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***Sfogliatella Riccia Napoletana:*
realization of a lard- and palm oil- free
"ready to eat" product**

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To my mother...

Abstract

In the last years, demand for “ready to eat” (RTE) products has exponentially increased because of lifestyle changing and new technologies introducing, such as microwave. This reflects a trend by consumers towards less time and effort in the preparation of food.

One of the most important sectors of the RTE product market is the baked confectionery segment.

Sfogliatella Riccia Napoletana (SRN), recognized as Traditional Agri-food Product (*Prodotti Agroalimentari Tradizionali* - PAT) of Campania Region, is worldwide known and appreciated for its goodness. But its production is limited to local markets.

In order to extend market area on an international basis, PhD research was divided into two steps focused on

- *Production of lard- and palm oil- free RTE-SRN*

Lard is traditionally used in SRN production for its technological properties and low cost, but there is a growing negative perception regarding the implication of animal fats on human health.

Palm oil is widely used as a cheaper vegetable alternative to lard, because of the high thermal stability and oil yield, however, it is nowadays under discussion, mostly related to sustainability of its cultivation.

So, the replacement of lard (traditionally used) in the formulation of SRN with palm oil- free vegetable blends was studied.

Besides, alternative baking methods -microwave and/or infrared- as substitute to traditional one for the production of RTE-SRN were assessed. According to data, a combined baking based on electric-infrared-microwave allowed baking time halving, with the advantage of combining time and energy saving properties of microwave baking with browning and crisping properties of infrared and electric heating.

The substitution of lard with palm oil-free vegetable blend, composed of shea butter, coconut oil and sunflower oil at different percentages, led to a reduction of oxidized compounds during cooking.

- *Storage evaluation*

SRN is characterized by high moisture and a_w content, so its shelf life is limited by microbial spoilage, mostly due to moulds and staling.

In order to achieve longer shelf life of RTE-SRN, frozen and refrigerated preservation combined with modified atmosphere packaging (MAP) were employed.

Results revealed that it is possible to obtain a RTE-SRN with a stable oxidative and moisture behaviour during prolonged frozen storage. Also substituting lard with palm oil-free fat, it is possible to obtain a product with sensory and textural qualities close to freshly baked SRN made with lard.

Indeed, modified atmosphere packaging (MAP) (CO₂/N₂ 50/50) combined with refrigerated storage revealed an energy saving alternative preservation method, ensuring acceptable sensory, physical and chemical stability to RTE-SRN stored at 5 °C for 49 days, extending to three times the shelf life compared to air packaging. Palm oil-free RTE-SRN preserved a better oxidative stability for the whole storage time.

The above findings can be applicable by baking industry to a large variety of confectionery bakery products.

Keywords: *Sfogliatella Riccia Napoletana*, ready to eat, palm oil- free, microwave, infrared, baking, MAP, freezing.

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PUBLICATIONS

Journals

Manzo N, Troise AD, Fogliano V, Pizzolongo F, **Montefusco I**, Cirillo C & Romano R, (2017) Impact of traditional and microwave roasting on chemical composition of hazelnut cultivar ‘Tonda di Giffoni’. *Quality Assurance and Safety of Crops and Food* (IN PRESS).

Peer-reviewed conference proceedings

Montefusco I, Blaiotta G & Romano R. Formulation and Technological Interventions for the Realization of “Ready to Eat” Sfogliatella Riccia Napoletana - XXI Workshop on Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, Portici, Italy.

Montefusco I, Blaiotta G & Romano R. Characterization and Technological Interventions for the Realization of the “Ready to Eat” *Sfogliatella Riccia Napoletana* - XX Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, Perugia, Italy.

Chapter 1 – State of the art

This chapter reviews the research which has been conducted over the last century with regards to bakery products (especially “ready to eat” bakery products) and the effect of different fat formulation on bakery items.

The different baking methods and their effects on textural, chemical and sensorial properties of the product were investigated. A section was particularly dedicated to a traditional bakery product, *Sfogliatella Riccia Napoletana*, its origin and processing.

The last part focuses on the spoilage concerns of bakery products and on traditional and novel methods of food preservation that can be used by the bakery industry to extend the shelf life and enhance the safety of products.

1.1 “Ready to eat” foods

In the last years, “ready to eat” (RTE) foods are increasingly popular with the consumer predominantly due to their convenience of consumption and ease of preparation and storage (Brennan *et al.*, 2013). Convenience, value, attractive appearance and texture are the main consumer appeal factors, causing the growing demand for RTE food (Harper, 1981).

According to the U.S. Food and Drug Administration, “ready to eat” product is a food that is in an edible form without any additional preparation to achieve food safety (to eliminate or reduce microorganisms of concern to an acceptable level) and may receive additional preparation for palatability or aesthetic, epicurean, gastronomic, or culinary purposes (FDA, 2009).

Under this definition, a number of processed foods can be regarded as RTE products including bakery item (biscuits, crisps, breads, pastries) (Fig. 1), for which further cooking is not required for food safety; but also dairy products (milk, cheese, spreads), sandwiches and rolls, prepared salads, vegetables and fruit. New products enter the RTE food market nearly every day, so the list is getting longer and longer (Fast, 1999).



Figure 1_ RTE bakery products.

A large number of causes may indeed be listed to explain the growing trend of this type of foods in various countries, including technological innovations such as the microwave, changing household structures with more single households and more women pursuing paid work, multicultural societies introducing new foods, and changing values and norms, appealing advertisement, ownership of kitchen technology, individualism, time usage, reticence, or (lack of) cooking skills (Brunner *et al.*, 2010; Buckley *et al.*, 2007; Sheely, 2008; Verriet, 2013). The catering business, including restaurants, schools, hospitals, nursing homes and prisons, has developed systems for the preparation and preservation of different items which can be quickly served without any reduction in the final quality (Murcia *et al.*, 2003).

RTE foods (also called *convenience food*) was produced prior to 1960 (Dipman, 1942), but the growth was highest in recent years (in the 2000s and 2010s about 7% per year, as against growth rates of about 4% prior to 2000). Between 2010 and 2014, this proportion reached 0.044%, or almost a doubling respect to the 2000s (Scholliers, 2015). Nowadays their value of the market exceed 81 billion dollars (Global Industry Analyst, Inc., 2010). The attitude of consumers towards food has changed since the 1990s and the demand to RTE foods, especially frozen ones, has increased exponentially.

In the 1970s, research on convenience food consumption started looking at the link between the employment status of the wife and the purchase of convenience products. The reasoning was that the more the wife worked outside the home, the less time was available for preparing food and the more she would depend on convenience products (Becker, 1965; Scholderer & Grunert, 2005). Another phenomenon which has contributed to the growth of the consumption of this kind of food has been the increase of the singles, that are willing to pay more to buy dishes whose preparation requires a minimum effort.

Nowadays RTE foods are omnipresent, and everybody uses them once in a while to make life a bit easier even if there is no time pressure or need to save time. They have been incorporated into daily life and that the term “homemade meal” has been reinterpreted to include the help of various convenience products (Brunner *et al.*, 2010). Consumers can eat everything and the representations of food in media have multiplied in the form of chef television shows, lifestyle sections of news representations and postings of food pictures and recommendations on social media.

Due to the increasing demand for and consumption of RTE foods, and owing to the fact that these are not further processed, the microbiological risks to the consumer from these products have also increased (Muriana *et al.*, 2002). The preparation of RTE involves human handling (such as cutting or slicing), which can easily contribute to cross-contamination. “Ready to eat” foods have been involved in numerous foodborne illness outbreaks (Gibbons *et al.*, 2006; Gilbreth *et al.*, 2005), especially due to cross-contamination of these products during processing and packaging. The most foodborne pathogenic bacteria found in RTE foods include *Listeria monocytogenes*, *Salmonella* Enterica, *Escherichia coli* O157:H7 and *Clostridium perfringens* (Hwang & Huang, 2010).

In the last ten years, demand for healthier and more nutritious convenient RTE product than those previously available is increasing because consumers have become more health conscious (Euromonitor, 2011). So food industry has been concentrating on ensuring a balanced nutritional status of RTE products.

This in itself presents numerous problems associated with ingredient formulation and processing parameters.

One of the most important sectors of the RTE product market is the bakery RTE segment.

1.2 Bakery products

Bakery products are one of the most consumed products in the world (Matsakidou *et al.*, 2010). They play very important roles in the diet of the modern consumer. A wide variety of bakery products can be found on supermarket shelves, such as breads, sweetened rolls, doughnuts, meat pies, dessert pies, pizza, quiche, crackers, cookies and other products. Several methods can be used to classify these products. Classification can be based on product type, i.e., unsweetened, sweetened, or filled goods, or on their method of leavening, e.g., biological, chemical or unleavened, or on the basis of their pH, moisture content and water activity (Smith *et al.*, 2004).

Today, the world production of bakery products generates billions of dollars in revenue and employs thousands of personnel. The global market for bakery products is projected to exceed US\$485 billion by 2020, driven by rising consumption of baked goods worldwide (Global Industry Analyst, Inc., 2010). In many countries, sales have increased in the last twenty years. In 1998, sales of bakery products in the United States exceeded 10 million metric tons and had a market value of approximately \$27 billion dollars, with a 14.5% increase over the previous four

years. In Canada, the bread and bakery industry shipped about \$2.3 billion dollars (Cdn) of products in 2000, an increase of 36.3% from 1988 levels, an amount which accounted for 4.2% of the total food and beverage processing sector shipments (Agriculture and Agri-Food Canada, 2000). Today, Europe represents the largest market worldwide and Italy is the leading EU country with US\$22.3 billion worth of retail value sales of bakery products, only in 2013 (Euromonitor International, 2014; Global Trade Information Services, 2014) (Fig. 2).

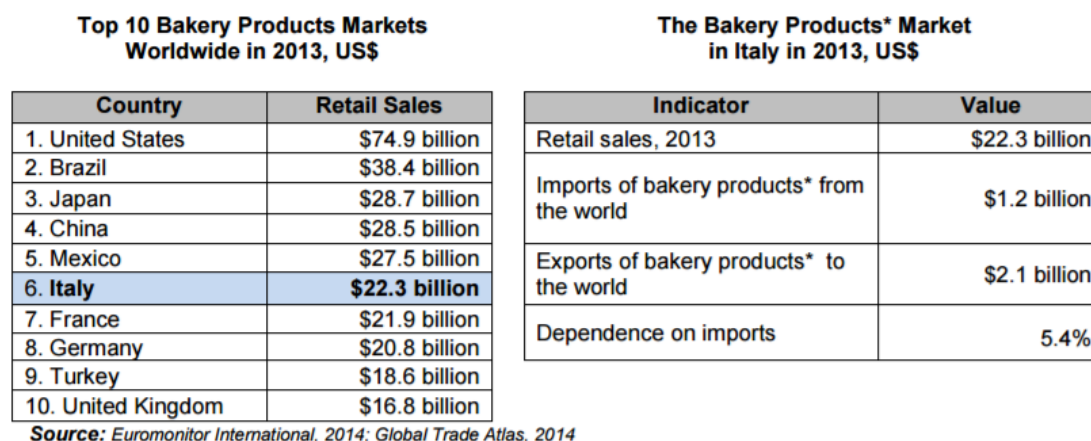


Figure 2_ Positioning bakery products in the world (Euromonitor International, 2014; Global Trade Information Services, 2014).

This sustained growth has been driven by consumer demand for convenient, premium baked goods which are fresh, nutritious, conveniently packaged and shelf stable. In Italy, several RTE products from cereal are available in the market. RTE bakery products are solving the constraints in food preparation, limited time for shopping due to change in lifestyles and the increasing consumers demand for fresh like products (Rosell, 2009). Growth in the market is attributed to various factors including availability of broad range of products at reasonable price points, convenience of “ready to eat” products, rapid urbanization, hectic lifestyles, vast product offerings with different tastes and textures, and growing health benefits of baked goods over other food options. Bread continues to remain the most consumed bakery product worldwide, but cakes and pastries represent the fastest expanding category in the market.

The term ‘pastry’ includes a broad range of food products, differing for ingredients and process conditions.

It is obtained from a batter, containing higher levels of water, sugar and, for some formulations, fat, compared to bread dough (Rogers, 2004). Formulation, process conditions and ingredient quality influence the final outcomes in terms of quality characteristics; in particular, the role of fat is a key aspect in pastries production.

Cereal based foods are very ancient products that have been employed by humans since prehistoric times. In fact, the first traces of bread can be tracked back to 30000 years ago but only with the beginning of agriculture, in the Neolithic age, bread was produced widely.

The introduction in the diet of a “cake or pastry” was a following step. At first, cakes were sweetened with honey and produced adding nuts and dried fruits. When cane sugar made its way from India to Arab countries, it became one of the main ingredients of this product. Furthermore, a key ingredient that differentiates a cake from bread is the high content of fat, making a cake a calorie rich food product.

Nowadays, in the developed and richer countries such as Europe, the act of eating has become not only essential for living but also, and in some cases mostly, a moment of indulgence. For this reason, many people suffer from pathologies related to the excess of food and calories intake deriving from sweet and fatty products.

Baked products are perishable foods that undergo severe physical, chemical, sensory and microbial changes during storage (Robertson, 1993). These changes result in the decrease of consumer acceptance for bakery products and in great economic losses. In order to achieve longer shelf life for bakery products and extend market area of a wide variety of bakery products, refrigerating conditions are employed, as well as new processing and packaging technologies are investigated.

RTE frozen pastries occupy an increasingly great portion of the food market covering sectors such as institutional and catering businesses, supermarkets, and restaurants (Kennedy, 2000). This technology offers advantages to the consumers, who can have freshly baked bread at any time of the day and in a variety of points of sale or even at home. Consumers want to spend less and less time for preparation of meals (just 34.9 minutes for lunch and 33.1 minutes for dinner) (Coldiretti, 2010), preserving the tradition.

At the same time, there has been an increase interest in artisan type bakery products. Traditional bakery products are produced from suitable materials, following the classic procedure and using the proper equipment.

1.3 Confectionery products: *Sfogliatella Riccia Napoletana*

Many consumers are becoming increasingly aware of rediscovering local traditions. As a consequence, a certain niche market for agrifood products, such as traditional products obtained by means of ancient recipes and processing technologies, has been established.

Confectionery products field in southern Italy is characterized almost entirely by traditional products, arising from ancient recipes.

Campania shows a secular tradition in the preparation of a large number of baked confectionery products, worldwide known and appreciated for their goodness.

Sfogliatella is recognized as Traditional Agri-food Product (Prodotti Agroalimentari Tradizionali - PAT) of Campania Region, according to MiPAAF (Gazzetta Ufficiale della Repubblica Italiana, 1999), into the category of bakery products and pastry.

