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The ability of ovine whey powder to improve quality of Sardinian bakery products

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1 Introduction

1.1 Typical bakery products in Sardinia

We not know with precision where and when man has begun to produce and consume bakery foods, but we have news that the Egyptians and the Babylonians were able to produce leavened bread and that our civilization has co-evolved along with these products. Only in last century it was proved that yeast is the leavening agent in bread-making.

The evolution and the characteristics of bakery products are linked to the environment, and to the social and economic background of each country, which in turn affects the selection of the raw material used to make bread.

In the Mediterranean area different types of bakery products with different flavours and tastes are produced, and they are consumed daily or prepared with different ingredients depending on the period and particular events. In particular, in Sardinia hundreds of bakery goods are produced, for different periods of the year: the "*papassinos*", a rectangular or rhomboidal sweet cookie, which can contain almonds or hazelnuts are prepared to celebrate the *all Hallows Day*; a typical bread named "*su pane de sos isposos*" is produced for marriage; in the central part of Sardinia typical heart breads are preferred during the winter season (named *moddizzosu, zicchi, chivalzu* or *pane russu*), whereas in summer and spring a typical flat crispy bread (*bistoccu* or *carasau*) is consumed. For Easter, a typical cheese cake named "*pardulas*" or "*casadinas*" are produced, whose main component is ovine ricotta cheese or ovine cheese, depending on the economic situation of the family or of the village, since the ricotta cheese is cheaper than ovine cheese. In North Sardinia the "*spianata*" bread a typical double round layered flat bread is produced; in western centre of the region, this bread with some modification in the recipe, and thickness of leaves becomes "*zichi ladu*" bread, whereas in eastern centre of Sardinia the *spianata* bread is split in two leaves which are toasted at very high temperature to obtain the "*carasau*" bread.

Nowadays the economic situation is different with respect to the past, and also the bakery products have changed their characteristics to meet the requirements of modern market: fresh bread must be available in the market every morning, then it needs to be packed, and a long shelf life and standard shape and weight are required. The needs in quality of raw materials are also changed, since the increasing production of bakery goods needs greater amounts than the past, and, to reach a standard level in the quality of the product, also a standard level of quality in raw materials is required. Addictives that often affect taste and flavour of the typical bakery products are used, turning them away from their original characteristics. Fortunately, local people know and remember "old" tastes and flavours of typical bakery products, so in the last decade, consumers became reluctant to buy products that contains additives and that are made in far countries and have no-typical or unknown ingredients.

This consumer behaviour is not a return to the past, and not must be seen as a step back toward a world of backwardness, of marginalization and poverty, even if it is from this context that our typical products were originated; but study them now, with modern techniques and modern means, being aware of the importance they have on the sustainability of natural resources and on their productions, on the food health and biodiversity of species cultivated or bred, will result in the development of the excellence in food productions where the rural matrix corresponds to modernity, to wealth and well-being. The link between the past and this kind of innovation will represent the added value of our food productions.

This PhD thesis has the general objective to test how two baked local products (spianata and ricottine) could meet today's market needs, by suggesting solutions with a strong imprint from our territory. In this context we propose the use of ovine whey powder (which will be discussed further below) and the use of an old variety of durum wheat (Senatore Cappelli), which now lives an increase of its use in the bakery productions in Sardinia.

1.2 The raw material for bakery products

In Sardinia the main raw material in bread-making is low-grade semolina from durum wheat (*Triticum turgidum* subsp. *durum*). It is worldwide used to make dry pasta, and this is the reason why durum wheat breeding programs have focused on improving pasta cooking quality, through improving gluten strength indices. This has caused the progressive replacement of the old landraces and the ancient varieties with the new cultivars. Moreover, as the world market demand has increased, many countries (table 1) increased their production of durum wheat (Canada, Turkey, Mexico), which is now imported and used in Sardinia to make bread, although it has developed to make high quality pasta. At the same time the cultivation of old varieties of durum wheat has become not competitive, due to their low yield.

Table 1 – World Durum wheat production (International Grain Council, 2015)

Country	2014/15 production	2015/2016 estimation	Country	2014/15 production	2015/2016 estimation
World	32.6*	36.1	Argentina	0.3	0.3
UE	7.1	7.5	Syria	0.8	1.4
France	1.5	1.8	Turkey	2.1	2.4
Greece	0.8	0.7	India	1.3	1.2
Italy	**3.7**	**3.9**	Algeria	1.3	2.5
Spain	0.8	0.9	Libya	0.1	0.1
Kazakhstan	2.0	2.1	Morocco	1.4	2.3
Canada	5.2	4.8	Tunisia	1.3	1.3
Mexico	2.3	2.3	Australia	0.5	0.5
USA	1.4	2.1	Others	5.7	5.5

*Millions of tons

The use of semolina milled from commercial cultivars of durum wheat and well suited to modern bread-making plants allows obtaining bread more quickly and easily than in the past.

The characteristic required to semolina to be defined of high quality, is the ability to obtain a strong visco-elastic matrix during dough mixing, with good rheological properties, that is a high resistance to extension (dough elastic and not sticky) combined with a good extensibility that should guarantee a high volume of the loaf during the baking process. Several studies (Liu *et al*., 1996) pointed out that many durum wheat cultivars do not have good bread-making quality, since they show very strong gluten, and that it would be necessary to develop varieties with less elastic and more extensible gluten. However, the use of durum wheat flours in bread making has many other advantages, as an intense colour (yellowish), a characteristic taste and a fresh baked flavour, a longer shelf life that is appreciated by consumers. Indeed, as described from Troncone and Auricchio (1991) semolina based bread seems to contain a lower quantity of toxic epitopies than bread wheat, and could be therefore considered healthier for people who suffer of intolerance to wheat gluten (this topic is under intense research).

Semolina is composed mainly by starch and protein. Starch is the main component in durum wheat flour (60-75%). After hydration of semolina and during the mixing phase starch granules swell, due to absorption of water. The higher the amount of damaged starch, the higher is the swelling of the granules. Starch granules are composed of amylose and amylopectin, which affect the nutritional value of bread and its shelf life, as a consequence of a physical phenomenon known as retrogradation of starch (this topic will be discussed later on). The average protein content in semolina from durum wheat ranges from 10 to 15%. The two main important proteins are gliadin and glutenin, which during mixing with water form a complex network known as gluten, whose formation confers to the dough its visco-elastic properties, a well balanced equilibrium between elasticity and extensibility. The interaction between these two properties is essential to form a network able to trap the gases during the fermentation and during baking in the oven. Gluten accounts from 75% to 85% of total protein content in wheat flour (Shewry *et al.*, 2009).

The microscopic structure of the gluten is still not known in deep, but it seems that the glutenins form an elastic network by means of strong covalent disulfide

bonds, whereas the gliadins interact with such basic structure via non-covalent forces (*i.e.*, hydrogen bridges and hydrophobic interactions), thus conferring viscosity and extensibility to the dough. For this reasons the gliadins play an important role in obtaining a good dough volume during the fermentation and a good loaf volume during baking, whereas the glutenins affect the elastic behaviour of the dough, giving strength to the dough. Other authors explain that the glutenin fraction also has a central role in cooking quality of semolina-based pasta, whereas variation in gliadin amount does not affect physical differences in dough properties (Liu *et al*., 1996). Gluten quality plays also a significant role on crumb characteristics, such as texture and cell size distribution (Fois *et al.*, 2012).
The quality of gluten matrix is the first parameter able to distinguish semolinas milled from new and old varieties of durum wheat. Indeed, the old genotypes show the HMG-20 (High Molecular Weight) glutenin patterns, which are well recognized as a marker for gluten weakness. Moreover, previous studies demonstrated that the differences in quality characteristics between durum wheat varieties containing γ -gliadin 45 and γ -gliadin 42 are a consequence of a different glutenin pattern, mainly related to low molecular weight glutenins (LMW), since γ -gliadin 42 is marker for the LMW-1 pattern, which is, again, related to weak gluten characteristics, whereas γ -gliadin 45 is marker for LMW-2, which is related to strong gluten properties **(**Shewry *et al.*, 2009). Due to their protein pattern, ancient varieties unfortunately show low suitability to be employed in modern plants; on the other hand, new varieties sometimes show too strong and tenacious gluten, which does not fit with bread-making technology.

1.3 The role of water in the bakery products

Another important element in durum wheat is water, whose amount ranges widely from 8 to 18%, and changes with environmental, agronomic and climatic grow conditions. Water is an essential ingredient for bakery products since it is needed for gluten development. Water is added to semolina during mixing of ingredients or is part of the same ingredients that are added to semolina. During bread-making the added water is needed to develop the dough, while during cheese cakes or cookies production the water is present in ingredients as ricotta cheese, cheese,

eggs. During mixing, water plays the important role to regulate the consistency of the dough, although during baking in the oven it is partially removed. The distribution of water in the final product explains several phenomena that will affect the quality and the shelf life of the final product. Moreover, during storage, due to free water availability, the quality of bakery products is threatened by microbial spoilage and chemical-physical alterations, as moisture loss and water redistribution or migration, both related to high values of water activity (a_w). The a_w is definite by the ratio:

$$a_w = p/p_0$$

where p is the actual partial pressure of water vapour and p_0 is the maximum possible water vapour pressure of pure water (saturation pressure) at the same temperature (Scott, 1957). The values range between 0, that means total absence of free water, and 1 that is a_w value of the pure distilled water. Moreover there is a relationship between the equilibrium relative humidity (ERH) and a_w values, as shown below:

$$\text{ERH} = a_w \text{ X } 100$$

where a_w is a fraction of 1, the equilibrium relative humidity is a percentage (Scott, 1957). Water activity is one of the most important parameters in the study of bakery foods, because it plays a critical role in the staling phenomena, particularly, it drives the redistribution of water under gradient between the centre and the crust of the product, and also determines the quality loss and microbiological degradation of food (Figura and Teixeira, 2007). The consequences of staling include both hardening of the crumb and loss of the fresh-baked flavour. Figure 1 shows the kinetic of different spoilage reactions as a function of a_w and the minimum critical a_w values required by microorganisms to growth on a sample material.

The knowledge of the critical values of water activity, for each microbiological species, is useful to preserve healthiness of food goods. In the past, many authors studied the correlation between the microbial growth in food and the water activity values of food matrix, and the results are summarized in table 2, where it

is clearly evidenced that for each species exist minimal conditions for growth or for influencing the toxin-forming ability.

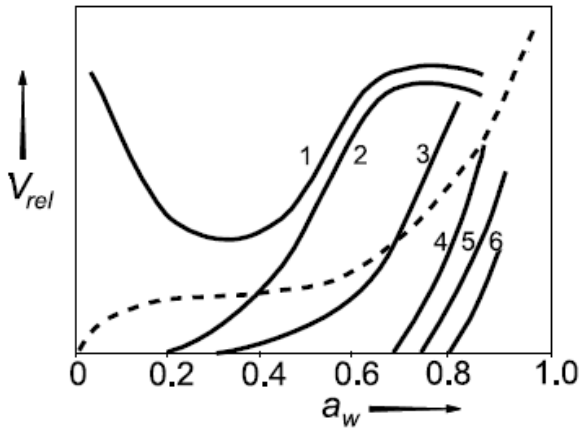

Figure 1 Relative rate (V_{rel}) of different spoilage reactions as a function of a_w in food. 1: lipid oxidation; 2: browning reactions; 3: enzymatic reactions; 4: moulds; 5: yeasts; 6: bacteria. The dashed line indicates the sorption isotherm of the sample material. (Figura and Teixeira, 2007)

This "hurdle effect" has a fundamental importance for food preservation, which can be improved to prevent food-related infections, food poisoning and deterioration due to microorganisms. It is very useful in monitoring fermentation process of food as dairy products, meat products (for example raw sausage and raw ham) or bread dough. There are different well known traditional food preservation methods (salting, sugaring, drying, freezing), which modify the concentration of the particles dissolved in the water, decreasing a_w values of the matrix (Rödel *et al.*, 1979). These methods delay or inhibit microbial growth with the aim to obtain a stabilizing or preserving effect on the food, although unfortunately they change textural and sensorial properties.

1.4 Techniques to preserve high quality in bakery products

Nowadays several non-invasive techniques are used in stabilization of bakery products (Smith *et al*., 2004), especially with food products that for their high values of a_w are very perishable, as cheese cakes or some kind of bread, such as spianata, which is part of the present study.

Table 2. Minimum a_w values for microorganisms growth associated with foods (Leister *et al*., 1981)

a_w	Bacteria	Yeasts	Moulds
0.98	*Clostridium, Pseudomonas*		
0.96	*Flavobacterium, Klebsiella, Shigella Lactobacillus, Proteus,Pseudomonas,*		
0.95	*Alcaligenes, Bacillus, Citrobacter, Clostridium, Enterobacter, Vibrio Escherichia,Propionibacterium, Proteus,Pseudomonas, Serratia, Salmonella,*		
0.94	*Bacillus, Clostridium, Lactobacillus, Micobacterium,Pediococcus, Vibrio, Streptococcus*		*Stachybotrys*
0.93	*Bacillus, Micrococcus, Lactobacillus, Streptococcus*		*Botrytis, Mucor, Rhizopus*
0.92		*Pichia, Rhodotorula, Saccharomyces*	
0.91	*Corynebacterium, Streptococcus*		
0.90	*Bacillus, Lactobacillus, Micrococcus, Staphyloccus, Vibrio*	*Hansenula, Saccharomyces*	
0.88		*Candida, Debaryomyces, Hanseniaspora*	*Cladosporium*
0.87		*Debaryomyces*	
0.86	*Micrococcus, Staphylococcus, Vibrio*		
0.84		*Paecilomyces*	*Alternaria, Aspergillus*
0.83	*Staphylococcus*	*Debaryomyces*	*Penicillium*
0.81		*Saccharomyces*	*Penicillium*
0.79			*Penicillium*
0.78			*Aspergillus, Emericella*
0.75	*Halobacterium, Halococcus*		*Wallemia*
0.70			*Aspergillus, Chrysosporium*
0.62		*Saccharomyces*	*Eurotium*
0.61			*Monascus*

The most employed technologies to extend the shelf life are modified atmosphere packaging (MAP) or active packaging (AP), product reformulation and the use of preservatives, alone or in combination. Nowadays MAP technology has become very popular and used by the bakery industries. MAP consists in substituting the ordinary atmosphere of the package with a mix of gases. Packaging materials (films or trays) must show gas and water vapour barrier characteristics to keep constant the concentrations of the mix of gases into the packaging and to prevent product dehydration. In bakery food MAP, in order to preserve the freshness and improve food safety, the O_2 concentration is reduced to 0% and CO_2 concentrations are kept at relatively high levels (20%), whereas N_2 is used as inert filler gas to prevent the packaging collapse. Bacteriostatic and fungistatic activities of high concentrations of CO_2 depend on several factors such as target microorganism, concentration, storage temperature and a_w of food (Smith *et al*., 1988; Farber, 1991). However, the combination of high CO_2 and low O_2 percentages in the package headspace does not warrant a mould-free shelf life of bakery products (Ellis *et al*., 1993, 1994). This is possible because, often O_2 is trapped into the food matrix and after 24 to 48 hours this gas mixes with gas headspace, increasing O_2 concentration up to 0.5% that is the critical value to obtain a mould free product (Tabak and Cook, 1978). The advantages of this packaging method can be summarized as follow: it is cheap, has the capacity to greatly increase the shelf life of products, provided that film permeability, package airtight sealing, gas flushing and mixing are made in a right way. The use of AP, through the use of iron-based oxygen absorbers, is a method to prevent O_2 presence into the packed tray and obtain a mould-free bakery product (Salminen *et al*., 1996; Guynot *et al*., 2003), but the concern of European (Italian) consumers and producers hinder a greater use of active packaging methods.

As mentioned above the use of preservative in bakery products is decreasing due to consumer concern, thus the reformulation of recipe (with the addition of *natural* ingredients where possible) to decrease the spoilage risk should be preferred, trying to not modify the sensory characteristic of the product, and

combined with MAP technology that is well accepted by both consumers and producer.

Among the different phenomena that affect staling in bread products, retrogradation of starch is considered a primary determinant (Piccinini *et al*., 2012). Moreover water loss and migration within the food matrix may lead to recrystalliation of sucrose resulting in the increase of hardness in cookies or cheese cakes (Secchi *et al*., 2011).

1.5 Retrogradation of starch

As summarized by Piccinini *et al*. (2012), staling is defined as the sum of physicochemical reactions and microbiological phenomena that leads to the loss of quality in bakery products. We do not know deeply all the mechanisms that are involved in the staling process, but we know that staling process leads to the hardening of the bread crumb, to the weakening of the crust, and to modifications in the texture of fresh baked bread, as a consequence of starch and gluten interactions and the concomitant movement of water from the crumb to the crust and between gluten and starch. In cookies or cheese cakes the staling process leads to movement of water between the different phases of the product, which consequently harden. The difference in the time needed for these physical phenomena to occur, together with the microorganism growth, represents the untimely shelf life of the product. As above mentioned, starch is the most abundant constituent in wheat flour, therefore any action devoted to reduce the retrogradation of starch, would directly impacts positively the shelf life of the product. Starch is composed of a mixture of two homopolymers: amylopectin and amylose, that are in turn composed of chains of α-1,4-linked D-glucose units, branched via α-1,6 glycosidic links. They differ in the number of branched chains, since amylase is almost linear or with few branched chains. During bread making process, this crystalline structure suffers important physical transformations: with the addition of water (mixing) starch granules swell, after that, during baking in the oven starch gelatinizes and during cooling and storage it recrystalises. The retrogradation of amylose occurs immediately after baking, whereas the retrogradation of amylopectin occurs later, during bread storage and represents the

principal cause of bread crumb firming (Gray and BeMiller, 2003; Fadda *et al*., 2014). Starch retrogradation can be monitored with several analytical methods but one of the most rapid and reliable is visible and near-infrared (NIR) spectroscopy, inasmuch it is able to search changes during storage time of the hydrogen bonding in the starch crystalline network (Wilson *et al*., 1991; Osborne 1996, 1998). Piccinini *et al*. (2012) and Xie *et al*. (2003) compared NIR spectroscopy analysis and texture analysis to detect changes in bread during storage; they verified that NIR FT-Raman spectroscopy can give direct information about starch molecular changes, indeed the spectra can be acquired in a few minutes, and the analyses can be performed on samples without any pre-treatment. This method as reported by Thygesen *et al*. (2003) has been applied to evaluate starch molecular changes in other food matrix as potato, almond, wheat grain, and barley, but Piccinini *et al*. (2012) were the first that applied Raman spectroscopy for the investigation of starch retrogradation in bread and particularly in the crumb of semolina-based bread.

1.6 Whey powders (WP) in bakery products

Whey is the main by-product of cheese making (Díaz *et al*., 2004). In Sardinia whey is the principal waste product of ovine dairy industry and reaches the amount of 250.000 tons per year. In the centre of Sardinia there is a plant that collects the 20% of liquid whey produced in the region, which comes from cheese factories, to convert it in spray dried ovine whey powder. The remaining amount of liquid whey is mainly used in ricotta cheese production and as liquid feed, but the management of this by-product represents a serious environmental problem. Whey products are produced in different forms: dry whole whey powder, which is obtained by evaporation and spray drying, whereas the whey protein concentrates (WPCs) are obtained by ultrafiltration and further spray drying. Protein in WPCs can range from 30% to 75%, whereas whey powder from 10% to 35% of protein. To obtain delactosed and demineralised dry whey, ion exchange or membrane technology prior to spray drying, are used (Kinsella and Whitehead, 1989).

Dry whey and whey powders are widely used in the food industry, in dairy, bakery, meat and beverages, as a food ingredient or an additive. The main whey

powder components are lactose and proteins, primarily β-lactoglobulin and α-lactalbumin (Zadow, 1993). In bakery these products can be a potential source of low cost functional proteins, giving an added value to the bread, due to the nutritional value of the proteins.

The addition of lactose and proteins from whey can modify the texture of food thanks to their gelling, film-forming, foaming and emulsifying properties. Indeed, the excellent gelling and foaming properties of whey have been exploited to replace the egg white in bakery products (Zadow, 1993). Whey products contribute to the functionality of the product relay in the fact that they are a good source of protein, carbohydrates and calcium (Zadow, 1993). Nevertheless, whey proteins are known to exert negative effects on bread quality, by depressing loaf volume, and increasing crumb firmness, although their denaturation seems to eliminate this effect (Erdogdu-Arnoczky *et al.*, 1996; Kadharmestan *et al.*, 1998). Indrani *et al*. (2007) studied the effect of replacement of wheat flour with different amount of WP on Parotta bread (Indian traditional unleavened flat bread product) to evaluate the influences on dough, pasting properties and quality of baked bread. They demonstrated a proportional increase in dough stability and resistance to extension and a decrease in water adsorption, extensibility and viscosity with addition of different WP levels. Secchi *et al*. (2011) demonstrated that the addition of 5% ovine WP in the formulation of amaretti cookies (Italian traditional cookies), resulted in delayed firming and increased shelf life of cookies, while it did not influence the sensory properties. The water-binding properties of ovine WP reduced the effect of the increase of a_w during storage as consequence of sugar re-crystallization. Büşra Madenci and Nermin Bilgiçli (2014) studied the effect of WP and buttermilk powder (BP) on rheological properties of bread dough. They concluded that WP and BP addition increased the mineral content and improved the dough rheological properties as dough stability and development time, thus strengthening the dough.

1.7 Objectives

This PhD thesis has been organized in three main chapters, devoted to face three different critical points in bakery industry. First of all, we tried to improve the leavening performance of innovative dough, prepared using both "modern" semolina, and semolina milled from the cultivar Senatore Cappelli. We expected that the second one would have given a bad performance in terms of handling properties, due to the stickiness of its gluten, then we tested six different formulations for each semolina, introducing the ovine whey powder as an improver in the dough, at different substitution rates. Two different powders were tested, differing in their protein content. The dough formulations were analyzed for their rheological characteristics, by means of a fitting procedure built on stress relaxation data. A spectroscopy technique has been used in order to study in deep molecular structure of gluten in the new formulation.

As far as the bakery products concern, we have chosen a typical double layered flat bread, named spianata, and a cheese cake, named ricottine, whose formulation contain a typical ovine ricotta cheese (ricotta gentile). The spianata was prepared in our pilot plant following the traditional recipe of our region, and it was tested for its quality and shelf life properties. Modern semolina and semolina milled from Senatori Cappelli were compared to check any difference between them, and the ovine whey powder was added to the formulation. Only a type of powder was used, at three different concentrations. Also a control pan-bread was prepared, to verify the better suitability to produce bread with crumb rather than a flat bread, of the new formulations. WP was added to study the effect on the texture, the sensory properties, and the staling phenomena on the breads. Raman spectroscopy has been used to monitor starch retrogradation,

Ricottine have been prepared in a small plant sited in the centre of Sardinia, following the traditional recipe. One type of WP was used at two different concentrations. They have been packed in ordinary and MAP atmosphere and shelf life was monitored, to check if the combination of the bacteriostatic and fungistatic effect of MAP together with the "hurdle effect" of WP, that is its capability to decrease a_w values, could extend the shelf life of the cakes.

