 Chemical and rheological analyses of durum wheat semolina*

Protein content was determined by means of Infratec 1241 Grain Analyzer (Foss Tecator, Höganäs, Sweden), based on Near Infrared Transmittance. A calibration curve (range 8.3-15.3) was previously set up on the results of Kjeldahl nitrogen method and validated according to ISO 12099:2010 method (ISO, 2010) on a large set of samples of durum wheat grain and semolina. Ash and moisture content were determined according to the AACC 44–19 and AACC 08–01 methods (AACC, 2000), respectively. Dry gluten and gluten index were determined by using a Glutomatic System consisting of Glutomatic 2200, Centrifuge 2015, Glutork 2020 (Perten Instruments AB, Huddinge, Sweden), according to the UNI 10690 method (UNI, 1979). The $\alpha$-amylase activity was determined by using the Falling Number 1500 apparatus (Perten Instruments AB, Huddinge, Sweden), following the ISO 3093:2009 method (ISO, 2009). The color parameters in the color space $L^*$, $a^*$, $b^*$ were determined by Chromameter CR-300 (Minolta, Osaka, Japan), under the illuminant D65. The farinograph indices were determined according to the AACC 54-21 method (AACC, 2000) by a farinograph (Brabender instrument, Duisburg, Germany), equipped with the software Farinograph®. Alveograph trials were performed according to the 10453 method (UNI, 1995) using an alveoconsistograph, equipped with the software Alveolink NG (Tripette et Renaud, Villeneuve-la-Garenne, France).

*6.2.5 Determination of moisture content and water activity of bread crumb*

Moisture content of bread crumb was determined by oven drying at 105 °C until constant weight according to AOAC method no. 945.15 (AOAC, 2000). Three bread slices (11±1 mm thickness) were used, and moisture was determined on a crumb cylinder (40 mm diameter) taken from the center of each slice. Water activity ($a_w$) was determined by Hygropalm 40 AW (Rotronic Instruments Ltd, Crawley, UK) according to manufacturers' instructions. Three bread slices (11±1 mm thickness) were used, after removal of the crust. For each set of determinations, separate loaves were considered.

*6.2.6 Texture Profile Analysis of bread*

The Texture Profile Analysis (TPA) of bread was carried out by means of an Universal Testing machine (model 3344, Instron, Norwood, MA, USA), equipped with a 5.0 cm diameter cylindrical probe and a 2000 N load cell. Data were acquired through Bluehill® 2 software (Instron, Norwood, MA, USA). Cyclic compression tests (30s gap between first and second compression) were set up: the crosshead speed was 3.3 mm/s, the force required to compress the samples by 40% was recorded on 5-cm side square portions of 24-mm thick slices, and the average value of five replicates was taken. Four primary TPA parameters (hardness, cohesiveness, springiness, and resilience), and two derived parameters (gumminess and chewiness) were calculated: hardness (N), defined as the peak force during the first compression cycle; cohesiveness, i.e. the adimensional ratio of the positive force area during the second compression cycle to the positive force area recorded during the first compression cycle, or Area 2/Area 1; springiness (mm), i.e. the elastic recovery that occurs when the compressive force is removed, defined as the height to which the food recovers during the time that elapses between the end of the first and the start of the second compression; resilience, defined as the adimensional ratio between the negative force input and the positive force input during the first compression, or Area 5/Area 4; chewiness (N mm), defined as the product of hardness, resilience and springiness.

*6.2.7 Headspace gas composition analysis*

The internal $O_2$ and $CO_2$ composition of packages was determined by means of Dansensor Checkpoint portable gas analyzer (Dansensor, Ringsted, Denmark). Ten mL of headspace were analyzed, with three replications.

### *6.2.8 Environmental scanning electron microscopy*

At 90 days of storage, bread crumb was submitted to environmental scanning electron microscopy (ESEM), carried out by means of the Inspect S50 electron microscope (FEI, Hillsboro, Oregon, USA) operating at an accelerating voltage of 15 kV. Before testing, the first layer of each sample was carefully removed. Then, the sample (small portions of bread cut with razor blade) were prepared for ESEM analysis. Bread samples were mounted on specific stubs using a thin spatula and transferred to the microscopy support. Digital images at 100× were acquired by using the Imix software (Princeton Gamma Tech, Princeton, USA).

### *6.2.9 Statistical analysis*

The statistical analyses were performed by using the CoStat Anova Statistic Software version 6.311 (Cohort, Monterey, CA, USA) for Windows. Textural properties, moisture content, $a_w$ and $pCO_2$ data were submitted to analysis of variance (ANOVA) using Duncan's multiple range test.

## **6.3. Results and discussion**

### *6.3.1 Characteristics of enzyme formulations and of durum wheat re-milled semolina*

Table 1 reports the main details regarding activity and composition of the five enzyme preparations tested. All the preparations were powdered blends of various ingredients mixed to the enzymes, such as refined soft wheat flour, NaCl, starch, maltodextrins, and vegetable oil, all aimed to facilitate the homogeneous dispersion in the bulk dough. Due to the intended aim of the work of giving useful information at industrial level, all the enzyme preparations were employed according to the recommended manufacturer's dosage, in agreement with similar studies (Barrett, Marando, Leung, & Kaletunç 2005; Purhagen et al., 2011). In particular, the amylase amount per bulk dough of 168.67 kg markedly varied from as low as 2250 U/dough in 3010, due to the compensating presence of lipase, to 12,500 U/dough in the trials involving the use of VxT and VMAC formulations, and to 30,000 U/dough in case of NM 15 and ML P15. The compositional data of durum wheat re-milled semolina are shown in Table 2. Protein content and farinograph parameters were in the ranges previously observed in

quality surveys about Southern Italian re-milled semolina used for bread-making (Pasqualone, Caponio, & Simeone, 2004; Raffo et al., 2003). The observed values of gluten content and gluten index, however, were borderline (Brescia et al., 2007; Pasqualone et al., 2004); in particular, gluten content was low and coupled to very high gluten index, so as to induce expecting a very compact bread. Also the alveograph parameters, especially the P/L ratio – high, although in line with durum milling products (Brescia et al., 2007; Pasqualone et al., 2004; Raffo et al., 2003) – further sustained these expectations. The high value of Falling Number indicated a limited fermentative activity, suitable to be supplemented by exogenous amylases. The bright yellow color, due to carotenoid pigments, is a typical feature of re-milled semolina that is partly transferred to final bread crumb (Pasqualone et al., 2004; Pasqualone, Summo, Bilancia, & Caponio, 2007) and is very appreciated by consumers. Similar yellow levels were previously observed (Brescia et al., 2007; Fadda et al., 2010; Pasqualone et al., 2004).

### *6.3.2 Crumb moisture content and water activity of bread*

The variations of moisture content of food are related to its stability and quality (Pomeranz & Meloan, 1994). Moistness is a favorable sensory attribute for baked goods because it is synonymous of soft and tender products. Moreover, moisture has a plasticizing effect on the crumb network, and its loss contributes to crumb hardening. This event, together with starch recrystallization, is one of the major factors involved in bread staling (Piazza & Masi, 1995). Usually, lower moisture variations correspond to slower firming rate. As shown in Table 3, all the samples exhibited a decrease of moisture content during storage, but at different extents. At the beginning, the highest moisture content was observed in ML P15-added bread. The control bread contained 36.37% moisture (not significantly different than 3010 and VxT-added breads), and showed a marked decrease during 90 days. The value reached after 7 days was in the range reported in literature for 1 kg-sized durum wheat sourdough-based bread after 9 days of unpackaged storage, without slicing (Raffo et al., 2003). The variations of moisture content during time were less pronounced in presence of the enzyme preparations. With the only exception of VxT-added bread, starting from 7 days of storage all the enzyme-added bread types appeared moister than control. However, bread supplemented by the 3010 $\alpha$-amylase-lipase formulation showed, starting from 68 days, a lower moisture content than the other enzyme-added samples, probably due to minor amylase activity of this formulation,

leading to lower formation of moisturizing dextrines. At the end of the storage period, NM 15-added bread showed the moister crumb, followed by VMAC, NM 15 and ML P15 samples – without significant differences among them – and by 3010-added bread. The changes of $a_w$, summarized in Table 4, basically paralleled the variations in moisture content. At the beginning of storage, control bread showed no significant differences respect to NM 15, ML P15, and VMAC-added breads while, starting from 7 days, it exhibited $a_w$ values significantly lower than those of the other samples. Among the enzyme-added breads, after 90 days the lowest values of $a_w$ were observed in bread supplemented by 3010 $\alpha$-amylase-lipase formulation.

#### *6.3.3 Textural characteristics of bread*

Texture is an important characteristic in consumer's perception of food and influences the purchasing decisions. Meilgaard, Civille, & Carr (1991) define food texture as the sensory manifestation for the structure of products in terms of their: (i) reaction to stress by the kinesthetic sense in the muscles of the hand, fingers, tongue, jaw, or lips (e.g. adhesiveness, cohesiveness, hardness, etc.), and (ii) tactile feel properties measured by the tactile nerves in the surface of the skin of the hand, lips, or tongue (e.g. oiliness, tenderness, moistness, etc.). The TPA results, obtained by means of double cycle compressions at 40% depth, evidenced the structural changes that affected the samples during storage and clearly differentiated control from the various enzyme-added samples (Figure 1). Crumb hardness showed significant differences ($p<0.01$) among the five treatments and the control. These differences, less evident at the beginning, were particularly marked starting from 7 days of storage. In fact, in spite of MAP, the control bread showed 80 N hardness – not easily acceptable by consumers – after only 7 days. This value was higher than those reported in literature for 1 kg-sized durum wheat sourdough-based breads after 9 days of unpackaged storage, without slicing (Raffo et al., 2003). After 28 days, control bread crumb even reached 180 N hardness, that was maintained for the rest of the storage period as it corresponded to complete hardening. The particularly high hardness values observed in control bread during storage were probably imputable also to the intrinsic characteristics of starting re-milled semolina that, in absence of $\alpha$-amylase corrections, coupled a low fermentative activity with low gluten content and excessive gluten tenacity. During the whole storage period considered, the enzyme-containing

breads were markedly softer than control and, compared to it, only a slight increase of hardness was observed, demonstrating the anti-staling effectiveness of all the enzyme formulations tested. The VxT-added bread even reported a decrease in hardness after 14 days. As regards to the 3010-added bread, its consistency was almost constant throughout the whole storage period, and was lower than that of the other breads, so that after 90 days it still showed a hardness value lower than 30 N. Therefore, in spite of the lower $\alpha$-amylase activity of 3010 formulation, our data show that the combined use of lipase and $\alpha$-amylase, although not very effective with regard to moisture keeping, potentiated the antistaling effect of $\alpha$-amylase itself, with special regard to the prevention of starch retrogradation and subsequent crumb firming. Siswoyo et al. (1999), in comparing the effects of lipase/$\alpha$-amylase to those of lipase alone, observed similar findings. They hypothesized that the starch-lipid complexes formed with the diacylglycerols and monoacylglycerols released by lipase, having an inhibiting effect on retrogradation, could be enhanced in amount and stability by the medium-sized starch-hydrolysis molecules released by $\alpha$-amylase at the same time. This hypothesis was further sustained by ascertaining amylose-lipid complexes with higher thermal stabilities in bread added of lipase and $\alpha$-amylase than in bread added of lipase alone (León et al., 2002; Siswoyo et al. 1999). The highest hardness values were showed, at any of the times considered, by VMAC-added breads. Besides having a lower activity than NM15 and ML P15, the enzyme formulation VMAC contained a maltogenic amylase of fungal origin, usually much less thermostable than those of bacterial origin (Błaszczak, Sadowska, Rosell, & Fornal, 2004; Palacios, Schwarz, & D'Appolonia, 2004). This could explain the worse performance of VMAC compared to VxT, having the same activity but of bacterial origin. Springiness did not provide information of great value as it did not present significant variations during storage (data not shown). On the contrary, the resilience, that shows how well a product 'fights to regain its original position after a stress' (Abdelghafor, Mustafa, Ibrahim, & Krishnan, 2011), was very informative. The resilience of control decreased significantly with storage time, indicating a marked tendency to become crumblier, with a less cohesive structure. At 28 days this kind of bread reached the lowest possible resilience value, accounting for about 0.35, and became extremely fragile and crumbly. The resilience of all the enzyme-supplemented bread loaves remained quite high during the whole storage period, again with a slightly worst ageing pattern for VMAC-added bread, but less pronounced than that observed for hardness. The

trends of variation of the derived parameter chewiness (hardness * resilience * springiness) paralleled those of hardness. Significant differences ($p<0.01$) were observed among all five treatments and control bread: after 14 days the latter showed values of chewiness markedly higher than those of enzyme-added breads, and after 28 days it reached the maximum possible value of chewiness. Moreover, chewiness was the parameter that better differentiated the effect of the various enzyme preparations. Again, the 3010 $\alpha$-amylase-lipase-added bread showed the lowest values of chewiness during the whole storage period, whereas VMAC-added breads showed the highest values. Significant correlations were observed among textural features, moisture content, and $a_w$ of bread samples (Table 5). In particular, crumb hardness and chewiness showed significant negative correlations with both moisture content and $a_w$. The inverse relationship between hardness and moisture content has been previously reported (Fik & Surowka, 2002; Rogers, Zeleznak, Lai, & Hosenei, 1988). Fessas & Schiraldi (1998) proposed that water acts as a plasticizer in bread. The decrease in moisture content favors the formation of hydrogen bonds among the starch polymers or between starch and proteins, yielding greater hardness. A significant negative correlation was observed between hardness and resilience ($r$ = -0.81), both considered as valuable bread quality indicators (Armero & Collar, 1998). In addition, hardness was positively correlated with chewiness ($r$ = 0.96), as expected being the latter a hardness-derived parameter with a trend similar to hardness during bread ageing. The results evidenced that all the enzymes tested had positive anti-staling effects on durum wheat bread. The addition of $\alpha$-amylase significantly slowed down the water loss and firmness increase, confirming the literature data (Błaszczak et al., 2004; Bollaín, Angioloni & Collar et al., 2005; Jiménez & Martínez-Anaya, 2001). The 3010 $\alpha$-amylase-lipase enzyme preparation showed synergistic interactions in preventing staling: it exhibited the most marked effect in slowing down both hardening and chewiness during storage. The VxT-supplemented bread also showed an interesting anti-staling effect and could be considered in future researches in combination with 3010 agent to achieve further improvement of durum wheat bread shelf-life.

*6.3.4 Headspace gas composition analysis*

The packaging material showed good barrier performances. In fact, $O_2$ was not detectable inside the packages through the whole duration of the shelf-life test. Also, the internal content

of $CO_2$ accounted for 31.46% at the beginning, and decreased to 24.26% after 90 days, probably mainly due to gas dissolution into the food matrix during storage. Furthermore, no significant differences were observed among $CO_2$ values in the different bread types (data not shown).