The list of PAT is yearly compiled by the Ministry of Agriculture (Gazzetta Ufficiale della Repubblica Italiana, 2016), that to be recognized as PAT is needed “to follow processing technologies, storage modalities and seasoning systems consolidated during time, homogeneous in all the area considered, according to traditional

practices, for a period of time not shorter than 25 years” (Gazzetta Ufficiale della Repubblica Italiana, 1999). Italian Traditional Agri-food Products are classified into different categories: dairy products, meat products, cereals, fruit and vegetables, bakery products and pastry, and alcoholic beverages.

In Neapolitan cuisine there are two different variants of the pastry: *Sfogliatella Riccia*, the "original" version, and *Sfogliatella Frolla*, a less labor-intensive pastry that uses a shortcrust dough and does not form the *Sfogliatella*'s characteristic layers.

Sfogliatella Riccia (Fig. 3) is a nonyeasted, shell-shaped filled Italian pastry native to Campania, distinctly layered pastry with a flaky texture. *Sfogliatella* means "small, thin leaf/layer", as the pastry's texture resembles stacked leaves. The outer puff pastry is a laminated bakery product made from a paste consisting of many thin layers of dough separated (laminated) by alternate fat layers. Lard is traditionally used in *Sfogliatella* production.



Figure 3_ *Sfogliatella Riccia Napoletana*.

1.3.1 Origin

Its origin dates back to 1600s, in the Monastery of St. Rose from Lima, between Furore and Conca dei Marini, on the Amalfi coast, thanks to a cook-nun. In fact, according to the legend (<http://www.sfogliatella.it/storia.htm>), she noticed some advanced semolina cooked in milk in the kitchen and, probably inspired by the need to not waste anything, she decided to prepare a mixture using semolina cooked in the milk, lemon liqueur, dried fruit and sugar. Then she added to the mixture white wine and lard and created a pocket like a nun's hood in which she put the first mixture. Once cooked, the nun garnished the new cake with pastry cream and raspberries. This delicious sweet was renamed "Santa Rosa", to glorify the Saint to which the monastery was dedicated. The sweet was put on the classic exiting wheel in change of currency.

In the early XIX century Pasquale Pintauro, a Neapolitan pastry chef, obtained the original recipe (probably from a nun aunt): he promptly changed it by removing the pastry cream and the raspberries and suppressing the bulge above hood of Munich. So he created the *Riccia* variant of the *Sfogliatella*: triangular-shaped, crunchy, composed of layers of thin puff pastry overlapping each other, filled with eggs, ricotta, semolina, sugar and candied fruit.

1.3.2 Processing

Sfogliatella Riccia Napoletana (SRN) making is a complicated process that involves traditional production practices and its making still remains an art.

The classical processing is composed of the following steps:

- dough mixing
- dividing of the dough in pieces
- laminating with roll-in fat
- relaxation
- stretching and cut of disks
- forming and filling
- baking

Flour, water, sugar and salt are mixed in one stage to obtain the base dough. After mixing the dough is divided in pieces and continuously sheeted and folded in a process commonly called ‘laminating’ or ‘layering’ the pastry and extruded into a dough sheet. During the mixing of flour with water, the proteins are developed into a continuous gluten matrix that binds starch granules, and subsequent processing ensures the required balance between cohesion, elasticity and extensibility of the dough; so doughs are usually mixed until the gluten structure is partially developed, the full development of the gluten structure occurs when the dough is sheeted for laminating. As there is still further gluten development during the lamination stage, the mixing of the dough should not be overdone.

In subsequent processing, the roll-in fat is deposited as a continuous layer of fat on top of a continuous layer of dough. Lard is traditionally used, because it is, tasteless and less expensive than butter. The roll-in fat is melted, applied to the dough sheet by a shortening extruder and absorbed by the dough giving it a soft texture and resistance to moisture gain which helps retain a crispness when wet fillings are used in the puff pastry. Finally the paste is rolled into a log (much like a Swiss roll, but with many more layers) and is allowed to rest. At present, the hand-shaping of the dough is usually replaced by a special equipment called “sfogliatrice” that is able to automatically shape rolled strips from a sheet of laminated dough to ensure greater productivity. The ability of the “roll-in-fat” to keep dough layers separated, primarily determines the quality of puff pastry. During the laminating process, dough layers are reduced to an average thickness of about 2-3 mm. At this thickness, the gluten structure in the dough assumes a two-dimensional orientation, as compared to the three-dimensional cell structure in most other doughs and batters. This process finally produces the flaky structure in the pastry during the baking process. The relaxation/retardation of paste is preferably done at ~ 4–8 °C to prevent temperature elevations in the paste and subsequent reduction in baking quality. An overnight retardation of paste at ~ 4–8 °C is sometimes applied before the final stretching step. Then disks are cut from the end, shaped to form pockets, and filled. Filling includes sugared ricotta, cooked semolina, eggs, candied orange cubes and flavorings. Important is to remember that the water activity of the filling has to be studied as a

function of the type of product, the expected shelf-life, stability during baking, packaging, and so on. Water migration from the filling to the dough has to be considered. The filling should contain sufficient sugar to avoid boiling (out) during baking.

The pastry is baked until the layers separate forming the *Sfogliatella's* characteristic ridges. As for all bakery products, baking time and temperatures are dependent on the type of oven, the size and shape of the product, and the type and quantity of the filling.

Bakeries have been responding to the consumers' demands for fresh like products. But traditional baking processes are sometimes too limited and inflexible to fully satisfy manufacturer's requirements and consumer's demands. One of the most significant technological developments in the bakery sector in the last few decades is the development of the technology applied to frozen or low temperature stored products.

SRN, as other bakery products, is characterized by a relatively short shelf-life, leading to loss of freshness and worsening of quality. One of the most efficient methods of slowing down the staling of these products is their freezing and frozen storage. Therefore, SRN is produced as raw frozen pastry in industrial scale. So the baking step is made by the final users, following the recommendations of the producers.

1.4 Bakery fats

Fats are among the main ingredients of *Sfogliatella*, responsible for its appreciated flaky structure. Fats cannot be absorbed by the dough layers; the fat film has to remain continuous during the rolling process. A low-melting unsaturated fat can melt and absorb into the dough quickly due to dough warm up during laminating. As a result, adjacent layers can stick together and the layering effect will be lost.

Fats play a key role in bakery products, providing desired rheological properties and specific sensory properties (aroma, flavor, softness, volume, palatability, bright appearance), as well as stabilizing the products toward oxidation reactions, stale, and moisture migration (Pagani *et al.*, 2010). In its loosest sense, the term "fat" is often used interchangeably with the term "shortening". "Shortening" is applied in the broader sense to edible fat used to shorten or tenderize baked products, with many functions in bakery foods (McClements & Decker, 2010; O'Brien, 2009; Ghotra *et al.*, 2002):

- tenderness and texture;
- mouthfeel;
- structural integrity;
- lubrication;
- air incorporation;
- heat transfer;
- shelf life extension.

Baardseth *et al.* (1995) using eleven different laminating fats made of milk fat or vegetable oil for Danish pastry production found the type of shortening influenced the taste, odour and colour using principle component analysis. Also laminating fat content in the formulae influenced the texture and colour (Wickramarachchi *et al.*, 2015).

Their functionality as bakery ingredients depends on several factors and is related to the final application of the product. Predetermined plasticity, firmness and solid fat content profile are the main requested quality attributes, due to their physical and chemical characteristics. The fats need to be on solid or semisolid state at room temperature to facilitate handling of the batter during the manufacturing, which implies an increase in the content of saturated fatty acids (SFA) (Tarancón *et al.*, 2013).

The suitability and application of a fat in bakery products depend on three parameters (Pareyt *et al.*, 2011):

1. the solid/liquid phase ratio at a given temperature, which determines the plasticity of the fat.
2. the crystal structure of the solid lipid.
3. oxidative stability, which is affected inter alia by FA composition and degree of unsaturation.

Plasticity of fat is of major importance for fat performances in baking.

In the material, both a solid and liquid phase must be present. The two phases must be in adequate solid- liquid proportion: the solid phase has to be in a fine dispersion, in order that the particles are held together by internal cohesive forces. Solid particles should be enough to prevent the flow of the mass, without forming a rigidly-interlocked structure (Pylar, 1988).

Another characteristic of fat is its crystalline nature. The three basic polymorphs (α , β , and β') (Marangoni *et al.*, 2012) influence its properties during baking and thus final outcomes in the product. In particular β' crystalline form is preferred for good baking (Wilderjans *et al.*, 2013). Smith & Johansson (2004) stated that shortenings containing small β' crystals are more effective in producing high quality bread than those containing larger β' crystals, even though the latter are more stable due to their higher melting points, so they may have a more pronounced positive impact on bread making (Pareyt *et al.*, 2011). Crystalline state is strictly related to the physical state of the fat and thus to its solid fat content (SFC). Short fatty acid (FA) chains, the presence of unsaturated FA and the cis configuration, all decrease the melting point (Manley, 2000), as the packing of the molecules becomes more difficult due to steric hindrance. Instead, FA chain length increase raises the melting point, as well as the presence of trans FA, as they show melting points closer to those of the corresponding saturates (Scrimgeour, 2005). A typical European standard for cake and pastry shortening is represented by blends that have slip melting point (SMP) between 35 and 38 °C and SFC of 20-35% at 20 °C (Klimes, 1989).

The texture, aroma and mouth sensation when consuming bakery foods are strongly dependent on their fat content and can eventually shape dietary choices in the long term (Drewnowski *et al.*, 1989).

The fats usually used in bakery products are butter and lard (animal fats), and hydrogenated and/or refined vegetable oils, which imply nutritional and environment problems.

Animal fats, especially lard, were originally fats used for preparation of bakery products.

1.4.1 Lard

Historically, lard was the first fat most widely used as shortening for the bakery industry. Lard is heat rendered from adipose tissues of pork (*Sus scrofa*) and subsequently refined. In the beginning, lard was chosen for its capability to produce an acceptable consistency, because is readily incorporated in bread, cakes, pastries, and other bakery products (Bodman *et al.*, 1951; Chrysam, 1985; Weiss, 1983).

Lard has many used in industrial applications because it has oxidative stability, thanks to its firmness (Gläser *et al.*, 2004), probably because lard crystallizes in the β -form (Ghotra *et al.*, 2002) for the high percentage of medium-melting disaturated, monounsaturated triglycerides (O'Brien, 2009).

As a by-product of the meat industry, lard became available in significant quantities. However, lard production is governed by the number and weight of hogs marketed. Consequently, the supply is not directly related to demand. Therefore, when world demand for edible fats increased, in the middle and later parts of the nineteenth century, serious attention was given to the development of alternatives for lard. The fatty acid composition of lard widely varies according to dietary intake, the climate in which it was raised. Typically it contains high levels in saturated fatty acid (~45%) and cholesterol (~97 mg/100g) (contributing to the development of cardiovascular disease) even if it shows a low oxidative stability due to absence of antioxidants (Conte *et al.*, 2003). However, there is a growing negative perception regarding the implication of animal fats on human health, so a significant trend toward utilization of vegetable-derived shortening as substitute of lard was revealed. Many reasons are attributed to this trend, including vegetable oil availability, oil processing capability, nutritional needs, cost, bulk handling, storage and labelling (Kamel, 1992); but studies dealing with the formulation of fat substitutes to mimic the physical characteristics of lard are still limited (Nur Illiyin *et al.*, 2013).

1.4.2 Vegetable fats

Towards the end of the nineteenth century, developments in refining technology and fat modification techniques allowed the use of a widening range of fats, such as coconut oil, soybean oil, palm oil and palm kernel oil as cheaper and zero-cholesterol substitutes for animal fats and in margarine and shortenings production.

Nowadays vegetable oils have great interest but most of them have to be transformed to reach the necessary functionality in such products. Partial hydrogenation,

interesterification, fractionation and bleaching, deodorization, winterization, and refining, are common fat modification processes (Dijkstra, 2007; Pajin *et al.*, 2011).

Sunflower, palm, soya bean and coconut are the major raw materials for production of vegetable oils in the world, although the usefulness of indigenous and semidomesticated plants and trees as vegetable oil source, such as *Vitellaria paradoxa*, is starting to percolate into the awareness and knowledge of agro-foresters, entrepreneurs and the public (Dianda *et al.*, 2009; Leakey, 1999; Kante *et al.*, 2009; Teklehaimanot, 2004). Vegetable oils can be used alone or in blend. Several studies show that formulation of blend is a key factor affecting the final product quality, but also the processing is as critical point (deMan *et al.*, 1989; Bongers & Almeida-Rivera, 2011; Chrysan, 2005; Miskandar *et al.*, 2005; Faur, 1980; Greenwell, 1981).

1.4.2.1 Palm oil

In the last few decades, palm oil has become the most produced vegetable oil in the world, also overtaking soybean oil (Oil World, 2013), because it is a low cost material, stable towards oxidation and versatile. Fig. 4 shows this emerging trend from 2004 to 2013 (MPOB, 2013).

Palm oil is extracted from the ripened mesocarp of the fruits of an ancient tropical plant (*Elaeis guineensis*) that originated from West Africa. Southeast Asia is the leading region for palm oil production but commercial plantations are on the rise in Latin America and West Africa. Indonesia, Malaysia, Thailand, Colombia and Nigeria are the leading producing countries (Mba *et al.*, 2015).

Oil palms are the highest yielding, least expensive of all vegetable oil crops; an estimated 58.431 million metric tons (MT) per year (MPOB, 2013). One hectare of oil palm plantation is able to produce up to 10 times more oil than other leading oilseed crops (Mba *et al.*, 2015). Worldwide, 90% of palm oil is undressed to the food industry, while the remaining 10% finds application in soap and oleochemical industries (Oil World, 2013).

Palm oil is suitable for numerous food applications because it has equal amounts of saturated and unsaturated fatty acids, a high smoke point of about 230 °C and a stronger resistance to thermoxidation than most other vegetable oils. The oil is semi-solid at room temperature and doesn't require hydrogenation. Melting temperature is in the range of 33-41 °C (Tarabukina *et al.*, 2009). Palm oil contains almost equal proportions of saturated (48% palmitic and 4% stearic) and unsaturated (37% oleic and 10% linoleic) fatty acids (Gunstone, 2005). It is essentially composed of three types of triglycerides: trisaturated (mainly tripalmitin), disaturated (mainly 2-oleodipalmitin, 30-40%) and monosaturated (mainly 1-palmito-2-3-diolein, 20-30%). Palm oil can be fractionated to palm olein, a liquid fraction and palm stearin, the solid fraction, having distinct physical and chemical properties.

Palm oil, palm olein and palm stearin are important constituents of several food and industrial products such as shortenings, ice cream, cosmetics, candles lubricants,

toothpaste and biodiesel (Barriuso *et al.*, 2013). Palm stearin is helpful in providing the solid fat functionality without the use of hydrogenation, thus reducing trans-fat intake in the diets (Kellens *et al.*, 2007).