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2 The role of ovine whey powder in improving the properties of a semolina-based dough for baking

2.1 Introduction

Semolina from durum wheat is used to make different types of local breads in the Mediterranean area, particularly in Southern Italy (Fadda *et al*., 2010a). The chemical and rheological characteristics of semolina greatly affect the handling properties of the dough, its leavening capacity, and the final quality of the finished bread. Semolina characterized by a strong gluten network, resulting in a high gluten index and P to L values, is suitable to produce soft bread with low specific weight (Armero and Collar, 1998) due to the high leavening and gas retention capacity. In contrast, semolina with a weak gluten network results in dough that is not able to expand well, producing bread that is heavy and hard with an inhomogeneous structure (Oates, 2001). In recent years, an increasing consumer desire for high food quality has led to renewed interest in ancient raw materials and traditional food production (Gallo *et al*., 2010). The importance of this topic was stated at the Universal Exhibition hosted in Milan in 2015 (EXPO, 2015) where "the best of the agri-food and gastronomic traditions of each of the exhibitor countries" were presented. In this context, local bread wheat landraces and old durum wheat varieties have recently been rediscovered, and there has been a general effort to reintroduce them into the bakery industry. However, the replacement process has been hindered by their poor agronomic characteristics and poor gluten quality, which renders them unsuitable for bread making in modern bakery plants. The functional properties of dough can be improved by the addition of food ingredients or additives such as whey powder, which is used as a food fortifier or as a source of low-cost proteins (Fadda *et al*., 2010b). Whey lactose and proteins can be added to introduce for food texture modifications (gelling, film-forming, foaming, and emulsifying) (Kinsella and Whitehead, 1989; Zadow, 1993). Whey proteins also have important nutritional and biological functions, since the protein components and their peptide fragments are bioactive; for example, they exert antimicrobial and antiviral actions as well as anticarcinogenic activity and they have the capacity to modulate the innate

immune system (Ko and Kwak, 2009). Moreover, 1 million tons of ovine whey is produced annually in Southern Italy (Sansonetti *et al*., 2010), resulting in a number of environmental problems that include pollution. Currently, the conversion of by-products from the dairy industry into dry products is one of the main industrial conversion processes. A number of scientific papers are available on the use of whey powders derived from bovine milk. In Mediterranean countries, whey powder from ovine milk is also readily available and has been used to increase the shelf life of cookies (Secchi *et al*., 2011).

The objective of this study was to investigate the role of ovine whey powder (OWP) on the leavening performance of semolina-based dough for making pan-bread, with particular emphasis on dough produced with semolina from the Senatore Cappelli cultivar, an old and tall wheat durum genotype characterized by weak gluten and sticky dough, features that poorly fit with the technological requirements of modern industrial plants. In addition, the effects of increasing substitution rates with two OWPs on two different types of dough were investigated. Specifically, dough was produced with commercial low-grade semolina derived from a blend of strong wheat cultivars or with semolina obtained from the Cappelli cultivar. The physical-chemical properties and rheological behaviour of the dough were evaluated and leavening trials were conducted. Potential molecular modifications in gluten conformation caused by the addition of OWPs were investigated using Fourier transform infrared (FTIR) spectroscopy.

2.2 Materials and Methods

2.1. Raw materials, dough preparation and analyses

Two commercial low-grade semolina (LGS) were used (Molino Galleu, Sardinia, Italy), referred to as 4T and 48T. The 4T was obtained by milling the Cappelli cultivar, whereas the 48T was derived from a blend of wheat cultivars that are commonly used to make pasta and typical semolina-based breads. Two commercial OWPs referred to as A15 (Alim21 A.SP1, Alimenta Srl, Sardinia, Italy) and A35 (Alim36 A.SP1, Alimenta Srl) were added to the dough

formulations at different substitution rates (0, 5, 10, and 15% w/w). Hereafter, dough samples will be denoted by the LGS name plus OWP name and relative percentage (e.g., 4T A15 10 or 4TC for the control sample). Raw material characterization was performed for: moisture (%), ash (%), protein content (%), calculated on a dry basis (d.b.), gluten index (%), and dry gluten content (% d.b.), using AACC Approved Methods: 44-15A, 08-12, 46-12, 66-20, 38-12A, and 54-30A (AACC, 2000) respectively. The latter method was adapted to durum wheat according to Dubois *et al*. (2008).

The Alveo-Consistograph (Chopin, France) was used to determine water adsorption capacity at a consistency of 2,200 mbar (fixed moisture basis of 15%), the pressure drop after 450 s of mixing (D450, mbar), and the time to reach the target consistency of 2,200 mbar (TPrMAX), according to the AACC Approved Method 54-50 (AACC, 2000).

The dough was prepared in the Consistograph mixer bowl at a fixed temperature (24°C) and at adapted hydration. OWP at different percentages, semolina, and yeast (1% w/w, total weight of LGS plus A15 or A35) were mixed in the Consistograph mixing bowl for 2 min before adding 2.5% saline solution. The chosen mixing time was the time needed to reach 75% of the maximum pressure (i.e., 2,200 ± 7% mbar) (figure 1), as measured on the Consistograph (Vinci *et al*., 2013). After reaching the desired consistency, the dough was sheeted and formed for alveographic analysis. Another dough was prepared for each treatment, and when the desired consistency was reached, it was extruded, sheeted and divided into five pieces that were used for a stress relaxation test. The third dough for each treatment was prepared in the same way in triplicate and used in the leavening trial.

2.2. Stress relaxation test

The TA.XT2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK) equipped with a 30 kg load cell and P50 probe was used for the stress relaxation test on the dough [15], and force (N) vs. time (s) curves were recorded using Texture Expert Exceed software version 2.64. The test speed was 1 mm s-1. The

instantaneous strain applied to the dough was 10%, and the resulting stress was recorded for 40 s. A generalized Maxwell model was used to interpret the stress relaxation data in accordance with Campus *et al*. (2010). The generalized Maxwell model is as follow:

$$\sigma(t) = \sum_{i=1}^{n} Ci(e^{-(t/\tau i)}) + \sigma e$$

where σ is the stress (N) at a given time, t is the time (s), Ci is the stress relaxation constant (N), σe is the equilibrium stress (N), and τi is the relaxation time of the Maxwell element (s). TableCurve 2D version 5.01 (Systat Software Inc., San Josè, CA, USA) was used to perform regression analyses on the stress relaxation data using the Levemberg-Marquardt method. R2 (i.e., the percentage of explained variation), and maximum relative difference (MRD) were used to evaluate the goodness of fit. Only the relaxation part of the curve was used for the fitting procedure (figure 2). A model with three Maxwell elements (n=3) was optimal to describe the visco-elastic behaviour of the dough (R2 ≤0.98 and MRD ≤7). The maximum force achieved in the stress relaxation test was the same in all dough, confirming that homogeneous dough were obtained in terms of consistency. The value of maximum force obtained was 3.8±0.3 N.

2.3. Leavening trial

Leavening tests were performed as described by Vinci *et al*. (2013). For each treatment, the dough was prepared in the Consistograph mixing bowl, as described above. After dough preparation, 100 g was transferred to graduated glass cylinders (250 mL capacity and 15 mm diameter) and left at 25°C until maximum leavening height was attained. The increase in dough height was recorded every 15 min.

2.4. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectral measurements were performed under vacuum condition using the Bruker infrared Vertex 70 interferometer (Ettlinger, Germany) equipped with a deuterated triglycine sulfate detector, and spectral data were processed by using Bruker OPUS 6.5 software. The dough samples were freeze dried before FT-IR analysis to avoid interference from water (Sivam *et al*., 2013). After freeze drying, each dough sample was homogenized using an agate mortar and pestle, after which an aliquot of 3 mg was mixed with 297 mg anhydrous potassium bromide (KBr, >99%; Sigma-Aldrich, St. Louis, MO, USA) and pelleted for the analyses. The spectra were recorded in the 400–4000 cm-1 range by averaging 128 scans at a 4 cm-1 resolution. The background was evaluated by measuring the KBr signals. Three replicates were measured for each treatment. Spectra were recorded between 1710 and 1580 cm-1 for studying the amide I band. After normalization, A15 and A35 spectra were subtracted from the spectrum for the dough, after being multiplied by their relative percentage in the dough. The peak positions, corresponding to the secondary structure of the proteins, were identified using the second derivative spectra and used to initialize the best fit of the amide I band in the spectral range of 1710 to 1580 cm-1. The best fit was obtained using the mixed Gaussian-Lorenzian function. The percentages of integrated peak areas were calculated. Each area corresponded to a different secondary structure, which were assigned as reported by Wang *et al*. (2016).

2.5. Statistical analysis

Principal component analysis (PCA) was performed on the correlation matrix of the variables including semolina and dough properties, mixing parameters, and height of leavening. The component loadings were calculated as simple correlations (using Pearson's r) between the components (i.e., the component scores for each dough) and the original variables. Data related to properties of dough samples (mixing time, volume of leavening, and protein content) were analyzed by one-way analysis of variance (ANOVA). When appropriate, mean separation was conducted according to the Duncan’s multiple range test at $p<0.05$.

2.3 Results and Discussion

3.1. Characteristics of LGSs and ovine whey powders

The chemical and physical properties of the LGS samples and OWPs are shown in table 1. The data show the differences between the two semolina samples with regard to protein content, gluten index, and alveographic parameters, the configuration ratio (P/L) and deformation energy (W). 48T had a higher protein content, gluten tenacity and extensibility than 4T, which was obtained from a cultivar that is well known for its poor gluten quality due to the pattern of the high molecular weight glutenin subunit 20 (HMW-GS 20), which is the predominant pattern in durum wheat landraces and old genotypes (Fois *et al*., 2011). However, it has been almost completely replaced by the 6+8 and 7+8 HMW patterns, which exhibit stronger dough properties and superior baking quality than HMW-GS 20 cultivars. There were evident differences in the chemical composition of the two OWPs, with A35 having a higher protein content and lower ash content than A15 (table 1).

3.2. Effect of ovine whey powders on rheological and leavening properties of the dough

The results of the PCA analysis on the dough samples are reported in figure 3 and in table 2. The first two PC axes explained 85.3% of the differences among samples (figure 3). Almost all of the variables contributed, both positively and negatively, to the first axes, whereas the original variable that contributed most to the second axis was the relaxation time of the Maxwell model τi. The position of the samples with respect to the first PC axis was consistent with the addition of whey proteins, which moved the samples to the positive part of the first principal component (PC1). The negative association between this first axis and the protein content of the dough ($r = -0.89$) highlights the negative effect of whey addition on the leavening performance of flour. Typically, the higher the protein content, the better the leavening capacity of the dough; however, in this case, although the addition of whey increased the protein content, it also strengthened the dough,

which probably led to greater resistance to extension. The correlations among the original variables confirmed this hypothesis, as strong associations among protein content and all of the indices of elasticity and strength of the dough (i.e., alveographic tenacity of the dough T, Maxwell decay force Ci, equilibrium stress σe) were found.

The strong correlations between the variables and PC1 established a strong relationship among them, notably between the leavening capacity of the dough and the consistographic parameters. Here, data from the Consistograph showed that the addition of OWP led to reduced water adsorption of the dough, similar to the study by Indrani *et al*. (2007), in which whey protein concentrate was added to a flour-based dough. Note that the available literature refers to flour from *Triticum aestivum* L., and no data have been published to date on the effects of the addition of whey protein to semolina-based dough. The effect of hardening on the dough was revealed by the increase in TPrMAX values (data not shown), which increased from 110.5 s in the 4TC to 385.0 s in the 4T A35 15, and the concomitant decrease in D450 values (from 880.5 s in the 4TC to 86.5 s in the 4T A35 15) showed an increase in dough stability as the OWP percentage increased, similar to the findings reported by Indrani *et al*. (2007). In a previous study Vinci *et al*. (2013), our group demonstrated the key role of technological mixing parameters (i.e., hydration level of the dough), as determined using the Consistograph, and mixing time in influencing the leavening performance of a semolina-based dough. We suggested that the optimum mixing time approximately corresponded with the time required to reduce the maximum pressure to 75% of its value (figure 1), which resulted in a softer and more elastic dough that was better suited to leavening. This procedure was applied in the current study to make sure all dough samples were mixed until the best consistency for leavening was attained, which explains why the mixing times were extremely variable among the samples, ranging from 330 s to 1,065 s (table 3, figure 4). As expected, the shortest mixing time was in the 4TC. The dough made from the stronger 48T showed a longer mixing time than the dough made from 4T in all treatments. We generally observed that the addition of OWP led to

an increase in mixing time, consistent with the degree of substitution of LGS with OWP. This is probably because whey proteins make it more difficult for the gluten network to form, causing the dough to take more time to reach the desired consistency. Interestingly, the leavening performance of 4TC was better than that for the 48T samples (table 3), although the corresponding LGS had a lower protein content and poorer gluten quality, in accordance with the conclusions of Vinci *et al*. (2013), who showed that the technological parameters used during dough preparation influence the leavening performance more than the semolina characteristics. Generally, the 4T performed better than the 48T in almost all of the treatments, and the addition of OWP had a negative effect on leavening height compared with the respective controls in both 4T and 48T. This result is in accordance with most studies, which have demonstrated that almost every milk fraction depresses loaf volume including whey proteins (powders or concentrates), casein, and lactose, as summarized by Erdogdu-Arnoczky *et al*. (1996). In our study, the depressing effect was greater in 4T than 48T, at the highest OWP concentrations (i.e., A15 at 15% and A35 at 10% and 15%; table 3). In fact, the loaf volume was not significantly different between 4T and 48T, but the volume in the 4T control was significantly higher than that in the 48T control. Conversely, at the lowest concentration of A15 (5%), dough made from 4T and 48T showed the same leavening height as their respective controls. Our data show that the addition of OWP leads to increased dough elasticity, as determined by the contribution of the stress relaxation constant Ci to PC1 (figure 3). We observed that the greater the reduction in leavening height, the higher the increase in Ci values; in the dough made from 4T and 48T with A35 at a 15% substitution rate, the C1 value increased by 151% and 81%, respectively, with respect to their controls. At the same time, the leavening height decreased by 56% and 52% in the 4T A35 15 and 48T A35 15 samples, respectively. These data suggest that the addition of OWP resulted in a gluten matrix that had a greater resistance to extension, which negatively affected leavening performance.

In the 4T dough, the 5% substitution rate with A15 (i.e., the lower concentration of whey proteins) led to an increase in leavening height (+6%), which can be

attributed to the weak gluten matrix of the Cappelli cultivar. In this case, the gluten matrix most likely benefited from the increased elasticity, which improved the leavening performance but did not make the dough too strong as to oppose expansion driven by fermentation, as occurred in the other samples. Indeed, Fois *et al*. (2012) reported that samples with a gluten network that is too strong and a P to L ratio that is too high tend to perform worse than samples with normal gluten strength.

In this study, the two kinds of semolina were well separated with respect to the second axes (figure 3), indicating that the 48T samples had a greater relaxation time than the 4T samples, regardless of the addition of whey protein. Relaxation time τi, which dominated PC2, clearly distinguished the 4T samples from the 48T samples. The relaxation times can be considered an indication of the relative rates of molecular motion in dissipating stress (Edwards *et al*., 2001), so the higher the relaxation time, the lower the chain mobility. Lower relaxation times may explain the higher leavening height in the 4T samples compared with the 48T samples at the lower concentrations of whey proteins (i.e., A15 at 5% and 10%, and A35 at 5%). Edwards *et al*. (2001) discussed the role of the relaxation time in the baking performance of common and durum wheat. The authors found that dough with lower relaxation times had higher loaf volumes, as in our case, and hypothesized that the strength of the dough is correlated with the relative molecular mobility. In the abovementioned substitution rates, the height of leavening was ~20% greater in the 4T samples than the homologous dough made from the strong commercial 48T, consistent with that of the latter samples, which moved up to the positive part of PC2.

3.2. Effect of whey powders on the secondary structure of gluten

FTIR spectra of 48TC and 4TC dough were collected and analysed to study the molecular differences among samples. FTIR spectra of 48TC and 4TC dough in the range of 1780–1440 cm^{-1} (figure 5) were similar to those found in other studies (Georget and Belton, 2006; Sivam *et al*., 2013), and revealed the C=O stretching of lipids at about 1750 cm^{-1}. Amide I and II are the most important

bands in the infrared spectrum of proteins. The most intense absorption band is amide I, which was centred at about 1660 cm^{-1} in this study, and is mainly derived from the C=O stretching of the peptide group combined with N-H bending. The amide II band was found in the range of 1580–1510 cm^{-1}; this band is more complex to study because it is derived from a combination of N-H bending, C-N, and C-C stretching. This is the reason why only the amide I band was studied in depth. The peak assignments to protein conformations in the amide I region were the same as reported by Wang *et al*. (2016): the bands located at 1650–1660 cm^{-1} were assigned to the α-helix, the bands located at 1618–1640 cm^{-1} and 1670–1690 cm^{-1} were assigned to the β-sheet, the bands at 1660–1670 cm^{-1} and 1690–1700 cm^{-1} were assigned to β-turns, and the band at 1645 cm^{-1} was assigned to random coils (Wang *et al*., 2016). The results of the curve fitting procedure are shown in figures 6–9, which show that about 50% of proteins in the dough adopted a β-sheet conformation in all of the samples analysed. Secondary structures were highly sensitive to the addition of ovine whey powder. In the 4T samples, the addition of OWP led to a slight increase in β-sheet and β-turn structures, at the expense of α-helix and random coil structures (figures 6 and 7). The contrary occurred in the 48T samples, in which there was a decrease in β-sheet and β-turn structures, but an increase in α-helix and random coil structures after the addition of whey powder (figures 8 and 9). The β-sheet structures are indicative of protein aggregation, which has been reported to decrease the volume of bread (Ravi *et al.,* 2009; Sivam *et al.*, 2013), and the increase of β-sheet and β-turn structures is an indicator of increased molecular rigidity and dough strength (Wang *et al*., 2016).

Generally the volume of leavening was reduced by the addition of OWP (table 3), indicating a detrimental effect on the dough, as previously explained. However, the hypothesis of excessive strengthening of the gluten matrix was consistent with the increase in β-sheet and β-turn structures in the 4T samples, but not in the 48T samples, in which the content of β-sheet and β-turn structures decreased. We suggest that other technological parameters may have influenced the relative percentage of the secondary structures. The 48T samples differed from the 4T samples with regard to hydration level and mixing time, which were calculated for

each dough using consistographic data. It is worth noting that the mixing time was significantly higher in the 48T samples compared with the 4T samples (table 3), which further increased with the addition of OWP. The longer mixing time may have been responsible for the partial disruption of the protein aggregates and a portion of the gluten network, thereby explaining the decreased leavening height in the 48T samples upon OWP addition, even though it was not accompanied by an increase in β-sheet and β-turn structures. This was reflected by the increase in random coil, unordered structures, which increased from 12% in the control 48TC sample to 20% in the 48T A15 15 (+ 66%) sample. Notably, the content of β-turn structures that are highly dependent on the mechanical history of the sample (Wellner *et al.*, 2005) decreased from 26% in the 48TC sample, to 19% in the 48TA1505 sample.

In our experiment, an interesting phenomenon was observed: the volume of bread followed a different trend than the height of leavening, but consistent with data from spectral analysis. As can been seen in the following section of this thesis (section 3, figure 3), the volume of bread in 4T samples significantly increased after addition of ovine whey powder, and this is consistent with our hypothesis that the increase of *β-sheet + β-turn* structures strengthened the gluten network in the dough, which could resist to the expansion of the alveoli during baking. On the contrary, the volume of bread in 48T samples decreased with the addition of whey powder, due to the supposed disruption of the gluten network during the prolonged mixing times.

2.4 Conclusions

In conclusion, the addition of OWP to the dough had a negative effect on leavening height, at all concentrations except the lowest (5%). Leavening height is generally recognized as a reliable indicator of bread volume (Fadda *et al*., 2014). In this study, the reduction in height occurred for different reasons. In the 4T samples, the addition of OWP led to an improvement in dough strength, as demonstrated by the increase in β-sheet and β-turn structures, which was revealed by analysis of the amide I band in the FTIR spectrum. On the contrary, in the 48T

samples, the addition of OWP led to the need for a longer mixing time to reach the desired consistency on the consistograph. The prolonged mixing time probably caused partial disruption of the gluten network, resulting in reduced leavening height and reduced bread volume after baking in the samples with OWP compared with the control. The significant increase in unordered protein structures confirms this hypothesis.

2.5 Figures and tables

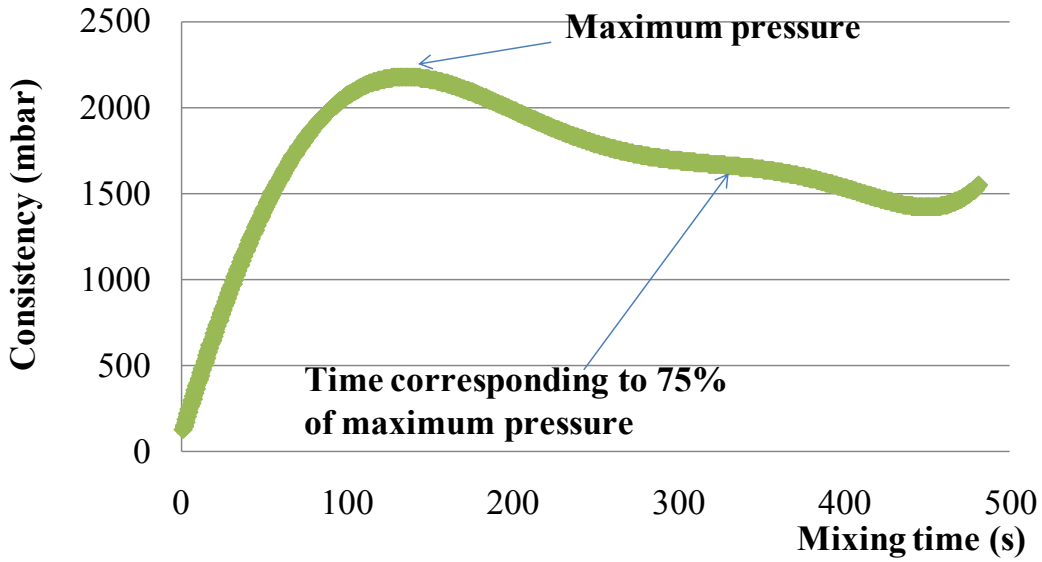

Figure 1. Curve from the consistograph: dough consistency *vs.* mixing time. The chosen mixing time was the time needed to reach 75% of the maximum pressure measured at the consistograph for each dough.

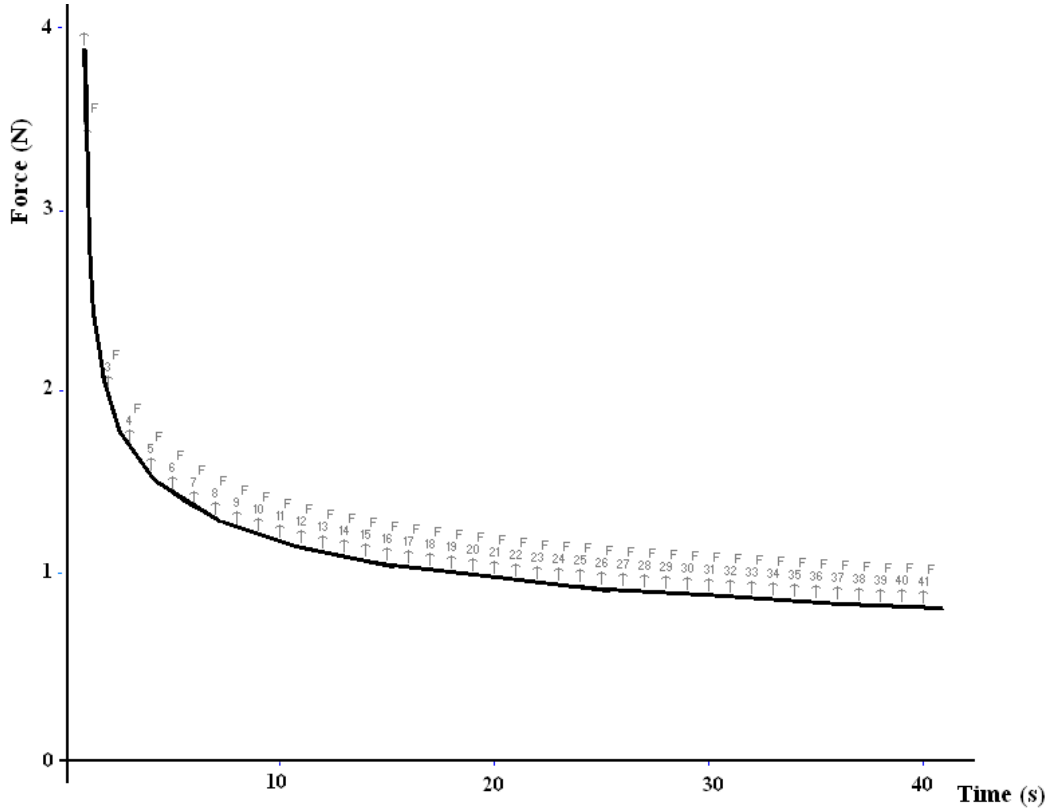

Figure 2. Relaxation part of the curve that was used for Maxwell model fitting.