*6.3.5 Morphological features of crumb pores*

The microstructure of bread samples was studied after 90 days of storage in order to visualize the effect of different enzymes used in bread formulation on the morphological features of crumb pores. The ESEM micrographs of crumb section (Figure 2), able to give qualitative information on pore structure (Datta, Sahin, Sumnu, & Keskin, 2007), showed that all breads were characterized by pores of different size, heterogeneously distributed. However, in presence of 3010, the pores appeared to be slightly smaller and more spherical as compared to other samples. This is consistent with the lower level of moisture and $a_w$ value observed in 3010 bread at the end of the storage period: water loss probably induced slightly greater pore shrinkage than in other samples. The pores of bread supplemented by NM 15 appeared to be the largest, while those of breads treated with ML P15 and VxT appeared to be elongated. The most irregular pore distribution was observed in VMAC-added breads, where a population of larger cells coexisted with smaller ones. Control bread appeared markedly different from the enzyme-supplemented breads: it showed a more opened structure, and a dry and opaque crumb, with rigid and fragile features typical of retrograded starch. Other authors (Błaszczak et al., 2004) observed similar structural differences, although less evident, between 5-days stored white breads added of $\alpha$-amylases of fungal and bacterial origin and samples obtained without the addition of enzymes. Finally, it has to be reported that, although sensory analysis was not carried out, at the end of storage period all the samples appeared devoid of anomalies, apart the unacceptable hardness of control, with no anomalous colors and/or odors (data not shown).

## 6.4. Conclusions

The evolution of textural properties, crumb moisture, and $a_w$ during bread storage confirmed that amylases are effective in slowing down bread staling also in durum wheat bread, and pointed out the significantly greater effect provided by the 3010 $\alpha$-amylase-lipase combination, that positively modified textural and crumb grain properties of bread. The

experimental data also indicated the close connection between moisture content and textural properties, with special regard to crumb hardness, resilience, and chewiness. Although further investigations are needed to achieve a better biochemical characterization of this new anti-staling formulation, the obtained results have an immediate practical application, since all the trials have been directly carried out at industrial level and accordingly to recommended dosages of each formulation. Hence, the producers may take advantage of the increases in shelf-life to enhance the diffusion and marketing of durum wheat bread far from the areas of production.

### 6.5 References


AACC International (2000). *Approved methods of analysis* (10th Ed.). St Paul, MN: American Association of Cereal Chemists.

AOAC (2000). *Official methods of analysis of the Association of Official Analytical Chemists* (17th Ed.). Gaithersburg, MD: Association of Official Analytical Chemists.

Abdelghafor, R. F., Mustafa, A. I., Ibrahim, A. M. H., & Krishnan, P. G. (2011). Quality of bread from composite flour of sorghum and hard white winter wheat. *Advance Journal of Food Science and Technology, 3*, 9–15.

Armero, E., & Collar, C. (1998). Crumb firming kinetics of wheat breads with anti-staling additives. *Journal of Cereal Science, 28*, 165–174.

Barrett, A. H., Marando, G., Leung, H., & Kaletunç, G. (2005). Effect of different enzymes on the textural stability of shelf-stable bread. *Cereal Chemistry*, *82*, 152–157.

Błaszczak, W., Sadowska, J., Rosell, C. M., & Fornal, J. (2004). Structural changes in the wheat dough and bread with the addition of alpha-amylases. *European Food Research and Technology*, *219*, 348–354.

Bollaín, C., Angioloni, A., & Collar, C. (2005). Relationships between dough and bread viscoelastic properties in enzyme supplemented samples. *Journal of Food Engineering, 77*, 665–671.

Boyacioglu, M. H., & D'Appolonia, B. L. (1994a). Characterization and utilization of durum wheat for breadmaking. III. Staling properties of bread baked from bread wheat flours and durum wheat flours. *Cereal Chemistry*, *71*, 34–41.

Boyacioglu, M. H., & D'Appolonia, B. L. (1994b). Characterization and utilization of durum wheat for breadmaking. II. Study of flour blends and various additives. *Cereal Chemistry, 71*, 28–33.

Brescia, M. A., Sacco, D., Sgaramella, A., Pasqualone, A., Simeone, R., Peri, G., & Sacco, A. (2007). Characterisation of different typical Italian breads by means of traditional, spectroscopic and image analyses. *Food Chemistry*, *104*, 429–438.

Castello, P., Baret, J. L., Potus, J., & Nicolas, J. (2000). Technological and biochemical effects of exogenous lipases in breadmaking. In T. Simoinen, & M. Tenkanen (Eds.), *2nd European symposium on enzymes in grain processing* (Vol. 207, pp. 193–200). Espoo: VTT Technical Research Centre of Finland.

Castello, P., Jollet, S., Potus, J., Baret, J. L., & Nicolas, J. (1998). Effect of exogenous lipases on dough lipids during mixing of wheat flour. *Cereal Chemistry*, *75,* 595–601.

Conde-Petit, B., Nuessli, J., Handschin, S., & Escher, F. (1998). Comparative characterization of aqueous starch dispersions by light microscopy, rheometry and iodine binding behavior. *Starch, 50*, 184–192.

Datta, A.K., Sahin S., Sumnu G., & Keskin, S. O. (2007). Porous media characterization of breads baked using novel heating modes. *Journal of Food Engineering, 79*, 106–116.

Fadda, C., Santos, E. M., Piga, A., & Collar, C. (2010). Innovative traditional Italian durum wheat breads: influence of yeast and gluten on performance of sourdough Moddizzosu breads. *Cereal Chemistry*, *87*, 204-213.

Fessas, D., & Schiraldi, A. (1998). Texture and staling of wheat bread crumb: effects of water extractable proteins and pentosans. *Thermochimica Acta, 323*, 17–26.

Fik, M., & Surowka, K. (2002). Effect of prebaking and frozen storage on the sensory quality and instrumental texture of bread. *Journal of the Science of Food and Agriculture, 82*, 1268–1275.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology, 16*, 12–30.

Gray, J. A., & Bemiller, J. N. (2003). Bread staling: molecular basis and control. *Comprehensive Reviews in Food Science and Food Safety, 2*, 1–21.

Hug-Iten, S., Escher, F., & Conde-Petit, B. (1999). Structural properties of starch in bread and bread model systems: influence of anti-staling alpha-amylase. *Cereal Chemistry, 78*, 421–428.

ISO (2009). *ISO 3093:2009. Wheat, rye and their flours, durum wheat and durum wheat semolina. Determination of the falling number according to Hagberg-Perten.* Geneva, Switzerland: ISO.

ISO (2010). *ISO 12099:2010. Animal feeding stuffs, cereals and milled cereal products. Guidelines for the application of near infrared spectrometry.* Geneva, Switzerland: ISO.

Jiménez, T., & Martínez-Anaya, M. A. (2001). Amylases and hemicellulases in breadmaking. Degradation by-products and potential relationship with functionality. *Food Science and Technology International, 7*, 5–14.

León, A. E., Durán, E., & Benedito de Barber, C. (2002). Utilization of enzyme mixtures to retard bread crumb firming. *Journal of Agricultural and Food Chemistry*, *50*, 1416–1419.

Martinez-Anaya, M., Devesa, A., Andreu, P., Escrivà, & Collar, C. (1999). Effects of the combination of starters and enzymes in regulating bread quality and shelf life. *Food Science and Technology International, 5*, 263–273.

Meilgaard, M., Civille, O. V., & Carr, B. T. (1991). Sensory attributes and the way we perceive them. In *Sensory evaluation techniques* (2nd ed.), (pp. 7–22). Boca Raton, FL: CRC Press Inc.

Olesen, T., Si, J. Q., & Donelyan, V. (2000). *Use of lipase in baking. U.S. Patent No. 6,110,508. August 29, 2000*. Washington, DC: US Patent and Trademark Office.

Palacios, H. R., Schwarz, P. B., & D'Appolonia, B. L. (2004). Effect of $\alpha$-amylases from different sources on the retrogradation and recrystallization of concentrated wheat starch gels: relationship to bread staling. *Journal of Agricultural and Food Chemistry*, *52*, 5978–5986.

Pasqualone, A. (2012). Italian durum wheat breads. In M. T. Pedrosa Silva Clerici (Ed.), *Bread consumption and health* (pp. 57–79). Hauppage, NY: Nova Science Publisher Inc.

Pasqualone, A., Caponio, F., & Simeone, R. (2004). Quality evaluation of re-milled durum wheat semolinas used for bread-making in Southern Italy. *European Food Research and Technology*, *219*, 630–634.

Pasqualone, A., Summo, C., Bilancia, M. T., & Caponio, F. (2007). Variations of the sensory profile of durum wheat Altamura PDO (Protected Designation of Origin) bread during staling. *Journal of Food Science*, *72*, S191-S196.

Piazza, L., & Masi, P. (1995). Moisture redistribution throughout the bread loaf during staling and its effect on mechanical properties. *Cereal Chemistry*, *72*, 320–325.

Pomeranz, Y., & Meloan, C. E. (1994). Determination of moisture. In *Food analysis: theory and practice* (3rd ed.), (pp. 575–583). New York: Chapman & Hall.

Purhagen, J. K., Sjöö, M. E., & Eliasson, A. C. (2011). Starch affecting anti-staling agents and their function in freestanding and pan-baked bread. *Food Hydrocolloids, 25*, 1656–1666.

Quaglia, G.B. (1988). Other durum wheat products. In G. Fabriani, & C. Lintas (Eds.), *Durum wheat chemistry and technology* (pp. 263–282). St. Paul, MN: American Association of Cereal Chemists.

Raffo, A., Pasqualone, A., Sinesio, F., Paoletti, F., Quaglia, G., & Simeone, R. (2003). Influence of durum wheat cultivar on the sensory profile and staling rate of Altamura bread. *European Food Research and Technology*, *218*, 49–55.

Rogers, D. E., Zeleznak, K. J., Lai, C. S., & Hoseney, R. C. (1988). Effect of native lipids, shortening, and bread moisture on bread firming. *Cereal Chemistry, 65*, 398–401.

Rosell, C. M., Haros, M., Escrivà, C., & Benedito de Barber, C. (2001). Experimental approach to optimize the use of $\alpha$-amylases in bread making. *Journal of Agricultural and Food Chemistry, 49*, 2973–2977.

Schaffarczyk, M., Østdal, H., & Koehler, P. (2014). Lipases in wheat breadmaking: analysis and functional effects of lipid reaction products. *Journal of Agricultural and Food Chemistry*, *62*, 8229–8237.

Siswoyo, T. A., Tanaka, N., & Morita, N. (1999). Effect of lipase combined with alpha-amylase on retrogradation of bread. *Food Science and Technology Research*, *5*, 356–361.

UNI (1979). *UNI method no. 10690. Durum wheat and semolina. Determination of gluten quality. Gluten index method.* Milan, Italy: UNI.

UNI (1995). *UNI method no. 10453. Durum wheat and semolina. Determination of rheological properties using an alveograph.* Milan, Italy: UNI.

**Table 1.**
Composition, activity, and dosage of the enzyme formulations tested.

| Formulation | Enzyme | Other ingredients | | Moisture content (g/100 g formulation) | Enzyme activity (U/g formulation) | Recommended dosage (g/100 kg flour) | Total units per dough[a] |
|---|---|---|---|---|---|---|---|
| | | Type | Amount (g/100 g formulation) | | | | |
| NM 15 | Maltogenic amylase | Refined soft wheat flour | 80 | 13.0 | 1500[b] | 20 | 30,000[b] |
| 3010 | Maltogenic amylase, lipase | Refined soft wheat flour | 70 | 7.5 | 150[b]; 190[c] | 15 | 2250[b]; 2850[c] |
| ML P15 | Bacterial glucan 1,4-$\alpha$-maltohydrolase | NaCl, starch, maltodextrins, palm oil[d] | 80 | 2.5 | 1500[b] | 20 | 30,000[b] |
| VxT | Bacterial $\alpha$-amylase | Maltodextrins, NaCl, sunflower oil, refined soft wheat flour[d] | 55 | 11.5 | 1000[b] | 12.5 | 12,500[b] |
| VMAC | Fungal $\alpha$-amylase | Maltodextrins, NaCl, sunflower oil[d] | 55 | 6.0 | 1000[b] | 12.5 | 12,500[b] |

[a]A bulk dough of 168.67 kg was produced from 100 kg flour for each trial.
[b]Units expressed as Maltogenic Amylase Novo Unit (MANU); 1 MANU is the amount of enzyme that decomposes 1 μmol of maltotriose per minute.
[c]Units expressed as Lipase Units (LU); 1 LU is the amount of enzyme, which releases 1 pmol butyric acid per minute.
[d]In decreasing quantitative order.

**Table 2.**
Quality attributes of durum wheat re-milled semolina used in the bread-making trials (n = 3). B.U. = Brabender Units

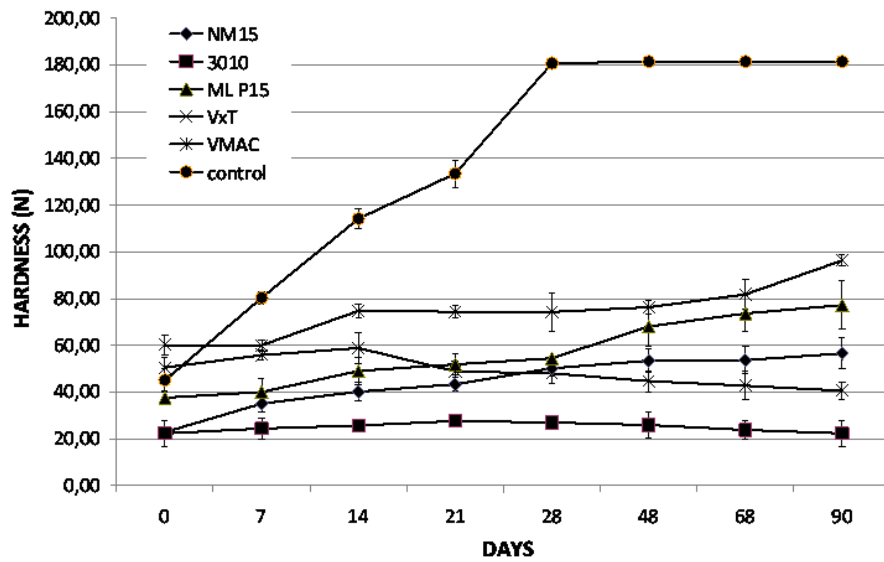
| Parameter | Value |
|---|---|
| *Chemical composition* | |
| Protein (% dry basis) | 11.05±0.07 |
| Ash (% dry basis) | 0.82±0.03 |
| | |
| Falling Number (s) | 487±1.41 |
| | |
| Gluten (% dry basis) | 7.77±0.26 |
| Gluten Index | 96±1.41 |
| | |
| *Colorimeter indexes* | |
| Luminosity (*L**) | 89.31 ±0.01 |
| Red index (*a**) | -2.38 ±0.05 |
| Yellow index (*b**) | 21.24 ±0.04 |
| | |
| *Farinograph parameters* | |
| Water absorption at 500 B.U. (%) | 54.70±0.28 |
| Development time (s) | 90±4.24 |
| Dough stability (s) | 342±4.24 |
| Softening index (B.U.) | 46±2.83 |
| | |
| *Alveograph trial* | |
| Deformation energy ($10^{-4}$ J) [W] | 159.00±1.41 |
| Configuration ratio curve (P/L) | 2.17±0.05 |