In many different areas of the world, low-*trans* blends are compounded by using only palm oil and palm oil fractions. 100% palm oil blends require special processing parameters due to the slow crystallisation rate of palm oil. As examples of different crystallisation rates, coconut oil needs only 3 min at 20 °C, whereas palm oil needs 27 min at 10 °C (Gerstenberg, 1996).

Blending palm oil and fractions with other oils with more unsaturated or monounsaturated oils can improve and enhance the commercial, functional, nutritional and technical attributes of the oil (Manorama & Rukmini, 1992). Blending is a desirable alternative to the negative effects associated with hydrogenation.

Also the interesterification of palm oil is an option to widen food applications. Interesterification can be used to incorporate essential polyunsaturated fatty acids in order to obtain oil rich in essential fatty acids and enhanced antioxidant properties (Hoffmann *et al.*, 2002; Naghshineh & Mirhosseini, 2010; Tiwari *et al.*, 2014). Global consumption rose from 14.6 million tons in 1995 to 61.1 million tons in 2015, making it the most consumed oil in the world (European Palm Oil Alliance, 2016).

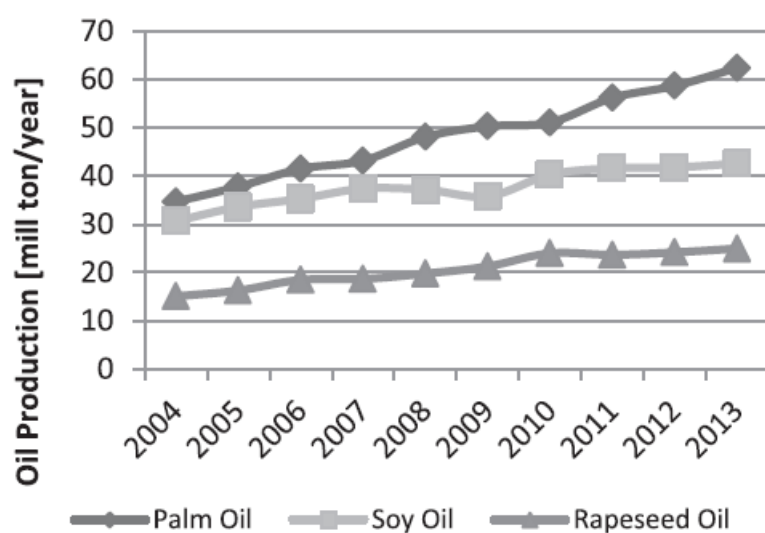


Figure 4_ Oil production of the three major vegetable oils (MPOB, 2013). Palm oil quantities include crude palm oil and crude palm kernel oil (Hansen *et al.*, 2015).

However, palm oil is nowadays under discussion, mostly related to sustainability of its cultivation.

Roughly 185 million tons of palm oil are produced in the tropics and then traded globally. Unfortunately, the highest concentrations of vegetable and animal species (or highest biodiversity) are also in the tropics, and so the doubling in palm oil production from 2003 to 2013 raises concerns about possible wildlife extinctions (Vijay *et al.*, 2016).

Deforestation has occurred. Farmers have cleared the forest quite recently. In these countries, palm plantations threaten iconic and endangered wildlife, including

orangutans, Bornean pygmy elephants, and the Sumatran tiger. Similarly, palm plantations in South America are also from recent deforestation. Moreover, tropical deforestation also releases the carbon stored in the tree tissues and in the soil, contributing to an estimated 10% of the greenhouse gas emissions that causing global warming. Demand for palm oil is increasing so there is concern that palm plantations will spread, deforesting other parts of the world, as Indonesia, Malaysia, Papua New Guinea, and Brazil (particularly in the Amazon), where animal species, like the Little Woodstar, the largest species of armadillo, and numerous brightly-colored poison frogs, could be threaten. In Indonesia, Malaysia, Ecuador and Peru, most of the plantations are on land deforested since 1990, and probably oil palm was the cause of much of this deforestation (Vijay *et al.*, 2016).

With the prospect of continued high demand in the largest importing countries (India, China and Europe) strategies for sustainable palm oil production represent an necessary step forward.

There also seems to be an emerging trend in palm oil alternatives in European countries by vegetable blends having the same technological properties.

1.4.2.2 Sunflower oil

Sunflower oil is seen as being a viable alternative to palm oil.

Sunflower (*Helianthus annuus L.*) is of North American origin and in XVI century was introduced in Europe.

Sunflower-seed oil accounts for approximately 14% of the world production of seed oils. Sunflower seeds production increased from 14 to 23 million tonnes between 1980 and 1991. The world's largest sunflower-seed producers are the former USSR (39% of world production), Argentina (13%), China (6%), U.S. (6%), India and France (both 5%).

It is annual plant and is commercially cultivated for vegetable oil extracted from the seeds. At room temperature is in a liquid state because of its low crystallization temperature, about 2-3 °C.

Fatty acid composition includes palmitic (4-9%); stearic (1-7%); oleic (14-10%); linoleic (48-74%) acid, with variations for both genetics and climate (British Pharmacopoeia, 2005).

Major triglycerides in the oil are typically tri-linolein (14%), 3-oleo-dilinolein (39%), 3-stearodilinolein (14%), 1-linoleo-diolein (19%), linoleo-oleo-stearin (11%), and others (3%) (Gunstone, 2005).

Due to the high content of unsaturated fatty acids, sunflower oil is liquid at room temperature. It is widespread in food industry for multiple purposes, in particular for frying processes, thanks to its high smoke point (around 230 °C).

1.4.2.3 Coconut oil

Coconut oil is an edible oil extracted from the kernel of harvested mature coconuts of the tree *Cocos nucifera*, Family *Aracaceae* (palm family).

All parts of the coconut palm are useful, in fact, it is produced in large scale for both food and non-food purposes especially in Pacific area (Kappally *et al.*, 2015). This is the reason the coconut is often referred to as the tree of life, or the king of tropical flora or the tree of abundance. World production exceeds 28 million tons.

Coconut oil is widely used in bakery item, especially for pastry products, due to high degree of saturation and good oxidative stability. The smoke point of refined oil is very high, about 232 °C. It has a hard consistency but fragile at low temperatures, having a low melting point (below 30 °C). This is caused by the low molecular weight of glycerides. It resembles coconut butter. Oil contains about 90% of saturated fatty acids, including lauric acids (44-52%), myristic (16-21%), and palmitic acid (7-10%) and caprylic acid (5-10%). On the other hand, the majority of fatty acids with high molecular mass are present in low levels, such as oleic acid (5-8%), linoleic acid (1-3%), linolenic acid (up to 0.2%) (Canja *et al.*, 2015). It can be used to replace solid fats produced through chemical or chemical-physical processes (interesterification of fats, fractionation, hydrogenation) in bakery and confectionery products (O'Brien, 2009).

Coconut oil is subjected to different operations for the purification of raw oil, such as neutralization, bleaching, deodorizing; to remove unpleasant volatile substances, including free fatty acids, phospholipids, pigments and traces of metals. These impurities are not supported because they give color to the oil, determine the appearance of the foam and precipitate in heat (Haji & Haji, 2012).

A number of health benefits have been attributed to this oil (Kappally *et al.*, 2015):

- the medium chain (C8-C12) fats in coconut oil are similar in structure to the fats in mother's milk that gives babies immunity from disease and have similar effects;
- coconut oil possesses anti-inflammatory, anti-microbial and antioxidant properties that work together to protect the arteries from atherosclerosis and the heart from cardiovascular disease;
- it is cholesterol-free, trans-fat free and heart-healthy;
- it boosts the immune system;
- it protects against heart disease by increasing high density lipoprotein which collects the excess or unused cholesterol in the body for excretion in the liver;
- it has antioxidant and antimicrobial properties, because the fatty acids under the influence of enzymes when coconut oil is ingested turn into compounds such as monolaurin, which provides protection against infectious diseases caused by lipid-coated microorganisms;
- in the digestive system, the medium chain fatty acids in coconut oil are rapidly absorbed, carried by the portal vein to the liver and then oxidised, thereby producing energy very rapidly;
- it represents a source of energy, recommended in case of tiredness because is digested easily without the need for bile and goes direct to the liver for conversion into energy;
- it helps to increase the rate of metabolism, helping in weight loss;

- it has beneficial effects on the gastrointestinal tract by increasing the absorption of vitamins, minerals and amino acids;
- it inhibits the action of cancer-forming substances.

1.4.2.4 Shea butter

Shea butter, also called Karite butter or Galam butter, is obtained from the kernels of a tree belonged to the family *Sapotaceae*, *Vitellaria paradoxa* C.F. Gaertner [synonym *Butyrospermum parkii* (G. Don) Hepper] mainly found in savannah belt of West Africa, between the equatorial rain forest and the Sahel zone bordering the Sahara (Hall *et al.*, 1996; Salle *et al.*, 1991). It occurs in 19 countries across the African continent, among which Benin, Burkina Faso, Cameroon, Ethiopia, Ghana and Guinea. *Vitellaria* density averages fifteen mature trees per hectare in Mali (Ruyssen, 1957), and comprises upwards of 70% of the woody vegetation in areas of Benin (Agbahungba & Depommier, 1989) and sometimes over 80% in northern Ghana (Lovett & Haq, 2000a) and Burkina Faso (Boffa, 1999). *Vitellaria* has been widely described as an unplanted, ecologically successful wild tree traditionally exploited for its pulp and fat (Chevalier, 1948; Boffa, 1999; Ruyssen, 1957). It was probably subjected to an unconscious domestication process (Lovett & Haq, 2000a; Maranz & Wiesman, 2003). Traditionally, the collection and processing of nuts into butter in Africa has been carried out by women (2 million women in 13 African countries produce shea butter both for cash and food), improving the incomes and living standards of rural farm families and the economies of exporting countries (Vermilye, 2004; Kante, 2007). Extraction has been usually done by boiling water and skimming off the released oil while commercial one is conducted by pressing or solvent extraction with further refining and deodorizing of shea butter (Alander, 2004). In recent years, the dried kernels have been exported to processing countries in Europe, Japan, and India where shea butter is extracted in large-scale industrial plants (Lovett, 2004).

The largest commercial use of shea butter is primarily as a cocoa butter equivalents for chocolates and confectionary (Maranz, 2004; Honfo *et al.*, 2014), due to its solid nature at room temperature; although it is increasingly popular in cosmetic product formulations.

The main fatty acids are stearic (S) and oleic (O) acids, which together contribute 85-90% of the fatty acids present, and its main TAGs are 1,3-distearoyl-2-oleoyl glycerol (SOS) and rac-1-stearoyl-2,3-oleoyl glycerol SOO (13-46% and 16-31%, respectively) (Maranz *et al.*, 2004). The stearin is principally SOS, which is stable in the β polymorph, which cause it to melt sharply close to body temperature and allow it to be compatible with cocoa butter (Moharram *et al.*, 2006; Lipp *et al.*, 1998). The high stearic acid content of the blends studied make the fats attractive on nutritional grounds, since stearic acid contributes to reduced plasma LDL concentrations and hence has no adverse effect on risk of cardiovascular disease (Mensink, 2005). 23 triglycerides were identified. SOS and SOO were shown to make up to 60% of the

fat (Swadogo & Bezar, 1982). It was used as a shortening (Badifu & Akaa, 2001) with comparable results to a control sample made with margarine.

The composition for interesterified products is given as an example in the patent application (Macrae, 1983).

The fatty acids of such products had been rearranged using a 1,3-specific lipase and yielded an olein fraction that consists of approximately 40% SOO and approximately 20% SOS, as well as a stearin fraction consisting of predominantly (>80%) SOS. The difference in fatty acid composition depends on the origin of the samples and the extraction method.

Shea kernels and shea butter have been found to have high levels of phenolic compounds (catechins) and tocopherols, with significant regional variation in the content of these antioxidants (Honfo *et al.*, 2014, Bail *et al.*, 2009); although refining process can lower these levels.

1.4.3 Fat structurization

Nowadays, the trend in animal oils alternatives with vegetable ones have gained interest but most vegetable oils have limited technological application in their original forms because of their specific physical and chemical properties. Thus, the vegetable oils have to be transformed to reach the desirable properties and enhance their commercial application, using different processes. Structured lipids include not only triacylglycerols, but also monoacylglycerols, diacylglycerols and glycerophospholipids that have been structurally modified from their natural form by changing the positions of fatty acids, or their profile, or synthesized to yield novel triacylglycerols through chemical or enzymatic processes (Akoh & Kim, 2008).

The common fat modification processes are fractionation, partial hydrogenation and interesterification (Pajin *et al.*, 2011; Dijkstra, 2007). They modify the solid-liquid balance of the triacylglycerols in the temperature range of interest in a given application. These processes may be used singly or in appropriate combinations.

In fractionation, fats/oils are separated into two fractions with different melting and textural properties (Kellens *et al.*, 2007), involving the use of solvents or dry processing (winterization and pressing techniques). This process is often used commercially to produce hard butters and specialty fats from oils such as palm and palm kernel. Fractionation can be an independent process or can be used as a pretreatment prior to hydrogenation, interesterification or blending (Shahidi, 2005).

Hydrogenation of vegetable has been used for a long time to get more solid a liquid fat.

Hydrogenation converts oil into plastic fat forms, with the consistency and handling characteristics required for functionality, such as creaming properties, frying stability, sharp melting properties and oxidative stability, necessary to maintain product acceptability for prolonged period after processing and packaging. This process has been used for years to produce margarine and shortening.

Double bonds of unsaturated fatty acids are saturated with hydrogen, using nickel as a catalyst, until the oil is at the desired consistency. Unfortunately, during hydrogenation some double bonds can be isomerized and converted from cis state to trans state. Trans-isomers are nowadays regarded as undesirable by nutritionists, and will be increasingly subject to product labelling regulations. 'Trans' fatty acids are implicated in increasing of LDL, decreasing of HDL, and increasing of total/HDL cholesterol (Mozaffarian & Clarke, 2009), the development of coronary heart disease (Iqbal, 2014) and cancer (Astorg, 2005).

Since the hydrogenation reaction can generate high levels of 'trans' fatty acids, there has been a trend toward reducing reliance on hydrogenated oils in formulations.

The technologies of production and raw materials choice have thus improved over the years, aiming to blend health aspects and technological requirements.

To accomplish this aim, another possibility of reorganizing fat structure, without altering its fatty acid profile, is interesterification.

1.4.3.1 Interesterification

The interesterification process consists in the rearrangement of the distribution of the fatty acids on the glycerol backbone, catalysed by enzyme (using lipase) or chemicals (by acidolysis and other ester exchange reactions) within and between the triglycerides (Wassel *et al.*, 2010). The rearrangement process does not change the degree of unsaturation or the isomeric state of the fatty acids as they transfer in their entirety from one position to another (Ribeiro *et al.*, 2009) (Fig. 5).