Table 1. Properties of low grade semolinas (LGS) and ovine whey powders (A).

Batch	**Ashes** (% d.b.*)	**Protein content** (% d.b.)	**Moisture** (%)	**Gluten index** (%)	**P**** (mm)	**L**** (mm)	**W**** ($J*10^{-4}$)	**P/L****
4T LGS	1,02±0.02	11.08±0.09	15.45±0.10	56.00±0.01	59.9±1.8	49.0±6.9	91.9±6.4	1.24±0.20
48T LGS	1.03±0.02	12.86±0.48	15.15±0.05	92.23±0.02	98.2±2.1	45.8±5.7	173.7±8.9	2.18±0.29
A15	8.50±0.05	15.11±0.15	4.05±0.12	-	-	-	-	-
A35	6.00±0.04	35.21±0.18	4.20±0.08	-	-	-	-	-

* d.b, dry basis. **Alveograph parameters at constant hydration: P, maximum overpressure; L, index of swelling; W, deformation energy; P/L, configuration ratio. Data were expressed as mean ± standard deviation.

Table 2. Proportion of total variation and eigenvectors of principal component (PC) axes in a principal component analysis performed on all the traits measured.

	Eigenvectors		Loadings	
	Axis 1*	Axis 2**	Axis 1	Axis 2
Moisture	0.91	0.66	0.29	-0.14
Ash	0.57	1.28	-0.29	-0.01
Protein	0.02	1.50	-0.31	0.08
HYDHA	-0.60	1.30	0.31	-0.03
D450	0.54	1.05	0.29	-0.19
T	-0.38	0.18	-0.31	0.19
Vol Leavening	-1.77	0.32	0.32	-0.03
C1	1.59	-0.74	-0.30	-0.16
$\tau 1$	0.96	-0.94	0.04	0.52
C2	0.27	-0.94	-0.30	-0.23
$\tau 2$	-0.45	-0.74	0.03	0.52
C3	0.67	-1.02	-0.33	-0.09
$\tau 3$	-0.45	-1.02	0.00	0.52
σ_e	-1.88	-0.90	-0.27	-0.04

Component loadings are the simple correlations between the component scores and the original variables.

*Proportion of total variation is 60.3%

**Proportion of total variation is 25.0%

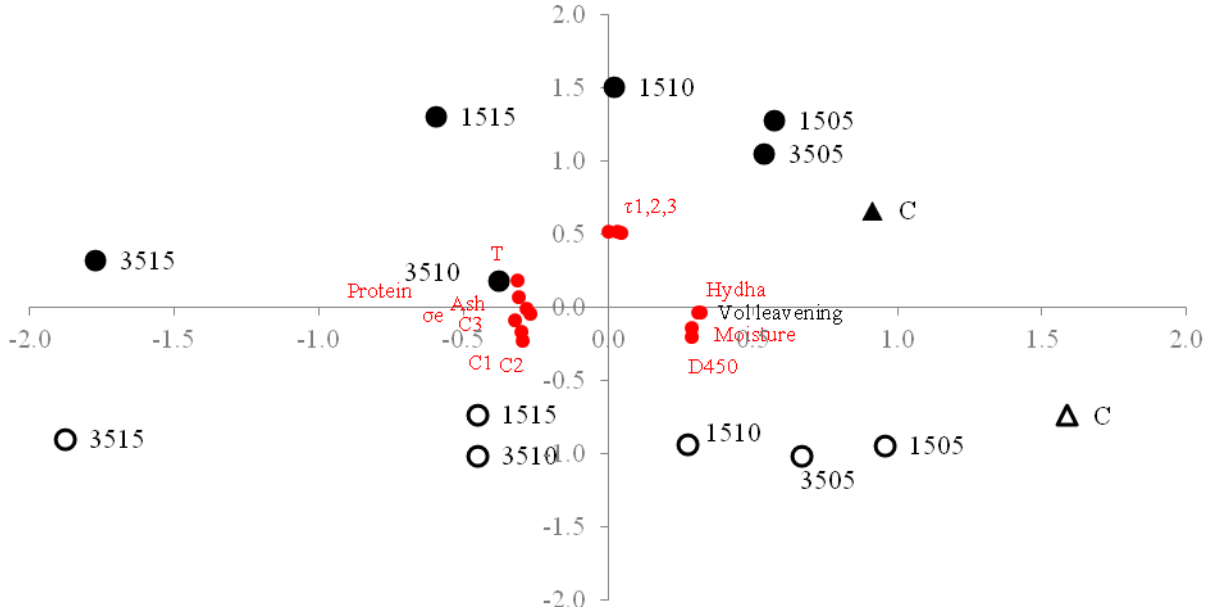

Figure 3. Principal component analysis (based on the correlation matrix) of dough samples. Biplot of factor scores (points). 4T control (△);48T control (▲); 4T doughs with ovine whey powders (A15,A35) (○);48T doughs with ovine whey powders (A15, A35) (●); loadings (●).

Stress relaxation test parameters: C1,C2,C3: decay force of Maxwell model; τ1,τ2,τ3: relaxation times of the Maxwell model; σ_e: the residual modulus that remained unrelaxed.
Alveograph parameters at adapted hydration: T: maximum overpressure (mm).
Consistograph parameters: HYDHA: actual hydration of dough used in the test (% 15% mb); D450 dough weakness at 450 s.
Vol leavening: leavening volume (mL).

Table 3. Properties of dough samples.

LGS	OWP	OWP (%)	Mixing time (s)	Volume leavening (ml)	Protein content (%)
4T	-	0	330 g	155 a	11.07 l
	A15	5	480 e	165 a	11.65 i
	A15	10	697 d	139 b	12.10 hi
	A15	15	930 b	115 de	12.72 g
	A35	5	480 e	142 b	12.70 g
	A35	10	765 c	86 f	15.08 cd
	A35	15	892 b	67 g	15.54 b
48T	-	0	420 f	135 bc	12.86 g
	A15	5	682 d	135 bc	12.53 gh
	A15	10	930 b	125 cd	12.97 fg
	A15	15	1050 a	115 de	13.72 de
	A35	5	675 d	119 de	13.43 ef
	A35	10	795 c	90 f	14.30 c
	A35	15	1065 a	65 g	16.61 a

Samples with different letters within each column are significantly different at $p<0.05$ according to Duncan's multiple range test.

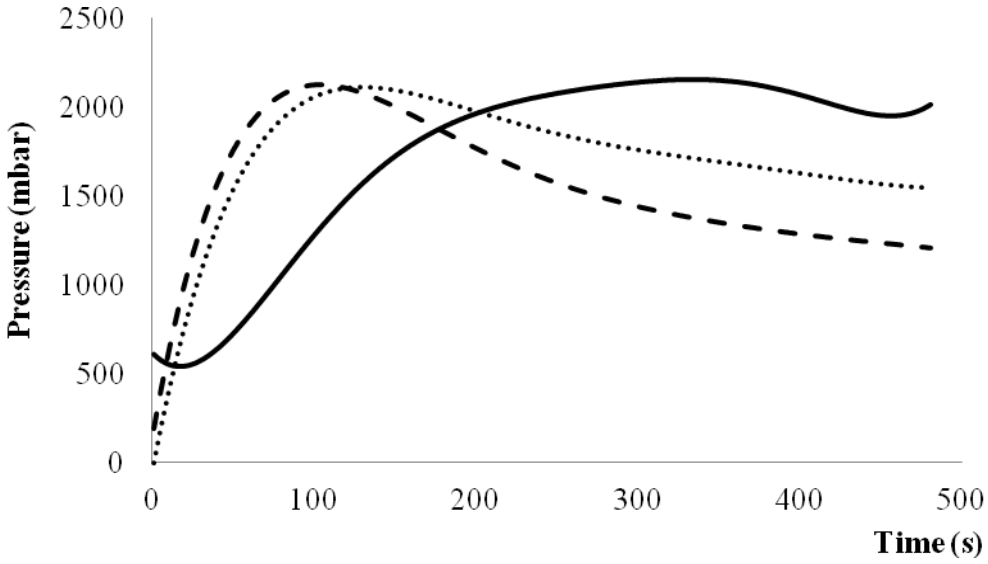

Figure 4. Evolution of mixing time during consistograph tests. 4TC dashed line; 4TA155 fine dotted line; 4TA1515 solid line.

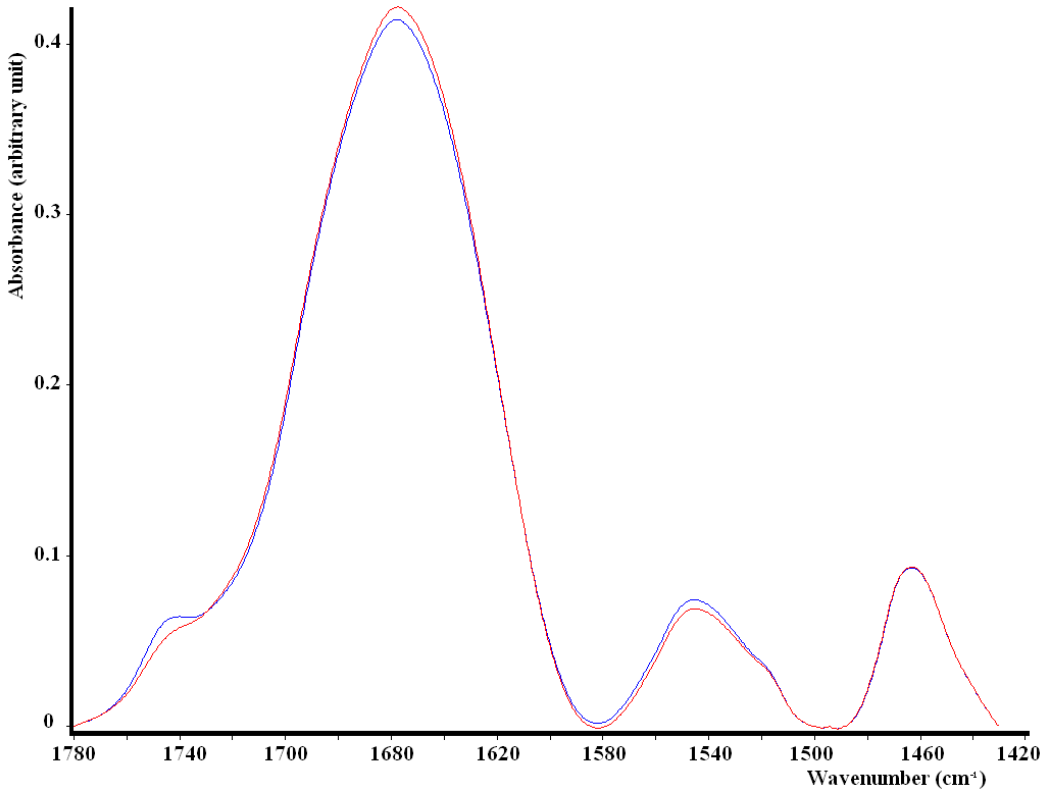

Figure 5. Typical FI-IR scan of 4TC (blu line) and 48TC (red line) in the range between 1780 and 1420 cm^{-1}.

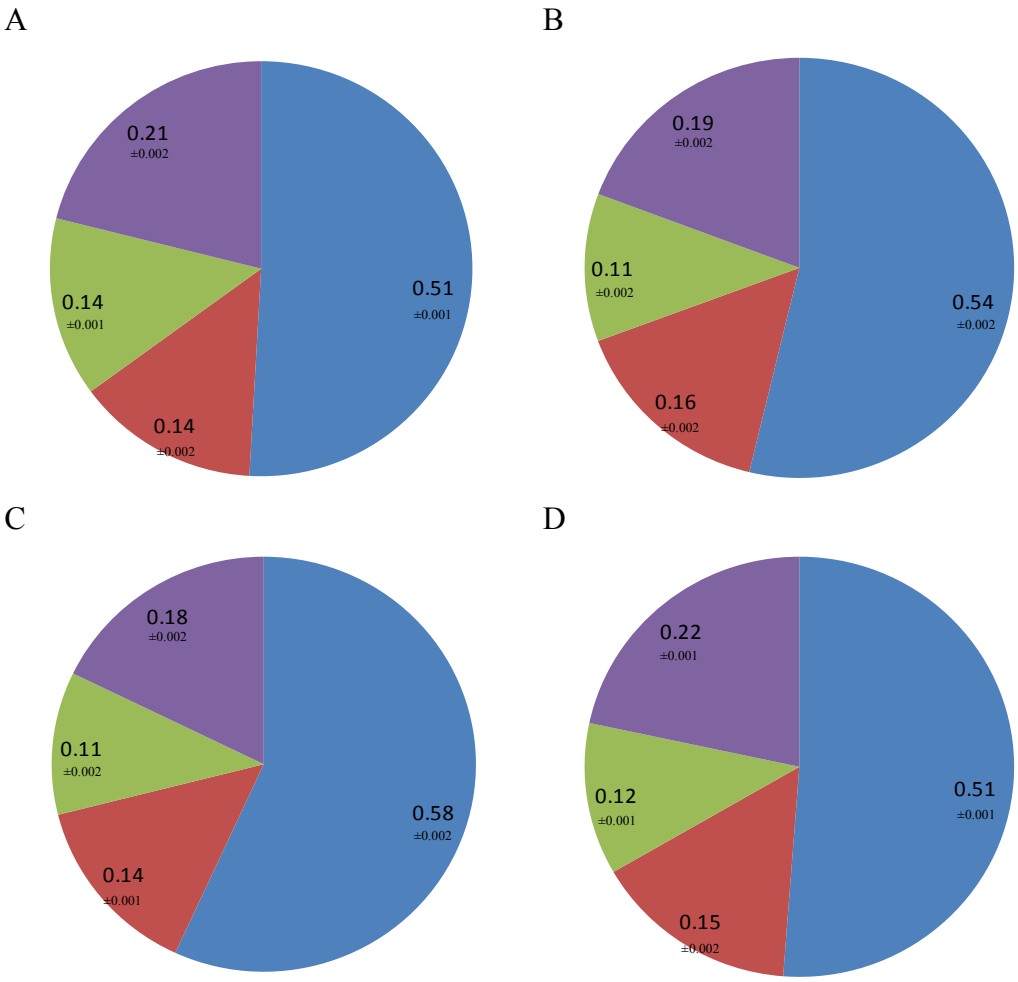

Figure 6. In the 4T-based dough, the effects of A15 on the secondary structure of gluten as revealed by FT-IR.

A=4TC; B=4TA155; C=4TA1510; D=4TA1515

β-sheet (■), random coil (■), α-helix (■), β-turn (■).

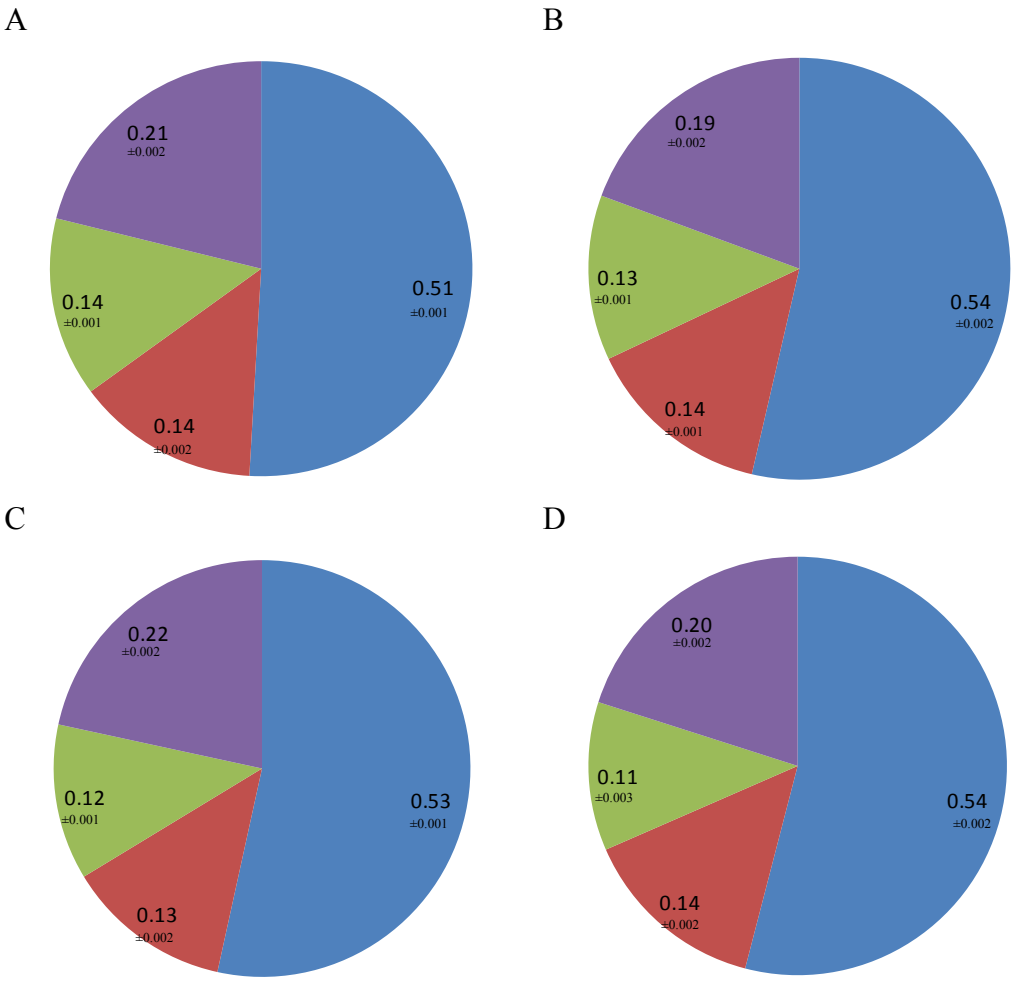

Figure 7. In the 4T-based dough, effects of A35 on the secondary structure of gluten as revealed by FT-IR.

A=4TC; B=4TA355; C=4TA3510; D=4TA3515

β-sheet (■), random coil (■), α-helix (■), β-turn (■).

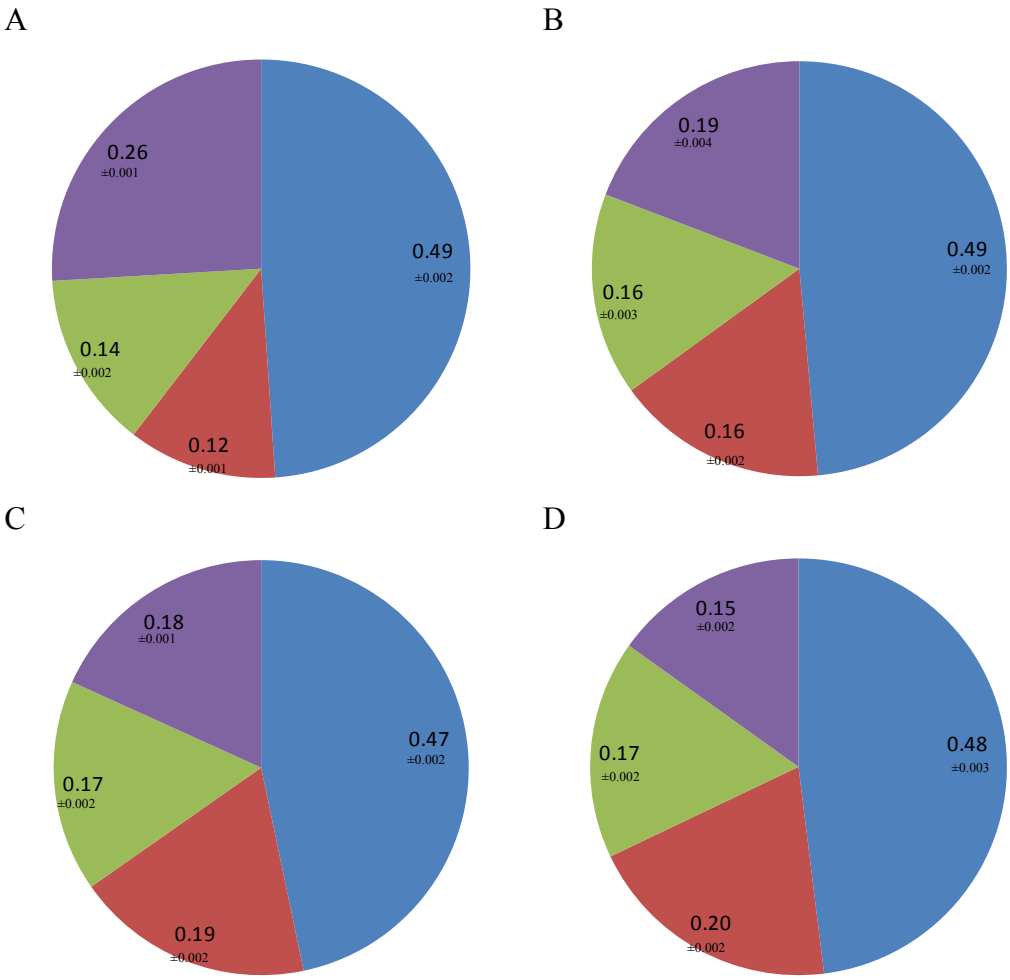

Figure 8. In the 48T-based dough, the effects of A15 on the secondary structure of gluten as revealed by FT-IR.

A=48TC; B=48TA155; C=48TA1510; D=48TA1515

β-sheet (■), random coil (■), α-helix (■), β-turn (■).

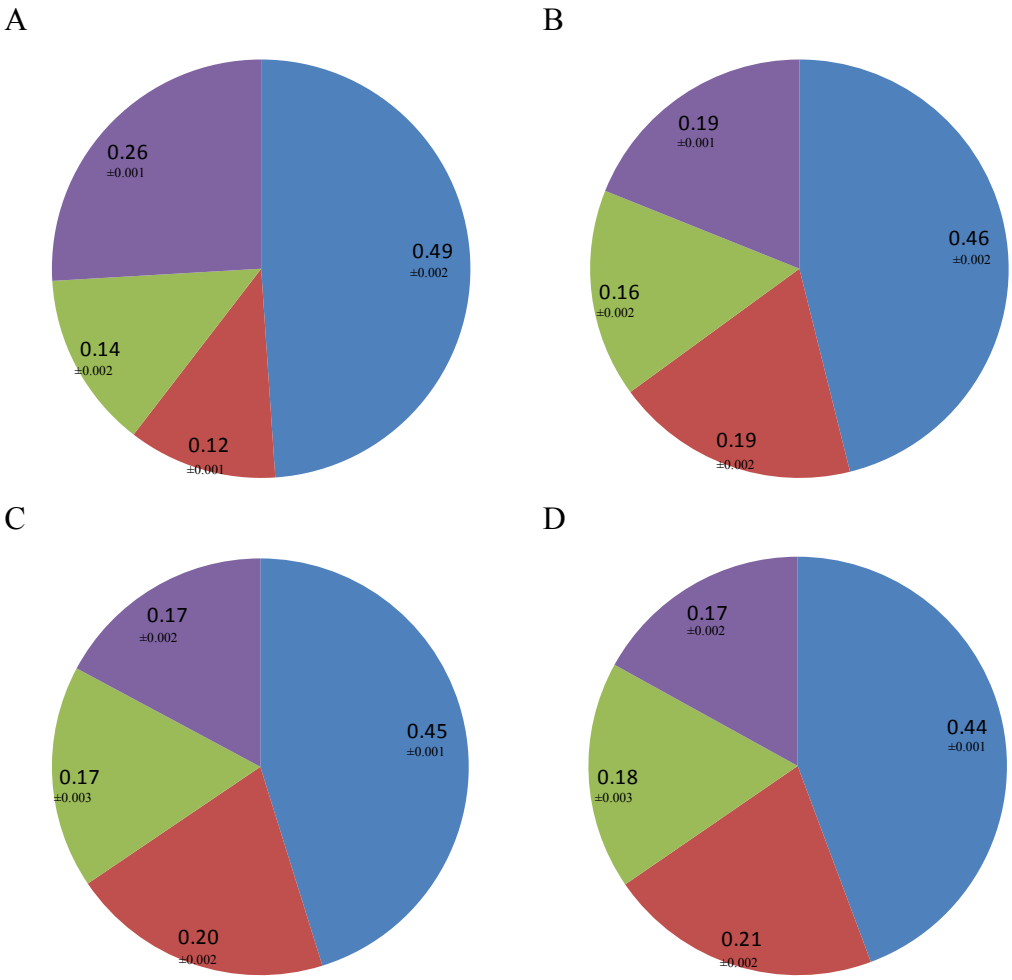

Figure 9. In the 48T based dough, tha effects of A35 on the secondary structure of gluten as revealed by FT-IR.