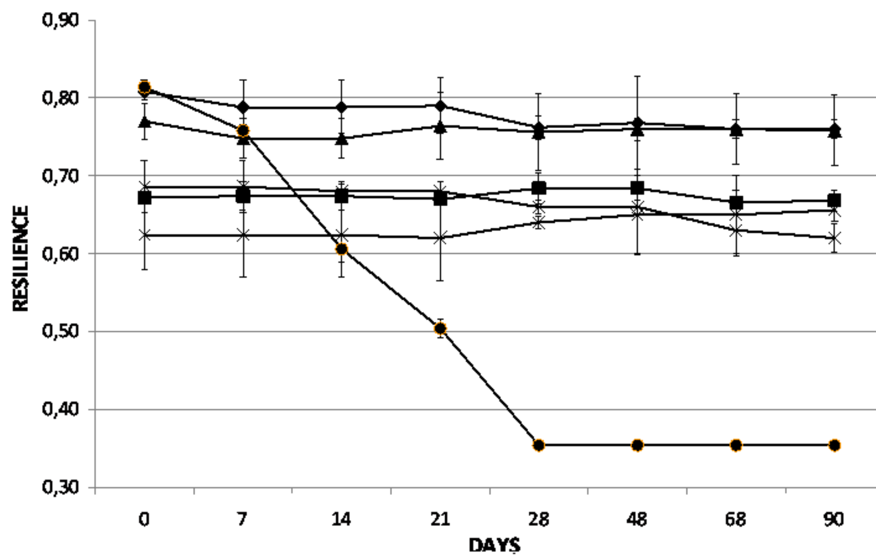
**Table 3.**
Crumb moisture content (%) of five enzyme-supplemented durum wheat breads and control (without enzymes) during 90 days of storage ($n$ = 3). Values followed by different lower case letters in column, and upper case letters in row, indicate significant differences ($p$<0.05)

| | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 48 | 68 | 90 |
| NM 15 | $37.49^{bA}$ ± 0.16 | $36.64^{aA}$ ± 0.08 | $36.97^{aAB}$ ± 0.74 | $36.70^{aAB}$ ± 0.23 | $35.87^{aBC}$ ± 0.36 | $35.05^{aC}$ ± 0.70 | $35.07^{aC}$ ± 0.64 | $34.11^{aD}$ ± 0.45 |
| 3010 | $36.26^{cA}$ ± 0.26 | $35.04^{cB}$ ± 0.17 | $34.86^{bcB}$ ± 0.54 | $33.99^{cC}$ ± 0.57 | $33.84^{cC}$ ± 0.29 | $32.10^{cD}$ ± 0.43 | $29.98^{dE}$ ± 0.08 | $29.94^{cE}$ ± 0.91 |
| ML P15 | $39.54^{aA}$ ± 0.23 | $36.49^{aB}$ ± 0.07 | $35.49^{bC}$ ± 0.35 | $35.49^{bC}$ ± 0.35 | $34.54^{bD}$ ± 0.00 | $32.68^{cE}$ ± 0.60 | $31.19^{cF}$ ± 0.58 | $31.31^{bF}$ ± 0.35 |
| VxT | $36.41^{cA}$ ± 0.26 | $34.29^{dB}$ ± 0.10 | $34.18^{cB}$ ± 0.38 | $32.91^{dC}$ ± 0.22 | $32.78^{dC}$ ± 0.24 | $32.02^{cC}$ ± 0.47 | $32.67^{bD}$ ± 0.61 | $31.13^{bE}$ ± 0.23 |
| VMAC | $37.18^{bA}$ ± 0.28 | $36.18^{bB}$ ± 0.24 | $34.31^{cC}$ ± 0.21 | $34.31^{cC}$ ± 0.21 | $34.25^{bcC}$ ± 0.08 | $33.91^{bC}$ ± 0.08 | $33.49^{bD}$ ± 0.08 | $31.25^{bE}$ ± 0.42 |
| Control | $36.37^{cA}$ ± 0.21 | $34.54^{dB}$ ± 0.08 | $33.24^{dC}$ ± 0.14 | $31.36^{dD}$ ± 0.17 | $28.15^{eE}$ ± 0.30 | $23.42^{dF}$ ± 0.10 | $23.42^{eF}$ ± 0.10 | $23.42^{dF}$ ± 0.10 |

**Table 4.**
Water activity ($a_w$) of five enzyme-supplemented durum wheat breads and control (without enzymes) during 90 days of storage ($n$ = 3). Values followed by different lower case letters in column, and upper case letters in row, indicate significant differences ($p$<0.05)

| | Storage time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 48 | 68 | 90 |
| NM15 | $0.946^{aA}$ ±0.001 | $0.940^{abB}$ ±0.001 | $0.940^{aB}$ ±0.002 | $0.938^{aB}$ ±0.001 | $0.935^{aC}$ ±0.001 | $0.931^{aD}$ ±0.001 | $0.922^{aE}$ ±0.003 | $0.920^{aE}$ ±0.001 |
| 3010 | $0.940^{bA}$ ±0.001 | $0.935^{cB}$ ±0.001 | $0.921^{cC}$ ±0.001 | $0.920^{dC}$ ±0.001 | $0.919^{cC}$ ±0.002 | $0.920^{bC}$ ±0.000 | $0.913^{bD}$ ±0.002 | $0.906^{bE}$ ±0.001 |
| ML P15 | $0.947^{aA}$ ±0.001 | $0.941^{aB}$ ±0.001 | $0.941^{aB}$ ±0.001 | $0.936^{bC}$ ±0.001 | $0.930^{abD}$ ±0.001 | $0.929^{aD}$ ±0.001 | $0.919^{aE}$ ±0.001 | $0.919^{aE}$ ±0.001 |
| VxT | $0.940^{bA}$ ±0.001 | $0.936^{bcAB}$ ±0.006 | $0.930^{bB}$ ±0.001 | $0.930^{cB}$ ±0.001 | $0.920^{cC}$ ±0.001 | $0.921^{bC}$ ±0.002 | $0.920^{aC}$ ±0.003 | $0.921^{aC}$ ±0.002 |
| VMAC | $0.946^{aA}$ ±0.001 | $0.940^{abB}$ ±0.001 | $0.930^{bC}$ ±0.001 | $0.930^{cC}$ ±0.001 | $0.924^{bcD}$ ±0.000 | $0.921^{bE}$ ±0.002 | $0.922^{aEF}$ ±0.002 | $0.919^{aF}$ ±0.001 |
| Control | $0.947^{aA}$ ±0.001 | $0.929^{dB}$ ±0.001 | $0.917^{dC}$ ±0.001 | $0.909^{eD}$ ±0.002 | $0.829^{dE}$ ±0.001 | $0.744^{cF}$ ±0.001 | $0.744^{cF}$ ±0.001 | $0.744^{cF}$ ±0.001 |



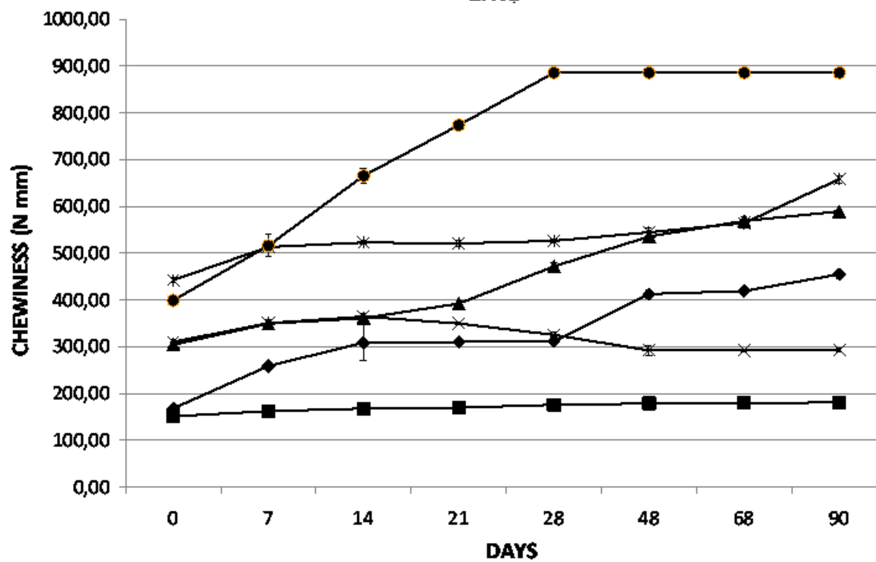




**Figure 1**. Significant effects on texture characteristics of five types of enzyme-supplemented durum wheat bread and control (without enzymes) [hardness (a); resilience (b); chewiness (c)]. Vertical bars denote 0.99 confidence intervals for means.

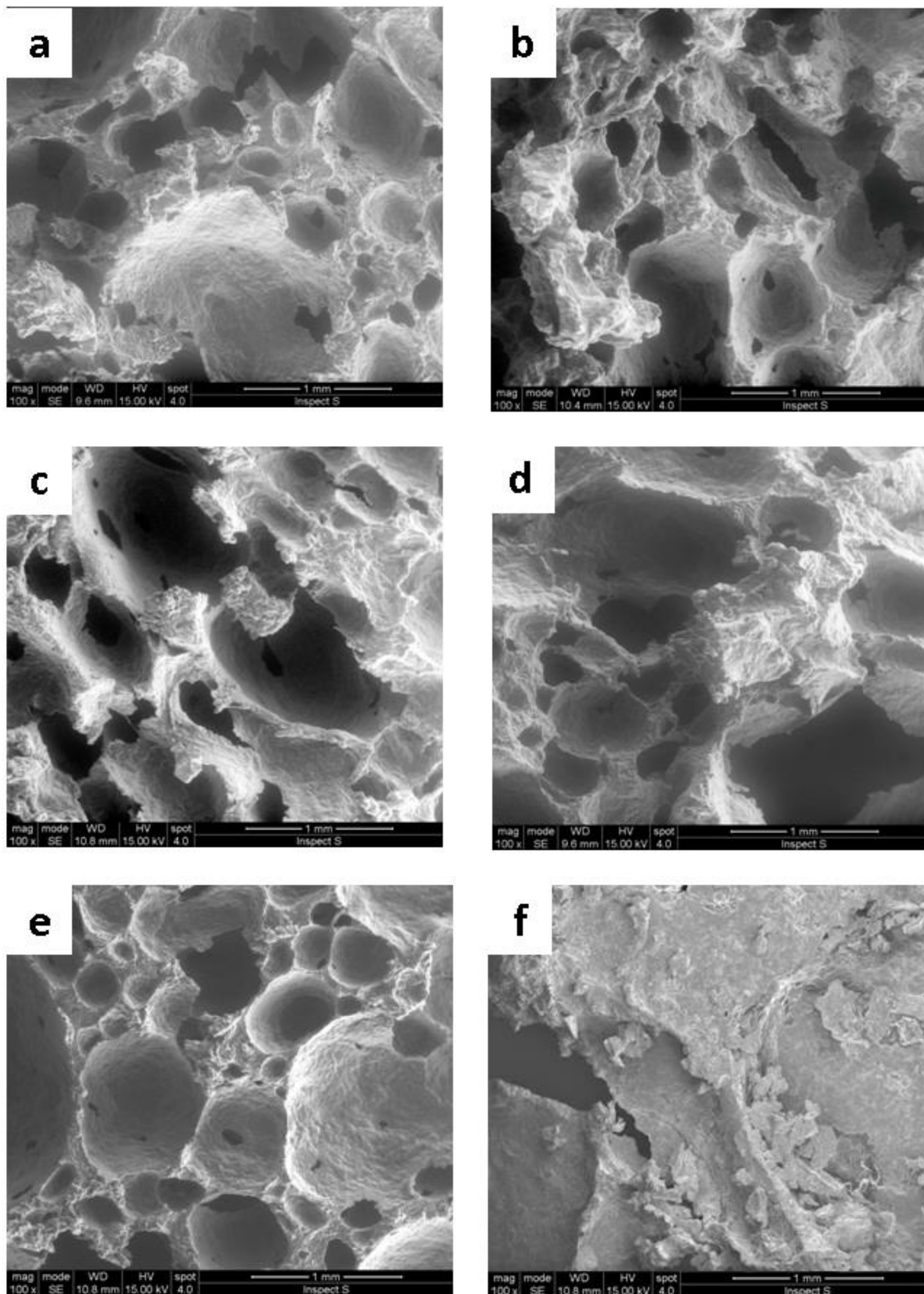



**Figure 2**. Environmental scanning electron micrographs of crumb of five enzyme-supplemented durum wheat breads and control (without enzymes) at the magnification of 100× [NM 15 (a); 3010 (b); ML P15 (c); VxT (d); VMAC (e); control (f)]. Horizontal white bars correspond to the length of 1 mm.

# Chapter 7

## Physico-chemical properties and sensory profile of durum wheat Dittaino PDO (Protected Designation of Origin) bread and quality of re-milled semolina used for its production

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**Abstract**

To help future quality checks, we characterized the physico-chemical and sensory properties of Dittaino bread, a sourdough-based durum wheat bread recently awarded with Protected Designation of Origin mark, along with the quality features of re-milled semolina used for its production. Semolina was checked for Falling Number (533-644 s), protein content (12.0-12.3 g/100 g d.m.), gluten content (9.7-10.5 g/100 g d.m.), yellow index (18.0-21.0), water absorption (59.3-62.3 g/100 g), farinograph dough stability (171-327 s), softening index (46-66 B.U.), alveograph W ($193\times10^{-4}$-$223\times10^{-4}$ J) and P/L (2.2-2.7). Accordingly, bread crumb was yellow, moderately hard (16.4-27.1 N) and chewy (88.2-109.2 N×mm), with low specific volume (2.28-3.03 mL/g). Bread aroma profile showed ethanol and acetic acid, followed by hexanol, 3-methyl-1-butanol, 2-phenylethanol, 3-methylbutanal, hexanal, benzaldehyde, and furfural. The sensory features were dominated by a thick brown crust, with marked toasted odor, coupled to yellow and consistent crumb, with coarse grain and well-perceivable sour taste and odor.