Intesterification, preferably lipase-catalyzed, can be considered an alternative process to partial hydrogenation, as no saturation or isomerization occurs (Hunter, 2004). This rearranged oil has an advantage that it does not contain *trans* fatty acid, because it is not made through partial hydrogenation (Sundram *et al.*, 2006; Noakes & Clifton 1998; Meijer & Westrate, 1997; Karabulut *et al.*, 2004; Kubow, 1996; Upritchard *et al.*, 2005); it has been used in many studies for producing modified solid fats with desired physical and chemical properties (Lee & Akoh, 1998; Zhang *et al.*, 2004).

The commercial application for interesterification is the production of specialty fats for the confectionery and vegetable dairy industries. This process permits further tailoring of triglyceride properties to achieve the required steep melting curves. Treating oils and fats with sodium methoxide as a catalyst at 80 °C causes intermolecule ester exchange, changing the molecular composition, while leaving the fatty acid composition unchanged. As a result, the oil changes its physical properties such as melting point and consistency.

It is applied to either an individual oil or a blend of oils, to produce triglycerides with different properties.

Blending interesterified exotic fats with liquid oils therefore can be a good solution to replace palm oil-based fats in some confectionary applications (Hinrichsen *et al.*, 2016). Commercial interest in enzymatic interesterification has increased

significantly in the past several decades since it doesn't create trans isomers (Lai & Lin, 2007). Several studies have been carried out using interesterification to produce *trans*-free margarine (Lumor *et al.*, 2007; Kim *et al.*, 2008; Adhikari *et al.*, 2010a, 2010b).

Previous researches highlighted that it is possible to improve the quality of the lipid fraction of bakery products through the preparation of *trans*-free margarines by interesterification and blending (Kok *et al.*, 1999) and *trans*-free shortenings by fractionation and blending (Reddy & Jeyaranin, 2001).

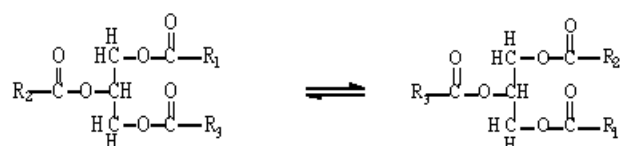


Figure 5_ Interesterification within triglycerides.

1.4.3.2 Health related issues

Bakery products are among the foods highest in fats; most of them contain hydrogenated fats with large quantities (as high as 20-40%) of trans fatty acids (Reddy & Jeyaranin, 2001).

In the last twenty years, health risks related to high fat consumption have been widely reported in literature. Epidemiological studies have identified the intake of saturated fatty acids (SFA) and trans fatty acid (TFA) as a risk factor in the increasing of blood lipids and low density lipoprotein (LDL) cholesterol concentrations, correlated with cardiovascular disease (Ansorena *et al.*, 2013).

In the US, the mean daily intake of TFA per person has been estimated at approximately 2.6% of total food energy (5.3 g/day), and the American Heart Association (AHA) recommended TFA intake of <1% of energy (Lichtenstein *et al.*, 2008). Partial hydrogenation is the main contributor of TFA (80%) in the U.S. diet (Eckel *et al.*, 2007).

TFA consumption should be as low as possible according to Dietary Guidelines for Americans (2010); the American Heart Association (2008) suggested that no more than 10% of daily energy should be consumed from trans and saturated fats (Rogers, 2009).

Thus, efforts for reducing the consumption of TFA has led to the prohibition of foods containing >2% TFA as a percentage of total fats in the Danish market (Stender & Dyerberg, 2003), and from January 2006 all foods containing ≥ 0.5 g *trans* fat/serving must be labelled accordingly in the US (FDA, 2003).

Currently, one of primary trends in bakery food is to reduce fat and cholesterol content (Ma & Boye, 2013).

Marangoni *et al.* (2007) suggested to shift fat consumption from saturated fats to unsaturated oils, eliminating TFA from the diet. In fact, consumption of polyunsaturated fatty acids lowers the total to high density lipoprotein (HDL)

cholesterol ratio, perhaps the best single lipid predictor of coronary heart disease risk (Prospective Studies Collaboration, 2007). Moreover, Salmeron *et al.* (2001) and Summers *et al.* (2002) found that polyunsaturated fatty acids consumption may also improve insulin resistance, while Mozaffarian *et al.* (2010) hypothesized a reduction in systemic inflammation.

This has raised the need to replace hydrogenated vegetable fats in cakes (Rios *et al.*, 2014) with other lipids (Sowmya *et al.*, 2009).

1.5 Baking

Baking is a complex process that brings about a series of physical, chemical and biochemical reactions in a product (Sumnu, 2001), such as:

- starch gelatinization,
- protein denaturation,
- liberation of carbon dioxide from leavening agents,
- volume expansion,
- evaporation of water,
- crust formation,
- browning reactions,

changing the structure, taste, color, and size of a dough piece (Acar *et al.*, 2012).

Baking temperature depend on the oven, but typically range from 200 to 250 °C, aiming to achieve a core temperature of approximately 92 to 96 °C by the end of the baking period (Cauvain, 2003).

During baking a simultaneous heat and mass transfer occur within the product and with the environment inside the oven (Megahey *et al.*, 2005).

In conventional baking, heat is transferred, mainly by convection, from the heating media and by radiation from oven walls to the product surface followed by conduction to the centre (Sablani *et al.*, 1998). There is also conduction from the product container and convection in the product by the movement of water vapour as the temperature rises (Sumnu, 2001). The driving force for heat transfer is the temperature gradient from regions near the surface (where the temperature is limited to the boiling point of water) to the center of product.

Migration of water to the surface occurs by capillary and diffusion mechanisms, both functions of temperature and concentration gradients (Yong *et al.*, 2002). The baking process consists of three phases, which overlap with one another. Firstly, expansion of the dough and moisture loss commences, while in the second phase both dough expansion and the rate of moisture loss reach a maximum. In the third phase, the structure of the air cells within the dough matrix collapses as a result of increasing vapour pressure, so the product height and rate of moisture loss decrease.

Pei (1982) has classified conventional baking into four baking stages:

1. formation of white crust;

2. heat transmission from crust to interior;
3. gelatinization or cooking process;
4. browning.

Baik *et al.* (2000a) characterized the baking conditions inside the baking chamber in two different multi-zone industrial ovens were by in order to understand the general industrial baking process, measuring the internal temperature profile, air velocity, absolute humidity and oven wall temperature and estimating heat and mass transfer parameters in each zone of two different tunnel type multi-zone industrial ovens (gas fired band oven and electric powered mould oven).

The rate of heat transfer, the amount of supplied heat, the humidity level within the baking chamber and the duration of the baking process influence the quality of the final product (Sani *et al.*, 2014).

In the last years, several authors have studied the influence of baking conditions on product quality for different bakery products. Unklesbay *et al.* (1981) determined the thermal conductivity of white bread during heat processing in forced air convection and analyzed the relationship among thermal conductivity and selected physical properties like moisture, volume, bulk density and porosity. They showed that thermal conductivity values were indirectly linearly dependent, $p \leq 0.05$ on volume and porosity and directly linearly dependent on bulk density and moisture loss.

Important quality parameters, such as texture, density, colour and viscosity of the cake batter were evaluated by Baik *et al.* (2000b) during baking in two different multi-zone industrial scale ovens. Lostie *et al.* (2002a) studied the texture evolution of sponge cake baked under natural convection at 200 and 240 °C. Vignali & Volpi (2013) evaluated different features (water activity, humidity, pH and sensorial judge) of a final baked product, Panettone. Shahapuzi *et al.* (2015) studied the effect of the airflow on the oven temperature profile, the internal cake temperature and quality. Recently, Ureta *et al.* (2016) elaborated an experimental characterization of the process (three convection modes, three oven temperatures) accompanied with a mathematical model simulating the heat transfer dynamics.

Temperature is the dominating factor in various physicochemical changes during baking. To reduce energy consumption and to improve product quality, optimization of oven operating condition is required. To achieve optimal baking, the common industrial practice is to bake bread in the oven controlled at a constant temperature (Mondal & Datta, 2008).

The texture and density of baked products such as bread and cakes are controlled by the way their rheology and vapor content change during the baking process. Colour development is a function of moisture content, baking time and baking temperature. They are very important factors determining the sensorial quality.

The formation of crust and browning during baking appear to be primary contributors to the formation of flavour.

The surface color of bakery product is an important quality associated with its aroma, texture and appearance characteristics that are important to consumers, and is often

used as an indicator of baking completion. The formation of color and aroma in the crust of all bakery goods during baking is a result of Maillard reaction or caramelization (Mondal & Datta, 2008). It involves condensation of amino groups and reducing sugars at temperature greater than 110°C, that result in the formation of intermediate chemical compounds which ultimately polymerize to form brown pigments. Unfortunately, one of the most significant consequences of the Maillard reaction is the formation of toxic compounds such as acrylamide (Mottram *et al.*, 2002; Stadler *et al.*, 2002; Zyzak *et al.*, 2003). Acrylamide is a neurotoxic and classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC 1994).

Brathen & Knutsen (2005) examined the effect of baking time and temperature on the formation of acrylamide in bread, flat bread, dry starch system and dried rye-based flat bread. Up to 10 g/kg acrylamide formation was reported. Ahrne *et al.* (2007) determined the effect of crust temperature and water content on acrylamide formation in bread crust. According to their findings relatively lower value of acrylamide was observed in the case of high temperature baking and low water content but that is unacceptable from a consumer stand point as the bread is too dark and other sensory attributes of the bread are unacceptable.

Moreover, bakery products are generally baked by conventional heating, with prolonged time for heat to reach the center of the batter (Bilgen *et al.*, 2004).

Thus, different baking technologies were developed to respond better to new market demands (Decock & Cappelle, 2005). The growing tendency to spend less time on food preparation, greater convenience and time saving has lead the food processors, confectioners and household users to go for microwave processing (Bhatt *et al.*, 2008). Furthermore, energy for baking ovens is becoming relatively more expensive and there is a growing concern to improve oven efficiency and to use less energy.

1.5.1 Microwave baking

Microwave baking is a promising alternative to traditional process, which bases its preservation properties in thermal reduction of the microbial counts in foods.

Microwaves are electromagnetic waves of radiant energy which stand on electromagnetic spectrum between the radio and infrared waves (frequency range between 300 MHz and 300 GHz) (Ahmed & Ramaswamy, 2007). Microwaves are usually generated by an electromagnetic device called a “magnetron” which produces an alternating electric field and produces heat as a result of orientation of the dipoles in alternating electromagnetic field (Geise, 1992).

Microwave is radiation without ionizing, in contrast with ionizing radiation (X-rays, gamma rays), which induces the formation of positively and negatively charged atoms or molecules (Parker, 2003).

Heating takes place due to the interaction of electromagnetic radiation (governed by Maxwell’s equations for electromagnetic waves) at certain frequencies with foods showing dielectric properties (Tang *et al.*, 2002). Since microwave frequencies are close to the frequencies of radio waves and overlap the radar range, microwaves can

interfere with communication processes, so the use of certain microwave frequencies comes under the regulation of governmental agencies. In the USA, the use of microwave radiation is regulated by the Federal Communications Commission. Typically microwave food processing uses two approved frequencies of 2450 ± 50 and 915 ± 25 MHz, but domestic microwaves usually operate at 2450 MHz, as they have a low penetration depth, while 915 MHz frequency is for the industrial heating (Fu, 2004). Similar frequencies are regulated worldwide through the International Telecommunication Union (Coronel *et al.*, 2008a; Singh & Heldman, 2009).

Microwave heating is also called “volumetric heating” due to microwave radiation directly penetrates the material causing volumetric heat generation in the material, resulting in high-energy efficiency and lower heating times, not achievable by any other conventional mechanisms (Zhu *et al.*, 2007).

Introduction of first microwave oven by Raytheon Co. in the late 1980s, claimed the novelty of microwave cooking (Hui, 1992). Nowadays there is a microwave generation of consumers and a high concentration of microwave ovens are in US, Canada, several European countries, Japan and Australia.

1.5.1.1 Mechanisms involved in microwave heating

Mechanism of microwave heating involves following sequential steps (Fig. 6).

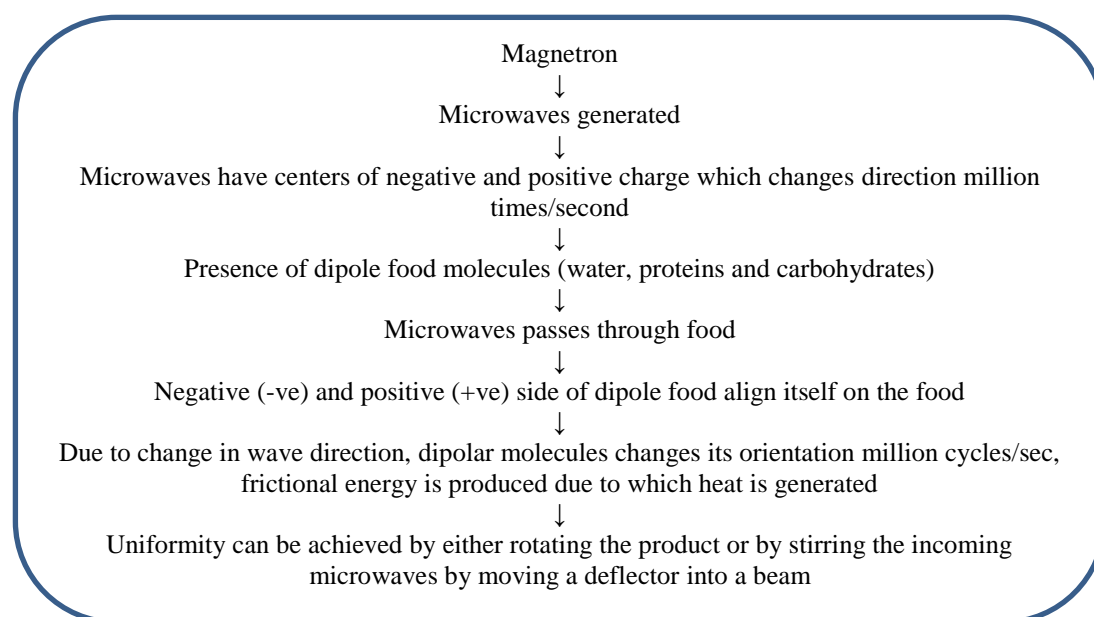


Figure 6_ Mechanism of microwave heating (Kaushal *et al.*, 2015).

There are two microwave heating mechanisms: ionic conduction and dipolar rotation, as shown in Fig. 7.

When microwaves penetrate foods produces a volumetrically distributed heat source and a dipolar interaction occurs; as water molecules and other polar molecules tend to align themselves with the electric field. The molecules attempting to oscillate at such frequencies generate intermolecular friction. In doing so, considerable energy is

extracted from the microwave field and heating takes place in all directions (Hossan *et al.*, 2010).