A=48TC; B=48TA355; C=48TA3510; D=48TA3515

β-sheet (■), random coil (■), α-helix (■), β-turn (■).

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3 The ability of ovine whey powder to improve quality of Sardinian breads

3.1 Introduction

In all over Mediterranean area and particularly in Southern Italy, semolina from durum wheat is widely used in bread making. Furthermore, durum wheat is worldwide used to make dry pasta, and this is the reason why durum wheat breeding programs have focused on improving pasta cooking quality, through improving gluten strength indices, rather than bread making quality. This has caused the progressive replacement of the old landraces and ancient varieties by new cultivars. Moreover, the old genotypes often show HMG-20 glutenin patterns, which are well recognized as a marker for gluten weakness in durum wheat. Sometime, in old genotypes, also γ -gliadin 42 type is present, which is marker for the LMW-2 glutenin pattern, and that is, again, related to weak gluten characteristics. Traditional varieties show poor adaptability to be employed in modern bakery plant, due to their protein pattern. On the other hand, new varieties sometimes show very strong and tenacious gluten that is inappropriate for bread-making technology. Whey, a by-product of the dairy industry, represents for baked products a potential source of low cost functional proteins that have emulsifying, foaming and gelling properties. The major whey proteins, *i.e.,* primarily *β*-lactoglobulin and secondarily *α*-lactalbumin, represent a well balanced source of essential amino acids, thus having a significant meaning as nutritional ingredients (Zadow JG, 1993). Nevertheless, whey proteins are known to exert negative effects on bread quality, by depressing loaf volume, and increasing crumb firmness, although their denaturation seems to eliminate this effect (Erdogdu-Arnocky *et al*., 1996; Kadharmestan *et al*., 1998). In general, as summarized by Erdogdu-Arnoczky *et al*. (1996), the effect of the addition of whey powder in the dough varies depending on the production conditions of the whey powder, on the quality of the flour, and on the technological parameters of baking.

The aim of this study was to investigate the effect of the addition of ovine whey powder (OWP) on the quality of two types of bread, *i.e.,* a conventional pan bread and a leavened flat bread (spianata), both obtained by a commercial low grade semolina (LGS) and a LGS milled from an old variety. Breads were evaluated in terms of colour, texture, cell size distribution in the crumb of the pan bread, staling properties and sensory determination.

3.2 Materials and methods

3.2.1 Bread making process

Two types of low grade semolinas (LGS) (Molino Galleu, Ozieri, Italy) were used: 48T milled from commercial durum wheat varieties; 4T from Senatore Cappelli variety, well known for its poor gluten quality. They were mixed with a commercial spray dried ovine whey powder, referred to as OWP (Alim21 A.SP1, Alimenta, Macomer, Italy), at increasing percentages (0, 5, 10 and 15% w/w), to make a spianata and a pan bread. Eight bread-making trials were carried out in a semi-automated bakery plant at Porto Conte Ricerche Srl (Alghero, Sardinia, Italy). Preliminary trials showed that the addition of Alim36 A.SP1 was not suitable for bread making due to excessive reduction of bread volume, even at the lowest concentration (data not shown). Hereinafter bread samples will be called using LGS name plus OWP with relative percentage (*i.e*. 4T OWP 10 or 4TC for control sample). The recipes are reported in table 1. In the formulations we reproduced the same conditions of the consistographic trial, with the aim to compare the results of the leavening trial on the bench scale (see section 2), with the results of the baking trail. This is the reason why water and salt content are different among samples: water content was calculated on the basis of Hydha values and salt percentage was 2.5% of water content, (Approved Method 54-30A, AACC, 2000). The relative percentages of the ingredients in the formulation varied, because the water addition was calculated as consistograph water absorption. The amount of baker's yeast was 1.0% of the total weight of LGSs plus OWP, on a as is basis. A total of 17 kg of dough for each sample were

prepared with a fork mixer. Ingredients were mixed for the time required to reach the maximum volume after leavening (Vinci *et al*., 2013), that occurred when the dough pressure value (mbar) reaches 75% of the maximum pressure measured at the consistograph (section 2.5 figure 1). Particularly dough at maximum pressure consistency (as registered in the consistograph) was found to be not ideal for baking purposes (Vinci *et al*., 2013), but a further mixing was needed to obtain a well-developed dough and to maximize the leavening performance. In section 3.2.3 we describe how we have found the optimum mixing time for the dough prepared in the fork mixer. The first proofing was conducted in a fermentation chamber set at 28 °C and 80% relative humidity for 35 min. To produce pan bread, six pieces of dough (1.2 kg for each piece) were manually introduced in aluminium pans and kept in the fermentation chamber (28 °C and 80% relative humidity) for 120 min and then baked at 230 °C for 35 min.

The bread was then set aside for cooling for 4h at room temperature and finally cut into slices 2 cm thick. To avoid moisture changes during storage due to dehydration of samples, three central slices per loaf were packaged individually using a water vapour and oxygen high barrier plastic film (oxygen transmission rates were: 4 cm^3 m^{-2} 24 h^{-1} bar^{-1} at 23 °C; water vapour transmission rates were: 9 g m^{-2} 24 h^{-1} at 38 °C). A sachet of oxygen absorber (Freshcare, O_2Control, The Netherlands) was placed inside the bags to avoid mould development. The slices were stored under controlled conditions (20 ± 0.5 °C), for textural analyses. The experiment was repeated twice. To produce spianata (a double layered leavened flat bread), the remaining dough (~9 kg) was divided by hand to obtain three pieces, which were passed through a mechanical dough sheeter with an initial gap of 25 mm. Each dough piece was sheeted 9 times, progressively reducing the gap between the rollers until a thickness of 1.5 mm had been reached. The sheeted dough were then shaped using a 22 cm diameter circular mould, placed in an aluminum tray and covered with cotton fabric. The final proofing was conducted under the same fermentation conditions as before for 90 min, and the breads were baked in a tunnel oven at 480 °C. During baking at high temperature the dough swells, due to vapour pressure inside the dough, until splitting into two layers.

The baking time ranged between 20 and 30 s to avoid burning of spianata. The bread was then set aside for cooling for 4 h at room temperature, spianata was packaged (3 breads *per* bag) and stored at the same conditions as pan bread, to avoid moisture changes during storage due to dehydration. Stored samples were used for textural analyses. The experiment was repeated twice.

3.2.2 Chemical-physical analyses

Ash (%), protein (% N 5.7), calculated on dry basis (d.b.), moisture (%), gluten index (GI) (%), dry gluten content (% d.b.), consistograph and alveograph profiles of the LGSs were analyzed as for AACC Approved Methods, 08-12, 46-30, 44-15A, 66-20, 38-12A, and 54-30A, respectively (AACC, 2000).

3.2.3 Texture analysis

A TA.XT2i Texture Analyzer (Stable Micro System Ltd., Godalming, Surrey, UK) equipped with a 30-kg load cell was used to perform the textural analyses on the dough, on pan bread and spianata.

To determine the optimum mixing time in the fork mixer, for each dough sample, a compression test was performed on the dough with a P/50 probe and 10% strain. The parameters set on the instrument were: pre-test, test and post-test speeds 2.0 mm/s, 1.0 mm/s and 10 mm/s, respectively. As soon as the dough started to develop in the mixer, a small sample of the dough (30 g) was collected for texture analysis. Before being analysed, the dough was sheeted and formed into 45 mm diameter discs, with the alveograph equipment (figure 1). Samples were collected at intervals of 1 minute, and analyzed until the value of 3.8±0.3 N as maximum force at the compression test, was measured. This value of force was the same measured on the dough samples mixed at the consistograph until optimum mixing time, as reported in section 2.2. When this value of force was measured on the dough, the mixing was stopped and recorded, and the dough was let to leavening. Stress relaxation tests were carried out on the pan bread slices after removal of the crust. The samples were compressed using an aluminium probe P 36R (36-mm

diameter) with a 10% strain for 40 s and the change in force (N) with time (s) was measured. A pre-test speed of 2 mm/s, test speed of 1 mm/s and a post-test speed of 10 mm/s were used. Force versus time plots were recorded for all data processing. Maxwell model was used to elaborate stress relaxation data (Campus *et al*., 2010). For details on data processing see section 2.2. The stress relaxation test was performed to monitor the crumb hardening over time, at 0, 1, 2, 3, and 6 days of storage. Six bread slices randomly selected were analyzed. For each sample maximum force value (Fmax, N) was detected and correlated with sensory analysis attributes (section 3.2.7.).

A biaxial extensibility test was performed to evaluate texture changes during storage of spianata. The test was carried out using a ball probe and the Tortillas/Pastry Burst Rig (HDP/TPD) platform (figure 2). The parameters set on the instrument were: pre-test 2.0 mm/s; test 1 mm/s; post-test 10 mm/s; distance of penetration 35 mm. Distance to break (mm) parameter was also measured. Analyses were carried out at 0, 1, 2, 3, 6, and 10 days. Six breads randomly selected were analysed.

3.2.4 NIR FT-Raman Spectroscopy

NIR FT-Raman analyses were performed using a back-scattering geometry with a Bruker RAM II FT-Raman module coupled to a Bruker Vertex 70v interferometer. A piece of pan bread crumb and a piece of the inner of the layers in the spianata (~5 g) were used for each measure. The instrument setting and the data analyses were done according to Piccinini *et al*. (2012): the laser excitation wavelength was 1,064 nm, and each spectrum was acquired by averaging 128 interferograms at a resolution of 4 cm^{-1} in the range of 250–3,500 cm^{-1} with a Blackmann-Harris 3-Term apodization function to more precisely determine the peak position and bandwidth of the pyranose ring breathing band that peaked at~480 cm^{-1}; this single band was selected by considering the spectral range of 460–510 cm^{-1}. As residual fluorescence was present, following baseline correction, the band intensity was normalized in this selected range. Finally, post

zero filling was applied to the 460–510-cm^{-1} spectral range, with a zero filling factor of 64, providing a very accurate band profile. With this spectral pre-processing and using the peak-picking function of the Bruker OPUS 6.5 software, the peak position and bandwidth were obtained with an accuracy of 0.01 cm^{-1}, as certified by Bruker Co. Spectrum of OWP was subtracted from the spectra of the breads.

3.2.5 Bread image analysis

Cell size distribution on the crumb of the pan bread was studied as for Fois *et al*. (2012). In summary, images of 6 slices of pan bread for each sample were recorded 4 h after baking using an Epson scanner (Expression 10000XL, Seiko Epson Corporation, Japan) supported by EpsonScan software, version 3.04E. An image in 24-bit colour at a resolution of 400 dots *per* inch, *i.e.*, 1 pixel=$(60)^2$ μm^2 for each slice of pan bread was obtained. Image analysis was performed using the Sigma Scan Pro 5.0 software (Systat Software, Inc., USA). The images were pre-treated by conversion into an 8-bit monochrome image. A threshold method was used for conversion to a binary image to optimize the image analysis. For each image a square (50 mm×50 mm) in the top half of the pan bread slice was selected. The number of gas cells and the area (mm^2) of each gas cell were determined. The cell size distribution was modelled by a log-normal distribution across the full range of cell areas, which ranged from 10^{-3} to 10^1 mm^2. Table curve 2.D v.5.01 (Systat Software Inc. San Josè, California, USA) was used to process data. The percent explained variation (R^2) was >0.96.

3.2.6 Colour determination

Browning index was determined on the surface crust of the pan bread samples. It was measured using a CM-700d spectrophotometer (Konica Minolta Sensing, INC, Osaka, Japan) with a D65 illuminant and a 10°CIE standard observer angle. L *(lightness), a*(redness) and b*(yellowness) coordinates were obtained. Before measurement, the spectrophotometer was calibrated against the white tile supplied

with the instrument. Measurements were taken after 1 day of storage; readings were performed on tree different points of each sample.

3.2.7 Sensory analysis

CATA test was performed on eight pan bread samples. The panel was composed by 56 consumers (57% male, 43% female). Each consumer evaluated all the breads using a questionnaire containing 18 attributes, most of them already used for bread sensory evaluation (Comendador 2012). The consumers were asked to select only the attributes they considered appropriate. In addition, consumers judged the overall liking and propensity to purchase using a 9-point scale (Giménez *et al*., 2013). The 18 attributes used are the following: sticky, good to accompany, smell of dairy products, very good for diet, unpleasant smell, salty, dry, soft, nice colour, soft to touch, healthy, innovative product, sweet, springy to touch, good for nutrition, smell of traditional bread, rubbery and ideal for breakfast.

For sensory analysis, pan breads, after cooling for 4 hours, were cut in slices of 20 mm of thickness, and slices were frozen. One hour before the sensory session, samples were thawed and 8 different slices were presented to the consumers, one after another at intervals of 5 minutes, and the evaluation was carried out in a randomised and balanced order (Ares *et al*., 2015) between and within participants. Consumers were provided with water as palate cleansers between samples.

3.2.8 Statistical analysis

Statistical analyses were performed using Statgraphics Plus, ver. 5.1 (StatPoint Technologies Inc, Warrenton, Virginia). The data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test ($p \leq 0.05$) was used to separate means. Pearson's correlation coefficient between variables, where pertinent, was calculated.

For sensory analysis the data were analyzed by using the Cochran's Q test ($p < 0.05$) to evaluate the significant differences among samples for each of the

attributes used. Correspondence analysis was performed to obtain a sensory map among samples and CATA attributes. One-way analysis of variance (ANOVA) was carried out to evaluate significant differences among samples for the overall liking and purchase intention attributes. Pearson's correlation coefficient between chemical and texture parameters and sensory attributes, where pertinent, was calculated. Data were performed with the software XLSTAT.

3.3 Results and Discussion

Chemical, physical and rheological properties of LGS and OWP are shown in table 2. As expected, 48T LGS had a higher gluten tenacity and extensibility than 4T LGS, as can be seen from gluten index and alveographic values. The time needed to reach maximum consistency at the consistograph (*i.e*., TPrMAX, s) and the level of hydration (HYDHA, %, 15% moisture basis) are keys parameters to maximize the leavening performance of the dough, which in turn should be correlated with bread volume. Mixing times and hydration values for the recipes used in the experiments are reported in table 3. In this experiment the optimum hydration level decreased with the addition of OWP, in agreement with what reported by other authors (Kenny *et al*., 2000; Indrani *et al*., 2007). TPrMAX was highly correlated with the percentage of OWP added, indicating that the presence of OWP complicated the formation of the gluten network in the dough thus affecting the mixing time (table 3). Erdogdu-Arnoczky *et al*. (1996) used heat treated whey protein in bread making trials and reported that the complete denaturation of whey protein eliminates the interference of native protein with gluten development. In this study no heat treatment on OWP was performed. Mixing time reported in table 3 refers to data collected during mixing at the fork mixer (section 3.2.3.). As we can note mixing time increased with the addition of OWP, and this is because the dough stability increased with the addition of OWP, as demonstrated by the decrease in the D450 (s) (figure 3 and 4 in section 2), therefore more time was needed to reach the sought after consistency.

Pan bread volumes are reported in figure 3. As expected, 4TC produced pan bread with a significantly lower volume than 48TC, due to the well known weakness of

gluten in Cappelli variety. In fact 4T LGS contained 100% of Senatore Cappelli variety. Moreover, 4TC showed a lower protein content than 48TC (minus 1.8%; table 2), and this can also explain the lower volume of bread. A significant volume reduction was observed in 48T pan breads supplemented with either 10 or 15% of OWP, with respect to their control, whereas the addition of 5% OWP did not have significant effects. On the contrary, 4T pan breads volume was significantly higher in 5% OWP sample, with respect to the 4T control (figure 3 and 4), whereas the addition of 10 and 15% of OWP did not decrease the volume of the bread, which was the same as the control.

The reason of such phenomenon can be found in the rheological properties of the dough. In fact, analyses of stress relaxation behaviour of the dough formulations showed that the stress relaxation constants (C1, C2, and C3) and the residual modulus (σ_e) increased with the OWP addition (section 2.3.2). This means that the dough became firmer, stiffer and more elastic and solid, whereas the relaxation times did not vary after the addition of OWP. As already discussed in section 2, the addition of OWP had a detrimental effect on the leavening process, because of its strengthening effect on the gluten matrix, which then showed a great resistance to the extension required by the gas production during the fermentation process. During baking, the gluten matrix suffers the stress of the oven spring that is a further expansion of the dough. Then, it can be suggested that in 48T samples the strengthening effect of the OWP addition on the gluten matrix was such that it did oppose to the oven spring. Such detrimental effect of extra strong gluten on dough expansion was already reported (Fois *et al*., 2012). On the other hand, 4T samples, which showed a less elastic and tenacious gluten than 48T samples, did benefit of the above mentioned strengthening effect of OWP on the gluten matrix. The OWP addition gave to 4T breads during baking enough strength and elasticity to sustain the oven spring without collapsing, as happened in the 4TC (figure 3 and 4).

Quality characteristics of breads were monitored over 6 days for pan bread, and over 10 days for spianata. Data of the moisture of bread, reported in table 4, refers only to the initial moisture, at T0, because, as expected moisture values did not

change during storage due to the packaging system. In pan bread, moisture values differ according to the percentage of OWP, since samples had different initial amount of water in recipe, according to optimum hydration calculated at the consistograph (table 3). On the contrary, in spianata, moisture values were not different among samples, despite the dough had been hydrated according to HYDHA values, probably due to the particular shape of the bread and to the baking at very high temperature. In fact, the bread is very thin and all the water within the two layers converts into vapour in few seconds due to the very high temperature.

Staling of pan bread and spianata was monitored with texture analyses and a Raman spectroscopy technique. Data of spianata are reported in table 5 and data of pan bread are reported in table 6. The Raman frequency of the band peaked at ~480 cm^{-1}, was used as indicator of bread staling. Its value, as measured in the crumb across the storage, decreased as a consequence of the retrogradation of amylopectin, in all treatments on spianata and in pan bread, according to what found by Piccinini *et al*. (2012). They reported that during storage the peak frequency of this band shifted towards lower wave numbers, reflecting the increasing cristallinity of starch.

Data of peak frequency in controls (4TC and 48TC) and OWP15 spianata samples are reported in figure 5, to give a better view of the retrogradation phenomenon. It can be observed that the retrogradation follows a linear pattern in the first 4 days for both samples (figure 5A and B). In 4T samples (figure 5A) at T0 the Raman frequency value was higher in the samples added with OWP (+0.22 wave numbers) than the control, indicating a lower level of retrogradation, and this effect was more evident at T1 (+0.32 wave numbers), and T2 (+0.23 wave numbers).

A different trend was observed by comparing data across 10 days of storage in 48TC and 48TOWP15 samples. 48TOWP15 showed a lower values of Raman frequency in the first 4 days of storage (figure 4B), thus indicating a higher level of amylopectin retrogradation.

Data from Raman spectroscopy were then compared with data collected with the texture analyzer and reported in table 5 for spianata. In a previous study done at our laboratory on spianata flat bread, it was found that when the distance to break falls below the value of 18 mm the bread was not accepted by the consumers (Secchi *et al*., 2008), because it is not more rollable without breaking. The addition of OWP had the effect of improving the texture in 4T samples, as can be seen by the increase of the distance to break, and improving the time of acceptability, which changed from 2 days in 4TC sample, to 4 days in 4TOWP5 and 4TOWP10, where the distance to break was higher than 18 mm (table 5). On the contrary the addition of OWP in 48T in spianata samples did not improve the time of acceptability, which remained above the value of 18 mm for three day in 48TC, and in 48TOWP5 and 48TOWP10.

In figure 6 the evolution of the distance to break over 10 days of storage for 4TC and 48TC and OWP15 are reported, to give a better view of differences between the samples at high concentrations of whey proteins. In 4T (figure 6A), the addition of OWP resulted in the significant increase in distance to break values at T0, T1 and T2, with respect to the control, due to the intrinsic extensibility properties of the gluten of Cappelli, which was strongly reinforced by the whey powder. In 48T samples the contrary was detected: 48TOWP15 sample showed lower values of distance to break (mainly at T1 and T2) and a lower time of acceptability than the 48TC (1 day instead of 2 days). Samples with and without OWP show a similar trend in distance to break values between 3 and 10 days of storage in 4T and at T6 and T10 in 48T.

In spianata samples, data from texture analyses are in accordance with data from Raman spectroscopy, since a lower value of distance to break corresponded to a lower value of frequency, indicating that the level of starch retrogradation was responsible for the texture properties of the spianata.

In pan bread, the shift of the Raman frequency over 6 days of storage for 4T and 48T control and OWP15 are reported in figure 7. Important differences were detected between 4T and 48T in the starch retrogradation pattern after the addition of OWP. 4TOWP15 and 4TC showed the same values of Raman frequency all

over the storage time (figure 7A). Retrogradation occurred with a linear pattern in the first 4 days of storage in all samples. Notably, the addition of OWP in the 48T pan bread (figure 7B) led to significantly lower values of Raman frequency *(i.e.*, higher level of retrogradation of the amylopectin), but the retrogradation has proceeded with a similar pattern in the 48TC and in the OWP samples. Previous work showed a strong correlation during storage between crumb hardness and Raman frequency (Piccinini *et al*., 2012) in a semolina based pan bread. In this study a generalized Maxwell model was used to describe stress relaxation behaviour of bread crumb in the pan bread samples, and stress relaxation constants C_i (N) were calculated, as for Campus *et al*. (2010) (table 6). The Maxwell model showed a significant good representation of experimental data (R^2>0.987). Trend of C1 value during storage for controls and OWP15 samples is shown in figure 8A (4T) and B (48T). In 4T pan bread differences between Ci values of control and OWP15 were not significant. On the contrary C1 values were significantly higher in 48TOWP15 samples with respect 48TC, indicating a firmer crumb. Data were consistent with data from Raman analyses, which showed no significant differences between control and OWP15 in 4T, and lower Raman frequency corresponding to a higher level of retrogradation in 48T samples added with OWP.