**Keywords:** durum wheat bread, re-milled semolina, quality control, sensory profile, texture, volatile compounds

## 7.1 Introduction

In many areas of the world, while taking into account the developments of new production methods and materials, farmers and food producers have tried to keep traditions alive, in terms of local artisanal processing methods. The cultural and gastronomic heritages are important factors contributing to the diversity of agricultural food productions and, besides the social aspects, a certain economic impact has been established. In fact, during the last decades, consumers have shown an increasing appreciation of traditional and typical foods, thus inducing the European Union to regulate this subject. According to the EU Regulation no. 1151/2012, "traditional" is the claim used for foods that historically – i.e. for a period of at least 30 years, that allows transmission between generations – are part of the cultural heritage of people living in a specific geographical area (European Parliament and European Council, 2012). "Typical" is the attribute of food whose quality features strictly depend on the geographical area of production, due to the combined effect of soil and water physico-chemical characteristics, climate, microflora, and local processing techniques (D'Amico, 2004). In particular, the "protected designation of origin" (PDO) identifies a product originated and totally produced in a specific geographical area, whereas to obtain the "protected geographical indication" (PGI) mark, less stringent than PDO, is sufficient that at least one of the production steps takes place in the defined geographical area (European Parliament and European Council, 2012). At European level, few breads have been awarded by PDO recognition: the Italian breads "Pane di Altamura" (Altamura bread), "Pagnotta del Dittaino" (Dittaino bread), and "Pane Toscano" (Tuscany bread), and the Swedish bread "Upplandskubb" (European Commission, 2016a), registered by the European Regulations nos. 1291/2003, 516/2009, 303/2016, and 843/2014, respectively (European Commission, 2003; 2009; 2014a; 2016b). Among them, Altamura PDO bread and Dittaino PDO bread, although being produced using different cultivars and in different areas, are both obtained from durum wheat re-milled semolina, according to a bread-making tradition consolidated in Southern Italy (Pasqualone, 2012). Altamura PDO bread has been extensively studied (Bianchi, Careri, Chiavaro, Musci, & Vittadini, 2008; Brescia et al., 2007; Chiavaro, Vittadini, Musci, Bianchi, & Curti, 2008; Raffo et al., 2003; Pasqualone, Summo, Bilancia, & Caponio, 2007; Pasqualone, Alba, Mangini, Blanco, & Montemurro, 2010). On the contrary, no research has been aimed until now to the quality characterization of Dittaino PDO bread, apart the inclusion of its sourdough in an array of samples for a survey on microbiotas used for traditional/typical Italian breads (Minervini et al., 2012). Starting from durum wheat cultivation, all

processing steps of Dittaino PDO bread take place within the area closely surrounding the Sicilian town of Enna (Italy), along the Dittaino river. Bread production follows a very simple and genuine recipe exclusively based on re-milled semolina, water, sourdough, and sea salt, without the addition of sugar, malt or malt extract, fats, anti-staling ingredients or any other additive. More specifically, durum wheat cultivars Simeto, Duilio, Arcangelo, Mongibello, Ciccio, Colosseo, Bronte, Iride, and Sant'Agata, grown in the Dittaino area, have to be used, alone or in combination, for at least 70% of the total semolina. The fermentation of dough is based on the dynamic equilibrium between yeasts and lactic bacteria of traditional sourdoughs (Type I) (De Vuyst & Neysens, 2005), with *Lactobacillus sanfranciscensis* (*Lactobacillus brevis* ssp. *lindneri*), *Candida milleri* and *Saccharomyces exiguus* as principal microbial species (Minervini et al., 2012; European Commission, 2014b). Dittaino PDO bread is finally baked at 230 °C for 60 min, traditionally as a round loaf of hearth bread weighing between 500 g and 1.100 g, characterized by a well-developed dark brown, highly consistent crust, and by pale yellow, uniformly porous crumb. The official technical sheet of Dittaino PDO bread reports the main physico-chemical characteristics of starting durum wheat, semolina and bread (European Commission, 2014b). In particular, re-milled semolina must have protein content ≥ 10.5% (d.m.), ashes = 0.70-0.90% (d.m.) and Falling number = 480-800 s. Bread loaves must have a 3-4 mm thick crust and a moisture content ≤ 38%. However, a more detailed quality characterization of the end-product and its raw material could improve technical awareness by producers, overcoming empirical knowledge, and could even enhance quality and consumer appreciation. In this framework, the aim of this research was to characterize the physico-chemical properties and sensory profile of Dittaino PDO bread, along with the quality features of re-milled semolina used for its production.

## 7.2 Materials and methods

### *7.2.1. Sample collection*

Samples of durum wheat Dittaino PDO bread, along with the starting re-milled semolina certified for PDO bread production, were collected in five samplings (coded A-E) that were carried out, within the period of two months, in local bakeries of the Dittaino area (Enna, Sicily, Italy). At each sampling, three bread loaves and two re-milled semolina samples were collected. Breads were produced according to the official procedure of Dittaino PDO bread (European Commission, 2014b),

that requires the use of natural sourdough (Type I) (De Vuyst & Neysens, 2005) derived from a daily renewed starter. The renewal procedure involves mixing sourdough starter, re-milled semolina, and water at 1:4:2 ratio (2.5 kg sourdough starter, 10 kg re-milled semolina, 5 L water), and resting for 12-14 h at approximately 15 °C, so as to double the volume. The final dough contained durum wheat re-milled semolina (100 kg), water (62.5 L), renewed sourdough (about 18 kg), and NaCl (2 kg). After 10-12 min mixing by means of diving arm mixers, the dough was rested in bulk for 1 h at room temperature, then was scaled into portions weighting about 1100 g (to take into account the weight loss due to water evaporation during baking). The portions were then shaped as round loaves and proofed for 2.5 h at 32-34 °C. Baking was carried out at 240 °C for 60 min in gas fueled ovens.

*7.2.2. Physico-chemical analyses of re-milled semolina*

Protein content was determined by means of Infratec 1241 Grain Analyzer 148 (Foss Tecator, Höganäs, Sweden), based on Near Infrared Transmittance. A calibration curve (range 8.3%-15.3%) was previously set up on the results of Kjeldahl nitrogen method and validated according to ISO 12099:2010 method (ISO, 2010) on a large set of samples. Ash and moisture content were determined according to the AACC 44–19 and AACC 08–01 methods (AACC, 2000), respectively. Dry gluten was determined by using a Glutomatic System consisting of Glutomatic 2200, Centrifuge 2015, Glutork 2020 (Perten Instruments AB, Huddinge, Sweden), according to the UNI 10690 method (UNI, 1979). The $\alpha$-amylase activity was determined by using the Falling Number 1500 apparatus (Perten Instruments AB, Huddinge, Sweden), according to the ISO 3093:2009 method (ISO, 2009). The color parameters in the color space $L^*$, $a^*$, $b^*$ were determined by Chromameter CR-300 (Minolta, Osaka, Japan), under the illuminant D65. Brown index was calculated as $100 - L^*$. The farinograph indices were determined according to the AACC 54–21 method (AACC, 2000) by a farinograph (Brabender instrument, Duisburg, Germany), equipped with the software Farinograph® (Brabender instrument, Duisburg, Germany). Water absorption needed to achieve the dough consistency of 500 ± 20 Brabender Units (B.U.) (A), dough development time (B), dough stability (CD), and consistency drop off after 12 min (E12) were measured. Alveograph trials were performed according to the AACC method 54–30A (AACC, 2000) using an alveoconsistograph, equipped with the software Alveolink NG (Tripette et Renaud, Villeneuve-la-Garenne, France). Damaged starch was determined enzymatically-spectrophotometrically according to AACC 76–31.01 method (AACC, 2000) using the Megazyme starch damage assay kit (Megazyme, Bray, Ireland), and was expressed

as percentage of flour weight on fresh weight (f.w.) basis. The particle size distribution was analyzed by a LabSifter (KBF7SN, Buhler, Switzerland). Re-milled semolina (100 g) was sifted for 5 min on sieves with opening of 300, 200, 180, and 160 μm. All the analyses were carried out in triplicate.

*7.2.3. Physico-chemical analyses of bread*

Moisture content of bread crumb was determined by oven drying at 105 °C until constant weight. Water activity ($a_w$) was determined by Hygropalm 40 AW (Rotronic Instruments Ltd, Crawley, UK) according to manufacturers' instructions. For these determinations, three bread slices (11±1 mm thickness) were used, and one square crumb sample (40 mm × 40 mm) was taken from the center of each slice. The Texture Profile Analysis (TPA) of bread was carried out by means of an Universal Testing machine (model 3344, Instron, Norwood, MA, USA), equipped with 5.0 cm diameter cylindrical probe, 2000 N load cell, and Bluehill® 2 software (Instron, Norwood, MA, USA), in the conditions reported in Giannone et al. (2016). Specific volume was determined by rapeseed displacement, as in AACC method 10-10 (AACC, 2000). Color parameters of crumb and crust in the color space $L^*$, $a^*$, $b^*$ were determined by Chromameter CR-300 (Minolta, Osaka, Japan), under the illuminant D65. Brown index was calculated as $100 - L^*$. Total carotenoid pigments were determined according to AACC approved method 14–50.01 (AACC, 2000) with slight modifications: bread crumb was lyophilized and ground in a mortar, then 1 g of each sample was extracted with 5 mL of water-saturated *n*-butyl alcohol on an orbital shaker for 3 h at 260 rpm. Samples were centrifuged for 7 min at 2400 × *g*, and the absorbance of water-saturated *n*-butyl alcohol extracts was measured at 435.8 nm by a Cary 60 UV-Vis spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA). Total carotenoid content was expressed as β-carotene, and calculations were made based on the extinction coefficient of 1.6632 for a solution of 1 mg β-carotene in 100 mL water-saturated *n*-butyl alcohol. All the analyses were carried out in triplicate.

*7.2.4. Sensory analysis of bread*

Quantitative Descriptive Analysis (QDA) of bread samples was performed by a sensory panel consisting of 8 members in the conditions described in a previous work (Pasqualone et al., 2007). The list of sensory terms included descriptors of appearance (crust color, crust thickness, crumb color, crumb grain), visual-tactile and chewing characteristics (crumb cohesiveness, crumb consistency), odor (semolina, sour, toast), and taste (sweet, salty, sour, bitter). The descriptors were

rated on an anchored line scale that provided a 0-9 score range (0 = minimum; 9 = maximum intensity). The definition of each descriptor and the scale anchors are reported in Table 1.

*7.2.5. Determination of volatile compounds of bread*

Volatile compounds of bread samples were determined by solid phase micro-extraction (SPME) coupled to gas-chromatography/mass spectrometry (GC/MS). Bread crust and crumb were cut into pieces of 2-3 mm and mixed in the crumb to crust ratio of 3:1, preliminarily determined in entire bread samples. Amounts of crumb and crust mixture of 400 ± 0.05 mg were then weighed in a 20-mL vial, and added of 4 mL of a 20% aqueous solution of NaCl (w/v). Vials were sealed by butyl rubber septa and aluminum crimp caps. Before volatile extraction, the sample was homogenized for 2 min using a laboratory vortex shaker. The extraction of volatile compounds was carried out by exposing a 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) in the headspace of the sample at 50 °C for 40 min. The fiber was then desorbed for 2 min in the injection port of the gas-chromatograph, operating in split-less mode. An Agilent 6850 gas-chromatograph equipped with an Agilent 5975 mass-spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) was used. The volatile compounds were separated on a HP-Innowax (Agilent Technologies Inc., Santa Clara, CA, USA) polar capillary column (60 m length × 0.25 mm i.d. × 0.25 μm film thickness), under the following conditions: injector temperature, 300 °C; flow of 2.0 mL/min. The oven temperature was held for 5 min at 35 °C, then increased by 5 °C/min to 50 °C and held in isothermal conditions for 5 min, then raised to 230 °C at 5.5 °C/min, and finally held constant at 230 °C for 5 min. The mass detector was set at the following conditions: interface temperature 230 °C; source temperature 230 °C; ionization energy 70 eV; scan range 33-260 amu. Peak identification was performed by computer matching with the reference mass spectra of National Institute of Standards and Technology (NIST) and Wiley libraries. Semi-quantitative data (peak areas expressed as total ion counts - TIC) were considered. The analysis was carried out in triplicate.

*7.2.6. Statistical analyses*

The statistical analyses were performed by using the CoStat Anova Statistic Software version 6.311 (Cohort, Monterey, CA, USA) for Windows. Data were submitted to analysis of variance (ANOVA) using Duncan's multiple range test.

## 7.3 Results and discussion

### *7.3.1. Re-milled semolina characteristics*

Several parameters are able to assess the bread-making quality of re-milled semolina. Fermentative aptitude (Falling Number), protein and gluten content, gluten strength (alveograph W), extensibility/tenacity balance (alveograph P/L ratio), water absorption capacity, mixing and kneading behavior (farinograph stability, dough development time, and softening index), and damaged starch content, are all useful parameters to predict the possibility to obtain voluminous breads with good yield. Therefore, re-milled semolina used in the production of Dittaino PDO bread was checked for the above mentioned parameters, as well as for color indices and particle size distribution (Table 2). The observed values of fermentative aptitude, expressed by Falling Number, although with significant differences among samplings, were always within the range 480-800 s required by the official sheet of Dittaino PDO bread (European Commission, 2014b). This range corresponds to a very limited amylase activity, usually more suitable for pasta than for bread-making. However, such limited values are needed because the use of sourdough causes a drop of pH which, in turn, increases the activity of enzymes, such as proteases and amylases (Arendt, Ryan, & Dal Bello, 2007). The lowest fermentative aptitude was found in sample set E. All re-milled semolina samples showed the majority of particles below 200 μm. D and E samples showed a significantly coarser particle size than the other samples, having higher amounts of particles > 300 μm and lower levels of < 160 μm particles. A reduction of the particle size increases starch damage, especially in hard materials as durum wheat. As a consequence, relevant amounts of damaged starch were observed, ranging from 14.9% to 20.2%. In agreement with coarser particle size, samples D and E showed significantly lower levels of damaged starch. Protein content was always above 10.5 g/100 g, as required by the official sheet (European Commission, 2014b). Similarly, ash content accomplished the basic legal requirements being in the range 0.70-0.90 g/100 g. Protein content has a positive effect on bread loaf volume (Goesaert et al., 2005). The observed protein levels were similar to those reported in a previous survey on the quality characteristics of durum wheat re-milled semolina from Southern Italy (11.0-12.9 g/100 g d.m.), the area where the majority of Italian durum wheat milling capability is concentrated (Pasqualone, Caponio, & Simeone, 2004). Gluten content was comprised between 9.7 g/100 g and 10.5 g/100 g, with slightly lower values than those observed in previous surveys (Pasqualone et al., 2004). Significant differences of gluten content were detected

among the collected semolina samples, related to significant differences observed also in protein levels. Mixing behavior was evaluated by farinograph. Dough development time, i.e. time needed to reach the maximum torque, was in the range 93-114 s, evidencing the absence of obstacles in gluten formation. Prolonged dough stability to mixing, and limited drop of consistency (softening index), both essential for sourdough propagation, were observed in particular in sample set A, which showed better values than those reported for starting re-milled semolina used in the production of another sourdough-based traditional bread, namely Altamura PDO bread (Raffo et al., 2003). The capacity to absorb water, determined by farinograph, depends on the content of protein and of damaged starch (Raffo et al., 2003), as well as on gluten strength, and is known to be positively related to bread yield. Water absorption was high in all the samples, but with significant differences among them and with the highest value in sample set A. An effect on farinograph data was exerted also by damaged starch, that is known to increase farinograph water absorption, and shorten farinograph development time (Sapirstein, David, Preston, & Dexter, 2007). Well-balanced visco-elastic properties and strong gluten are essential to allow optimal bread development. In soft wheat, the alveograph P/L ratio should range from 0.4 to 0.8. A tenacious gluten, instead, is expected in durum wheat re-milled semolina, and alveograph P/L values higher than 1.0 are tolerated. In particular, values up to 2.5 were observed in commercial re-milled semolina in previous investigations (Pasqualone et al., 2004). In re-milled semolina destined to the production of Dittaino PDO bread were ascertained P/L values from 2.2 to 2.7, indicating a highly tenacious dough for all the samples, expected to increase in volume very slightly during leavening. P/L, in fact, is known to be negatively correlated with bread specific volume (Pasqualone et al., 2004). On the other hand, the alveograph index W was comprised between $193 \times 10^{-4}$ J and $223 \times 10^{-4}$ J, indicating the presence of sufficiently strong gluten, able to bear the prolonged leavening times required by sourdough-based bread-making. However, the differences observed in dough rheological properties (mainly P/L) were measured at constant water absorption (as alveograph protocol requires a constant hydration), so that were influenced also by damaged starch content (Hatcher, Anderson, Desjardins, Edwards, & Dexter, 2002). Another quality trait of durum wheat re-milled semolina is the amber-yellow color, partly transferred to bread and due to carotenoid pigments. Yellowish crumb is highly appreciated by the consumers of durum wheat breads, therefore yellow index of starting re-milled semolina should be high. Rather low values of yellow index were observed in sample sets C, D and E, whereas a remarkably high value was found in sample set A. In any case, the negligible contribution of red

index, coupled to values of brown index considerably lower than in whole-meal semolina (Pasqualone et al., 2015), allowed to perceive a brilliant and luminous color in all the samples. Overall, the quality characteristics of semolina samples were variable, but remained within the ranges required by the official sheet of Dittaino PDO bread (European Commission, 2014b). However it has to be pointed out that some parameters, such as the alveograph and farinograph indices, as well as the yellow index, although being strongly related to bread quality, are not included in the official list of pre-requisites for the production of this kind of bread. Their future inclusion would be very useful to enhance quality and keep it more constant. In this perspective, the "A" semolina could be assumed as a superior quality reference, having the highest values of yellow index, protein and gluten content, and optimal values of alveograph and farinograph indices.