Moreover, when ionic solutions are exposed to a microwave field, the ions are obligated to flow first in one direction, and then in the opposite one with the rapidly alternating field. The resulting collisions between ions and other molecules cause the conversion of kinetic energy of the moving ions into thermal energy (Suárez *et al.*, 2000; Singh & Heldman, 2009).

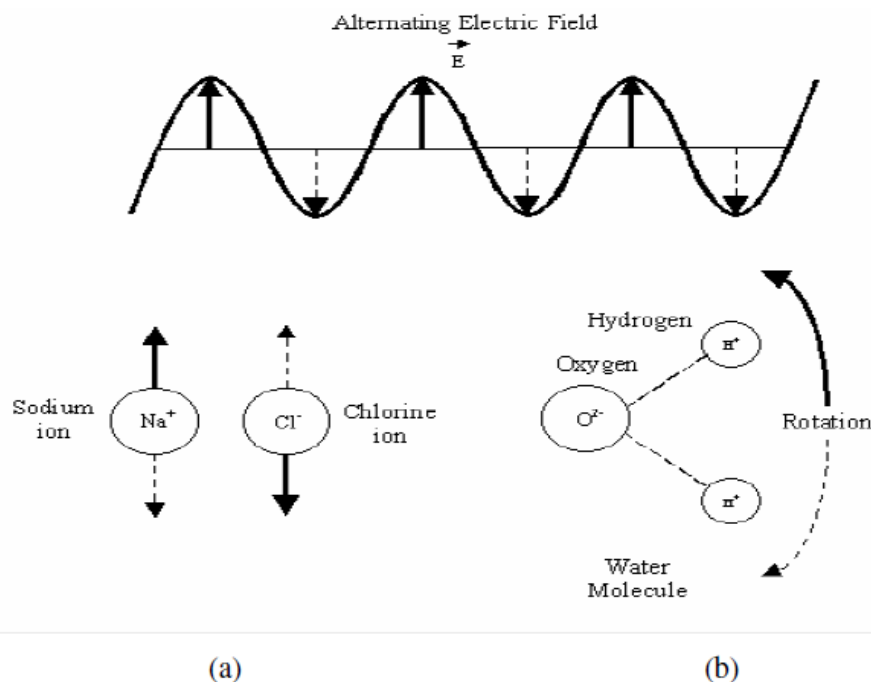


Figure 7_ Microwave heating mechanisms (a) Ionic conduction (b) Dipolar rotation (Sahin & Sumnu, 2006).

Governing equation for heat transport in microwave heating is given in equation (1.1):

$$\frac{\partial T}{\partial t} = \alpha \nabla^2 T + \frac{Q}{\rho C_p} \quad (1.1)$$

where T is temperature (K), t is time (s), α is thermal diffusivity (m^2/s), ρ is density (kg/m^3), C_p is specific heat of the material ($J/kg \cdot K$) and Q is the rate of heat generated per unit volume of material ($J/s \cdot m^3$).

The heat generated per unit volume of material per unit time (Q) represents the conversion of electromagnetic energy. Its relationship to the electric field intensity (E) at that location can be derived from Maxwell's equation of electromagnetic waves, as shown by Metaxas & Meredith (1983):

$$Q = 2\pi \epsilon_0 \epsilon'' f E^2 \quad (1.2)$$

where the magnetic losses of the food material have been ignored, ϵ_0 is the dielectric constant of free space, ϵ'' is the dielectric loss factor of the food, f is the frequency of oven and E is the electric field intensity.

Heat transfer by conduction, convection and evaporation are the main causes of time-temperature profiles within the product when heated by microwave (Mudgett, 1982).

Since there is a lack of ambient heat and the cooling effects of evaporation in the microwave oven, the interior temperature of a food heated by microwave energy is hotter than the surface temperature (Decareau, 1992).

1.5.1.2 Food properties affecting microwave heating

Knowledge of dielectric properties of foods is essential for understanding of heating behaviour of foods in microwave oven. Dielectric properties are dielectric constant (ϵ_0) and dielectric loss factor (ϵ'').

Dielectric constant is an ability of a material to store microwave energy while dielectric loss factor is the ability of a material to dissipate microwave energy into heat.

According to Venkatesh & Raghavan (2004), temperature, moisture content, composition, physical structure, density and frequency are the factors influencing the dielectric properties of the related food product.

The research about dielectric properties of bakery products is limited in literature. Goedeken *et al.* (1997) searched the change in dielectric properties of bread with and without salt. They found that as the bread was heated, the dielectric constant increased until the sample reached 60°C, once the temperature reached 60 °C the dielectric constant remained constant. In the case of dielectric loss factor, for samples containing salt, it increased with temperature but for the ones without salt, dielectric loss factor decreased with increasing temperature. Kim *et al.* (1998) developed models which predicted the dielectric properties of baked biscuit dough at different moisture content, density and high temperature at 27 MHz.

Kim & Cornillon (2001) studied the variation of dielectric properties of wheat flour dough with respect to temperature and mixing time. They found that temperature was effective on both dielectric constant and dielectric loss factor. The dielectric constant was found to remain constant as temperature increased up to 60 °C, and then decreased. Whereas, it was observed that dielectric loss factor increased with increasing temperature.

Sumnu *et al.* (2007) found that dielectric properties of breads baked with different heating modes of microwave plus infrared and microwave plus jet impingement, were affected by the decrease in moisture content and increase in porosity.

Keskin *et al.* (2007) investigated the effects of different gums on dielectric properties of doughs and breads baked in infrared-microwave combination oven were by. They concluded that the dielectric properties and quality parameters of breads baked in infrared-microwave combination oven were dependent on gum type.

1.5.1.3 Advantages and disadvantages of microwave heating

This technology has found many applications in the food industry, as it offers important advantages over conventional heating (Marra *et al.*, 2010), such as:

- ✓ less start-up time and quick heat penetration: the heating time for many materials is about one quarter of the time used in conventional heating, thus preserving nutrient and vitamin contents as well as flavor, sensory characteristics, and color of foods (Ahmed & Ramaswamy, 2007). For example, for reheating marsecuite from 38 to 54 °C, the process in heat exchangers using hot water takes from 1 to 5 h, while with a continuous microwave system, the residence time can be reduced to less than 1 min (Bento *et al.*, 2006);
- ✓ easy to use: the heating systems can be turned on or off instantly (Datta, 2003);
- ✓ energy saving and much shorter process time: overall microwave energy efficiency is approximately 50%;
- ✓ higher quality products and ecology friendliness: microwave heating avoids degradation of product strength and surface properties caused by exposure to high temperatures;
- ✓ microwave equipment is suitable for cleaning-in-place system, low cost system maintenance, and environmentally clean processing, since microwave generation does not produce exhaust gas or toxic waste (Fu, 2004; Ahmed & Ramaswamy, 2007);
- ✓ selective heating and food with high nutritional quality: since different materials absorb microwave energy at different rates, a product with many components can be heated selectively;
- ✓ space savings;
- ✓ combination with conventional methods: microwave energy may be added before, after, or inside conventional heating or drying units to decrease processing time by 75%.

The major disadvantage is the non-uniform temperature distribution in microwave heating resulting in hot and cold spots in microwave-heated products (Vadivambal & Jayas, 2010), associated mainly to solid foods. To resolve this limitation a rotating turntables in domestic ovens is used (Geedipalli *et al.*, 2007).

Microwave processing has been successfully used in food industry, and one of the potentially important growth areas is microwave-baked products.

1.5.2 Microwave and microwaves - infrared combination baking

The application of microwave heating in baking industry is an interesting alternative to conventional one because of the time and energy savings (Reiger & Schubert, 2001).

Megahey *et al.* (2005) investigated the microwave baking characteristics of Maderia cakes and microwave baking was found to allow for up to a 93% reduction in baking time, in comparison with conventional baking.

The use of microwave heating for final baking of cookies results in a more uniform moisture distribution (Schiffmann, 1992).

Bernussi *et al.* (1998) investigated the effects of microwave baking on the moisture gradient and overall quality of the cookies. The moisture gradient was significantly reduced in microwave baking and also the total moisture content of the cookies, avoided cracking but produced slight darker cookies.

Sumnu (2000) studied the effects of emulsifier, water, shortening and starch on microwave-baked cake quality and developed a cake formulation which produced cake with comparable quality to convective-baked cake.

On the other hand, microwave-baked products may develop some quality problems like lack of browning and flavour development, no homogeneous heating, reduced height of the product, dense or gummy texture and crumb hardness, rapid staling after heating mainly caused by re-crystallization of starch an undesirable moisture gradient along a vertical axis in the final baked product (Sumnu *et al.*, 2005; Sumnu, 2001).

Maillard browning reactions, which are responsible for the production of many flavored and colored compounds, can't occur due to low surface temperature (Decareau, 1992; Hegenbert, 1992). Brown surfaces, produced by the Maillard reaction and caramelization of sugars, are a result of high temperatures accompanied by dehydration (Burea *et al.*, 1987). During microwave baking, Since the ambient temperature inside the microwave oven is cool, the product can't experience the browning reactions.

Moreover, quality problems occur because some physico-chemical changes (starch gelatinization, starch conversion by enzymes) and interactions of major ingredients, which would normally occur over a lengthy baking period in a conventional system, cannot always be completed during the short baking period of a microwave system (Hegenbert, 1992). Also the flavor compounds may not have the opportunity to develop as they would under conventional baking. Different chemical reactions took place during microwave baking, so different flavors are produced (Hegenbert, 1992).

Moisture vapour generation inside the food material increases during microwave heating, due to relatively larger amounts of interior heating than conventional heating, and this creates significant interior pressure and concentration gradients. This causes higher rate of moisture losses during microwave heating, creating an outward flux of rapidly escaping vapour (Datta, 1990). In fact, breads and cakes baked in microwave oven were shown to lose more moisture as compared to conventionally baked ones (Sumnu *et al.*, 1999; Zincirkiran *et al.*, 2002).

There have been many studies in order to solve the problems observed in microwave baked products and to improve the quality of these products (Sumnu, 2001).

Effects of methylcellulose gums on texture of microwave cakes were investigated by Bell & Steinke (1991). They found that these gums favoured the volume, texture and moisture holding capacity of microwave baked cakes.

Ozmutlu *et al.* (2001a) found that gluten content was the significant factor in affecting the firmness of microwave baked breads. Breads formulated with low

gluten flour were softer and had higher volume as compared to the ones formulated with high gluten flour.

Seyhun *et al.* (2003) investigated the effects of different types of emulsifiers and gums and fat contents on the retardation of staling of microwave baked cakes. It was found that use of emulsifiers and gums helped to retard the staling of microwave baked cakes. It was also concluded that fat content was a significant factor in affecting variation of firmness and weight loss of the cakes during storage.

Sahin *et al.* (2002) examined the effects of different browning treatments, in order to achieve the brown colour and crisp crust of microwave baked breads.

Zuckerman & Miltz (1992) found that when dough was placed on top of susceptors, desired browning and hardness were obtained at the bottom surfaces of the breads. Susceptors metallized, generally aluminized, biaxially oriented polyester films laminated to paperboard on top of which, or within which, the product, they have the property of absorbing the microwave energy and converting it to heat, which is transferred to the product by conduction and radiation (Main *et al.*, 2007).

Therefore, combining microwaves with other heat source can be a solution to prevent quality problems and promote cake surface browning, such as hot air assisted microwave heating and infrared (IR) (Bilgen *et al.*, 2004).

According to Decareau (1992), microwave-baked products showed a typical surface browning and flavour development with the combination of microwave and forced convection heating.

Microwaves have been combined with conventional heating for baking of biscuits (Ahmad *et al.*, 2001; Bernussi *et al.*, 1998).

Bilgen *et al.* (2002) investigated the effects of baking parameters on the white layer cake quality baked by combined conventional and microwave ovens. Conventional baking was applied for crust formation and later microwave baking was continued. They had concluded that using a combination of conventional and microwave baking produced products with quality equivalent to the quality of cakes baked in conventional oven.

Studies showed that breads baked in combination oven had comparable quality with the conventionally baked ones in terms of specific volume, color, texture, and porosity (Demirekler *et al.*, 2004).

The microwave-IR combination oven combines the browning and crisping advantages of near infrared heating with the time saving advantages of microwave heating. Halogen lamps provide near infrared radiation (wavelength range is 0.7 μm – 5 μm) to achieve surface browning and to prevent soggy surface. IR radiation is the part of the electromagnetic spectrum responsible for the heating effect of the sun (Ranjan *et al.*, 2002). It is found between the visible light and microwaves (Sepulveda & Barbosa-Canovas, 2003) and can be divided into three different categories, namely, near-infrared radiation (NIR), mid-infrared radiation (MIR) and far-infrared (FIR) radiation (Ranjan *et al.*, 2002).

The infrared source has a high temperature (500-3000 °C), the heating effect of infrared radiation has an impact only on the surface of the body and heat transfer through the body proceeds by conduction or convection (Sepulveda & Barbosa-Canovas, 2003).

Datta & Ni (2002) studied the temperature and moisture profiles for infrared and hot air assisted microwave heating of food using a multiphase porous media transport model. It has been concluded that for foods with small infrared penetration, the large power flux available with infrared heat could reduce moisture and increase surface temperature. Hot air could reduce surface moisture and increase surface temperature, but not as effectively as infrared heat, perhaps due to the lower surface heat flux for hot air as compared to the infrared energy.

Keskin *et al.* (2004) showed that IR-microwave combination oven reduced the conventional baking time of breads by 75% with develop of colour and crust formation.

Demirekler *et al.* (2004) optimized the baking conditions of bread in an infrared-microwave combination oven by reducing the conventional baking time of breads by 60 %. It was found that the breads baked at optimum condition had comparable quality with the conventionally baked ones.

Keskin *et al.* (2005) investigated the gelatinization of cookies baked in microwave and infrared-microwave combination ovens. They found that combining microwaves with infrared resulted in comparable gelatinization levels with those of conventional baking.

The processing conditions during infrared-microwave baking of cake were optimized by using Response Surface Methodology (Sevimli *et al.*, 2005). It was possible to obtain high quality cakes by reducing the conventional baking time by about 79% by utilizing near infrared-microwave combination ovens.

Sumnu *et al.* (2005) and Turabi *et al.* (2008) focused on the optimization of rice cake baking using a combination of microwave and infrared technology, considering different quality parameters as weight loss, specific volume, firmness and colour.

Sakiyan *et al.* (2007) evaluated the effects of different formulations on color and texture of different cakes during baking in microwave and near infrared-microwave combination ovens.

Sánchez-Pardo *et al.* (2007) investigated the effects of microwave and conventional oven on starch characteristics of pound cake. The microwave oven with a power of 240 W was employed, and conventional baking was performed at 180 °C for 40 min. They reported less starch gelatinization in microwaved pound cake, attributed to the limited water availability during microwave heating.