Pan bread crumb image analysis was performed to compare cell size distribution among samples. Data of controls and OWP15 samples are reported in figure 9 and 10, where *x* (cell area in mm^2), and *y* (the complementary cumulative distribution function) were plotted on a log-log scale. Data of the areas and the numbers of the gas cells were used to calculate the complementary cumulative distribution function (CCDF), as described in Fois *et al*. (2012). In summary, the CCDF = 1-CDF (cumulative distribution function) is the integral of the probability distribution function. No significant differences (p>0.05) were found between the log-normal parameters of the 4TC and the 4TOWP15 sample, indicating that the gas cell size distribution was the same. In figure 9A we can see that the curves completely overlap. This is in agreement with data previously reported. In fact the volume of the bread and the texture of the crumb in 4TC and 4TOWP15 was the

same (figure 3 and 8A). On the contrary in 48T the addition of OWP at 15% had an effect on the cell size distribution. As it can be observe in figure 9B the 48TC and 48TOWP15 curves overlap only in the first part, which regard the small cells having an area <1.E-01. After that value of *x*, the curves are different, and in the 48TC curve the probability (*y*) of finding a cell size greater than a given x value across the curve was higher than in the OWP15, this indicates that in the 48TC sample the crumb structure was less dense and compact, with a great probability of finding medium and great cell sizes. Again these data are in agreement with the volume of bread, which in 48OWP15 was significantly lower than 48TC (figure 3), and with crumb texture, which in 48OWP15 was firmer than 48TC (figure 8B). Note that the addition of 5% of OWP had a different effect on the cell size distribution (figure 10). In both 4T and 48T cell size distributions, the control and the OWP5 curves overlap, as before, with the difference that the 4TC and 4TOWP5 overlap only in the first part (figure 10A), whereas the 48TC and 48TOWP5 overlap for a greater range of x values (figure 10B), and differ mainly for the biggest cell sizes, that is in the tail of the curve. Unlike what seen before for OWP15, the 4TC and 48TC curves remained well below the OWP5 curves, indicating that the medium-great size distribution was different, but in a way that in the controls there were a lower probability of finding medium and great cell size than OWP5 samples. This means that the addition of 5% of OWP improved crumb structure, as was also demonstrated by the volume of pan bread and the values of Ci (figure 4 and table 6), mainly in the 4T sample.

The CATA test (Check All That Apply) (Varela *et al*., 2012), a recent rapid methodology, was performed to obtain sensory profiles of the eight samples of pan bread produced. Moreover a judgment, expressed by the consumers, of overall liking and purchase to intention, was associated to the CATA data. Cochran's Q test highlighted significant differences for 11 out of 18 attributes, among the pan bread samples (table 7). 4T OWP5 sample was evaluated less rubbery and dry and softer to touch, of nice colour, soft and good to accompany than the other samples. Correspondence analysis (figure 11) confirmed this behaviour, since OWP samples were distributed along the PC1 and were

described by the following attributes: unpleasant smell, smell of dairy products, sticky, spring to touch, rubbery and sweet.

4TOWP5 sample was differentiated from the PC2, confirming the results obtained from Cochran's Q test. Consumer overall liking and propensity to purchase data revealed that 4TOWP5 was the most preferred (table 8). Pearson's correlation between textural data and sensory analysis showed that textural parameter (Fmax) was positively correlated with rubbery and sticky whereas salty and was negatively correlated with good for nutrition (table 9).

Finally, crust index of browning was positively correlated with smell of dairy products, which are considered not good qualities for bread products. No correlation was found between the crumb colour and the sensory descriptors. High values of moisture are positively correlated with smell of traditional bread and very good for diet, on the contrary low values of moisture are correlated with negatives attributes as unpleasant smell and smell of dairy products. At last, as expected, overall liking is positively correlated with the propensity to purchase, whereas negative descriptors (rubbery, dry, unpleasant smell, smell of dairy products, sticky) are negatively correlated with it.

3.4 Conclusions

The addition of OWP to the dough formulation had different effects on bread, depending on the quality characteristics of the raw material, the kind of bread produced, and the amount of OWP added. LGS with a weak gluten, as was the case of our 4T LGS, produced a bread with a lower volume than the low grade semolina characterized by a strong and tenacious gluten (48T LGS), as expected. The addition of OWP led to a strengthening effect on the gluten matrix, with a positive effect on 4T samples, with the weak gluten, and a negative effect on the strong gluten of 48T samples, which became too strong. Therefore, the volume of pan bread in 4T sample was higher with the addition of a low percentage of OWP (5%), and was not affected by the addition of higher concentration of OWP (10 and 15%). The contrary happened in 48T samples, where the addition of OWP

had a detrimental effect on bread volume. Crumb characteristics, measured in term of firmness and cell size distribution, also were improved in 4T samples with 5% of OWP, and were not affected at higher concentrations of OWP. 4TOWP5 was also the most preferred samples by consumers. In the typical Sardinian flat bread spianata, the addition of OWP improved the shelf life in terms of textural characteristics. This study demonstrates that the use of OWP was able to improve the bread making quality of the variety characterized by weak gluten, as Cappelli is. This result could be exploited to verify the effect of the addition of OWP to old durum wheat varieties, which are generally characterized by having weak and sticky gluten.

3.5 Figures and tables

Table 1. Dough formulations (% on dough basis)

Samples	LGS (%)*	OWP (%)	Water (%)	Salt (%)
4T C	67.25	0.00	31.93	0.82
4TOWP 5	65.57	3.45	30.21	0.77
4TOWP 10	63.60	7.07	28.60	0.73
4TOWP 15	61.33	10.82	27.15	0.70
48T C	67.79	0.00	31.41	0.81
48TOWP 5	65.82	3.50	29.94	0.77
48TOWP 10	64.05	7.10	28.11	0.72
48TOWP 15	61.28	10.80	27.21	0.70

* =% on dough weight-basis

Figure 1. Compression test performed on dough samples.

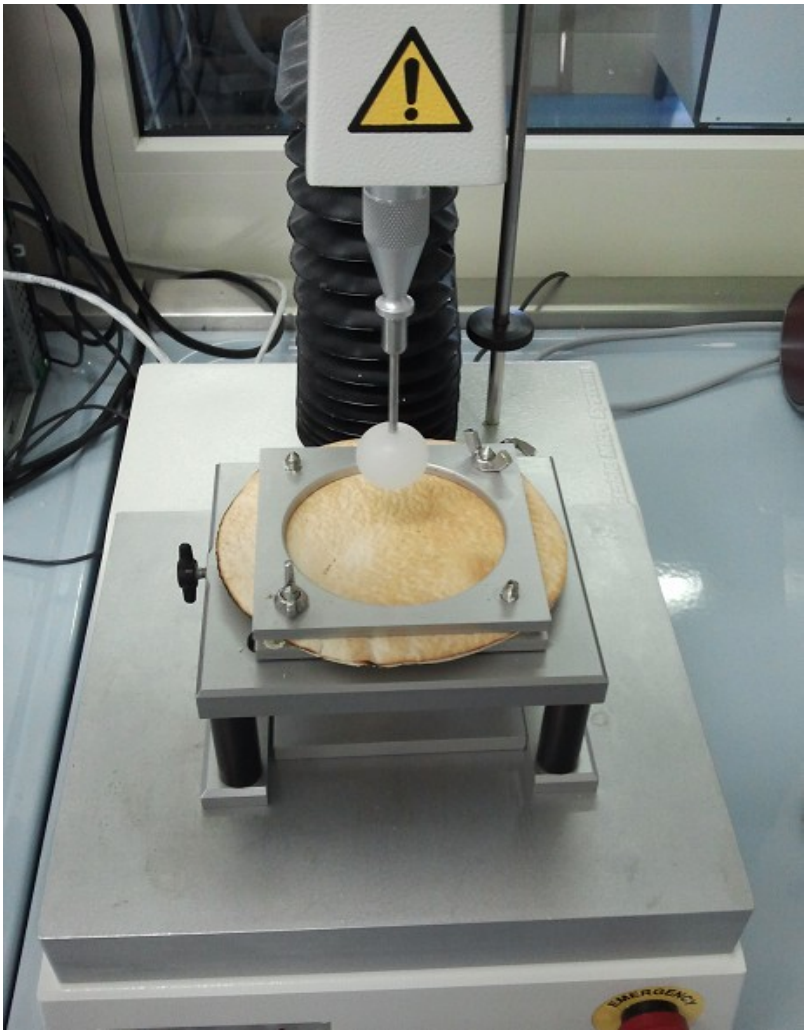

Figure 2. Extensibility test performed on spianata flat bread.

Table 2. Properties of Low Grade Semolina (LGS) and ovine whey powder (OWP) used in experimental mixing (Secchi, 2015).

Batch	Ashes (% d.b.*)	Protein content (% d.b.)	Moisture (%)	Gluten index (%)	P** (mm)	L** (mm)	W** ($J*10^{-4}$)	P/L**
LGS4T	1.02±0.02	11.08±0.09	15.45±0.10	56.00±0.01	59.9±1.8	49.0±6.9	91.9±6.4	1.24±0.2
LGS48T	1.03±0.02	12.86±0.48	15.15±0.05	92.23±0.02	98.2±2.1	45.8±5.7	173.7±8.9	2.18±0.3
OWP	8.50±0.05	15.11±0.15	4.05±0.12	-	-	-	-	-

* d.b, dry basis. **Alveograph parameters at constant hydratation: P, maximum overpressure; L, index of swelling; W, deformation energy; P/L, configuration ratio. Data were expressed as mean ± standard deviation.

Table 3. Properties of dough samples.

Sample	TPrMAX (s)	HYDHA (% 15% mb)	Mixing time (min)	Protein content (%)
4T C	110.5 d	50.3 a	17 e	11.07 e
4TOWP 5	147.5 cd	45.0 c	20 d	11.65 d
4TOWP 10	234.5 bc	39.9 e	23 c	12.10 cd
4TOWP 15	334.5 ab	36.8 g	30 b	12.72 b
48T C	150.0 cd	48.5 b	21 d	12.86 b
48TOWP 5	218.5 c	44.2 d	27 b	12.53 bc
48TOWP 10	398.0 a	39.3 f	35 a	12.97 ab
48TOWP 15	381.5 a	36.1 h	38 a	13.72 a

Samples with different letters are significantly different at $p<0.05$ according to Duncan's multiple range test.

Table 4. Initial moisture values in bread samples.

Sample	Pan bread moisture (%)	Spianata bread moisture (%)
4TC	42.45 a	26.43 a
4T15 5	39.83 b	27.14 a
4T1510	37.85 c	25.88 a
4T1515	37.05 d	25.43 a
48TC	41.40 a	27.17 a
48T15 5	39.66 b	27.72 a
48T1510	38.61 c	26.53 a
48T1515	37.24 d	26.44 a

Samples with different letters are significantly different at $p<0.05$ according to Duncan's multiple range test.

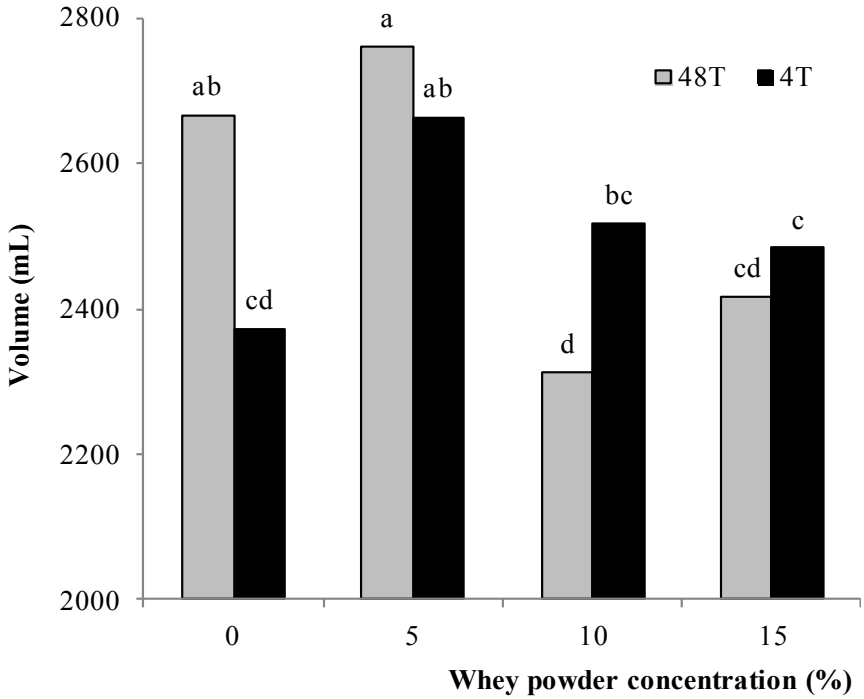

Figure 3. Volume of pan bread (mL).

Samples with different letters are significantly different at $p<0.05$ according to Duncan's multiple range test.

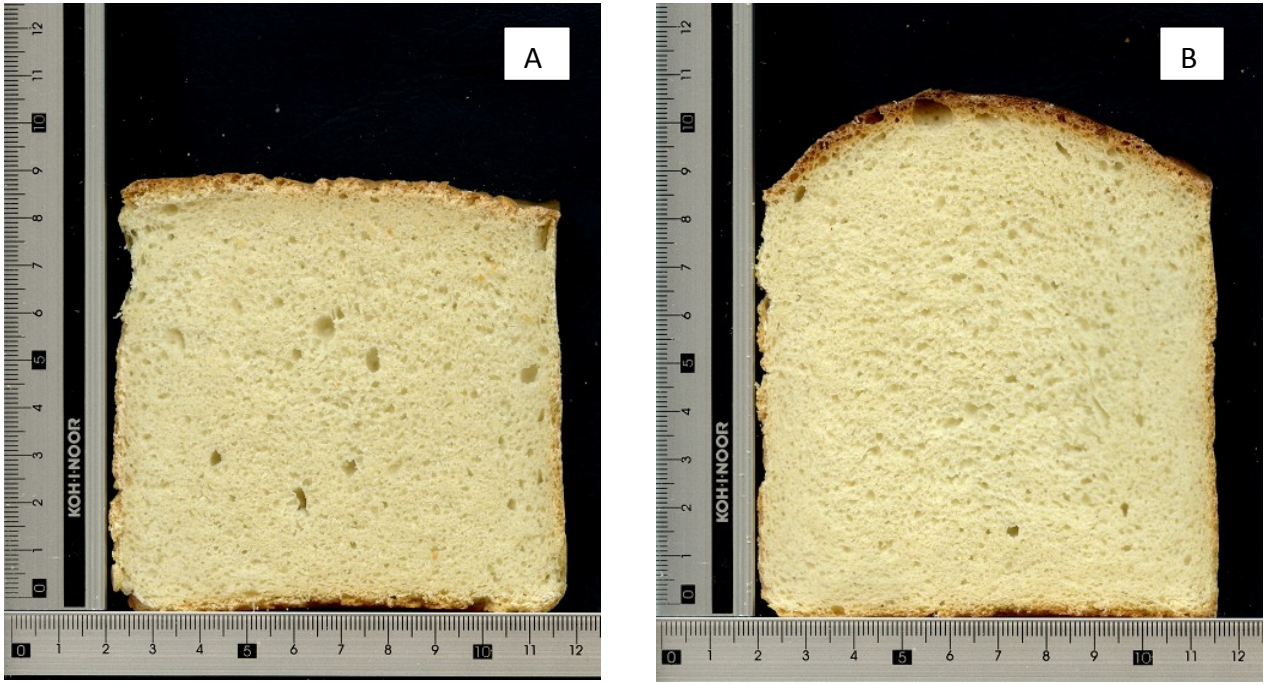

Figure 4. Pan bread sections. A= 4TC; B=4TOWP5.

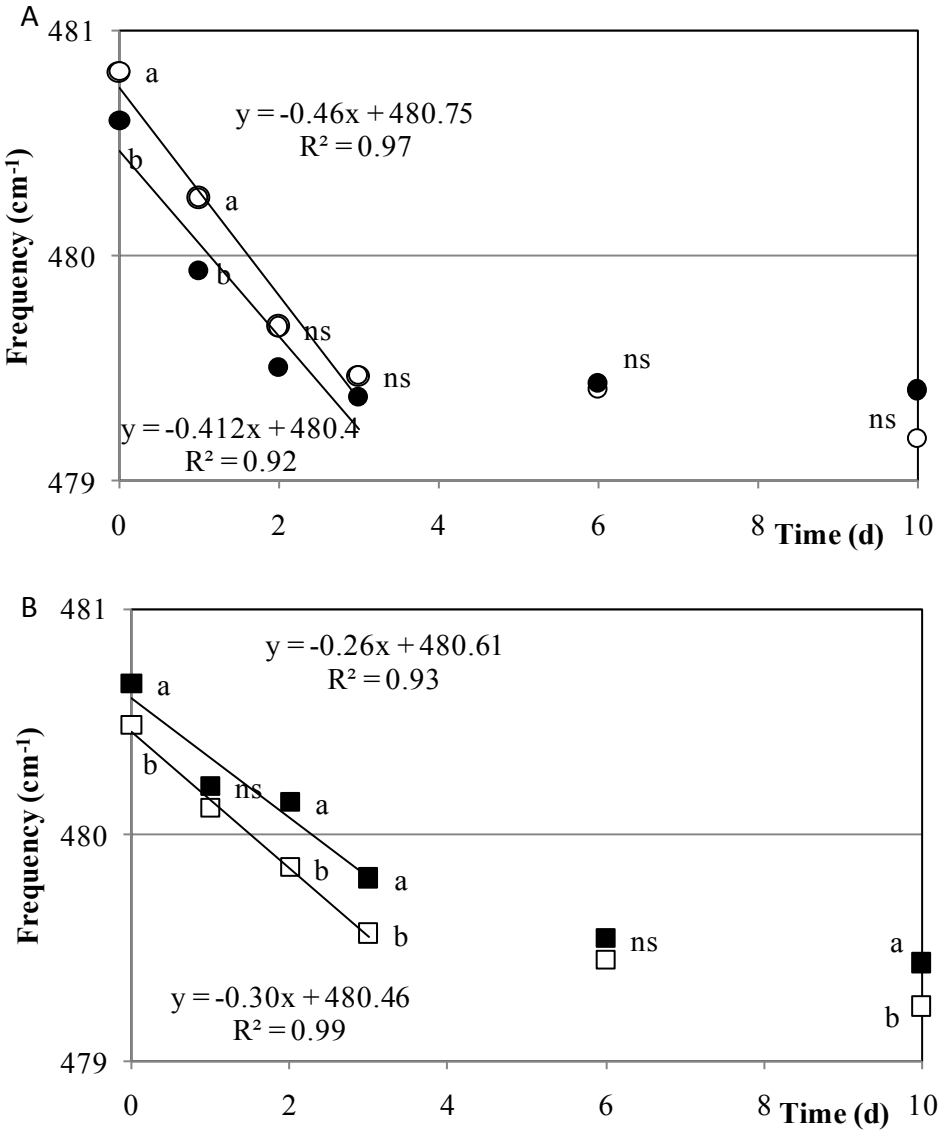

Figure 5 A, B. Variations in peak frequency of the Raman band at ~480 cm^{-1} during storage in spianata. 4T control (●), 4TOWP15 (○),48T control (■), 48TOWP15 (□).

Samples with different letters for each time, are significantly different at $p<0.05$ according to Duncan's multiple range test. ns: not significant at $p<0.05$.

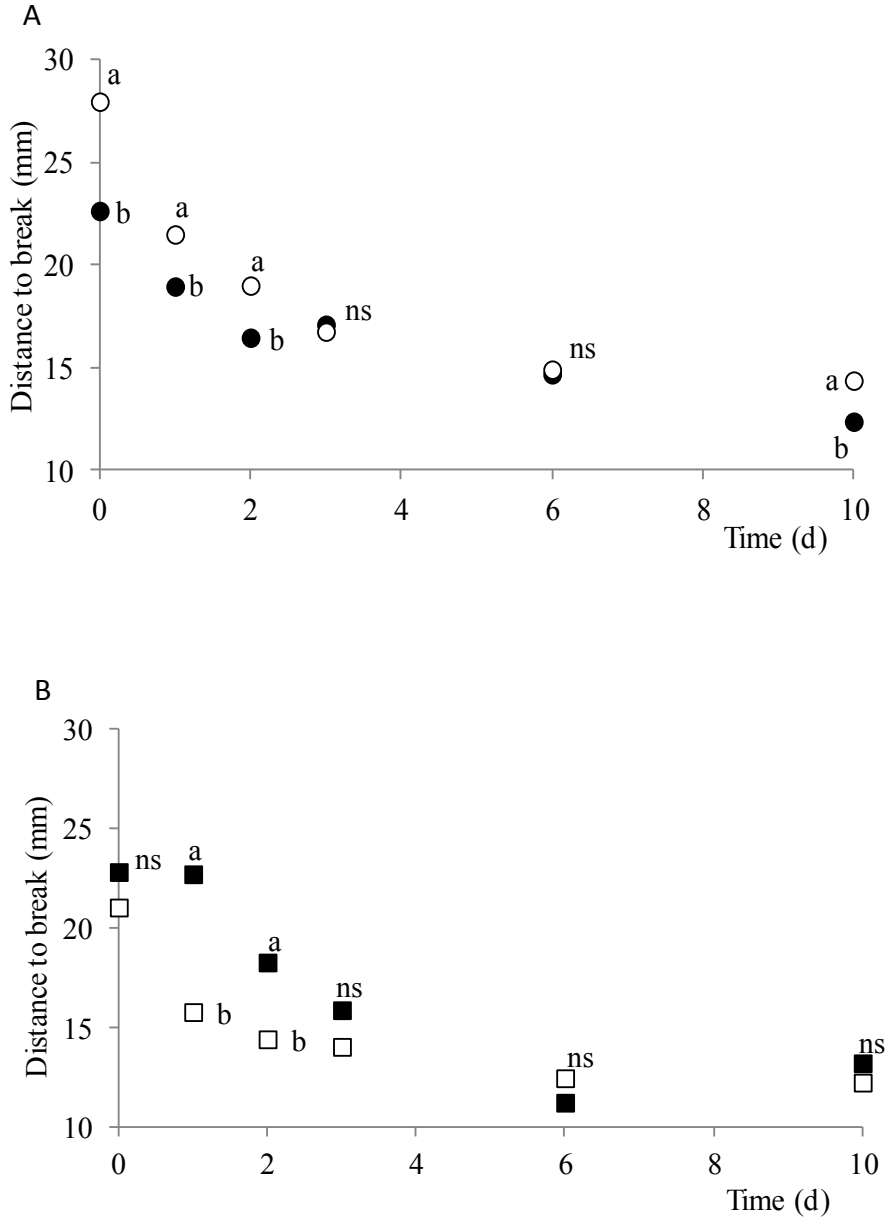

Figure 6 A, B. Texture evolution in spianata during storage. 4T control (●), 4TOWP15 (○),48T control (■), 48TOWP15 (□) .

Samples with different letters for each time are significantly different at $p<0.05$ according to Duncan's multiple range test. ns: not significant at $p<0.05$.