### *7.3.2. Dittaino PDO bread characteristics*

Crumb moisture and $a_w$ (Table 3) were within the typical range for 1-kg hearth bread loaves (Pasqualone et al., 2007; Raffo et al., 2003; Licciardello et al., 2017), but with significantly higher values in A and B than in the other samples. According to the official procedure of Dittaino PDO bread (European Commission, 2014b) the amount of water added to semolina has to be 62.5 L, therefore the difference in crumb moisture and $a_w$ was attributable to different water absorption capacity of semolina samples (with the highest value in sample set A) and to slight variations in the thermal effects of baking. It would be useful to specify in the official procedure that water should be added on the basis of farinograph-determined absorption, instead of indicating a fixed water amount. Crumb color was nearly yellow and reflected semolina color, with sample set A showing the highest yellow index. Yellow color is typical, with varying intensity, of all durum wheat breads, such as "Pane di Altamura" (Pasqualone et al., 2007; Brescia et al., 2007), "Pane di Laterza", and "Pane di Matera" (Brescia et al., 2007). Carotenoid pigments were detected in levels between 2.30 and 3.65 mg/kg, in the range observed in other durum wheat breads (Pasqualone et al., 2004). Crust color was dark brown, with some red reflexes, due to the prolonged baking process needed to allow heat to reach the inner part of big-sized loaves. The sample sets A, B and C had a darker crust than the other samples. In fact, the combined presence of damaged starch and amylase determines an increase of reducing sugars and a more reactive system towards Maillard reaction. The specific volume of Dittaino bread was rather low (2.28-3.03 mL/g), as expected, due to the combined effect of sourdough (Martínez-Anaya, Pitarch, Bayarri, & Benedito de Barber, 1990) and tenacious gluten.

Bread sample set E showed the lowest value of specific volume, probably due to further negative effect of low fermentative aptitude of semolina. Textural data, obtained by means of double cycle compressions at 40% depth, evidenced moderately high values of hardness and chewiness, with significant differences among samplings. In particular, hardness ranged from 16.4 N to 27.1 N, and chewiness from 88.2 N × mm to 109.2 N × mm. The differences were in accordance with specific volume: bread sample sets A and C, that were the softest and less chewy, also had the highest specific volume. The observed values of resilience and springiness indicated a good ability of all bread samples to regain the original position after compression, again with significantly better values in sample sets A and C. Resilience and springiness are known to decrease with storage time, with a tendency of bread to become crumblier and to lose its cohesive structure. The volatile compounds of Dittaino PDO bread included alcohols, aldehydes, ketones, carboxylic acids, furan compounds, pyrazines, and sulfur compounds (Table 4). In fact, bread aroma results from the complex combination of many volatile compounds derived from semolina and originated or modified during leavening and baking steps. Ethanol and acetic acid, derived from fermentation reactions, were by far the most abundant volatiles. The latter, in particular, together with ethyl acetate, was typical of sourdough-based leavening (Rehman, Paterson, & Piggott, 2006). Among alcohols, also hexanol, 3-methyl-1-butanol (isoamyl alcohol) and 2-phenylethanol were quantitatively relevant. Ruiz, Quilez, Mestres, & Guasch (2003) observed the same alcohols in baguette and ciabatta crumb. Isoamyl alcohol was found in important amounts by Chang, Seitz, & Chambers IV (1995) in white pan and whole wheat breads. Short-chain alcohols and fatty acids derive from sugar fermentation, whereas higher molecular weight alcohols arise from aminoacid metabolism (Rehman et al., 2006). In particular, 2-phenylethanol derives from phenylalanine. Carbonyl compounds such as 3-methylbutanal, hexanal, benzaldehyde and furfural, important components of bread volatiles (Chang et al., 1995; Ruiz et al., 2003), were found in high amounts in Dittaino PDO bread. Hexanal (as well as nonanal) takes its origin in lipid oxidation (Frankel, 1983). 3-Methylbutanal is a Strecker's aldehyde arising from leucine, and is responsible for a malty note (Pozo-Bayón, Guichard, & Cayot, 2006). Benzaldehyde can derive from metabolic or thermal degradation of phenylalanine (Pripis-Nicolau, De Revel, Bertrand, & Maujean, 2000). Pyrazines and furan compounds raised from thermal reactions such as Maillard reaction and caramelization (Martínez-Anaya, 1996). These reactions are more intense at loaf surface, generating the crust and its typical odor notes. In particular, were detected: methylpyrazine (associated to popcorn odor), 2-ethyl-3-methylpyrazine (toasted, nutty,

crust-like), 2,6-dimethylpyrazine (nutty), ethylpyrazine (musty, nutty), furfural (brown), and 2-furanmethanol (burnt) (Chang et al., 1995). Overall, pyrazines were more abundant in sample sets A, B and C than in D and E, indicating a more intense thermal effect during baking, that agreed with colorimetric data of crust and with analytical data of starting semolina, in terms of amylase activity and damaged starch content. Pyrazines, however, were less abundant than furan and furan-derivatives. In fact, at pH lower than 7 (such as in sourdough breads) the formation of furan compounds is favorite over pyrazines (Jousse, Jongen, Agterof, Russell, & Braat, 2002). Moreover, also furan compounds were more abundant in sample sets A-C than in D and E. Among sulfur compounds only dimethyl disulfide was detected, in very low amounts. It derives from methionine via the decomposition of its Strecker aldehyde, namely methional. The sensory profile of Dittaino PDO bread, presented in Table 5, largely agreed with instrumental data. Sensory properties are a key factor in food marketing. For some food products, such as extra virgin olive oil, sensory descriptors are even included in the list of legal parameters to be checked for quality categorization. Although bread sensory properties are not ruled by current laws, their evaluation is very useful to discriminate among bread types and quality levels. Durum wheat breads, in fact, have peculiar sensory features, different from those of common wheat breads (Pasqualone, 2012). Crust was always scored as brown and very thick, with a moderately crispy consistency, as expected in 1-kg bread loaves submitted to prolonged baking. This result accomplished the requirements of the official technical sheet of Dittaino PDO bread (European Commission, 2014b). Sample sets A, B and C showed significantly darker and thicker crust than D and E, as already indicated by colorimetric data. Also crumb color, perceived as yellow (score range 5.0-6.7), agreed with the colorimeter determination and carotenoid content of bread crumb. Crumb was highly consistent (score range 4.6-5.7) and cohesive (score range 4.6-5.9), in agreement with instrumental evaluations of hardness and resilience, respectively. Crumb grain was rather coarse (score range 4.7-5.8) due to the process of leavening based on sourdough, which causes a slower and more gradual production of $CO_2$ allowing small alveoli to merge into larger ones. Among the taste attributes, the most markedly perceived was sour taste, scored from 2.2 to 3.9 and largely overcoming sweet, salty and bitter taste. Sour taste was due to the sourdough-based leavening procedure, that also influenced bread odor. Sour taste and sour odor (the latter scored from 2.7 to 5.3) were perceived with stronger intensity in sample sets D and E, according to the determination of volatile compounds (acetic acid, in particular). Also toasted odor was markedly perceived (score range 3.1-4.7), with significantly

higher values in sample sets A, B and C, reflecting the level of volatiles of thermal origin (furans and pyrazines). Overall, the physico-chemical and sensory properties of bread were related to quality features of re-milled semolina. In particular, bread volume was directly related to protein content ($R = 0.6077$, $p < 0.05$), gluten content ($R = 0.5892$, $p < 0.05$), and gluten strength ($R = 0.9032$, $p < 0.001$), whereas was inversely correlated to P/L ($R = -0.7251$, $p < 0.001$). Increases in damaged starch content and amylase activity positively influenced brown crust formation and increased the content of aromatic volatiles, such as furans and pyrazines. In detail, brown index of crust was significantly correlated with damaged starch ($R = 0.8607$, $p < 0.001$) and with amylase activity ($R = 0.7183$, $p < 0.001$). The sum of furans was significantly correlated with damaged starch and with amylase activity ($R = 0.8288$, $p < 0.001$; $R = 0.6300$, $p < 0.001$, respectively). The sum of pyrazines was significantly correlated with damaged starch ($R = 0.8628$, $p < 0.001$), although the correlation with amylase activity was below significance ($R = 0.4350$). As a consequence, the observed differences among breads were largely imputable to differences in the physico-chemical features of the re-milled semolina used.

### 7.4 Conclusions

The obtained results allowed to characterize in detail the quality level of Dittaino PDO bread and its starting semolina. In particular, these data also allowed to define the width of the quality variations, related to raw material variability and to the intrinsic nature of an artisanal productive process. It is a matter of fact that a certain niche market for artisanal agri-food products, obtained according to traditional recipes and processing technologies, with features of high quality and genuineness, has been established. However, an effort in keeping quality as much constant as possible has to be made, even in an artisanal process. With this aim, some well-established commercial quality indices of re-milled semolina, such as farinograph and alveograph parameters and color indices, could be helpful in setting up a voluntary quality standard for the producers of Dittaino PDO bread, without the need of modifying the basic official technical sheet approved at European level. These suggestions could enhance quality and further increase the appreciation of end-users. Finally, the results of the sensory evaluation of Dittaino PDO bread were in agreement with the instrumental physico-chemical analyses and allowed to point out the distinctive characteristics of this kind of bread. In particular, although a certain variability among samples was observed, the sensory profile was dominated by a thick brown crust with a marked toasted odor note, coupled to yellow and consistent

crumb with coarse grain and with well-perceivable sour taste and sour odor notes. These sensory features could be highlighted and communicated to consumers for further increasing product knowledge and appreciation.

### 7.5 References


AACC International (2000). *Approved methods of analysis* (10th Ed.). St Paul, MN: American Association of Cereal Chemists.

AOAC (2000). *Official methods of analysis of the Association of Official Analytical Chemists* (17th Ed.). Gaithersburg, MD: Association of Official Analytical Chemists.

Arendt, E. K., Ryan, L. A., & Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*, *24*, 165–174.

Bianchi, F., Careri, M., Chiavaro, E., Musci, M., & Vittadini, E. (2008). Gas chromatographic–mass spectrometric characterisation of the Italian protected designation of origin "Altamura" bread volatile profile. *Food Chemistry*, *110*, 787–793.

Brescia, M. A., Sacco, D., Sgaramella, A., Pasqualone, A., Simeone, R., Peri, G., & Sacco, A. (2007). Characterisation of different typical Italian breads by means of traditional, spectroscopic and image analyses. *Food Chemistry*, *104*, 429–438.

Chang, C. Y., Seitz, L. M., & Chambers IV, E. (1995). Volatile flavor components of breads made from hard red winter wheat and hard white winter wheat. *Cereal Chemistry*, *72*, 237-242.

Chiavaro, E., Vittadini, E., Musci, M., Bianchi, F., & Curti, E. (2008). Shelf-life stability of artisanally and industrially produced durum wheat sourdough bread ("Altamura bread"). *LWT-Food Science and Technology*, *41*, 58–70.

D'Amico, A. (2004). The enhancement of the typical products value: from commodity to experience. The case of Esperya.com. *British Food Journal*, *106*, 793–805.

De Vuyst, L., & Neysens, P. (2005). The sourdough microflora: biodiversity and metabolic interactions. *Trends in Food Science and Technology*, *16*, 43–56.

European Commission. (2003). EC Council Regulation no. 1291/2003 of 18 July, 2003 supplementing the Annex to Regulation (EC) No 2400/96 on the entry of certain names in the 'Register of protected designation of origin and protected geographical indications' provided for in Council Regulation (EEC) no. 2081/92 on the protection of geographical indications and designations of

origin for agricultural products and foodstuffs (Pane di Altamura). *Official Journal of the European Union*, *L181*, 12–19.

European Commission. (2009). EC Council Regulation no. 516/2009 of 17 June, 2009 entering a name in the register of protected designations of origin and protected geographical indications [Pagnotta del Dittaino (DOP)]. *Official Journal of the European Union*, *L155*, 7–8.

European Commission. (2014a). Commission Implementing Regulation (EU) no. 843/2014 of 23 July 2014 entering a name in the register of protected designations of origin and protected geographical indications - Upplandskubb (PDO). *Official Journal of the European Union*, *L232*, 1–1.

European Commission (2014b). Commission Implementing Regulation (EU) no. 613/2014 of 3 June 2014 approving a minor amendment to the specification for a name entered in the register of protected designations of origin and protected geographical indications [Pagnotta del Dittaino (PDO)]. *Official Journal of the European Union*, *L168*, 68–72.

European Commission. (2016a). Agriculture and Rural Development, Agriculture and food DOOR Portal. Website http://ec.europa.eu/agriculture/quality/door/list.html Accessed on May, 16th, 2016.

European Commission. (2016b). Commission Implementing Regulation (EU) no. 2016/303 of 1 March 2016 entering a name in the register of protected designations of origin and protected geographical indications - Pane Toscano (PDO). *Official Journal of the European Union*, *L58*, 24–27.

European Parliament and European Council. (2012). Regulation (EU) no. 1151/2012 of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs. *Official Journal of the European Union*, *L343*, 1–29.

Frankel, E. N. (1983). Volatile lipid oxidation products. *Progress in Lipid Research*, *22*, 1–33.

Jousse, F., Jongen, T., Agterof, W., Russell, S., & Braat, P. (2002). Simplified kinetic scheme of flavor formation by the Maillard reaction. *Journal of Food Science*, *67*, 2534–2542.