A formulation study in cakes baked by infrared-microwave combination was performed by Turabi *et al.* (2010). The effects of different gums on macro and micro structure of gluten-free cakes baked in conventional and combination ovens were determined.

Another research on combination oven was performed by Demirkesen *et al.* (2011). The main objective of their study was to design gluten free Breads with chestnut and rice flour and xanthan-guar gum blend in the formulation. Different formulations were baked in infrared-microwave combination oven to find the optimum formulation.

Infrared–microwave combination baking is also a promising method to solve insufficient starch gelatinization in microwave baked products. It was found that cakes baked in infrared–microwave combination oven had similar gelatinization degree with that of the conventionally baked ones (Sakiyan *et al.*, 2011).

Al-Muhtaseb *et al.* (2013) examined the effect of baking mode (microwave and convective), baking time, and batter formulation on the textural properties (hardness, springiness, cohesiveness, gumminess, chewiness) of microwave-baked and convective-baked Madeira cake. Madeira cake microwave-baked at 250 W resulted to have the most favourable textural properties in terms of springiness and cohesiveness. In contrast, cake microwave-baked at 900 W exhibited the least favourable hardness, gumminess and chewiness characteristics.

Purlis (2014) analysed transport phenomena and physicochemical changes occurring during the conventional bread baking process. The use of infrared heating gives good results in comparison with conventional baking, improving process outputs such as baking time, weight loss, thermal input, and energy input.

Chhanwal *et al.* (2015) studied the use of infrared radiation to achieve a more efficient baking process in terms of bread quality attributes: moisture content, volume, crumb firmness, colour and sensory analysis. The results showed that breads baked with infrared had higher moisture content, higher volume, lower crumb firmness and similar overall quality score compared with conventional baking process.

Sakiyan (2015) focused on the optimization of the formulation of a functional cake (soy-cake) baked in infrared-microwave combination oven by the application of response surface methodology.

As a result of an extensive and comprehensive literature review, there is no information about the application of microwave or its combination heating on filled bakery products, such as Sfogliatella, yet.

1.6 Shelf-life

Bakery products are characterised by a relatively short shelf-life.

The term “shelf-life” refers to the period during it a food will retain in an acceptable level of eating quality from a safety and organoleptic point of view, depends on four main factors, namely formulation, processing, packaging and storage conditions (Galic *et al.*, 2009).

Bakery products are subject to physical, chemical, and microbiological spoilage, making a food less palatable at the time of consumption. This limited stability leads to great economic losses throughout the world (Karaoglu *et al.*, 2005).

The spoilage problems of bakery products can be sub-divided into:

- 1) physical spoilage (moisture loss, staling),
- 2) chemical spoilage (rancidity),
- 3) microbiological spoilage (yeast, mould, bacterial growth).

The shelf life of low and intermediate moisture bakery products is limited by physical and chemical spoilage problems, while microbiological spoilage is the main concern of intermediate and high moisture products.

Several factors influence the spoilage, such as storage temperature, relative humidity, level of preservatives, pH, packaging material and gaseous environment surrounding the product and, most importantly, the moisture content and a_w .

Physical spoilage

Many bakery products undergo during storage to moisture loss or gain, that can result in textural changes and may even promote chemical and microbiological spoilage in low and intermediate moisture products. However, both moisture loss or gain can be overcome by packaging products in materials with selective moisture and gas barrier properties, such as low density polyethylene (LDPE).

A more serious physical spoilage problem in bakery product is staling. Staling has been defined as “almost any change, short of microbiological spoilage, that occurs in bread or other products, during the post baking period, making it less acceptable to the consumer” (Aureli *et al.*, 1996).

Several studies have suggested that staling is due to moisture migration from the crumb to the crust; other studies reported that the degree and rate of crystallization (association) of starch components, specifically of the non-linear amylopectin fraction, is mainly responsible for staling. But complex formation between starch polymers, lipids, and flour proteins is thought to inhibit the aggregation of amylose and amylopectin (Kulp, 1979). Thus, reformulation with lipids and shortenings, surfactants, emulsifiers, gums, and mono- and diglycerides can be used to delay the staling of bakery products.

Chemical spoilage

Bakery products, especially those with a high fat content, can undergo to chemical spoilage or rancidity. Rancidity is lipid degradation resulting in off-odors and off-flavors, which render products unpalatable (Smith *et al.*, 2004). Two types of rancidity problems can occur: oxidative and hydrolytic.

Oxidative rancidity results in the breakdown of unsaturated fatty acids by oxygen through an autolytic free-radical mechanism. Consequently, malodorous aldehydes, ketones, and short chain fatty acids are formed. These free radicals and peroxides, formed during lipid oxidation, may lead to even more detrimental effects on food

quality by bleaching pigments (e.g., lycopene in tomato paste in pizza), destroying certain vitamins, such as vitamin A and E, and protein degradation.

Instead, hydrolytic rancidity occurs in the absence of O₂ and results in the hydrolysis of triglycerides and the subsequent release of glycerol and malodorous fatty acids. This type of rancidity is enhanced by the presence of moisture and endogenous enzymes, such as lipases and lipoxigenases.

The addition of antioxidants can prevent this type of spoilage.

Microbiological spoilage

Microbiological spoilage is the major cause of economic loss to the bakery industry. It has been estimated that in the U.S. alone, losses due to microbiological spoilage are ~1 to 3% or over 90 million kg of products each year (Ooraikul, 1991). The most important factor influencing microbiological spoilage of bakery products is water activity (a_w). For low moisture baked products ($a_w < 0.6$), microbiological spoilage is not a problem. In intermediate moisture products ($a_w 0.6-0.85$), osmophilic yeasts and moulds are the predominant spoilage microorganisms. In high moisture products ($a_w 0.94-0.99$), almost all bacteria, yeasts, and moulds are capable of growth (Smith, 1992).

Moreover, many fillings can support the growth of food pathogens, especially if they contain egg or dairy products (Seiler, 1978).

SRNs are high moisture confectionery bakery products. Their shelf life is limited by microbial spoilage, mostly due to moulds and staling.

Microbiological spoilage can be controlled using different strategies, such as the control of post baking contamination (Smith *et al.*, 2004). In fact, special attention must be given to the storage conditions of bakery products. They can be stored at room temperature, under a modified atmosphere, under refrigeration or frozen.

1.6.1 Frozen storage

Nowadays frozen storage is the most used process to preserve bakery products, such as pre-baked bread. It occupies an increasingly great portion of the bakery market covering sectors such as supermarkets, restaurants, institutional and catering businesses, because allows for storage for long periods (Kennedy, 2000; Rouille *et al.*, 2000; Stear, 1990);

Freezing converts the water present in a food into a non-active compound, and, together with the low temperature, prevents the growth of microorganisms and the development of the chemical and enzymatic reactions responsible for food deterioration (Barcenas & Rosell, 2006b).

A coupling of heat transference with moisture diffusion occurs during freezing, further complicated by transition of the water into ice (Hamdami *et al.*, 2004).

Frozen storage is one of the most efficient methods to retard the staling process (Mandala & Sotirakoglou, 2005) and moisture equilibration between the crust and

the crumb of bread. Moreover, it preserves the aroma of freshly baked product (Sluimer, 2005).

Nevertheless, it causes some changes in the food structure which result in defects, such as the reduction/contraction in specific volume, the collapse (shrinkage) of the structure, the appearance of discoloration immediately under the crust, commonly known as snow-white discoloration, separation of the crust from the crumb and flaking of the crust.

These defects occur especially after a relatively long time in the freezer (Sluimer, 2005).

Not all bakery products can be frozen with success. This is especially true for crisp bakery products where there is a considerable difference in moisture content between the crust and the crumb.

However, the success of freezing bread also requires care and attention in all aspects of the operation. Inadequate freezing, frozen storage, and thawing of the bakery products, as bread, can increase staling (Inoue & Bushuk, 1996), which was, indeed, confirmed by Cauvain (2004), Kennedy (2000) and Vulicevic *et al.* (2004).

On the other hand, this method is an expensive process due to the high energy consumption during the production, storage and distribution chain of the frozen product (Lainez *et al.*, 2008). A comparison between the frozen par-baked bread process and conventional bread showed that, under industrial conditions, the frozen pre-baked bread process demands about 2.2 times more electrical energy than the conventional process (without considering the energy used to cool the par-baked bread, for frozen storage and for thawing of the frozen par-baked bread before the final baking (Le Bail *et al.*, 2010).

Refrigeration is an adequate alternative storage (Lainez *et al.*, 2008), since it extends the shelf-life with a lower energy requirement when compared to frozen storage (Barcnas & Rosell, 2006a; Rosell & Santos, 2010). Combined effects with other preservation method, such as modified atmosphere packaging (MAP) represent a valid packaging technology to preserve bakery products.

1.6.2 Modified atmosphere packaging (MAP)

An alternative method to chemical preservatives controlling mould spoilage problems in bakery products, is Modified Atmosphere Packaging (MAP).

MAP is defined as the enclosure of a perishable product in packages comprised of gas barrier materials that rely on mixtures of gases in concentrations different to those of air, to retard deterioration processes in foods extending the shelf life of product (Rodriguez-Aguilera & Oliveira, 2009). This technology offers several advantages to consumers and food products, such as development of new polymeric barrier packaging materials, extended market areas, consumer concerns about preservatives, decreasing energy costs associated with traditional methods of food preservation, such as freezing, and consumer perception of MAP (Floros & Murray, 2005).

Many consumers favored MAP technology over other methods of preservation and were willing to pay a premium for MAP food products (Smith & Simpson, 1996).

It has been estimated that MAP can reduce energy consumption by 18–20%, compared to freezing for the shelf life extension of bakery products (Aboagye *et al.*, 1986).

The production, storage and transportation costs of frozen crumpets was 45% more energy intensive than MAP crumpets. This was reduced to an overall cost advantage of 14% over freezing, due mainly to the higher cost of packaging materials used in gas packaging. Therefore, for a bakery with an annual production of 1,000,000 kg of crumpets, a saving of \$34,730 could be achieved by using MAP instead of freezing (Aboagye *et al.*, 1986).

The most used gases in MAP of bakery products are nitrogen (N₂) and carbon dioxide (CO₂). The use of O₂ concentrations is below atmospheric levels.

Nitrogen is a relatively inert gas with no odor, taste, or color, it has no antimicrobial activity and acts to displace oxygen during gas flushing and to prevent pack collapse (Ooraikul, 2003; Sivertsvik *et al.*, 2002).

Carbon dioxide has the ability to selectively inhibit the growth of microorganisms, specifically yeasts and moulds (Cauvain & Young, 2000), but it is highly soluble in water and fats, where it forms carbonic acid, which may result in a reduction of pH of product (Smith & Simpson, 1996). Furthermore, CO₂ can be absorbed by the food, resulting in package collapse (Farber, 1991).

CO₂ is most effective against aerobic bacteria and moulds. Concentrations of CO₂ as low as 5–10% are being used to suppress growth of these microorganisms (Ooraikul, 1991; Smith, 1992).

However, a concentration of 50% CO₂ was needed for complete inhibition of mould growth on bread. Generally, gram-negative bacteria are more sensitive to CO₂-enriched atmospheres than gram-positive types. However, certain gram-positive bacteria (e.g., lactic acid bacteria and certain *Bacillus* species) are very resistant to CO₂ and can tolerate and even grow in atmospheres containing 75–100% CO₂. Anaerobic bacteria, such as *Clostridium botulinum*, are not inhibited by elevated levels of CO₂ in the package atmosphere, and indeed, their growth may actually be stimulated by CO₂.

For most products, a minimum of 20–30% CO₂ (v/v) is required to inhibit the majority of aerobic spoilage microorganisms, while for longer shelf life extensions, a concentration of ~60% should be used (Ooraikul, 1982).

Storage temperature also affects the antimicrobial activity of CO₂. It has been shown that CO₂ is a very effective antimicrobial agent at low storage temperatures, due to greater dissolution of CO₂ in the aqueous phase of products and resultant changes in the intracellular pH and enzymatic activities of microorganisms.

MA-packaging, together with adequate refrigeration, inhibits the growth of aerobic micro-organisms, proteolytic bacteria, yeasts and fungi (Swiderski *et al.*, 1997). Even if, Francis & O'Beirne (1998) suggested that the gas atmospheres present within

packages of vegetables may increase the growth of *Listeria*. In particular, five pathogenic bacteria are known to grow on food below 5 °C (*Clostridium botulinum*, *Listeria monocytogenes*, *Yersinia enterocolitica*, enterotoxigenic *Escherichia coli* and *Aeromonas hydrophila*) and another five at temperatures just above 5 °C (*Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp., *Vibrio parahaemolyticus* and *Bacillus cereus*) (Devlieghere *et al.*, 2000; Farber, 1991).

Some of the disadvantages of MAP technology include the initial cost of packaging equipment, slower throughput of product, secondary fermentation problems caused by CO₂ resistant microorganisms, and the potential growth of microorganisms of public health concern, particularly *Clostridium botulinum*.

Numerous studies have been performed to evaluate the influence of MAP, along with refrigeration, on the extension of shelf life of different bakery products (Smith *et al.*, 1986, 1988; Seiler, 1989; Ooraikul, 1991; Smith & Simpson, 1995). The effect of different concentrations of CO₂ (0–100%) on the shelf life of a variety of bakery products (Madeira cake, crumpets, par-baked bread, rye bread, and fruit pies) stored at ambient temperature was investigated (Karaoglu *et al.*, 2014; Seiler, 1989). Despite the known effect of MAP to prevent the microbial spoilage of bread, conflicting results have been reported on the effect of atmosphere inside a package on staling and physio-chemical quality changes occurring during bread storage (Rasmussen & Hansen, 2001). Minor problems encountered were related to the texture, color and taste of the gas packaged products.

The results in a study by Knorr & Tomlins (1985) suggest reducing crumb firmness of white and whole meal bread during storage in CO₂. Similar results were obtained by Cencic *et al.* (1996), who found a relevant decrease of bread firmness packed in 100% CO₂. On the other hand, Black *et al.* (1993) and Rasmussen & Hansen (2001) showed that bread firming is not affected by atmosphere. A different type of shortening was recommended to overcome this textural problem in these gas packaged products. Cake doughnuts also became sticky during storage, probably due to moisture migration from the inside of the doughnut. This problem could be overcome by using a less hygroscopic sugar or sweetening agent or anti-caking agent (Ooraikul, 1991). However, as far as we know, there are no reports dealing with shelf life extension of this type of heterogeneous confectionery product with a dry crispy puff pastry and a moist filling based on ricotta and semolina.

Chapter 2 – Aim of the study

In the last years, demand for “ready to eat” (RTE) products has exponentially increased because of lifestyle changing and new technologies introducing, such as microwave. This reflects a trend by consumers towards less time and effort in the preparation of food.

One of the most important sectors of the RTE product market is the baked confectionery segment.