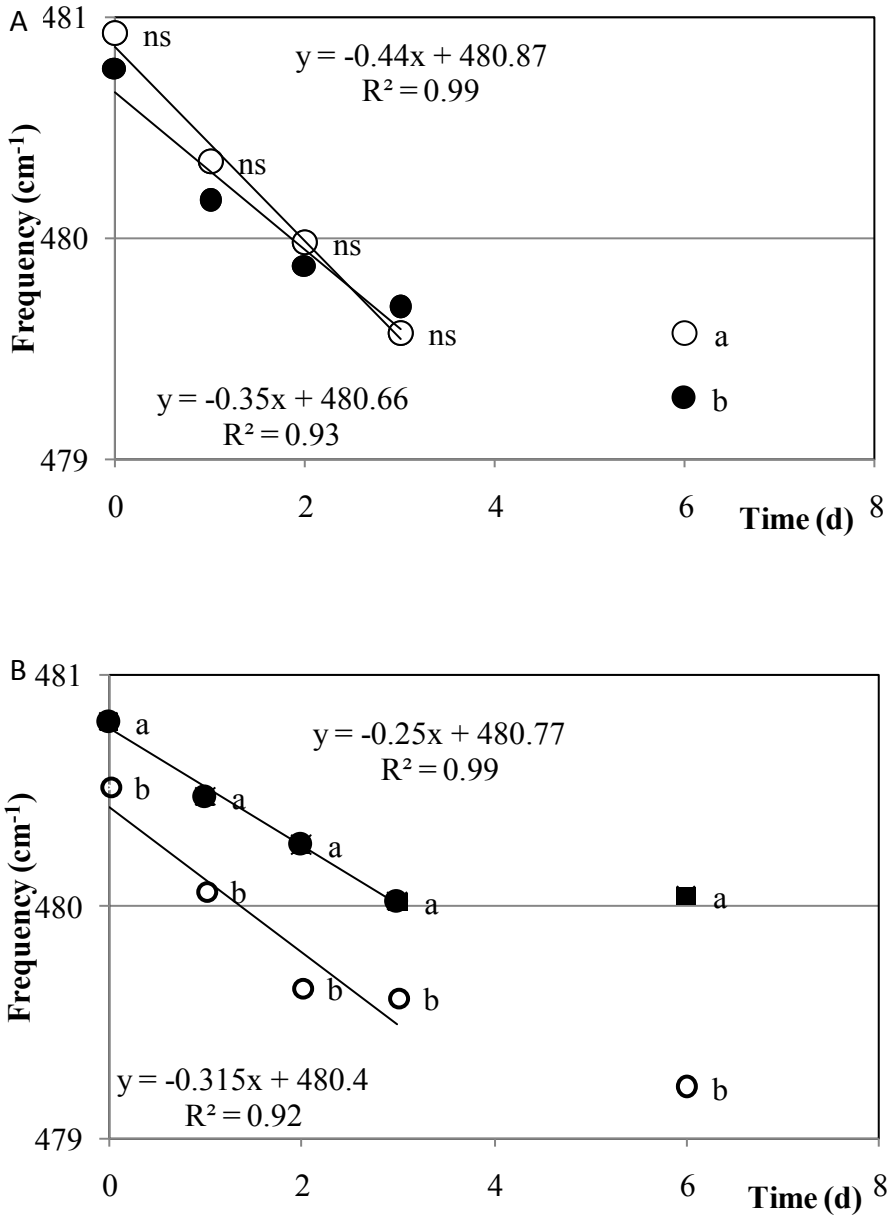

Figure 7 A, B. Variations in peak frequency of the Raman band at ~480 cm^{-1} during storage in pan bread. 4T control (●), 4TOWP15 (○),48T control (■), 48TOWP15 (□).

Samples with different letters for each time are significantly different at $p<0.05$ according to Duncan's multiple range test. ns: not significant at $p<0.05$.

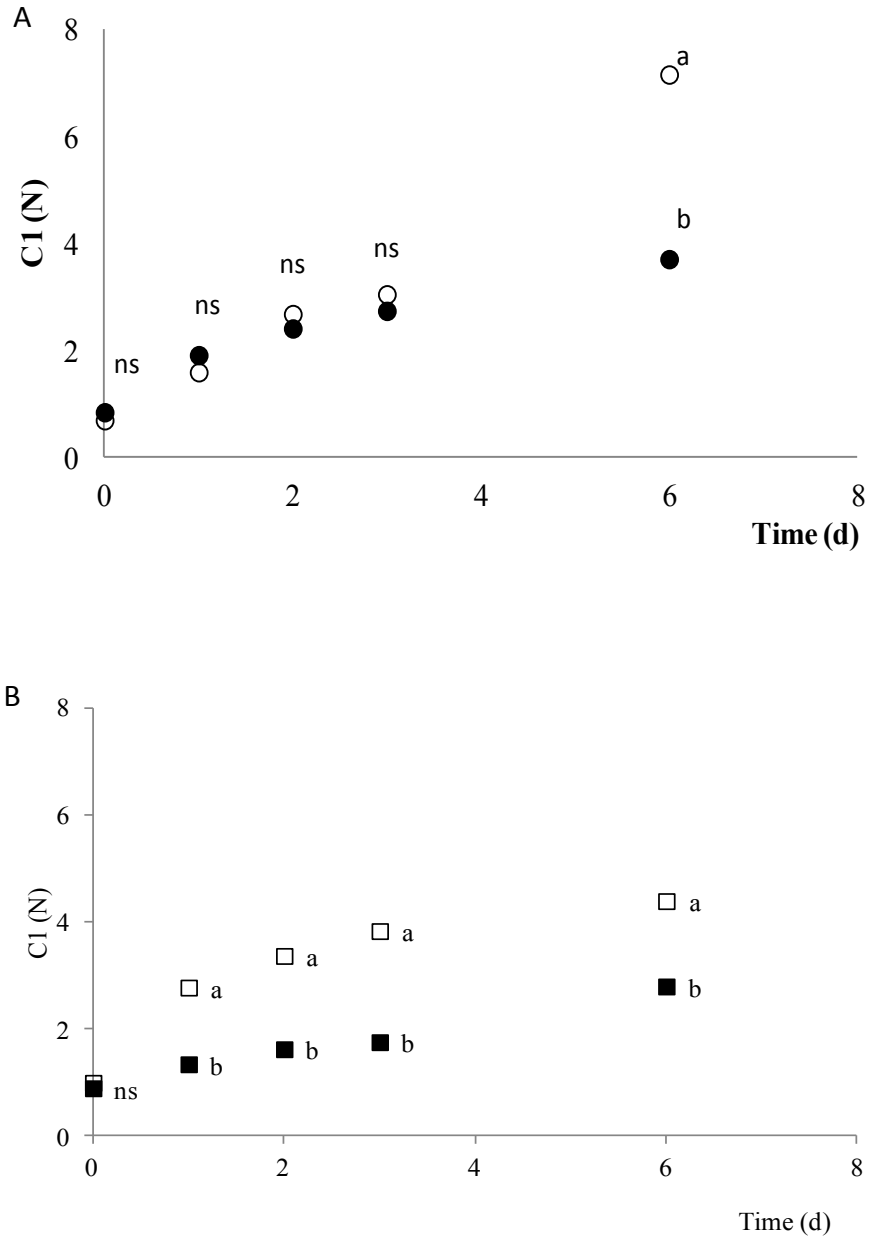

Figure 8 A, B. Texture expressed as decay force of Maxwell model during storage in pan bread. 4T control (●), 4TOWP15 (○),48T control (■), 48TOWP15 (□).

Samples with different letters for each time are significantly different at $p<0.05$ according to Duncan's multiple range test. ns: not significant at $p<0.05$.

Table 5. Staling of spianata: data from texture analysis and Raman spectroscopy.

Sample	Storage time (d)	Distance to break (mm)	Raman Frequency (cm^{-1})	Sample	Storage time (d)	Distance to break (mm)	Raman Frequency (cm^{-1})
4TC	0	22.64aBC*	480.60aC	48TC	0	22.14aBC	480.67aBC
	1	18.96bB	479.93bCD		1	23.06aA	480.21abBC
	2	16.68cC	479.45bC		2	18.28bBC	480.14acA
	3	16.88cAC	479.37bB		3	15.88cBC	479.81bdA
	6	14.69cdB	479.43bNS		6	11.22dD	479.54cd
	10	12.38dB	479.40bNS		10	13.21cdAB	479.43d
4TOWP5	0	27.45aA	480.73aBC	48TOWP5	0	21.52abC	480.29aD
	1	23.90bA	480.40abAB		1	21.68abA	479.66bD
	2	19.04cAB	479.85bcB		2	24.93aA	479.77bBC
	3	18.02cAB	479.77bcB		3	16.94cdAC	479.55bB
	6	13.89dCD	479.70c		6	16.88bcA	479.57b
	10	14.35dA	479.58c		10	13.60dAB	479.48b
4TOWP10	0	27.06aA	481.05aA	48TOWP10	0	25.49aAB	480.31abD
	1	21.60bA	479.80bCD		1	19.71bAB	480.52aA
	2	19.51bAB	479.76bBC		2	20.36bA	479.90bcB
	3	19.00bA	479.49bB		3	15.86cBC	479.40dB
	6	16.25cA	479.51b		6	13.31dCD	479.36d
	10	14.94cA	479.34b		10	12.69dB	479.72cd
4TOWP15	0	27.96aA	480.82aB	48TOWP15	0	21.05aC	480.48aD
	1	21.50bA	480.25abAB		1	15.79bC	480.11abBC
	2	19.01bAB	479.68bcBC		2	14.42bcD	479.85acB
	3	16.77cdAC	479.47cB		3	14.03cC	479.56bcB
	6	14.92dB	479.40c		6	12.46dD	479.44bc
	10	14.38dA	479.19c		10	12.24dB	479.24c

* Data for each column followed by different letters within each batch (lower case letter) and within each storage time (capital letters) differ significantly according to Duncan's Multiple Range Test at $P<0.05$.

Table 6. Staling of pan bread: data from texture analysis and Raman spectroscopy.

Sample	Storage time (d)	C1 (N)	Raman Frequency (cm^{-1})	Sample	Storage time (d)	C1 (N)	Raman Frequency (cm^{-1})
4TC	0	0.69dBC*	480.70aA	48TC	0	0.90dB	480.80aA
	1	1.59cdB	480.07bB		1	1.34cBC	480.47aA
	2	2.68bcB	479.93bB		2	1.63bD	480.27bA
	3	3.05bAB	479.68 bB		3	1.76bD	480.02bA
	6	7.17aA	479.27cC		6	2.80aE	480.05bA
4TOWP5	0	0.70dBC	480.34aB	48TOWP5	0	0.88dB	480.57aB
	1	1.40cBC	479.75bBC		1	1.40cBC	479.64bC
	2	2.53bB	479.70bBC		2	2.12bC	479.66bBC
	3	2.83bB	479.53bB		3	2.18bC	479.69bB
	6	4.35aB	479.58bB		6	3.05aD	479.61bB
4TOWP10	0	0.65eBC	480.59aB	48TOWP10	0	1.33dA	480.71aA
	1	1.57dB	479.70bBC		1	2.51cA	480.31bcA
	2	2.54cB	479.52bC		2	2.68cB	479.81bcB
	3	3.11bAB	479.56bB		3	3.41bA	479.25cC
	6	4.61aB	479.32bC		6	4.67aB	479.27cC
4TOWP15	0	0.84dB	480.92aA	48TOWP15	0	1.00dAB	480.52aB
	1	1.91cAB	480.14bB		1	2.78cA	480.07abB
	2	2.41bB	479.98bcB		2	3.37bA	479.65bcBC
	3	2.74abB	479.57cB		3	3.83bA	479.61bcB
	6	3.71aC	479.57cB		6	4.71aB	479.22cC

* Data for each column followed by different letters within each batch (lower case letter) and within each storage time (capital letters) differ significantly according to Duncan's Multiple Range Test at $P<0.05$.

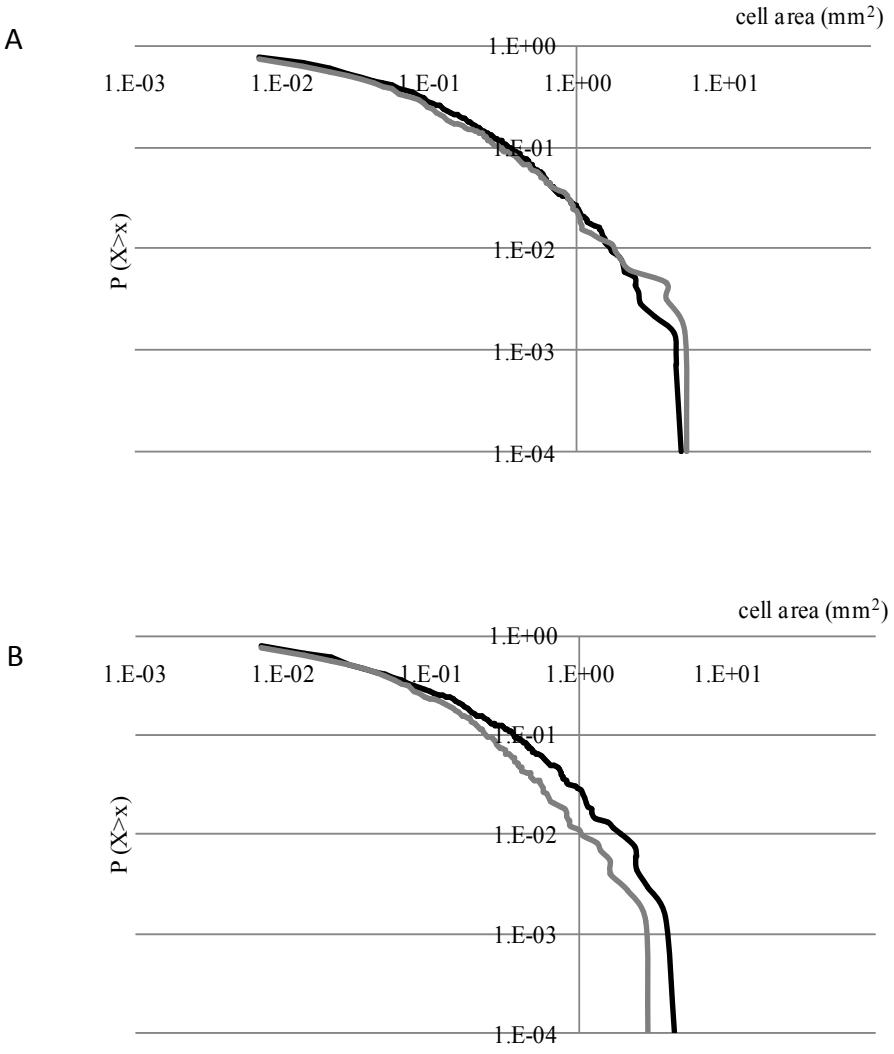

Figure 9 A, B. Complementary cumulative density function analysis in pan bread crumb. A: 4T control (▬), 4TOWP15(▬); B: 48T control (▬), 48TOWP15 (▬).

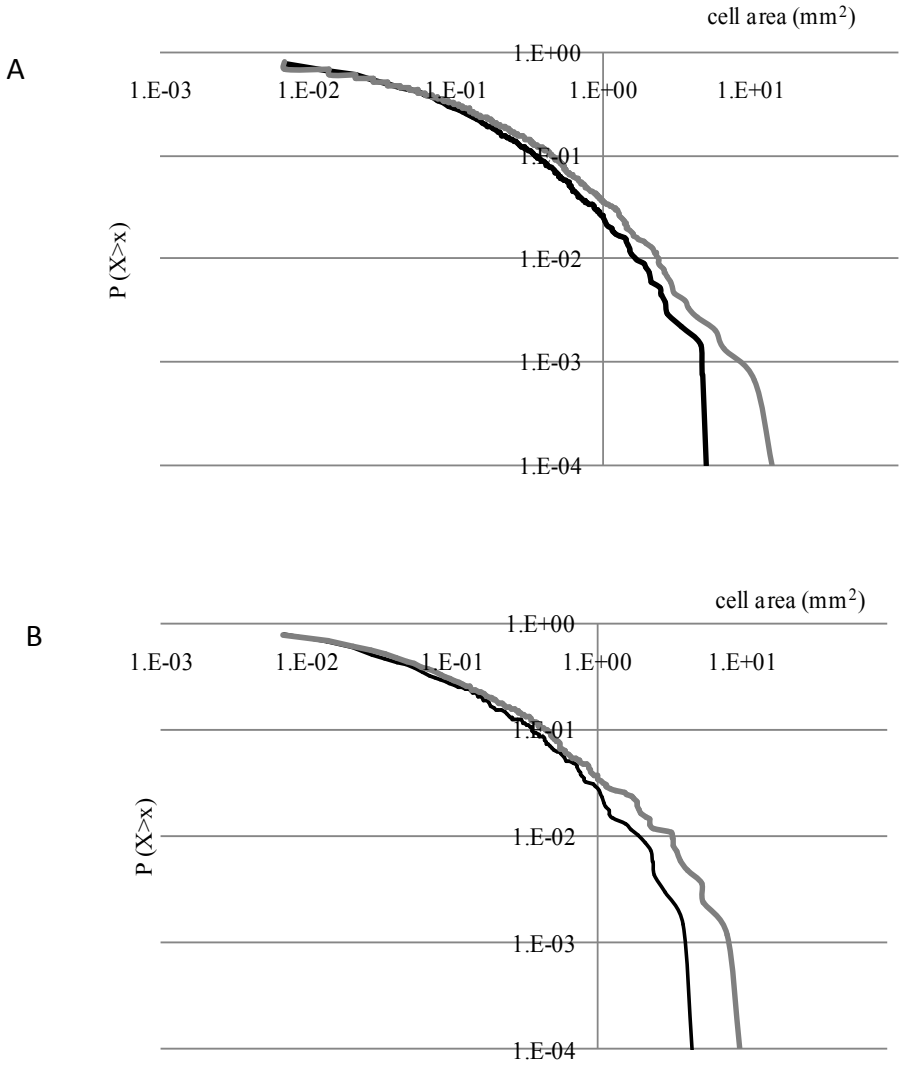

Figure 10 A, B. Complementary cumulative density function analysis in pan bread crumb. A: 4T control (━), 4TOWP5(━); B: 48T control (━), 48TOWP5 (━).

Table 7. Cochran's Q test results in bread attributes.

Attribute	P-VALUE
Sticky	0.596
Good to accompany	**0.003***
Smell of dairy products	**< 0.0001**
Very good for diet	0.116
Unpleasant smell	0.061
Salty	0.429
Dry	**0.000**
Soft in the mouth	**< 0.0001**
Nice colour	**0.013**
Soft to touch	**< 0.0001**
Healthy	0.982
Innovative product	0.130
Sweet	**< 0.0001**
Springy to touch	**0.031**
Good for nutrition	0.497
Smell of traditional bread	**< 0.0001**
Rubbery	**0.009**
Ideal for breakfast	**0.013**

* Attributes are significantly different among the bread samples at $p<0.05$ according to Cochran's Q test.

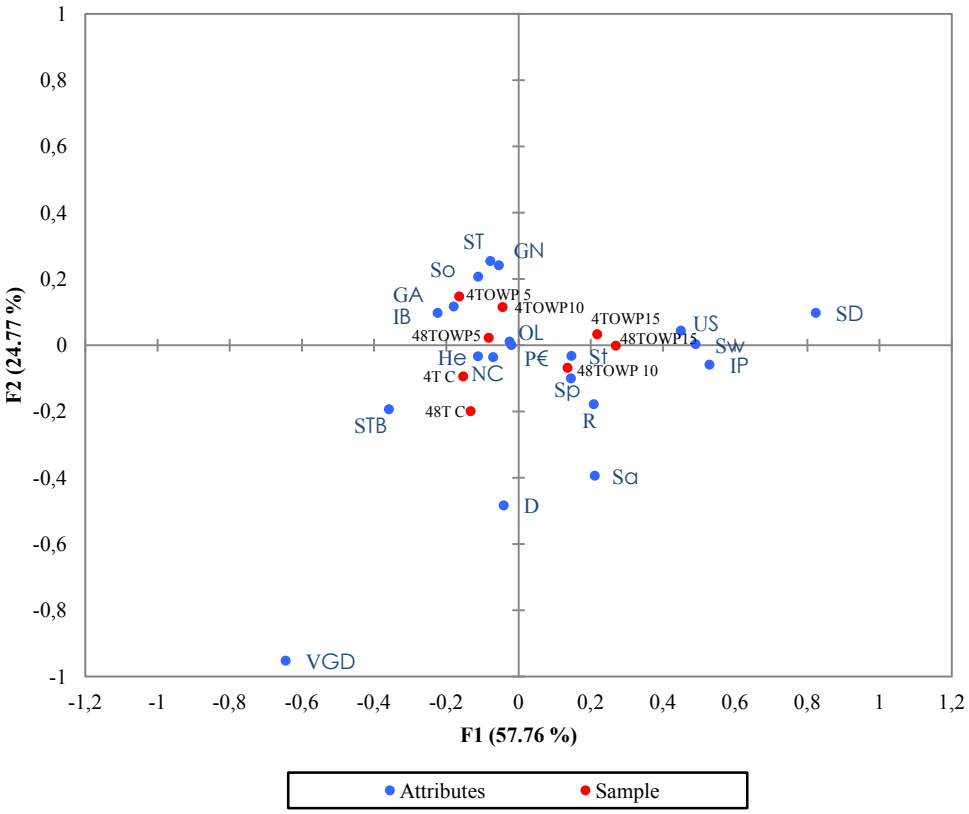

Figure 11. Principal component analysis of bread attributes.

Attributes code: S=sticky; GA=good to accompany; SD= smell of dairy products; VGD=very good for diet; US=unpleasant smell, Sa=salty; D=dry; So=soft; NC=nice colour; ST=soft to touch; H=healthy, IP=innovative product; Sw=sweet, Sp=springy to touch; GN=good for nutrition; STB=smell of traditional bread; R=rubbery; IB=ideal for breakfast; P€=propensity to purchase; OL=overall liking.

Table 8. Propensity to purchase and overall liking in pan bread.

Sample		Propensity to purchase	Overall liking
4T	Control	4.21 b*	4.36 b
	OWP 5	5.21 a	5.33 a
	OWP 10	4.57 ab	4.71 ab
	OWP 15	4.00 b	4.17 b
48T	Control	4.00 b	4.28 b
	OWP 5	4.63 ab	4.71 ab
	OWP 10	4.13 b	4.44 b
	OWP 15	3.93 b	4.17 b

* Samples with different letters are significantly different at $p<0.05$ according to Duncan's multiple range test, $p<0.05$.

Table 9. Pearson correlation between textural data and CATA attributes.

Attributes	Moisture	Crust pan bread color indeces				Fmax (N)
		L	a	b	Browning index	
overall liking	0.176	-0.012	0.380	0.057	0.012	-0.502
sticky	-0.522	-0.270	-0.052	-0.239	0.270	0.234
good to accompany	0.196	-0.007	0.353	0.085	0.007	-0.549
smell of dairy products	**-0.882***	**-0.772***	-0.504	**-0.799***	**0.772***	0.429
very good for diet	**0.827***	**0.765***	0.407	**0.738***	**-0.765***	0.172
unpleasant smell	**-0.788***	**-0.727***	-0.572	**-0.728***	**0.727***	0.295
salty	0.072	0.276	0.335	0.305	-0.276	**0.838***
dry	0.597	0.673	-0.021	0.544	-0.673	0.155
soft a	0.036	-0.235	-0.057	-0.228	0.235	-0.686
nice colour	0.392	0.284	**0.781***	0.395	-0.284	0.047
soft to touch	-0.122	-0.361	-0.113	-0.331	0.361	**-0.722***
healthy	0.480	0.479	0.557	0.451	-0.479	0.016
innovative product	-0.455	-0.251	-0.135	-0.350	0.251	0.527
sweet	**-0.771***	-0.655	-0.552	**-0.720***	0.655	0.336
springy to touch	-0.377	-0.221	0.279	-0.108	0.221	**0.817***
good for nutrition	-0.284	-0.460	-0.378	-0.422	0.460	**-0.854***
smell of traditional bread	**0.846***	**0.725***	0.691	**0.791***	**-0.725***	-0.109
rubbery	-0.399	-0.162	-0.121	-0.168	0.162	**0.775***
ideal for breakfast	0.306	0.251	0.569	0.312	-0.251	-0.396
propensity to purchase	0.152	-0.043	0.313	0.036	0.043	-0.587

* Significant correlations according to Pearson's correlation are reported in bold ($p<0.05$).