Giannone, V., Lauro, M. R., Spina, A., Pasqualone, A., Auditore, L., Puglisi, I., & Puglisi, G., (2016). A novel $\alpha$-amylase-lipase formulation as anti-staling agent in durum wheat bread. *LWT-Food Science and Technology*, *65*, 381–389.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K. & Delcour, J. A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science and Technology*, *16*, 12–30.

Hatcher, D. W., Anderson, M. J., Desjardins, R. G., Edwards, N. M., & Dexter, J. E. (2002). Effects of flour particle size and starch damage on processing and quality of white salted noodles. *Cereal Chemistry*, *79*, 64–71.

ISO (2009). *ISO 3093:2009. Wheat, rye and their flours, durum wheat and durum wheat semolina. Determination of the falling number according to Hagberg-Perten.* Geneva, Switzerland: ISO.

ISO (2010). *ISO 12099:2010. Animal feeding stuffs, cereals and milled cereal products. Guidelines for the application of near infrared spectrometry.* Geneva, Switzerland: ISO.

Licciardello, F., Giannone, V., Del Nobile, M. A., Muratore, G., Summo, C., Giarnetti, M., Caponio, F., Paradiso, V.M., & Pasqualone, A. (2017). Shelf life assessment of industrial durum wheat bread as a function of packaging system. *Food Chemistry*, *224*, 181–190.

Martínez-Anaya, M. A. (1996). Enzymes and bread flavor. *Journal of Agricultural and Food Chemistry*, *44*, 2469–2480.

Martínez-Anaya, M. A., Pitarch, B., Bayarri, P., & Benedito de Barber, C. (1990). Microflora of the sourdoughs of wheat flour bread x interactions between yeasts and lactic acid bacteria in wheat doughs and their effects on bread quality. *Cereal Chemistry*, *67*, 85–91.

Minervini, F., Di Cagno, R., Lattanzi, A., De Angelis, M., Antonielli, L., Cardinali, G., Cappelle, S., & Gobbetti, M. (2012). Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical Italian breads: interactions between ingredients and microbial species diversity. *Applied and Environmental Microbiology*, *78*, 1251–1264.

Pasqualone, A. (2012). Italian durum wheat breads. In M. T. Pedrosa Silva Clerici (Ed.), *Bread consumption and health* (pp. 57–79). Hauppage, NY: Nova Science Publisher Inc.

Pasqualone, A., Alba, V., Mangini, G., Blanco, A., & Montemurro, C. (2010). Durum wheat cultivar traceability in PDO Altamura bread by analysis of DNA microsatellites. *European Food Research and Technology*, *230*, 723–729.

Pasqualone, A., Caponio, F., & Simeone, R. (2004). Quality evaluation of re-milled durum wheat semolinas used for bread-making in Southern Italy. *European Food Research and Technology*, *219*, 630–634.

Pasqualone, A., Piarulli, L., Mangini, G., Gadaleta, A., Blanco, A., & Simeone, R. (2015). Quality characteristics of parental lines of wheat mapping populations. *Agricultural and Food Science*, *24*, 118–127.

Pasqualone, A., Summo, C., Bilancia, M. T., & Caponio, F. (2007). Variation of the sensory profile of durum wheat Altamura PDO (Protected Designation of Origin) bread during staling. *Journal of Food Science*, *72*, S191–S196.

Pozo-Bayón, M.A., Guichard, E., & Cayot, N. (2006). Flavor control in baked cereal products. *Food Reviews International*, *22*, 335-379.

Pripis-Nicolau, L., De Revel, G., Bertrand, A., & Maujean, A. (2000). Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. *Journal of Agricultural and Food Chemistry*, *48*, 3761–3766.

Raffo, A., Pasqualone, A., Sinesio, F., Paoletti, F., Quaglia, G., & Simeone, R. (2003). Influence of durum wheat cultivar on the sensory profile and staling rate of Altamura bread. *European Food Research and Technology*, *218*, 49–55.

Rehman, S., Paterson, A., & Piggott, J. R. (2006). Flavour in sourdough breads: a review. *Trends in Food Science & Technology*, *17*, 557–566.

Ruiz, J. A., Quilez, J., Mestres, M., & Guasch, J. (2003). Solid-phase microextraction method for headspace analysis of volatile compounds in bread crumb. *Cereal Chemistry*, *80*, 255–259.

Sapirstein, H. D., David, P., Preston, K. R., & Dexter, J. E. (2007). Durum wheat breadmaking quality: Effects of gluten strength, protein composition, semolina particle size and fermentation time. *Journal of Cereal Science*, *45*, 150–161.

UNI (1979). *UNI method no. 10690. Durum wheat and semolina. Determination of gluten quality. Gluten index method.* Milan, Italy: UNI.

UNI (1995). *UNI method no. 10453. Durum wheat and semolina. Determination of rheological properties using an alveograph.* Milan, Italy: UNI.

**Table 1.**
Descriptive terms used for sensory profiling Dittaino PDO bread samples.

| Descriptor | Definition | Scale anchors min (0) | max (9) |
|---|---|---|---|
| *Visual appearance* | | | |
| Crust color | Color tone and intensity of crust | Same color of crumb | Dark brown |
| Crust thickness | Crust depth | Very thin (1 mm) | Very thick (>5 mm) |
| Crumb color | Color tone and intensity of crumb | Whitish | Light yellow |
| Crumb grain | Cell structure of crumb grain | Thin and very homogeneous (1-2 mm pores) | Coarse and poorly homogeneous (the biggest pore > 30 mm) |
| *Visual-tactile and chewing characteristics* | | | |
| Crumb consistency | Consistency of crumb, evaluated by fingers and during chewing | Soft | Tough |
| Crumb cohesiveness | The way the crumb reacts when broken by fingers | Poorly cohesive, it crumbles | Very cohesive, it sticks |
| *Odor attributes* | | | |
| Semolina (evaluated in crumb) | Intensity of typical semolina odor | None | Strong |
| Sour (evaluated in crumb) | Intensity of the aromatics associated with sourdough fermentation | None | Strong |
| Toasted (evaluated in crust) | Intensity of the aromatics associated with toasted bread | None | Strong |
| *Taste attributes* | | | |
| Salty | Primary sensation produced by sodium chloride | None | Strong |
| Sweet | Primary sensation produced by sugars | None | Strong |
| Bitter | Primary sensation produced by caffeine | None | Strong |
| Sour | Primary sensation produced by acid substances | None | Strong |

**Table 2.**
Chemical and rheological characteristics of durum wheat re-milled semolina used in the production of Dittaino PDO bread. Two re-milled semolina samples were collected at each sampling.

| Parameter | Sampling | | | | |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** |
| Falling Number (s) | $562 \pm 3^{C}$ | $533 \pm 3^{D}$ | $586 \pm 4^{B}$ | $556 \pm 5^{C}$ | $644 \pm 6^{A}$ |
| Damaged starch (g/100 g fresh weight) | $20.2 \pm 4^{A}$ | $17.1 \pm 2^{B}$ | $19.6 \pm 2^{A}$ | $15.5 \pm 3^{C}$ | $14.9 \pm 3^{C}$ |
| Protein (g/100 g dry basis) | $12.3 \pm 0.1^{A}$ | $12.2 \pm 0.1^{AB}$ | $12.2 \pm 0.1^{AB}$ | $12.0 \pm 0.1^{B}$ | $12.1 \pm 0.1^{AB}$ |
| Dry gluten (g/100 g dry basis) | $10.5 \pm 0.1^{A}$ | $10.0 \pm 0.1^{B}$ | $10.0 \pm 0.1^{B}$ | $9.7 \pm 0.1^{C}$ | $9.9 \pm 0.1^{B}$ |
| Ash (g/100 g dry basis) | $0.88 \pm 0.01^{AB}$ | $0.87 \pm 0.01^{BC}$ | $0.89 \pm 0.01^{A}$ | $0.87 \pm 0.01^{BC}$ | $0.86 \pm 0.01^{C}$ |
| *Alveograph parameters* | | | | | |
| Tenacity/extensibility ratio | $2.4 \pm 0.1^{BC}$ | $2.7 \pm 0.1^{A}$ | $2.2 \pm 0.2^{C}$ | $2.6 \pm 0.1^{AB}$ | $2.7 \pm 0.1^{A}$ |
| Deformation energy $\times 10^{-4}$ (J) | $223 \pm 4^{A}$ | $208 \pm 5^{B}$ | $210 \pm 5^{B}$ | $212 \pm 4^{B}$ | $193 \pm 4^{C}$ |
| *Farinograph parameters* | | | | | |
| Water absorption at 500 B.U. (g/100 g) | $62.3 \pm 0.4^{A}$ | $60.9 \pm 0.7^{AB}$ | $60.1 \pm 0.2^{B}$ | $59.6 \pm 0.2^{C}$ | $59.7 \pm 0.2^{BC}$ |
| Dough development time (s) | $93 \pm 4^{C}$ | $108 \pm 1^{B}$ | $101 \pm 2^{C}$ | $114 \pm 1^{A}$ | $111 \pm 4^{AB}$ |
| Dough stability (s) | $327 \pm 14^{A}$ | $306 \pm 16^{A}$ | $197 \pm 14^{B}$ | $171 \pm 15^{B}$ | $180 \pm 14^{B}$ |
| Softening index (B.U.) | $46 \pm 1^{C}$ | $59 \pm 1^{B}$ | $59 \pm 1^{B}$ | $66 \pm 2^{A}$ | $61 \pm 2^{B}$ |
| *Color indices* | | | | | |
| Yellow index ($b^*$) | $21.0 \pm 0.1^{A}$ | $19.2 \pm 0.2^{B}$ | $18.4 \pm 0.2^{C}$ | $18.0 \pm 0.1^{C}$ | $18.1 \pm 0.1^{C}$ |
| Red index ($a^*$) | $-2.3 \pm 0.1^{C}$ | $-1.9 \pm 0.1^{B}$ | $-1.6 \pm 0.3^{AB}$ | $-1.5 \pm 0.1^{A}$ | $-1.4 \pm 0.1^{A}$ |
| Brown index ($100 - L^*$) | $10.7 \pm 0.1^{C}$ | $10.6 \pm 0.1^{C}$ | $11.0 \pm 0.2^{BC}$ | $11.2 \pm 0.1^{B}$ | $11.6 \pm 0.1^{A}$ |
| *Particle size distribution* | | | | | |
| > 300 µm (g/100 g) | $10 \pm 2^{BC}$ | $10 \pm 1^{BC}$ | $9 \pm 1^{C}$ | $13 \pm 2^{AB}$ | $15 \pm 2^{A}$ |
| 200-300 µm (g/100 g) | 27 ± 4 | 25 ± 4 | 25 ± 5 | 27 ± 5 | 27 ± 5 |
| 180-200 µm (g/100 g) | 22 ± 4 | 20 ± 3 | 21 ± 4 | 24 ± 4 | 24 ± 5 |
| 160-180 µm (g/100 g) | 21 ± 3 | 20 ± 3 | 22 ± 4 | 19 ± 3 | 18 ± 2 |
| < 160 µm (g/100 g) | $20 \pm 2^{AB}$ | $25 \pm 3^{A}$ | $23 \pm 3^{A}$ | $17 \pm 2^{B}$ | $16 \pm 2^{B}$ |

Different letters in the same row indicate significant differences at $p<0.05$.

B.U. = Brabender Units.

**Table 3.**
Main physicochemical characteristics of Dittaino PDO bread. Three bread loaves were collected at each sampling.

| Parameter | Sampling | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Crumb moisture (g/100 g) | 46.2± 0.4$^{A}$ | 45.6 ± 0.6$^{A}$ | 41.9 ± 0.2$^{BC}$ | 41.4 ± 0.4$^{C}$ | 42.5 ± 0.3$^{B}$ |
| Crumb $a_w$ | 0.964 ± 0.002$^{A}$ | 0.963 ± 0.003$^{A}$ | 0.929 ± 0.003$^{B}$ | 0.924 ± 0.003$^{B}$ | 0.930 ± 0.002$^{B}$ |
| Carotenoid pigments (mg/kg) | 3.65 ± 0.14$^{A}$ | 2.87 ± 0.19$^{B}$ | 2.36 ± 0.11$^{C}$ | 2.41 ± 0.15$^{C}$ | 2.30 ± 0.13$^{C}$ |
| Specific volume (mL/g) | 3.03 ± 0.12$^{A}$ | 2.67 ± 0.15$^{B}$ | 2.97 ± 0.17$^{AB}$ | 2.69± 0.16$^{B}$ | 2.28 ± 0.21$^{C}$ |
| *Crumb color indices* | | | | | |
| Yellow index (*b**) | 21.6 ± 0.5$^{A}$ | 20.2± 0.6$^{B}$ | 19.3 ± 0.5$^{B}$ | 19.9± 0.7$^{B}$ | 19.2 ± 0.4$^{B}$ |
| Red index (*a**) | -2.5 ± 0.1$^{A}$ | -3.1 ± 0.1$^{B}$ | -2.6 ± 0.1$^{A}$ | -3.0± 0.2$^{B}$ | -3.2 ± 0.2$^{B}$ |
| Brown index (100 - *L**) | 27.4 ± 0.5$^{B}$ | 25.6 ± 0.4$^{C}$ | 25.3 ± 0.9$^{C}$ | 24.2 ± 1.3$^{C}$ | 29.2 ± 1.0$^{A}$ |
| *Crust color indices* | | | | | |
| Yellow index (*b**) | 26.8 ± 0.2$^{A}$ | 26.0 ± 1.9$^{AB}$ | 28.5 ± 0.7$^{A}$ | 23.6 ± 0.9$^{B}$ | 26.9 ± 0.6$^{A}$ |
| Red index (*a**) | 10.1 ± 0.6$^{B}$ | 12.8 ± 0.8$^{A}$ | 13.1 ± 0.5$^{A}$ | 12.8 ± 0.8$^{A}$ | 9.2 ± 0.1$^{C}$ |
| Brown index (100 - *L**) | 52.8 ± 2.0$^{AB}$ | 55.6 ± 2.5$^{A}$ | 54.6 ± 2.1$^{A}$ | 49.7 ± 1.8$^{BC}$ | 47.1 ± 3.0$^{C}$ |
| *Textural parameters* | | | | | |
| Hardness (N) | 17.3 ± 0.5$^{C}$ | 27.1 ± 1.0$^{A}$ | 16.4 ± 0.4$^{C}$ | 23.7 ± 0.6$^{B}$ | 26.0 ± 0.1$^{A}$ |
| Springiness (mm) | 5.7 ± 0.2$^{A}$ | 4.5± 0.4$^{C}$ | 5.6 ± 0.1$^{A}$ | 5.3 ± 0.1$^{AB}$ | 4.6 ± 0.5$^{BC}$ |
| Resilience | 0.95 ± 0.01$^{A}$ | 0.89 ± 0.02$^{B}$ | 0.96 ± 0.01$^{A}$ | 0.87 ± 0.02$^{B}$ | 0.89 ± 0.02$^{B}$ |
| Chewiness (N × mm) | 93.7 ± 7.1$^{B}$ | 108.5 ± 4.8$^{A}$ | 88.2 ± 5.3$^{B}$ | 109.2 ± 5.7$^{A}$ | 106.4 ± 8.8$^{AB}$ |

Different letters in the same row indicate significant differences at $p<0.05$.