Sfogliatella Riccia Napoletana (SRN), recognized as Traditional Agri-food Product (*Prodotti Agroalimentari Tradizionali* - PAT) of Campania Region, is worldwide known and appreciated for its goodness. But its production is limited to local markets, any research has never been aimed to optimization of technological production of this product, in order to extend market area of traditional product on a national and international basis.

To achieve this goal, PhD research was divided into two steps focused on the

1. formulation and processing,
2. shelf life

of SRN, by the application of alternative technologies.

Recently, there is an increasing trend for healthier and more nutritious food than those previously available, as consumers have become more health conscious. So food industry has been concentrating on ensuring a balanced nutritional status of products. This in itself presents numerous problems associated with ingredient formulation and processing parameters.

Lipids play an important role on final bakery product characteristics. Lard is traditionally used in SRN production. However, there is a growing negative perception regarding the implication of animal fats on human health, but studies dealing with the formulation of fat substitutes to mimic the physical characteristics of lard are still limited (Nur Illiyin *et al.*, 2013). In the last few decades, palm oil has become one of the most important oils globally in food applications because of the high thermal stability and oil yield, however, it is nowadays under discussion, mostly related to sustainability of its cultivation. There also seems to be a growing interest in palm oil alternatives in European countries by vegetable blends.

So, the replacement of lard (traditionally used) in the formulation of SRN with mixture of palm oil free vegetable fats, obtained by enzymatic interestification, having the same technological properties of lard, was studied in order to obtain a SRN low in cholesterol and trans fatty acid contents.

Besides, the second objective of first phase was to realize a RTE-SRN, with the same quality as conventionally baked one, but with reducing the baking time.

To realize RTE-SRN, a pre-cooking phase followed by a heating phase were chosen, with application of a baking based on electrical-infrared-microwave combination method, using a response surface methodology.

Due to the short processing times and specific interaction of food components with microwaves, quality problems in microwave baked products can be observed. In order to overcome these problems; infrared-microwave and electric-microwave combinations ovens can be used with advantage of combining time and energy saving properties of microwave baking with browning and crisping properties of infrared and electric heating.

No information are reported in literature about the application of microwave or combined baking methods on this type of filled product.

Therefore, the effects of different fat blend formulations on quality of SRN baked in different methods were investigated.

The final scope is to obtain an optimized palm oil free fat blend formulation for RTE-SRN, resembling as much as possible to the lard SRN reference.

The second phase of the research aimed to study the behaviour of RTE-SRN, with different fat formulations, during storage.

SRN, as many bakery products, is characterized by high moisture and a_w content, so its shelf life is limited by microbial spoilage, mostly due to moulds and staling.

In order to achieve longer shelf life, frozen or refrigerated preservation is employed to bakery products.

Nowadays frozen storage is the process most used to preserve bakery products, such as pre-baked bread. It occupies an increasingly great portion of the bakery market covering sectors such as supermarkets, restaurants, institutional and catering businesses, because allows the storage for long periods (Kennedy, 2000; Rouille *et al.*, 2000; Stear, 1990); but is an expensive process due to the high maintenance costs of the cold chain (Lainez *et al.*, 2008).

Refrigeration is an adequate alternative storage (Lainez *et al.*, 2008), since it extends the shelf-life with a lower energy requirement when compared to frozen storage (Barcnas & Rosell, 2006a; Rosell & Santos, 2010). Combined effects with other preservation method, such as modified atmosphere packaging (MAP) represent a valid packaging technology to preserve bakery products, with energy saving respect to freezing.

The mold-free shelf life of crumpets produced by Forecrest Foods of Calgary, Alberta, was extended from ~4 days to ~1 month at ambient temperature, through product reformulation and gas packaging in 60% CO₂. This allowed the distribution of crumpets throughout Canada and into U.S. markets. Consequently, sales of crumpets quadrupled (Smith *et al.*, 1996).

In particular, the first goal of the second phase was to evaluate the influence of frozen storage time on chemical-physical, textural and microbiological characteristics of RTE-SRN made with different fat formulation.

As second goal, evaluation of chemical-physical and microbiological stability of pre-cooked SRN during storage under MAP at refrigeration and room temperature condition was considered, in order to reduce costs of RTE-SRN production.

2.1. Experimental plan

First phase_Production of lard- and palm oil- free RTE-SRN

Firstly, with the aim of replacing the traditionally used lard in the formulation of *Sfogliatella Riccia Napoletana* (SRN) with reduced cholesterol content, palm oil-free vegetable fat blends, with low trans fatty content (<1% of total fatty acids), were attempted.

The second goal is to create more added value for SRN pastry, creating a RTE product.

So, alternative baking methods - microwave and/or infrared - were evaluated as substitute to traditional one for the production of RTE. The aim was to find a correct combination of traditional and innovative characteristics, which would meet the expectations of potential clients who were looking for a convenient, RTE product, but at the same time, wanted to respect traditional flavours and other features.

Therefore, different time/thermal power combinations were experimented to identify the products most similar to traditional one. Experimental cooking methods were composed of a pre-cooking phase followed by a heating phase. To optimize the baking process parameter, a Response Surface Methodology (RSM) was applied and the optimal treatments were analysed in order to compare the theoretical optimum values with control sample.

The experimental design is shown in Fig. 8.

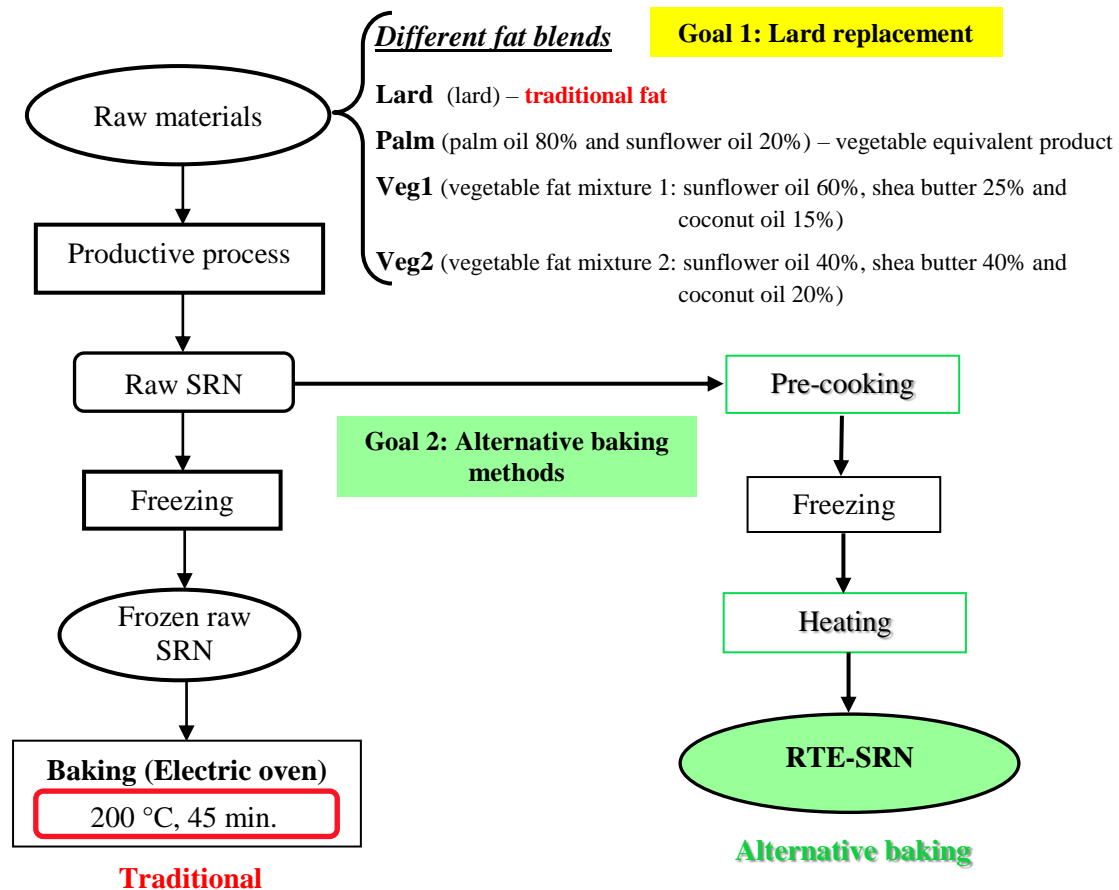


Figure 8_ Experimental plan of first phase.

Second phase _Storage evaluation

In order to evaluate RTE-SRN storage, the pre-cooked SRN, formulated with different fat blends (L, P, V1 and V2) were stored under:

- freezing condition
- modified atmosphere packaging and refrigerated or room temperature condition.

The first goal was to investigate the quality characteristics of frozen pre-cooked SRN through moisture, a_w , color, texture and microbiological measurements, in order to predict product's behaviour and shelf-life after prolonged storage. Thus, pre-cooked SRN were frozen at -30 ± 2 °C and stored in freezer at -22 ± 2 °C for 12 months. Every month, frozen pre-cooked SRNs were withdrawn and heated (according alternative method) and subjected to analysis.

As second goal, pre-cooked SRNs were stored in modified atmosphere (MAP), under different $N_2:CO_2$ ratios (70:30 and 50:50). The control sample was packaged under atmospheric composition. Changes in in-package gas composition, microbial growth, chemical–physical parameters including texture and sensory attributes were monitored for 49 days at 5 and 20 °C.

The experimental design is shown in Figure 9.

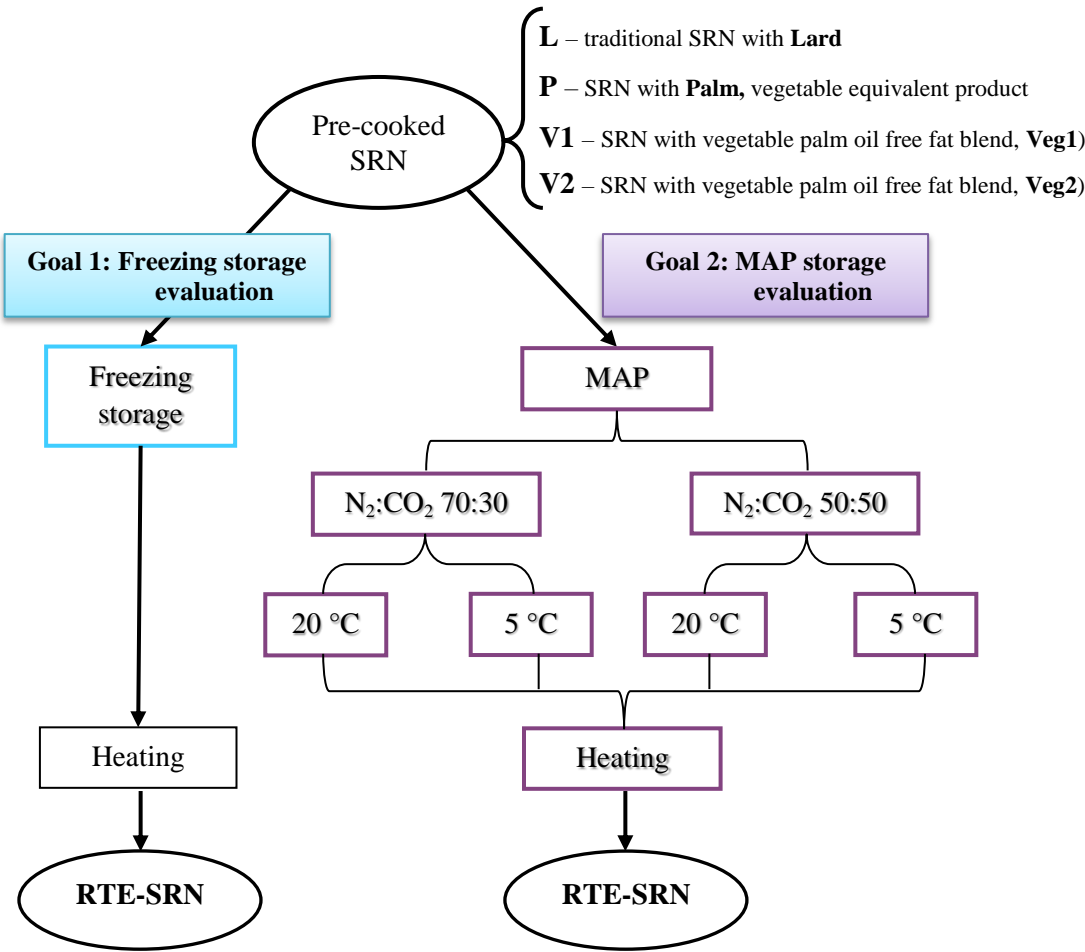


Figure 9_ Experimental plan of second phase.

Chapter 3 - Materials and Methods

3.1 SRN fats

Palm oil (PO), sunflower oil (SO), coconut oil (CO), shea butter (SB) blended in different proportions in order to modulate the melting characteristics, were used in experimental SRN formulations. Lard (Ingra Brozzi S.p.A., Cogozzo di Viadana, Italy) (coded Lard), was used in the reference SRN as it is the fat traditionally used in this kind of products.

The fat blends with similar levels of saturation than lard (39.4 ± 0.19 %) as seen in Tab. 1, were provided through an enzymatic interesterification process by Senna S.r.l. (Stockhamern-Gasse, Austria). They were stored at 20 °C in a closed container until use.

Table 1_ Fat blends composition.

Blend	PO (%)	SO (%)	CO (%)	SB (%)	Saturation (%)
Palm	80	20	-	-	42.9
Veg1	-	60	15	25	40.4
Veg2	-	40	20	40	42.4
Lard	-	-	-	-	39.4

3.1.1 Lipid analyses

Fat blends were subjected to different chemical-physical analyses: free acidity (FFA), peroxide value (PV), fatty acid (FA) composition, triacylglycerols (TAG) analysis and solid fat content (SFC).

3.1.1.1 Free acidity

FFA is determined by the amount (measured by weight, in grams) of free fatty acids, expressed as oleic acid, present in 100 g of oil (Grossi *et al.*, 2014). Measured by quantitative analysis, acidity is a measure of the hydrolysis of the oil's triglycerides: as the oil degrades, more fatty acids are freed from the glycerides, increasing the level of free acidity and thereby increasing hydrolytic rancidity.

Free acidity was determined according to the European official methods of analysis (EECR 2568/1991) by titration of a solution of oil dissolved in ethanol–ether (1:2) with sodium hydroxide.

3.1.1.2 Peroxide value

Another measure of the fat's chemical degradation is the peroxide value (Grossi *et al.*, 2015) which measures the formation of intermediate hydroperoxides in milliequivalents of active oxygen per kilogram of oil (meq O₂/kg).