3.6 References

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4 Effectiveness of modified atmosphere packaging and ovine whey powder addition in extending the shelf life of whey cheese cakes

4.1 Introduction

The shelf life of food strictly correlates with the rate at which degradation reactions occur. One main factor that, in general, is positively correlated with the impairment of food quality is the water activity (aw) value; products with higher values are more likely to have a shorter shelf life than those with lower values (Barbosa-Canovas *et al*., 2007). Among high-moisture foods (HMFs), which are characterised by aw values higher than 0.90 (Tapia *et al*., 2007), cheese cakes show the microbial spoilage as the critical descriptor of shelf life (Smith *et al.*, 2004). Approaches to reduce the microbiological alterations in bakery foods have relied on the application of strategies to prevent, stop, or control post-baking contamination (Smith *et al*., 2004). While the first two approaches are seldom used, control of post-baking contamination is the preferred means used by the food industry and includes the use of preservatives (Grundy, 1996; Saranraj and Geetha, 2012), recipe reformulation, or packaging conditions such as modified atmosphere packaging (MAP) (Kotsianis *et al*., 2002; Sanguinetti *et al*., 2009) or active packaging (AP) (Gutierrez *et al*., 2011; Siro, 2012). The use of preservatives is actually decreasing due to consumers' reluctance to buy products that contain them. Thus, the food/bakery industry is focusing on extending the shelf life of products by combining product reformulation and the use of MAP and/or AP, especially in HMFs such as baked goods in which a well-calibrated ingredient reformulation could result in a significant reduction of aw values without changing the sensory properties. Product reformulation can act as a hurdle to microbial growth when paired with the well-known antimicrobial activity of MAP at O_2 and CO_2 concentrations lower than 1% and higher than 20%, respectively (Daniels *et al*., 1985; Ellis *et al*., 1993; Ellis *et al*., 1994; Guynot *et al*., 2003; Guynot *et al*., 2003; Ooraikul, 1991; Sanguinetti *et al*., 2009; Smith *et*

al., 1988; Smith *et al*., 1986; Smith and Simpson, 1995; Seiler, 1989). One of the ingredients used in the bakery industry as a nutritional or texture enhancer is obtained from bovine whey, a by-product of cheese and casein production. Most whey products come in dry powder form and are produced by evaporation and spray drying or as concentrates obtained by ultrafiltration and spray drying. Lactose and proteins are the main constituents and result in texture modifications such as gelling, film formation, foaming and emulsifying (Kinsella and Whitehead, 1989; Zadow, 1992). Whey products are exclusively obtained from bovine milk, as ovine whey is used for the production of a whey cheese specialty, ricotta cheese (Diaz *et al*., 2004). Recently, Secchi *et al*. (2011) used sorption isotherms and magnetic resonance imaging to show that ovine whey powders (OWPs) have good water-binding properties that delay the firming of cookies compared with control samples, without impairing the sensory properties. Sanguinetti *et al*. (2009) showed that MAP extended the shelf life of cheesecakes packaged under 30% or 80% CO_2 to 14 and 34 days, respectively, whereas air-packaged cakes were spoiled by mould after only 7 days. Although 80% CO_2 allowed a considerable extension in mould-free shelf life, the use of high CO_2 concentrations may result in other problems such as packaging collapse or sensory alterations due to the strong acidification of food. The goal of this study was to determine whether the combined use of low CO_2 MAP (30%) and OWP would extend the shelf life of whey cheesecakes, which is normally 10 days.

4.2 Materials and methods

4.2.1 Sample preparation

Control ovine whey cheese cakes were produced in a local plant (Esca Dolciaria Snc; Dorgali, Sardinia, Italy) according to the formulation and methodology reported by Sanguinetti *et al*. (2009), but with the substitution of the ovine cheese filling with ovine whey cheese. Two other batches were prepared by substituting 4% or 8% of the ovine whey cheese with OWP (Alim21 A.SP1, Alimenta Srl; Macomer, Sardinia, Italy). All of the batches and relative codes are reported in table 1. Freshly prepared cheesecakes were cooked at 180 C in a rotor oven for 12

min, and after cooling at room temperature in air, were packaged inside multilayer (EVOH/PS/PE) gas barrier trays (six cheese tarts/tray) (Aerpack B5-30, Coopbox Italia; Reggio Emilia, Italy) that were hermetically closed with a multilayer (EVOH/OPET/ PE) gas and water barrier film with a thickness of 54 mm (EOM 360B, Sealed Air; Charlotte, NC, USA). The gas transmission rates for the tray were: O_2, 1.07 cm^3 m^{-2} 24 h^{-1} bar^{-1} at 23 °C; CO_2, 5.35 cm^3 m^{-2} 24 h^{-1} bar^{-1} at 23 °C; water vapour, 6.3 g m^{-2} 24 h^{-1} at 38 °C. The gas transmission rates for the film were: O_2, 4 cm^3 m^{-2} 24 h^{-1} bar^{-1} at 23 °C; CO_2, 13 cm^3 m^{-2} 24 h^{-1} bar^{-1} at 23 °C: water vapour, 9 g m^{-2} 24 h^{-1} at 38 °C. Each batch was packed in ordinary atmosphere (ORD) and in MAP with CO_2:N_2 at a 30:70 gas ratio using a packaging machine (Mod. Reetray 250, Reepack Srl; Seriate, Italy). Storage was done under controlled temperature conditions (20°C ± 0.5). An appropriate number of samples were prepared in order to have a sufficient number of cakes for the six batches. Analyses were performed at 0,10, 20, 40, and 60 days.

4.2.2 Microbiological analysis

Three cheese cakes (one from three different trays) were homogenised, and 10 g was added to 90 mL sterile water and mixed for 2 min in a Stomaker Lab blender 80 (PBI; Milan, Italy). Decimal dilutions were obtained with sterile 0.1% peptone solution and used on specific microbiological media to enumerate the total microbial count (TMC), as well as the presence of Staphylococci, mould and yeast. TMC was determined in Plate Count Agar (PCA) medium (Oxoid; Milan, Italy) after incubation at 30°C for 48 h, whereas Staphylococci were detected and enumerated on Baird Parker Agar (BPA) (Oxoid) after 48 h at 37°C. Mould and yeast were detected on Yeast Peptone-Dextrose (YPD) Agar incubated for 4 days at 25°C. Counts were expressed as colony-forming units per gram (CFU/g). Three repetitions of each analysis were performed at the inspection times reported.

4.2.3 Chemical-physical analyses

Homogenised samples of whole cheesecake were used to check for changes in a_w and moisture content (%) over time. The a_w was determined using an AQUALAB

instrument (Series 3, Decagon; Pullman, WA, USA) calibrated in the range of 0.1 e 0.95 with LiCl, NaCl and KCl solutions of known activity (Labuza *et al*., 1976). The moisture content was obtained after the sample was dehydrated in a vacuum oven for 12 h at 70°C (AOAC, 2012). Triplicate determinations were performed (one cheesecake sample from three different trays) and each measurement was repeated five times.

4.2.4 Texture and colour determination

The texture of the freshly prepared cakes and changes during storage were evaluated using a texture analyser (mod. TA-XTplus Texture Analyser, Stable Microsystems; Godalming, UK) fitted with a 30 kg load cell. The Texture Expert program (version 1.21) was used for data processing. A puncture test was carried out using a 5mm diameter cylinder probe (mod. P/5). The contact plate of the texture analyser was replaced with a confectionery holder supplied with a top and bottom hole of 6 mm in diameter. The following speed parameters were set on the instrument: pre-test, test and post-test speeds of 2 mm/s, 1 mm/s and 5 mm/s, respectively. After reaching a penetration depth of 40 mm, the probe returned to its start position. Two parameters were taken into account to evaluate changes in texture: the maximum force (N) reached during puncturing and hardness (N*s), defined as the area under the curve up to the maximum force (N) divided by the time (s) between the two points. The surface colour of the cheesecakes was determined with a CM-700d spectrophotometer (Minolta; Osaka, Japan) using a D65 illuminant and a CIE 10° standard observer angle. L*(lightness), a*(redness) and b*(yellowness) were acquired, and a* and b* were used to compute the hue angle. Before measurement, the spectrophotometer was calibrated against a white tile supplied with the instrument. Measurements were taken from six cheesecakes after 1 day of storage to check for any change occurring due to a probable enhanced Maillard reaction derived by the use of OWP. Readings were performed on two different points of each sample.

4.2.5 Gas analysis

The headspace gas evolution of MAP trays during storage, with O_2, CO_2 and N_2 components, was determined using a gas analyser (Mod. Combi Check 9800-1, PBI-Dansensor; Ringsted, Denmark). Gas analysis was performed on five trays immediately after packaging and at every sampling time. 2.6. Sensory evaluation Sensory analysis was used to check for differences in the preference of freshly baked cakes and to assess changes in acceptability during storage. To evaluate preference, a ranking preference test was used to compare the three batches (control, 4% OWP, 8% OWP) according to a single attribute. The test was performed with 70 consumers, namely, 30 women and 40 men aged between 25 and 60 years. Samples were presented in a randomised and balanced order at room temperature. Data were analysed using the Friedman's test, which is a non-parametric statistical test used to detect differences among groups when the dependent variable being measured is ordinal. Thus, for the attribute being measured (in this case preference) it established, with a defined confidence level, if two or more different populations had the same distribution. The critical value F was calculated with the following formula:

$$F = \frac{12}{b \cdot k(k+1)} \cdot \sum SR^2 - 3b(k+1)$$

where:

b = number of consumers

k = number of samples

R^2 = sum of the ranks for each sample

If the F value obtained is the same or higher than that present in the χ^2 table for the a priori defined confidence level (in this case 95%) and for the degrees of freedom of k^{-1}, then the difference between at least two samples is significant for the attribute analysed. In order to establish significant differences among the other samples it was necessary to calculate Fisher's least significant difference (LSD).

To assess changes in acceptability, an acceptability test was performed by 32 untrained consumers who evaluated the acceptability of the samples using a hedonic scale that ranged from 1 to 7 (1, extremely dislike; 4, acceptable; 7,

extremely like) for colour, odour, taste and firmness. An overall acceptability score was also computed as the mean of the four attributes.

4.2.6 Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) using the Statistica 6.0 software for Windows. To evaluate changes within each batch, storage time was selected as the factor; and to assess significance differences between treatments, batch was chosen as the factor. Means, when required, were separated according to the Duncan's Multiple Range Test with a significance level of $p \leq 0.05$. Pearson's correlation coefficient between variables was calculated.

4.3 Results and discussion

4.3.1 Changes in tray gas concentrations over time

One of the main problems with MAP technology is ensuring that the gas mixture concentration that was introduced at the time of packaging is maintained, and in particular, controlling the O_2 level in the packaging headspace during storage of the food products. The plastic materials and technology used in our study allowed the O_2 concentration inside the trays to be maintained at 0% for up to 20 days of storage, and only a slight increase up to 0.1% was noticed at 40 and 60 days (table 2). On the other hand, control trays that were packaged in an ordinary atmosphere showed a slight but not significant decrease in O_2 concentration after 10 days of storage, probably due to absorption by the cakes because of their porous structure. The control of O_2 concentration build up inside the trays was better than that reported in other studies that used similar bakery products (Guynot *et al*., 2003; Guynot *et al*., 2004; Guynot *et al*., 2003; Sanguinetti *et al*., 2009); thus, using optimal materials for the barrier film and trays and proper operation of the packaging machine are of paramount importance for the success of MAP. With regard to CO_2 levels, a significant decrease was noted during storage inside the MAP trays with losses ranging from 3% to almost 7% (table 2), and the highest loss was detected in cakes supplemented with whey powder. However, the final

CO_2 concentration did not decrease to levels below 20%, which is the minimum value needed to inhibit the growth of aerobic spoilage microorganisms like mould (Smith *et al*., 2004).

4.3.2 Chemical-physical and microbiological analysis

Our data on chemical-physical parameters showed that the control batch had an aw value of 0.933 and a moisture content of 36.57%; the partial substitution of whey cheese with OWP resulted in a significant reduction in aw, with a mean value of 0.91 in 8% OWP supplemented cheesecakes and consistently lower moisture values, the lowest value recorded being 32.90% (table 3). In general, a_w values and moisture content did not significantly change over time, although there were some exceptions. These data show that all of the samples were very susceptible to mould growth, but had a sufficiently low aw value to prevent pathogen growth, apart from Staphylococci, which are known to grow in substrates with an a_w as low as 0.85. The PCA medium showed very low microbial contamination on the freshly baked cakes as well as a negligible number of viable cells in both YPD and BP culture media (table 4); thus, massive post-process contamination was avoided. The PCA counts increased during storage up to 10^6 CFU. Control cakes had mould growth after 11 days in storage, whereas the use of OWP at 4% or 8% more than doubled the shelf life of the samples, which began to spoil 21 days after baking. MAP and 4% MAP samples were mould-free up to about 45 days in storage, whereas the combined effects of MAP and the highest concentration of OWP (8%) prevented visible mould growth for 60 days. The reduction of both aw and moisture content obtained using OWP is probably what caused the delayed mould growth on the cake's surface. Moulds are aerobic spoilage microorganisms that are sensitive to very high CO_2 concentrations (>60%) inside MAP packaging, even if the O_2 headspace content is close to or higher than 1% (Ooraikul, 1991; Seiler, 1989; Stollman *et al*., 1994). However, it has been reported that a decrease in CO_2 levels may hasten mould growth, even at very low oxygen concentrations (Abellana *et al*., 2000; Bogadtke, 1979). In our study, CO_2 levels decreased below the starting level of 30%, and thus, although

we noticed a very low concentration of O_2, the decrease in CO_2 may have resulted in mould development even after a long lag phase, as has been previously reported (Abellana *et al*., 2000; Bogadtke, 1979). Moreover, the combined effects of MAP and aw reduction by the addition of 8% OWP permitted a consistent mould-free shelf life extension of up to 60 days. These results were better than those reported by Sanguinetti *et al*. (2009), who worked with a very similar product and MAP conditions, thereby confirming the beneficial effects of OWP substitution. Staphylococci were not detected in any sample.

4.3.3 Texture, colour and sensory analysis

The puncture test allowed complete penetration of the samples by the probe, resulting in a curve with a double peak; the first peak corresponded to the maximum force needed to puncture cut the surface, and the second one corresponded to the maximum force applied to penetrate the base of the cake, which was greater than the force applied to the upper portion of the cake (figure 1). The maximum peak was obtained at 20 e 25 mm from the beginning of penetration, which correlated to the thickness of the filling. The second index obtained from the puncture test regarded hardness (table 3), which refers to the entire cake and indicates loss of elasticity of the samples during storage. The maximum force was increased by the addition of OWP in both the control and MAP samples, but no difference was evident between the batches containing 4% or 8% OWP (table 3). The maximum force required increased in all batches during storage, probably due to the loss of moisture in the cheesecake base rather than for staling; indeed, the high fat content of the base helps prevent the staling phenomenon, as previously reported (Sanguinetti *et al*., 2009).

The hardness index generally showed the same trend of maximum force, which can be ascribed to the loss of moisture of both the base and the filling. No significant difference in colour existed between cheesecake batches (data not shown); thus the addition of OWP did not influence colour as previously reported in a study on amaretti cookies (Secchi *et al*., 2011). In fact, the expected change in colour should have been caused by onset of the Maillard reaction due to the

presence of amino acids and lactose in the whey cheese, but it is probable that the additional lactose from the OWP was not enough to significantly change the colour. Sensory analysis added an important contribution to previously reported data, and the results of the ranking preference test are shown in table 5. No significant differences were found among the three samples analysed, that is, the 70 consumers did not reveal a preference for the control samples compared to those supplemented with OWP. Therefore, the addition of OWP did not impair the sensory attributes of the whey cheesecakes. The acceptability test showed that after 10 days of storage, there was a significant decrease in all of the parameters (table 6), which did not subsequently change and did not go below the acceptability threshold set at 4 points; thus the acceptability score remained above the threshold throughout the storage period. No significant differences were detected among batches, thereby confirming that the addition of OWP did not result in changes in sensory properties during the storage period.

4.4 Conclusions

The early critical event of shelf life limitation in most bakery foods is the appearance of mould on the product's surface. In our study, a combination approach was used to extend the mould-free shelf life of an ovine whey cheesecake, namely, using MAP technology and changing the product's formulation. MAP alone was able to extend the shelf life of the ovine whey cheesecakes from 11 to about 45 days, but the combined effects of MAP with addition of the highest concentration (8%) of OWP resulted in a significant extension of the shelf life period up to 60 days while maintaining the product acceptability. On the other hand, the use of OWP substitution alone without MAP managed to double the shelf life of air-packaged samples. The beneficial effect of OWP is most probably due to the reduction in water activity, which changed the instrumental texture parameters without impairing the sensory attributes. Thus, the use of OWP seems to be a promising technique for extending the shelf life of food products, especially if associated with MAP technology, as it has no impact

on sensory acceptability, it is cheap and easy to use, and may also improve the nutritional quality of the food product.

4.5 Figures and tables

Table 1. Codes used for the 6 batches of cheese cakes.

Ovine whey powder (%)	Packaging	Code
0	ORD[a]	Ctr
	MAP	MAP
4	ORD	4%
	MAP	4%MAP
8	ORD	8%
	MAP	8%MAP

[a] ORD= ordinary atmosphere packaging; MAP = modified atmosphere packaging

Table 2. Changes in gas concentration[x] inside trays containing whey cheese cakes during 60 days of storage.

Gas %	Experimental batches	Storage time (days) 0	10	20	40	60
O_2	Ctr	21.0a	20.8a	-[y]	-	-
	MAP	0.0	0.0	0.0	0.1	-
	4%	21.0a	20.8a	20.7a	-	-
	4%MAP	0.0	0.0	0.0	0.1	-
	8%	21.0a	20.8a	20.7a	-	-
	8%MAP	0.0	0.0	0.0	0.1	0.1
CO_2	Ctr	0.5a	0.6a	-	-	-
	MAP	28.70a	25.35b	24.65b	25.10b	-
	4%	0.5a	0.6a	0.7a	-	-
	4%MAP	28.65a	23.85b	22.90b	22.90b	-
	8%	0.5a	0.6a	0.7a	-	-
	8%MAP	28.90a	24.20b	23.35b	22.95b	23.45b
N_2	Ctr	78.5a	78.6a	-	-	-
	MAP	71.30b	74.65a	75.35a	74.90a	-
	4%	78.5a	78.6a	78.6a	-	-
	4%MAP	71.35b	76.15a	77.10a	77.10a	-
	8%	78.5a	78.6a	78.6a	-	-
	8%MAP	71.10b	75.80a	76.65a	77.05a	76.45a

[x] MAP70/30= 70% N_2 and 30% CO_2.
[y]Gas readings have not been carried out due to visible mould growth on tarts.

Table 3. Changes in chemical-physical and texture parameters of whey cheese cakes during 60 days in storage

Experimental batches	Storage time (days)	Parameter			
		a_w	Moisture content (%)	Maximum force (N)	Hardness (N)
Ctr	0	0.933Aa*	36.57Aa	2.20Cb	1.27Bb
MAP		0.933Aa	36.57Aa	2.20Cd	1.27Bc
4%		0.925Ba	34.28Ba	2.48Ac	1.11Cb
4%MAP		0.925Ba	34.28Ba	2.48Ad	1.11Cc
8%		0.910Cb	32.90Ba	2.68Ab	1.39Ab
8%MAP		0.910Cb	32.90Ba	2.68Ac	1.39Ac
Ctr	10	0.928Bb	35.94Aa	2.90Ba	1.71Aa
MAP		0.933Aa	36.01Aa	2.93Bc	1.70Ab
4%		0.923Ca	34.47Ba	3.33Ab	1.36Aa
4%MAP		0.921Ca	33.63Ca	3.30Ac	1.42Ac
8%		0.913Db	32.84Da	3.56Aa	1.65Aab
8%MAP		0.911Dab	32.48Da	3.63Ab	1.86Ab
MAP	20	0.935Aa	35.38Aa	3.42Bb	1.97Ab
4%		0.927Ba	33.84Ba	3.83Aa	1.40Ba
4%MAP		0.919Ca	32.37Cb	3.88Ab	2.17Ab
8%		0.921Ca	33.34Ba	3.99Aa	1.77Ba
8%MAP		0.913Da	32.14Ca	4.11Aa	2.37Aa
MAP	40	0.931Aa	34.17Aa	3.83Ba	2.70Aa
4%MAP		0.917Ba	31.72Bb	4.52Aa	2.80Aa
8%MAP		0.913Ba	32.23Ba	4.26Aa	2.85Aa
8%MAP	60	0.909b	30.44a	4.27a	2.54a

* Data for each column followed by different letters within each batch and storage time (lower case letter) and within each storage time (capital letters) differ significantly according to Duncan's Multiple Range Test at $P<0.05$.

Table 4. Microbial development on whey cheese cakes (cfu/g) packaged under different conditions. Total bacterial count (PCA)[x], yeast and mould (YPD) and Staphylococci (BP) growth has been reported

Microbial media	Experimental batches	Storage time (days) 0	10	20	40	60
PCA	Ctr	$<1.0 \times 10^1$	2.5×10^3	-[y]	-	-
	MAP	7.3×10^2	1.2×10^3	2.8×10^6	2.7×10^6	-
	4%	$<1.0 \times 10^1$	3.5×10^3	9.0 x 103	-	-
	4%MAP	1.4×10^2	1.0×10^2	5.0×10^5	6.5×10^6	-
	8%	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	-	-
	8%MAP	5.0×10^1	4.0×10^2	1.8×10^4	4.1×10^6	3.6×10^3
YPD	Ctr	$<1.0 \times 10^2$	6.0×10^2	-	-	-
	MAP	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$	2.0×10^3	-
	4%	$<1.0 \times 10^2$	1.0×10^2	8.0×10^2	-	-
	4%MAP	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$	6.0×10^2	-
	8%	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$	-	-
	8%MAP	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$< 1.0 \times 10^2$
BP	Ctr	$<1.0 \times 10^1$	5.0×10^1	-	-	-
	MAP	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	-
	4%	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	-	-
	4%MAP	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	-
	8%	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	-	-
	8%MAP	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$

[x] PCA, Plate count agar; YPD, Yeast peptone dextrose agar; Bp, Baird Park Agar.
[y] Sampling has been stopped due to visible mold growth on cakes.

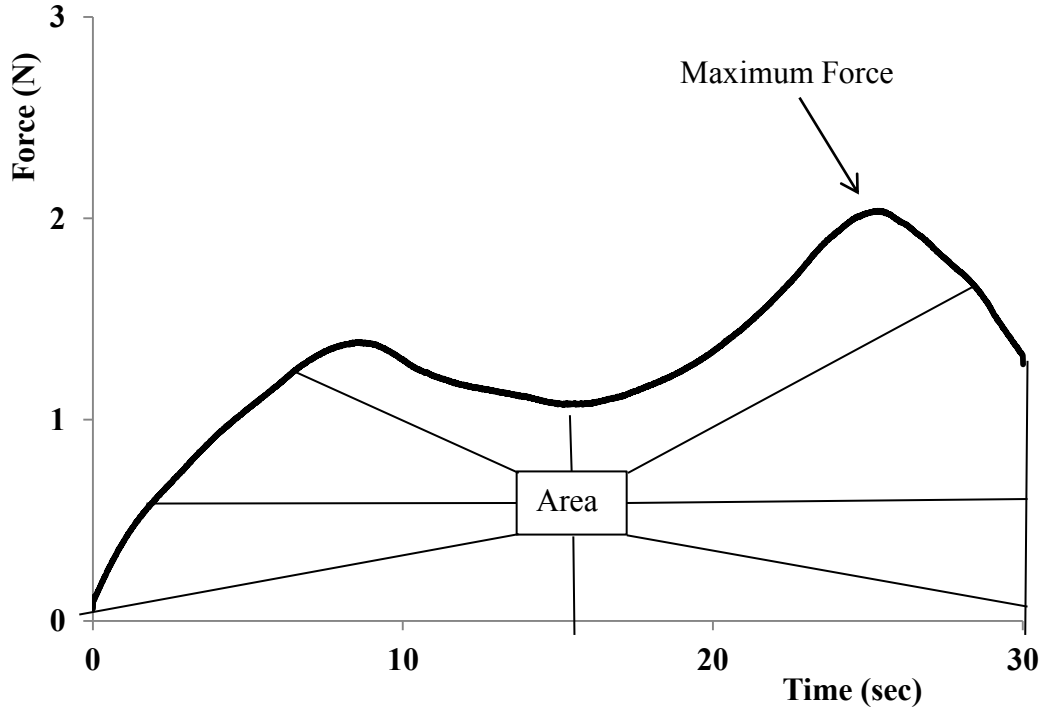

Figure 1. Representative graph of a puncture test.

Table 5. Results of the Ranking preference test obtained on freshly baked whey cheese cakes.

Samples	Ctr	4%	8%
Rank	136	137	135
Significance ($p \leq 0.05$)	a	a	a

Table 6. Changes in sensory attributes of whey cheese cakes during 60 days in storage as revealed by an acceptability test.