**Table 4.**
Volatile compounds (peak areas expressed as total ion chromatogram × $10^6$) of Dittaino PDO bread. Three bread loaves were collected at each sampling.

| | Sampling | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| *Alcohols* | | | | | |
| Ethanol | 245.76 ± 19.24$^A$ | 257.13 ± 11.06$^A$ | 196.07 ± 9.18$^B$ | 137.98 ± 27.61$^C$ | 146.42 ± 7.41$^C$ |
| Propanol | n.d. | 1.03 ± 0.13 | n.d. | n.d. | n.d. |
| 3-Methyl-1-butanol | 3.08 ± 1.54$^C$ | 3.17 ± 0.81$^C$ | 1.67 ± 0.75$^C$ | 106.13 ± 3.44$^B$ | 135.03 ± 3.95$^A$ |
| Pentanol | 0.95 ± 0.19$^C$ | 1.07 ± 0.11$^C$ | 0.79 ± 0.18$^C$ | 6.48 ± 0.27$^B$ | 8.86 ± 0.34$^A$ |
| Hexanol | 6.14 ± 0.82$^C$ | 7.75 ± 1.43$^C$ | 7.57 ± 1.25$^C$ | 37.28 ± 2.58$^B$ | 45.22 ± 3.21$^A$ |
| Heptanol | 1.32 ± 0.87$^B$ | n.d. | n.d. | 3.33 ± 0.30$^A$ | n.d. |
| 1-Octen-3-ol | n.d. | 1.08 ± 0.21$^C$ | n.d. | 2.19 ± 0.31$^A$ | 1.62 ± 0.05$^B$ |
| 2-Phenylethanol | 5.58 ± 0.03$^A$ | 2.72 ± 1.03$^C$ | 5.08 ± 1.25$^{AB}$ | 4.11 ± 1.03$^{BC}$ | 4.97 ± 0.09$^B$ |
| *Aldehydes* | | | | | |
| 2-Methylpropanal | 1.33 ± 0.05$^B$ | 1.27 ± 0.06$^B$ | 1.04 ± 0.02$^C$ | 1.25 ± 0.05$^B$ | 2.34 ± 0.22$^A$ |
| Butanal | 1.67 ± 0.16$^B$ | 2.08 ± 0.17$^A$ | 0.73 ± 0.33$^C$ | n.d. | n.d. |
| 2-Methylbutanal | 3.73 ± 0.15$^C$ | 4.03 ± 0.32$^B$ | 4.36 ± 0.21$^B$ | 4.11 ± 0.14$^B$ | 4.85 ± 0.04$^A$ |
| 3-Methylbutanal | 10.66 ± 0.10$^C$ | 10.88 ± 2.11$^{BC}$ | 11.76 ± 2.33$^{BC}$ | 18.68 ± 2.44$^B$ | 25.14 ± 1.40$^A$ |
| Pentanal | 1.01 ± 0.07$^C$ | 1.12 ± 0.15$^C$ | 0.76 ± 0.13$^C$ | 4.68 ± 0.44$^B$ | 6.32 ± 0.20$^A$ |
| Hexanal | 13.76 ± 1.95$^C$ | 18.09 ± 1.56$^B$ | 13.69 ± 3.67$^C$ | 49.32 ± 0.94$^A$ | 51.50 ± 1.74$^A$ |
| 2-Hexenal | 1.94 ± 0.87$^B$ | 1.33 ± 0.14$^B$ | 5.01 ± 2.45$^A$ | 1.27 + 0.08$^B$ | n.d. |
| Heptanal | 5.20 ± 1.12 | 4.86 ± 1.13 | 5.77 ± 1.63 | 5.02 ± 1.18 | 5.27 ± 1.61 |
| 2-Heptenal | 2.47 ± 0.40 | 2.85 ± 0.21 | 2.99 ± 0.58 | 2.64 ± 0.33 | 2.57 ± 0.31 |
| Octanal | 3.49 ± 0.48 | 3.69 ± 1.29 | 3.23 ± 0.23 | n.d. | n.d. |
| 2-Octenal | 2.37 ± 0.25$^A$ | 2.38 ± 0.39$^A$ | 2.35 ± 0.11$^A$ | 1.52 ± 0.18$^B$ | 1.38 ± 0.28$^B$ |
| Nonanal | 14.87 ± 3.37$^A$ | 7.70 ± 0.25$^B$ | 13.01 ± 2.42$^A$ | 3.78 ± 0.19$^C$ | 2.49 ± 0.18$^C$ |
| 2-Nonenal | 4.26 ± 0.22$^B$ | 3.67 ± 0.22$^C$ | 5.19 ± 0.17$^A$ | 3.04 ± 0.14$^D$ | 1.98 ± 0.05$^E$ |
| Benzaldehyde | 10.77 ± 0.32$^B$ | 10.00 ± 0.37$^B$ | 10.41 ± 0.87$^B$ | 12.19 ± 0.58$^A$ | 12.80 ± 0.75$^A$ |
| Phenylacetaldehyde | 1.08 ± 0.11$^B$ | 0.95 ± 0.08$^B$ | 2.52 ± 0.19$^A$ | 1.03 ± 0.10$^B$ | 0.86 ± 0.21$^B$ |
| *Ketones* | | | | | |
| 2-Butanone | 3.72 ± 0.19$^C$ | 3.21 ± 0.11$^D$ | 4.36 ± 0.17$^B$ | 5.22 ± 0.21$^A$ | 5.45 ± 0.61$^A$ |
| 2,3-Butanedione | 0.57 ± 0.05$^D$ | 1.64 ± 0.08$^B$ | 0.71 ± 0.10$^C$ | 2.98 ± 0.26$^A$ | 3.03 ± 0.25$^A$ |
| 2-Pentanone | n.d. | 0.55 ± 0.01 | n.d. | n.d. | n.d. |
| 2,3-Pentanedione | 2.17 ± 0.19$^B$ | 2.51 ± 0.30$^B$ | 2.63 ± 0.27$^B$ | 2.82 ± 0.24$^B$ | 3.23 ± 0.09$^A$ |
| 2-Octanone | 0.96 ± 0.11 | 0.93 ± 0.04 | 0.96 ± 0.10 | n.d. | n.d. |
| *Carboxylic acids* | | | | | |
| Acetic acid | 81.14 ± 4.34$^C$ | 90.77 ± 6.46$^C$ | 82.91 ± 3.81$^C$ | 101.29 ± 3.61$^B$ | 168.22 ± 0.66$^A$ |
| Propanoic acid | 17.71 ± 1.93$^A$ | 16.86 ± 5.32$^{AB}$ | 9.62 ± 1.36$^B$ | 8.54 ± 2.41$^B$ | 10.31 ± 2.37$^B$ |
| Butanoic acid | n.d. | n.d. | 1.35 ± 0.09 | n.d. | n.d. |
| Hexanoic acid | 4.96 ± 0.47$^A$ | 1.93 ± 0.16$^B$ | 1.94 ± 1.01$^B$ | 1.67 ± 0.80$^B$ | 1.43 ± 0.71$^B$ |
| Heptanoic acid | 0.81 ± 0.32 | n.d. | n.d. | 0.80 ± 0.20 | n.d. |
| Octanoic acid | n.d. | n.d. | 1.88 ± 0.82 | n.d. | n.d. |
| Nonanoic acid | 2.55 ± 0.47$^B$ | 3.89 ± 1.01$^A$ | n.d. | 2.41 ± 0.36$^B$ | 2.12 ± 0.27$^B$ |
| Decanoic acid | 3.28 ± 0.78$^{AB}$ | 3.76 ± 0.96$^{AB}$ | 4.27 ± 0.40$^A$ | 3.10 ± 0.17$^B$ | 4.12 ± 0.22$^A$ |
| *Esters* | | | | | |
| Ethyl acetate | 1.82 ± 0.12$^B$ | 2.06 ± 0.21$^B$ | 1.50 ± 0.24$^B$ | 10.96 ± 0.26$^A$ | 10.00 ± 2.06$^A$ |
| *Furan compounds* | | | | | |
| 2-Ethyl-4-hydroxy-5-methyl-3(2H)furanone | 27.93 ± 8.79$^A$ | 31.10 ± 5.98$^A$ | 13.25 ± 4.34$^B$ | 10.03 ± 2.12$^B$ | 14.21 ± 3.22$^B$ |
| 2-Furanmethanol | 11.94 ± 3.85$^{ABC}$ | 14.61 ± 5.51$^A$ | 15.91 ± 4.10$^A$ | 10.21 ± 0.14$^B$ | 8.21 ± 0.42$^C$ |
| 2-Furanmethanol acetate | 1.05 ± 0.04 | 0.90 ± 0.34 | 1.37 ± 0.83 | n.d. | n.d. |

| | | | | | |
|---|---|---|---|---|---|
| 2-Furanmethanol propionate | 1.12 ± 0.83$^{B}$ | 0.89 ± 0.65$^{B}$ | 2.73 ± 0.13$^{A}$ | 0.71 ± 0.11$^{B}$ | 0.56 ± 0.04$^{B}$ |
| 2-Furanylethanone | n.d. | n.d. | n.d. | 2.31 ± 0.11$^{A}$ | 1.69 ± 0.14$^{B}$ |
| 3-Methylfurfural | 1.87 ± 0.09 | 1.57 ± 0.67 | n.d. | n.d. | n.d. |
| 5-Pentylfuran | 4.86 ± 0.96$^{A}$ | 4.40 ± 1.01$^{A}$ | 5.03 ± 1.10$^{A}$ | 1.70 ± 0.14$^{B}$ | 2.69 ± 0.35$^{B}$ |
| Acetylfuran | 2.10 ± 0.44$^{B}$ | 2.28 ± 0.63$^{B}$ | 3.33 ± 0.97$^{A}$ | n.d. | n.d. |
| Furfural | 36.29 ± 1.74$^{B}$ | 54.32 ± 2.70$^{A}$ | 29.38 ± 2.16$^{C}$ | 15.83 ± 1.37$^{D}$ | 16.98 ± 1.16$^{D}$ |
| *Pyrazines* | | | | | |
| 2,3,5-Trimethylpyrazine | n.d. | 1.02 ± 0.16 | n.d. | n.d. | n.d. |
| 2,6-Dimethylpyrazine | n.d. | 0.57 ± 0.12$^{B}$ | 0.83 ± 0.01$^{A}$ | n.d. | n.d. |
| 2-Ethyl-3-methylpyrazine | n.d. | n.d. | 1.26 ± 0.05 | n.d. | n.d. |
| Ethylpyrazine | 2.39 ± 0.49$^{A}$ | 2.03 ± 0.38$^{A}$ | 2.59 ± 0.69$^{A}$ | 0.96 ± 0.21$^{B}$ | 0.73 ± 0.10$^{B}$ |
| Methylpyrazine | 1.93 ± 0.67$^{A}$ | 2.63 ± 1.10$^{A}$ | 2.76 ± 0.97$^{A}$ | 0.71 ± 0.09$^{B}$ | 0.65 ± 0.11$^{B}$ |
| Pyrazine | n.d. | 0.93 ± 0.06 | n.d. | n.d. | n.d. |
| *Sulfur compounds* | | | | | |
| Dimethyl disulfide | 0.74 ± 0.09$^{B}$ | 1.44 ± 0.44$^{A}$ | n.d. | n.d. | n.d. |

Different letters within the same row indicate significant differences at $p<0.05$; n.d. = not detected.

**Table 5.**
Sensory characteristics of Dittaino PDO bread. Three bread loaves were collected at each sampling.

| Descriptor intensity | Sampling | | | | |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** |
| *Visual appearance* | | | | | |
| Crust color | $5.9 \pm 0.6^{A}$ | $5.5 \pm 0.4^{AB}$ | $5.6 \pm 0.5^{AB}$ | $4.8 \pm 0.4^{AB}$ | $4.6 \pm 0.5^{B}$ |
| Crust thickness | $5.8 \pm 0.4^{A}$ | $5.4 \pm 0.6^{AB}$ | $5.7 \pm 0.4^{A}$ | $4.7 \pm 0.5^{B}$ | $4.8 \pm 0.3^{B}$ |
| Crumb color | $6.7 \pm 0.6^{A}$ | $6.0 \pm 1.3^{AB}$ | $5.5 \pm 0.4^{B}$ | $5.0 \pm 0.5^{B}$ | $5.3 \pm 0.5^{B}$ |
| Crumb grain | $5.8 \pm 0.6^{A}$ | $5.1 \pm 0.4^{AB}$ | $5.7 \pm 0.5^{A}$ | $5.0 \pm 0.2^{AB}$ | $4.7 \pm 0.4^{B}$ |
| *Visual-tactile and chewing characteristics* | | | | | |
| Crumb consistency | $4.6 \pm 0.3^{B}$ | $5.7 \pm 0.6^{A}$ | $4.6 \pm 0.3^{B}$ | $5.0 \pm 0.6^{AB}$ | $5.6 \pm 0.1^{A}$ |
| Crumb cohesiveness | $5.9 \pm 0.6^{A}$ | $5.3 \pm 0.7^{AB}$ | $5.9 \pm 0.5^{A}$ | $4.6 \pm 0.4^{B}$ | $5.0 \pm 0.4^{AB}$ |
| *Odor attributes* | | | | | |
| Semolina odor (crumb) | $3.2 \pm 0.6$ | $3.1 \pm 0.7$ | $2.8 \pm 0.8$ | $2.4 \pm 0.5$ | $2.2 \pm 0.6$ |
| Sour odor (crumb) | $2.7 \pm 0.9^{B}$ | $3.0 \pm 0.2^{B}$ | $3.1 \pm 0.7^{B}$ | $5.1 \pm 0.9^{A}$ | $5.3 \pm 1.1^{A}$ |
| Toasted odor (crust) | $4.7 \pm 0.9^{A}$ | $4.3 \pm 0.6^{A}$ | $4.4 \pm 0.7^{A}$ | $3.1 \pm 0.4^{B}$ | $3.3 \pm 0.3^{B}$ |
| *Taste attributes* | | | | | |
| Salty taste | $1.8 \pm 0.9$ | $2.0 \pm 0.7$ | $1.9 \pm 0.6$ | $2.2 \pm 0.5$ | $2.4 \pm 0.7$ |
| Sweet taste | $1.6 \pm 0.5$ | $1.2 \pm 0.2$ | $1.4 \pm 0.2$ | $0.8 \pm 0.1$ | $1.1 \pm 0.1$ |
| Bitter taste | $0.7 \pm 0.1$ | $0.7 \pm 0.4$ | $0.5 \pm 0.2$ | $0.7 \pm 0.1$ | $0.5 \pm 0.1$ |
| Sour taste | $2.2 \pm 0.3^{B}$ | $2.2 \pm 0.2^{B}$ | $2.3 \pm 0.5^{B}$ | $3.8 \pm 0.4^{A}$ | $3.9 \pm 0.7^{A}$ |

Different letters in the same row indicate significant differences at $p<0.05$.