Experimental batches	Storage time (days)	Sensory attribute				
		Colour	Olfactory intensity	Taste	Consistency	Overall acceptability
Ctr	0	4.91a*	5.03a	5.06a	4.94a	4.98a
MAP		4.91a	5.03a	5.06a	4.94a	4.98a
4%		4.94a	5.00a	5.03a	5.03a	5.00a
4%MAP		4.94a	5.00a	5.03a	5.03a	5.00a
8%		4.91a	4.98a	5.00a	5.00a	4.97a
8%MAP		4.91a	4.98a	5.00a	5.00a	4.97a
Ctr	10	4.87a	4.66b	4.50b	4.38b	4.60b
MAP		4.90a	4.50b	4.51b	4.41b	4.58b
4%		4.87a	4.56ab	4.47b	4.47b	4.59b
4%MAP		4.88a	4.57bc	4.44b	4.44b	4.58b
8%		4.90a	4.56b	4.51b	4.41b	4.59b
8%MAP		4.90a	4.56b	4.47b	4.44b	4.5b
MAP	20	4.87a	4.59b	4.47b	4.38b	4.58b
4%		4.80a	4.31b	4.41b	4.41b	4.48b
4%MAP		4.85a	4.59ab	4.44b	4.34b	4.55b
8%		4.88a	4.53b	4.47b	4.31b	4.55b
8%MAP		4.88a	4.50b	4.44b	4.34b	4.54b
MAP	40	4.82a	4.50b	4.41b	4.41b	4.53b
4%MAP		4.84a	4.47b	4.32b	4.31b	4.48b
8%MAP		4.85a	4.44b	4.38b	4.37b	4.51b
8%MAP	60	4.82a	4.30b	4.40b	4.41b	4.48b

* Data followed by different letters within each thesis and column differ significantly according to Duncan's Multiple Range Test at $P<0.05$. Means for each column and within each storage time were not statistically different.

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5 Final conclusions and future perspectives

In conclusion, the addition of OWP to the dough had a negative effect on the leavening height, at all concentrations except the lowest (5%). Leavening height is generally recognized as a reliable indicator of bread volume. In this study, the reduction in height occurred for different reasons. In the 4T samples the addition of OWP led to an improvement in dough strength, as demonstrated by the increase in β-sheet and β-turn structures, which was revealed by analysis of the amide I band in the FTIR spectrum. On the contrary, in the 48T samples, the addition of OWP resulted in a longer mixing time to reach the desired consistency on the consistograph. The prolonged mixing time probably caused partial disruption of the gluten network, resulting in reduced leavening height and reduced bread volume after baking of the samples with OWP, if compared with the control. The significant increase in unordered protein structures confirms this hypothesis. The addition of OWP to the dough formulation had different effects on bread, depending on the quality characteristics of the raw material, the kind of bread produced, and the amount of OWP added. LGS with a weak gluten, as was the case of our 4T LGS, produced a bread with a lower volume than the low grade semolina characterized by a strong and tenacious gluten (48T LGS), as expected. The addition of OWP led to a strengthening effect on the gluten matrix, with a positive effect on 4T samples, with the weak gluten, and a negative effect on the strong gluten of 48T samples, which became too strong. Therefore, the volume of pan bread in 4T sample was higher with the addition of a low percentage of OWP (5%), and was not affected by the addition of higher concentration of OWP (10 and 15%). The contrary happened in 48T samples, where the addition of OWP had a detrimental effect on bread volume. Crumb characteristics, measured in term of firmness and cell size distribution, also were improved in 4T samples with 5% of OWP, and were not affected at higher concentrations of OWP. 4TOWP5 was also the most preferred samples by consumers. In the typical Sardinian flat bread spianata the addition of OWP improved the shelf life in terms of textural characteristics. This study demonstrates that the use of OWP was able to improve

the bread making quality of the variety characterized by weak gluten, as Cappelli is. This result could be exploited to verify the effect of the addition of OWP to old durum wheat varieties, which are generally characterized by having weak and sticky gluten. The early critical event of shelf life limitation in most bakery foods is the appearance of mould on the product's surface. In our study a combination approach was used to extend the mould-free shelf life of an ovine whey cheesecake, namely, using MAP technology and changing the product's formulation. MAP alone was able to extend the shelf life of the ovine whey cheesecakes from 11 to about 45 days, but the combined effects of MAP with addition of the highest concentration (8%) of OWP resulted in a significant extension of the shelf life period up to 60 days, while maintaining the product acceptability. On the other hand, the use of OWP alone without MAP managed to double the shelf life of air-packaged samples. The beneficial effect of OWP is most probably due to the reduction in water activity, which changed the instrumental texture parameters without impairing the sensory attributes. Thus, the use of OWP seems to be a promising technique for extending the shelf life of food products, especially if associated with MAP technology, as it has no impact on sensory acceptability, it is cheap and easy to use, and may also improve the nutritional quality of the food product.

Bakery product have a strategic role for agri-food economy in Mediterranean areas, particularly in Sardinia, where, as reported in the Smart Specialization Strategy (S3) of Regione Autonoma della Sardegna, the small and medium enterprises operating in the production of bakery products (both sweets and salted ones) are the most widespread with 1,424 companies out of a total of 1,994 (71%).

The solutions proposed in this PhD dissertation could be easily exploited due to their immediate transferability into the layout of production process in the bakery enterprises. Nowadays, market choices are more and more oriented to the rediscovery of typical products made with old and traditional raw materials, in order to reintroduce old-time flavours, unfortunately lost in the products coming from big industrial production: the producers are therefore interested to the

reintroduction of ancient and autochthonous cultivars of cereals. This commercial strategy has received growing interest from consumers. Old varieties and local landraces of durum wheat are often characterized by poor gluten quality, as is the cultivar Senatore Cappelli which we used in these studies, therefore we could speculate that the positive effect that we found in our results could be also found when the ovine whey powder is added to semolinas milled from such old varieties.

6 Posters and papers

Article

Secchi N, Fadda C, Sanna M, Conte P, Del Caro A, Catzeddu P, Piga A (2017) Effectiveness of modified atmosphere packaging and ovine whey powder in extending the shelf life of whey cheesecakes. Food Science and Technology 75; 373-378.

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Effectiveness of modified atmosphere packaging and ovine whey powder in extending the shelf life of whey cheesecakes

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ABSTRACT

The goal of this study was to determine if the combined use of modified atmosphere packaging (MAP) and substitution of 4% and 8% whey cheese with ovine whey powder (OWP) would extend the shelf life of whey cheesecake, compared with samples packaged in air and without OWP. To this end, whey cheesecakes were packaged under a N_2/CO_2 ratio of 70/30 and with air and stored for 60 days at 20 °C. Changes in microbial growth, in-package gas composition, chemical-physical parameters, texture, and sensory attributes were evaluated.

The use of OWP and MAP extended the shelf life to 21 and 45 days, respectively, compared with the control samples that spoiled after 11 days. The combined use of MAP and OWP at the highest concentration further increased the mould-free shelf life of the cakes to 60 days. All of the samples underwent a significant increase in hardness during storage, but sensory acceptability was not impaired. Panellists did not find differences in sensory properties between control and OWP samples and gave an acceptability score over the threshold during the entire storage period.

1. Introduction

The shelf life of food strictly correlates with the rate at which degradation reactions occur. One main factor that, in general, is positively correlated with the impairment of food quality is the water activity (a_w) value; products with higher values are more likely to have a shorter shelf life than those with lower values (Barbosa-Cánovas, Fontana, Schmidt, & Labuza, 2007). Among high-moisture foods (HMFs), which are characterised by a_w values higher than 0.90 (Tapia, Alzamora, & Chirife, 2007), cheese cakes show the microbial spoilage as the critical descriptor of shelf life (Smith, Daifas, El-Khoury, Koukotsis, & El-Khouri, 2004). Approaches to reduce the microbiological alterations in bakery foods have relied on the application of strategies to prevent, stop, or control post-baking contamination (Smith et al., 2004). While the first two approaches are seldom used, control of post-baking contamination is the preferred means used by the food industry and includes the use of preservatives (Grundy, 1996; Saranraj & Geetha, 2012), recipe reformulation, or packaging conditions such as modified atmosphere packaging (MAP) (Kotsianis, Giannou, & Tzia, 2002; Sanguinetti et al., 2009) or active packaging (AP) (Gutiérrez, Batlle, Andújar, Sánchez, & Nerín, 2011; Siro, 2012). The use of preservatives is actually decreasing due to consumers' reluctance to buy products that contain them. Thus, the food/bakery industry is focusing on extending the shelf life of products by combining product reformulation and the use of MAP and/or AP, especially in HMFs such as baked goods in which a well-calibrated ingredient reformulation could result in a significant reduction of a_w values without changing the sensory properties. Product reformulation can act as a hurdle to microbial growth when paired with the well-known antimicrobial activity of MAP at O_2 and CO_2 concentrations lower than 1% and higher than 20%, respectively (Daniels, Krishnamurthi, & Rizvi, 1985; Ellis, Smith, Simpson, & Ramaswamy, 1993; Ellis, Smith, Simpson, Ramaswamy, & Doyon, 1994; Guynot, Marin, Sanchis, & Ramos, 2003; Guynot, Sanchis, Ramos, & Marin, 2003; Ooraikul, 1991; Sanguinetti et al., 2009; Smith et al., 1988; Smith, Ooraikul, Koersen, & Jackson, 1986; Smith & Simpson, 1995; Seiler, 1989).

One of the ingredients used in the bakery industry as a nutritional or texture enhancer is obtained from bovine whey, a by-

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Oral communication

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The ability of ovine whey powder to improve quality of Sardinian breads

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The objective of this PhD study was to investigate the ability of ovine whey powder to improve the shelf life of some Sardinian traditional bakery foods. To reach this aim two kinds of bread were made (pan bread and *Spianata* flat bread) using 2 low grade semolinas that were mixed with ovine whey powder at 4 different concentrations (0, 5, 10, 15%), control samples were prepared with no whey. Physical chemical and textural properties of breads were evaluated.

L'impiego del siero di latte ovino in polvere nel miglioramento qualitativo dei pani tradizionali della regione Sardegna

Il presente progetto di tesi di dottorato ha lo scopo di valutare l'efficacia del siero di latte ovino in polvere nell'estensione della shelf life di alcuni prodotti da forno tipici della regione Sardegna. A questo scopo sono state preparate due tipologie di pane (pane in cassetta e pane Spianata) utilizzando due differenti tipologie di semolato, miscelate a concentrazioni crescenti di siero di latte ovino in polvere, mentre i campioni di controllo sono stati preparati senza l'aggiunta di siero di latte. Per ciascuna tesi analitica sono state valutate le proprietà chimiche, fisiche e reologiche.

Key words: NIR FT-Raman Spectroscopy, semolina, bread making, crumb texture, cell size distribution, staling.

1. Introduction

In all over Mediterranean area and particularly in Southern Italy, semolina from durum wheat is widely used in bread making. Furthermore, durum wheat is worldwide used to make dry pasta, and this is the reason why durum wheat breeding programs have focused on improving pasta cooking quality, through improving gluten strength indices. This has caused the progressive replacement of the old landraces and ancient varieties by the new cultivars. Moreover, the old genotypes often show HMG-20 glutenin patterns, which is well recognized as a marker for gluten weakness in durum wheat. Sometime, in old genotypes, also γ-gliadin 42 type is present, which is marker for the LMW-2 pattern, and which is, again, related to weak gluten characteristics. Due to their protein pattern, traditional varieties show poor adaptability to be employed in modern plant, on the other hand, new varieties sometimes show very strong and tenacious gluten, which does not fit with bread making technology. Whey, a by-product of the dairy industry, represents for baked products a potential source of low cost functional proteins, which show emulsifying, foaming and gelling properties. The major whey proteins, i.e., primarily beta-lactoglobulin and secondarily alpha-lactalbumin, represent a well balanced source of essential amino acids, and then possess a significant meaning as nutritional ingredients (Zadow JG, 1993). Nevertheless, whey proteins are known to exert negative effects on bread quality, by depressing loaf volume, and increasing crumb firmness, although their denaturation seems to eliminate this effect (Erdogdu-Arnocky et al., 1996; Kadharmestan et al., 1998). The aim of this study was to investigate the effect of the addition of ovine whey powder on the quality of two types of bread, i.e., a conventional pan bread and a leavened flat bread (*Spianata*), both obtained by a commercial low grade semolina (LGS) and a LGS milled from an old variety.

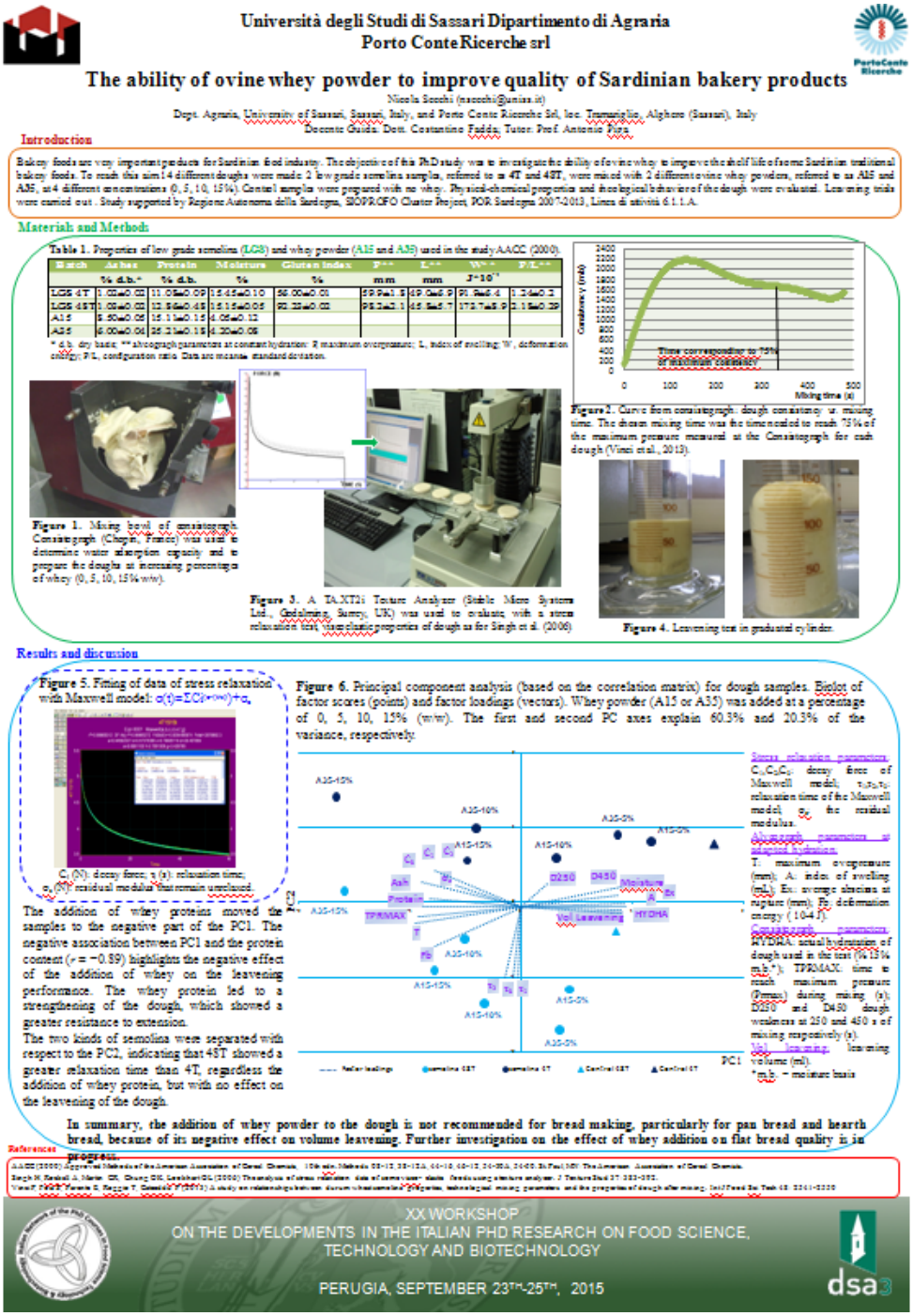
2. Materials and Methods

2.1 Bread making process

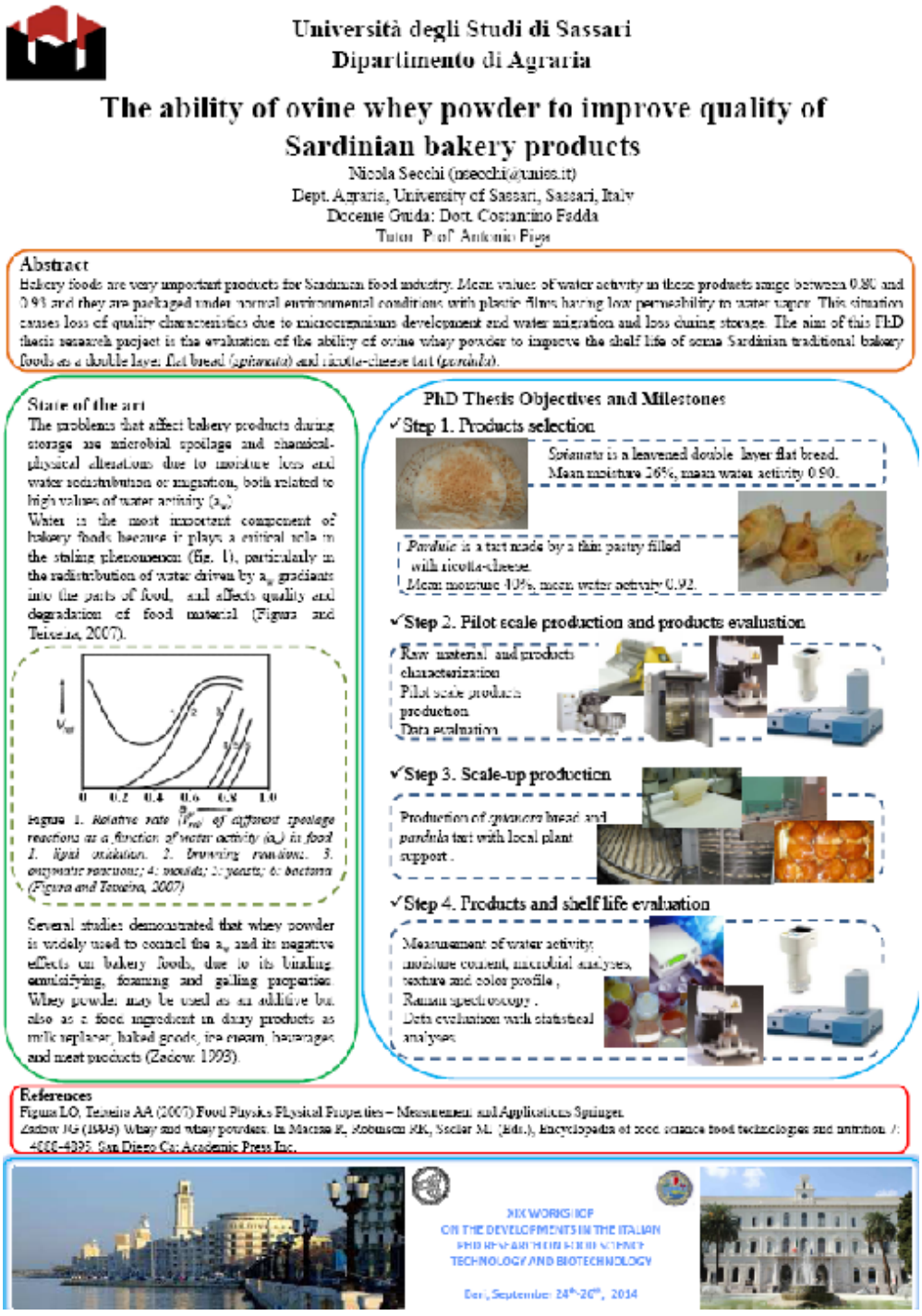
Two types of LGS (Molino Galleu, Ozieri, Italy) were used: 48T milled from commercial durum wheat varieties; 4T from Senatore Cappelli variety, well known for its poor gluten quality. They were mixed with a commercial spray dried ovine whey powder, referred to as OWP (Alim20 A.SP1, Alimenta, Macomer, Italy), at increasing percentage (0, 5, 10 and 15% w/w), to make a flat bread and a pan bread. Eight bread making trials were carried out in a semi automated bakery plant at Porto Conte Ricerche Srl. The recipes are reported in table 1. The amount of baker's yeast was 1.0% on the total weight of LGSs plus OWP. Seventeen kilograms of dough for each sample were mixed, in a fork mixer, for the time required to reach the maximum volume after leavening, as reported by Vinci et al., (2013). First proofing was conducted in a fermentation chamber at 28 °C and a relative humidity of 80% for 35 min. To produce pan bread, six pieces of dough (1.2 kg for each piece) were manually introduced in aluminum pans and kept in the fermentation chamber (28 °C and 80% relative humidity) for 120 min and then baked at 230 °C for 35 min. The bread was then set aside for cooling for 4 h at room temperature and finally cut into slices 2 cm thick. Three central slices per loaf were packaged individually using a water vapor and oxygen high barrier plastic film. A sachet of oxygen absorber (Freshcare, O_2 Control, The

Posters

Secchi N (2015) The ability of ovine whey powder to improve quality of Sardinian bakery products. 20th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Perugia, Perugia September 23rd -25th, 2015.



Secchi N (2014) The ability of ovine whey powder to improve quality of Sardinian bakery products. 19th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, University of Bari, Bari September 24rd -26th, 2014.



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The ability of ovine whey powder to improve quality of Sardinian bakery products

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Abstract
Bakery foods are very important products for Sardinian food industry. Mean values of water activity in these products range between 0.80 and 0.93 and they are packaged under normal environmental conditions with plastic films having low permeability to water vapor. This situation causes loss of quality characteristics due to microorganisms development and water migration and loss during storage. The aim of this PhD thesis research project is the evaluation of the ability of ovine whey powder to improve the shelf life of some Sardinian traditional bakery foods as a double layer flat bread (*spianata*) and ricotta-cheese tart (*pardula*).

State of the art
The problems that affect bakery products during storage are microbial spoilage and chemical-physical alterations due to moisture loss and water redistribution or migration, both related to high values of water activity (a_w).
Water is the most important component of bakery foods because it plays a critical role in the staling phenomenon (fig. 1), particularly in the redistribution of water driven by a_w gradients into the parts of food, and affects quality and degradation of food material (Figura and Teixeira, 2007).

Figure 1. Relative rate (V_{rel}) of different spoilage reactions as a function of water activity (a_w) in food 1. lipid oxidation. 2. browning reactions. 3. enzymatic reactions; 4: moulds; 5: yeasts; 6: bacteria (Figura and Teixeira, 2007)

Several studies demonstrated that whey powder is widely used to control the a_w and its negative effects on bakery foods, due to its binding, emulsifying, foaming and gelling properties. Whey powder may be used as an additive but also as a food ingredient in dairy products as milk replacer, baked goods, ice cream, beverages and meat products (Zadow, 1993).

PhD Thesis Objectives and Milestones

✓**Step 1. Products selection**

Spianata is a leavened double layer flat bread. Mean moisture 26%, mean water activity 0.90.

Pardula is a tart made by a thin pastry filled with ricotta-cheese. Mean moisture 40%, mean water activity 0.92.

✓**Step 2. Pilot scale production and products evaluation**

Raw material and products characterization
Pilot scale products production
Data evaluation

✓**Step 3. Scale-up production**

Production of *spianata* bread and *pardula* tart with local plant support.

✓**Step 4. Products and shelf life evaluation**

Measurement of water activity, moisture content, microbial analyses, texture and color profile, Raman spectroscopy.
Data evaluation with statistical analyses

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XIX WORKSHOP
ON THE DEVELOPMENTS IN THE ITALIAN PHD RESEARCH ON FOOD SCIENCE TECHNOLOGY AND BIOTECHNOLOGY
Bari, September 24th-26th, 2014