## 8. General conclusions

The PhD work addressed the investigation of new strategies for improvements of baked goods. To achieve this main goal, two fields were followed: new technologies applied to the industrial processes and to the develop of new products.

The results of case study 1, showed that the **sanitizing treatments** in order to reduce the microbial contamination (spoilage yeasts) on the finished product were very efficient. In particular the effects of Hydrogen Peroxide and Silver solution 12% (HPS) treatment in the leavening cells and cooling chambers, in comparison with the conventional Ortho-Phenylphenol (OPP) fumigating treatment, showed a significant reduction on the incidence of chalk defects of the commercialized products. One-hundred percent of the isolated yeasts were identified as *S. fibuligera*, strain that growths in a wide range of water activity aw (0.922-0.940), moisture content values (33.40-35.39%) and environmental conditions (temperature between 11.5 to 28.5 °C and relative humidity between 70.00 to 80.17%). The company, after this experimentation, performs this treatment with weekly frequency as adequate preventive process strategies against chalk mold defects.

Case II has developed the possibility to achieve the shelf life of durum wheat **reducing the packaging wastes**. The monitoring of 100gg shelf life, among different packaging systems, showed that the film with high barrier features keep the best physico-chemical and sensorial properties and volatile compounds of sliced bread during 90 days of storage. Based on the whole data set, and results of the PCA analysis, an overall comparison of the three packaging systems point to a significant influence on bread characteristics in the initial phase of storage, when the conventional system TF1 showed the best performance, allowing only slight changes compared to the fresh product. However, storage times longer than 15 days, which correspond to the period when the majority of product is generally purchased, tended to smooth the differences induced by packaging. Both TF2 – thermoformed package with lower thickness – and FP -flowpack package-could be valid alternatives to TF1: while the former would not jeopardize the standard shelf life of 60 days, the latter could be adopted when the expected product turnover is within 30 days without compromising the standard commercial life of industrial bread and allowing to save packaging material. The adoption of TF2 or FP systems would carry a significant reduction of packaging consumption which, in turn, results in environmental and economic improvements.

Case III has ascertained the great potential of **almond processing by-products** as food ingredients, with the aim of improving the health value of cereal-based bakery products. The research included three phases: i) optimization of skin drying; ii) optimization of quali-quantitative determination of phenolic compounds, by comparing three extracting protocols; iii) assessment of the impact of by-products on the rheology of composite doughs with wheat flour. The least time-consuming drying mode (at 60 °C for 30 min) retained better odor notes, higher content of phenolics (814 μg/g d.m. by HPLC, with the most effective extracting method) and greater antioxidant activity than sun-drying. Blanching water showed 917 μg/mL phenolics. Dried almond skins altered alveograph and farinograph indices of dough at doses higher than 30 and 50 g/kg, respectively, whereas blanching water did not cause significant changes. Therefore, almond skins could be used in products tolerating weak gluten network, such as cookies in which formulation usually other ingredients are included, able to mitigate the negative effect of skins on dough rheology, whereas blanching water could be added to any bakery good. Blanched skins show the advantage of being, after proper drying, relatively stable to oxidation and easy to store and manage. Blanching water, instead, should be necessarily used fresh, soon after production, so that an ideal layout would see an almond-processing industry joined, on place, with a second plant exploiting the liquid by-product. The treatment A (almond skins dried at 60 °C for 30 min) could be added at the productive cycle catching the temperature of drying of the hulled almonds to produce semi-finished row materials rich in bio-compounds for food industry.

In addition, industrial tests were performed for the development of **functional foods** (Case IV and V). A low-salt bread with salt content according to the fixed limits of REG (EU) 1924/2006 on nutrition and health claims was developed. Saltwell (natural Na-K sea salt, Atacama, Chile), with 65% of NaCl, 30% KCl, 1.5% $SO_4$ and traces of other elements was used in order to obtain functional durum wheat bread with low in sodium (<0.12g/100g) and very low sodium claim (< 0.04/100 g). Results of physicochemical and textural attributes of bread showed significant differences in terms of loaf volume, height and weight and crust thickness. Significant differences in loaf base diameter were also observed. Textural attributes (hardness, resilience and chewiness) of 0.15% sea salt-supplemented bread were significantly higher compared to bread containing 1.70 % and 0.35% sea salt. 1.70% Na-K supplemented bread showed the greatest improvement in terms of increased softness and crumb moisture. 0.35% of sea salt supplementation allowed obtaining functional bread with low sodium content without affecting the overall of physicochemical and textural properties of

the product. Considering the increasing interest on durum wheat bread from consumers and the guidelines for a healthy diet, these results showed the possibility of producing durum wheat bread with low salt content.

An **amylase and lipase formulation for staling reduction** and the improvement of the shelf life was carried out in this PhD program. Chemical-physical, rheological parameters and the microstructure evaluations were realized out to monitor the effects of several enzymes on the finished product. The $\alpha$-amylase-lipase enzyme preparation showed synergistic interactions in preventing staling. In particular, bread added of these two enzymes in mixture was always softer and more chewable than either control or samples added of other enzymes. Moreover, $\alpha$-amylase-lipase exhibited the most marked effect in slowing down both hardening and chewiness changes during time. Starting from 7 days of storage, both water activity and moisture content of bread added of $\alpha$-amylase-lipase were higher than in control. Starting from 68 days the moisture content of $\alpha$-amylase-lipase-added bread became lower than that of other enzyme-added breads, and at the end of storage also water activity was significantly lower. Pore morphology of $\alpha$-amylase-lipase-added bread appeared markedly different from that of control bread. The evolution of textural properties, crumb moisture, and $a_w$ during bread storage confirmed that amylases are effective in slowing down bread staling also in durum wheat bread, and pointed out the significantly greater effect provided by the 3010 $\alpha$-amylase-lipase combination, that positively modified textural and crumb grain properties of bread. The experimental data also indicated the close connection between moisture content and textural properties, with special regard to crumb hardness, resilience, and chewiness. Although further investigations are needed to achieve a better biochemical characterization of this new anti-staling formulation, the obtained results have an immediate practical application, since all the trials have been directly carried out at industrial level and accordingly to recommended dosages of each formulation. Hence, the producers may take advantage of the increases in shelf-life to enhance the diffusion and marketing of durum wheat bread far from the areas of production.

The sixth case study performed the physico-chemical properties and sensory profile of **durum wheat Dittaino PDO** (Protected Designation of Origin) bread and quality of re-milled semolina used for its production. Semolina was checked for Falling Number (533-644 s), protein content (12.0-12.3 g/100 g d.m.), gluten content (9.7-10.5 g/100 g d.m.), yellow index (18.0-21.0), water absorption (59.3-62.3 g/100 g), farinograph dough stability (171-327 s), softening index (46-66 B.U.), alveograph W ($193\times10^{-4}$-$223\times10^{-4}$ J) and P/L (2.2-2.7). Accordingly, bread crumb was yellow, moderately hard (16.4-

27.1 N) and chewy (88.2-109.2 N×mm), with low specific volume (2.28-3.03 mL/g). Bread aroma profile showed ethanol and acetic acid, followed by hexanol, 3-methyl-1-butanol, 2-phenylethanol, 3-methylbutanal, hexanal, benzaldehyde, and furfural. The sensory features were dominated by a thick brown crust, with marked toasted odor, coupled to yellow and consistent crumb, with coarse grain and well-perceivable sour taste and odor. The obtained results allowed to characterize in detail the quality level of Dittaino PDO bread and its starting semolina. In particular, these data also allowed to define the width of the quality variations, related to raw material variability and to the intrinsic nature of an artisanal productive process. It is a matter of fact that a certain niche market for artisanal agri-food products, obtained according to traditional recipes and processing technologies, with features of high quality and genuineness, has been established. However, an effort in keeping quality as much constant as possible has to be made, even in an artisanal process. With this aim, some well-established commercial quality indices of re-milled semolina, such as farinograph and alveograph parameters and color indices, could be helpful in setting up a voluntary quality standard for the producers of Dittaino PDO bread, without the need of modifying the basic official technical sheet approved at European level. These suggestions could enhance quality and further increase the product knowledge and appreciation of end-users.

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## LIST OF PUBLICATIONS

The research papers (accepted) to reviewed national/international journals, and the works presented at national and international conferences as oral and poster presentations during the study period are listed below.

**2015:**

- **Virgilio Giannone**, Serena Muccilli, Aldo Todaro, Vincenzo Alfeo, Diego Planeta, Onofrio Corona and Alfio Spina (2015). *Effect of natural sea salt on physicochemical and textural properties of low sodium durum wheat bread*. ICC/AISTEC Conference Proceedings "Grains for feeding the world", EXPO 2015, Milan (Italy). 267-272, ISBN: 978-88-906680-4-3.
- Alfeo V., **Giannone V**., Planeta D., Corona O., Muccilli S., Spina A., Schimmenti E., Todaro A. (2015). *Use of microbial enzyme on brewing*. ICC/AISTEC Conference Proceedings "Grains for feeding the world", EXPO 2015, Milan (Italy). 203-206, ISBN: 978-88-906680-4-3.
- **Giannone V. (**2015**)** *Study and Development of Functional Food based on Almond (Amygdalus communis L.) By-Product from Sicilian Industry***.** In Proc.s of 20th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology**,** Perugia (Italy), 23-25 September, 2015, pp 66-67.
- M. R. Lauro, L. Crascì, **V. Giannone**, C. Carbone, T. Musumeci, F. Sansone, A. Panico, R. P. Aquino, G. Puglisi. (2015). *Formulative and stability studies of a Citrus extract to produce novel "healthy food"*. Proceedings XXIII National Meeting on Medicinal Chemistry (NMMC2015), 6-9 September, Salerno (Italy), 121.

**2016:**

- **Giannone V.**, Lauro M.R., Spina A., Pasqualone A., Auditore L., Puglisi I., Puglisi G. (2016) *A novel α-amylase-lipase formulation as anti-staling agent in durum wheat bread*. LWT - Food Science and Technology, Vol. 65, Pag. 381–389. ISSN: 0023-6438, doi: 10.1016/j.lwt.2015.08.020.

- E. Arena, S. Brighina, A. Mazzaglia, A. Spina, S. Muccilli, **V. Giannone**, B. Fallico (2016). *Use of a natural low Na+ salt in durum wheat bread*. Italian Journal of Food Science, 77-81. ISSN: 1120-1770.
- A. Spina, S. Muccilli, E. Arena, S. Brighina, B. Fallico, **V. Giannone**, P. Rapisarda (2016) *Durum wheat breads enriched with citrus fruits pectin and flavonoids*. Italian Journal of Food Science, 72-76. ISSN: 1120-1770.
- S. Brighina, E. Arena, A. Mazzaglia, Spina, S. Muccilli, **V. Giannone**, S. Fabroni, P. Rapisarda, B. Fallico (2016). *Results of industrial breadmaking trials of durum wheat wholegrain flour enriched with citrus fruits fibre*. Italian Journal of Food Science, 67-71. ISSN: 1120-1770.
- Alessandra Carrubba, Andrea Comparato, Andrea Labruzzo, Serena Muccilli, **Virgilio Giannone**, Alfio Spina (2016). *Quality Characteristics of Wholemeal Flour and Bread from Durum Wheat (Triticum turgidum L subsp. durum Desf.) after Field Treatment with Plant Water Extracts*. Journal of Food Chemistry, Vol. 81(9), Pag. C2158-2166. doi: 10.1111/1750-3841.13400.
- **Virgilio Giannone**, Iole Pitino, Biagio Pecorino, Aldo Todaro, Alfio Spina, Maria Rosaria Lauro, Filippo Tomaselli, Cristina Restuccia (2016). *Effects of innovative and conventional sanitizing treatments on the reduction of Saccharomycopsis fibuligera defects on industrial durum wheat bread*. International Journal of Food Microbiology 235, 71–76. doi.org/10.1016/j.ijfoodmicro.2016.07.009.
- Rosa Palmeri, Julieta I. Monteleone, Antonio C. Barbera, Carmelo Maucieri, Aldo Todaro, **Virgilio Giannone**, Giovanni Spagna (2016). *Production of Functional Crackers Enriched with Olive (Olea europaea L.) Leaf Extract*. Proceedings ICFPTS 2016: 18th International Conference on Food Processing Technology and Supplements, July 28-29, London (United Kingdom).

**2017:**

- Fabio Licciardello, **Virgilio Giannone**, Matteo Alessandro Del Nobile, Giuseppe Muratore, Carmine Summo, Mariagrazia Giarnetti, Francesco Caponio, Vito Michele Paradiso, Antonella Pasqualone (2017). *Shelf life*

*assessment of industrial durum wheat bread as a function of packaging system.* Food Chemistry 224 (2017) 181–190. doi.org/10.1016/j.foodchem.2016.12.080

- Alfio Spina, Carlo Amato, Sebastiano Blangiforti, **Virgilio Giannone**, Paolo Guarnaccia, Gianfranco Venora, Paolo Caruso, Umberto Anastasi (2017). *Popolazioni locali siciliane di frumento tenero (Triticum aestivum L.): caratteristiche morfo-fisiologiche, merceologiche e qualitative***.** Conference Proceedings 11° Convegno AISTEC, I cereali per un sistema agroalimentare di qualità, 22-24 November, Roma (Italy).
- Carlo Amato, Umberto Anastasi, Sebastiano Blangiforti, **Virgilio Giannone**, Paolo Guarnaccia, Gianfranco Venora, Paolo Caruso, Alfio Spina (2017). *Caratteristiche bioagronomiche e qualitative di popolazioni locali siciliane di frumento duro.* Conference Proceedings 11° Convegno AISTEC, I cereali per un sistema agroalimentare di qualità, 22-24 November, Roma (Italy).
- Maria Rosaria Lauro, Lucia Crascì, **Virgilio Giannone,** Gabriele Ballistreri, Simona Fabroni, Francesca Sansone, Paolo Rapisarda, Anna Maria Panico, Giovanni Puglisi (2017). A*n alginate/cyclodextrin spray drying matrix to improve shelf life and antioxidant efficiency of a blood orange by-product extract rich in polyphenols: mmps inhibition and antiglycation activity in dysmetabolic diseases.* Oxidative Medicine and Cellular Longevity, Volume 2017, Article ID 2867630, 12 pages, doi.org/10.1155/2017/2867630.

**2018:**

- **Virgilio Giannone**, Mariagrazia Giarnetti, Alfio Spina, Aldo Todaro, Biagio Pecorino, Carmine Summo, Francesco Caponio, Vito Michele Paradiso, Antonella Pasqualone (2017). *Physico-chemical properties and sensory profile of durum wheat Dittaino PDO (Protected Designation of Origin) bread and quality of re-milled semolina used for its production*. Food Chemistry 221 (2018) 242-249. doi.org/10.1016/j.foodchem.2017.08.096.
- Antonella Pasqualone, Barbara Laddomada, Alfio Spina, Aldo Todaro, Carlos Guzmàn, Carmine Summo, Giovanni Mita, **Virgilio Giannone** (2018). *Almond by-products: Extraction and characterization of phenolic compounds and evaluation of*

*their potential use in composite dough with wheat flour*. LWT - Food Science and Technology, 89, 299-306. doi.org/10.1016/j.lwt.2017.10.066.

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