The peroxide value of lard was determined according to annex III in the Reg. CE n. 1989 (2003). An aliquot of fat (1.5 g) was transferred into a dry 125-mL Erlenmeyer flask and 15 mL of acetic acid and chloroform solution (3:2) were added. The flask was swirled and 0.25 mL saturated potassium iodide solution was added. After allowing the solution to remain for 10 min in a dark place, 15 mL of distilled water was added. This solution was titrated against 0.1 N sodium thiosulfate after the addition of 1 mL of starch indicator (1% w/w) until the blue color of the solution had disappeared.

3.1.1.3 FA composition

FA composition analysis was performed by gas chromatograph (GC) following derivatization to FAMES with 2N KOH in methanol according to the IUPAC standard method no. 2.301 (IUPAC 1990). Briefly, 50 mg of fat in a 1 mL glass-stoppered test-tube was dissolved in 1 mL n-hexane. A solution of 2N potassium hydroxide in methanol (300 μ L) was added. The tube was vortexed for 30 s and allowed to react for 4 min at room temperature. A 1 μ L aliquot of the upper organic phase was analyzed by high-resolution gas chromatography (HRGC).

A DANI Master gas chromatograph (Dani Instrument SPA, Milan, Italy) equipped with a programmed temperature vaporizer (PTV), a flame ionization detector (FID) and a SP-2380 fused silica capillary column (Supelco Inc., Bellefonte, USA) with dimensions of 50 m \times 0.25 mm. i.d. and a film thickness of 0.25 μ m, were used, as previously described by Romano *et al.* (2014). The oven temperature was programmed at 80 $^{\circ}$ C for 5 min, 5 $^{\circ}$ C/min ramp-up to 165 $^{\circ}$ C for 5 min, and then 3 $^{\circ}$ C/min ramp-up to 230 $^{\circ}$ C for 1 min. The carrier gas, helium, was introduced at a flow rate of 20 cm/s. An aliquot of 1 μ L was injected into the injection port with a split ratio of 1/60 (v/v) and the FID temperature was set at 260 $^{\circ}$ C and a 8:1 ratio of air:hydrogen. The PTV operating conditions were 50 $^{\circ}$ C for 0.1 min, increased at 400 $^{\circ}$ C min⁻¹ up to 230 $^{\circ}$ C and held for 1 min.

Identification of peaks was performed using an external standard (SupelcoTM 37 component FAME MIX). The sample concentrations were calculated through a comparison with the pure standard retention time and were based on response factors to convert peak areas into weight percentages (mg/100 g of fatty acids) as suggested by Molkentin (2009).

3.1.1.4 TAG and cholesterol analysis

1 μ L of a solution of 4% (w/w) of fats in hexan was injected into the gas chromatograph. The TAG solution was analyzed on a GC Dani 1000 gas chromatograph equipped with a flame ionization detector (FID) held at 360 $^{\circ}$ C and a temperature programmed vaporizer (PTV) injector. An Rtx 65TG (mod. 17008) capillary column (30 m \times 0.25 mm i.d.; 0.10 μ m film thickness) from Restek (Bellefonte, USA) was used with high-purity helium as a gas carrier at 1.5 mL/min. The injection was performed with a split ratio of 1/30 (v/v) and a heating program of 50 $^{\circ}$ C for 0.3 min, followed by a 500 $^{\circ}$ C/min increase to 300 $^{\circ}$ C with a 7 min hold.

The column oven temperature program was 250 °C for 2 min, followed by an increase of 6 °C/min to 360 °C for 10 min, as described in Romano *et al.*, (2011). The flow-rate of the gas carrier, He, was 1.2 mL min⁻¹.

TAGs were identified by comparing their retention times with those of a triglyceride standard (Sigma, USA). These samples allowed response factors (Rfs), to be calculated, which convert peak areas into weight percentages. The reference standard for cholesterol was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3.1.1.5 Solid fat content

Solid fat content was measured by a rapid and convenient method, pulsed NMR (Nuclear Magnetic Resonance) with a Bruker Minispec mq-one instrument (Bruker, Karlsruhe, Germany) following the official ISO 8292-1:2008 method of analysis.

The SFC was determined in the range of 10-40 °C at 5 °C intervals. Melted fat was put in a NMR tube; thermal pre-treatment which followed was characterised by the following phases: 15 min in 60°C water bath, 90 min in 0 °C water bath; 40 h in 20 °C water bath; 90 min in 0 °C water bath; 60 min in water baths of chosen temperatures (10, 15, 20, 25, 30, 35, 40 °C). Measurements were then taken in a stabilised, temperature-controlled NMR apparatus.

3.2 SRN formulation

SRNs were performed at a confectionery company in Naples with the following traditional recipe: short pastry was prepared mixing first together water (11%), salt (1%) and sugar (1%) then adding wheat flour (33%) [15.4% protein, 14.0% moisture and 0.65% ash, 420 W, supplied by Molino Sul Clitunno srl (Trevi, Italy)] with a fork mixer (INM 200, Pietroberto s.r.l, Marano Vicentino, Italy) at 22 rpm for 15-20 min.

The short pastry was laminated with adding of melt fat (8% total fat) and pastry circles of 9 cm in diameter were obtained.

The filling was obtained by mixing cooked semolina (23%), sugared ricotta (19.5%), pasteurized eggs (1.8%), candied orange cubes (1.5%) and cinnamon, vanilla and orange as flavourings (<1%) in a spiral mixer and batted until a homogeneous mix was obtained.

An adequate amount of filling was layered in the centre of the short crust circle, which was subsequently shaped to obtain a shell-shaped pastry with a weight of approximately 110 g.

Traditional SRN was produced with use of Lard (indicated with **L**). In the experimental formulations, lard was replaced with the same weight by different fat blends according to the experimental design previously described, keeping constant the ratios among the other ingredients. The SRNs, made with the other fat blends, were named:

- **P**, SRN made with Palm (palm oil 80% and sunflower oil 20%);
- **V1**, SRN made with vegetable fat blend 1 (Veg1), (composed of sunflower

- oil 60%, shea butter 25% and coconut oil 15%);
- **V2**, SRN made with vegetable fat blend 2 (Veg2), (composed of sunflower oil 40%, shea butter 40% and coconut oil 20%).

3.3 Baking process

SRN formulated with different fat blends were subjected to different experimental baking methods.

3.3.1 Conventional baking

Conventional baking was performed in a commercial electric oven (Electrolux Rex, Porcia, Italy), at 200 °C for 45 min. The oven was preheated to the set temperature before placing the frozen sample into it. SRN was cooled down at room temperature (20-22 °C), during 1 h and placed in sealed plastic containers until analysis. Conventionally baked cakes were used as the control.

3.3.2 Microwave and/or infrared baking

A microwave oven GW72V Samsung (Seoul, South Korea) with 2.45 GHz of radiation equipped with a near infrared lamp to toast the surface of SRN was used (Fig. 10). There was a rotary table in the oven to improve heating uniformity of sample. Sample was placed at the centre of the turn table which was previously marked. Halogen lamps at the top and bottom were operated at the same power as during infrared (IR) baking. The halogen lamp at the top was located 15 cm above the bread surface while the halogen lamp at the bottom was just under the rotary table.

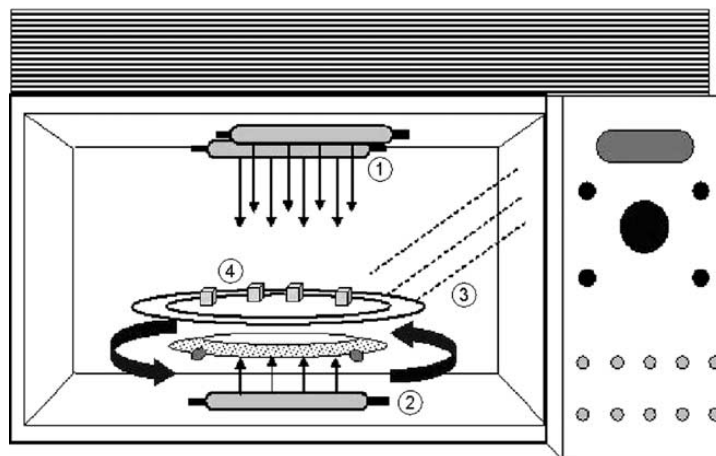


Figure 10_ Illustration of IR-microwave combination oven. (1) Upper halogen lamps, (2) Lower halogen lamp, (3) Microwaves, (4) Turntable (Sumnu *et al.*, 2005).

3.3.3 Freezing

Freezing was conducted in a blast freezer at -30 ± 2 °C and 1.5 m/s of air speed, at conditions that are typically found in the baking industry. The endpoint of freezing was established when the thermocouple inside the core of some pieces indicated -18 °C.

After freezing, the pre-cooked SRNs were packed in polyethylene bags and stored in a freezing room at -22 ± 2 °C.

3.4 Experimental design

To realize the RTE, the experimental cooking methods were composed of a pre-cooking phase followed by a heating phase.

The optimum level of the pre-cooking process parameters (microwave time, A; electric oven time, B) and heating process parameters (microwave power, A; microwave-IR combination time, B) were determined by using response surface methodology (RSM) (Myers *et al.*, 2016). To study the main effects and interactions of the pre-cooking process parameters, a D-optimal design having 20 experimental runs with different combination of factors and one block was conducted. The coded and uncoded (actual) levels of the independent variables used in the experiments are given in Table 2. The microwave power was fixed at 300 W and oven temperature at 200 °C.

Instead, to study the main effects and interactions of the heating process parameters, a User Defined Response Surface experimental design having 17 experimental runs with different combination of factors and one block was conducted. The coded and uncoded (actual) levels of the independent variables used in the experiments are given in Table 3. The IR power was fixed at 950 W.

The effects of extraneous variables were minimized by randomizing the experiments. Pre-cooked SRNs were stored at freezing conditions for 30 days before heating.

The response variable measured on the product were: moisture of filling and puff pastry, CIE $L^*a^*b^*$ parameters of upper and lower puff pastry, texture and core temperature. These responses were expressed individually for each cooking step as a function of independent variables. Data were analysed by multiple regression analysis, reporting analysis of variance, lack of fit test, effect of each variable and regression analysis, in order to adjust the variables to a mathematical model.

The experimental order design and data analysis was obtained from the Design-Expert 7.0 for Windows-software, for design of experiments (Stat-Ease Inc., Minneapolis, USA). Response surface plots were generated with the same software.

In order to obtain the best process conditions of the two-phases of baking process, the equations used to describe experimental data were processed with the numerical optimization function of the Design Expert software using as target value of the response variable the control cake variable value.

Table 2_ Response surface experimental design for pre-cooking phase.

Factor	Code	Levels		
		-1	0	+1
Microwave time (min)	A	2.00	4.50	7.00
Electric oven time (min)	B	10	15	20

Table 3_ Response surface experimental design for heating phase.

Factor	Code	Levels		
		-1	0	+1
Microwave power (W)	A	100	425	750
Microwave-IR time (min)	B	2.00	4.50	7.00

3.4.1 Response variable

Colour measurements

Color of pastry was determined with a colorimeter tristimulus (Chroma Meter, model CR- 300; Minolta, Osaka, Japan) with a circular measurement area (D 8 mm), following the method described by Xiao *et al.* (2009) with some modifications, on upper and lower puff pastry expressing the results in the CIELab system coordinates (Fig. 11):

- L^* which is the brightness and varies between 0 (black) to 100 (white),
- a^* which ranges from -60 (green) to +60 (red),
- b^* ranging between -60 (blue) to +60 (yellow).

The parameter a^* takes positive values for reddish colours and negative values for the greenish ones, whereas b^* takes positive values for yellowish colours and negative values for the bluish ones. L^* is an approximate measurement of luminosity, which is the property according to which each colour can be considered as equivalent to a member of the grey-scale, between black and white (Granato & Masson, 2010).

The colorimeter was calibrated with a white standard plate ($L^* 100$).

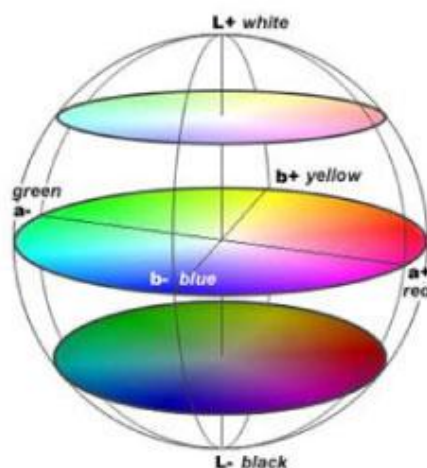


Figure 11_ CIELAB colour space.

Moisture content measurement

The moisture content of samples was determined by the gravimetric method (AACC 2000; method 44-15A). Puff pastry and filling samples were oven-dried at 105 °C and accurately weighed at regular time intervals until constant weight was reached. Three measurements were performed for each sample and the average was determined.

The moisture content was expressed as grams of water over grams of total weight (g/100 g):

$$(b - a)/p * 100$$

in which:

a = weight of cap with dried sample;

b = weight of cap with not-dried sample;

p = weight of sample in grams.

Texture measurement

A Texture Analyser (TMS-Pro Stable Micro Systems, Godalming, Surrey, UK) (Fig. 12) was used to study the textural properties of SRN in order to determine the effect of baking time and heating mode on the textural properties of SRN.

Five replicate samples were baked at each baking condition and then allowed to cool to room temperature (approx 20 °C) before testing.

The experiment was performed with a compression test. The sample was placed on the platform and tested under vertical compression. The test was conducted at pretest speed of 30 mm/s until to the surface of sample, test speed of 50 mm/s and strain deformation of 10 mm of its initial height under the influence of a load cell of 50 N force by an stainless steel spherical probe with diameter of 25 mm.

From each force–time curve, maximum force required to accomplish a given deformation (10 mm of its original width) during the compression was taken as pastry firmness (Steffe, 1996). The compression level was selected on the basis of preliminary experiments.

The test was conducted on four different points of SRN and the main was determined.

The data was processed using Texture Expert Excede v. 1.0 (Stable Micro Systems Software).



Figure 12_ Texture analyser.

Core temperature

Core temperature was measured during baking in the center of the sample with a Temperature Logger (EBI IF 100-1, Ebro, Ingolstadt, Germany) with a measuring range of -40 up to +150 °C, an accuracy of ± 0.1 °C and a resolution of 0.01 °C.

3.5 Alternative vs. traditional baking - Quality measurements of SRN

Once optimized the process parameters, SRNs formulated with different fats and baked with the optimal treatments were analysed in order to compare the theoretical optimum values (obtained with the power and time obtained as the optimal from the response surface methodology analysis) with real ones and thus with control samples (baked in the conventional oven).

Changes in microbial growth, chemical–physical parameters including texture and sensory attributes and fat characterization were determined.

Energy costs of treatments were estimated in terms of energy density, according to power and oven capacity information provided by oven producers and time of treatments.

3.5.1 Microbiological analysis

The microbiological limits for RTE food in general consist of three components:

- process indicators (counts of mesophilic and psychrotrophic germs, moulds and yeasts, Sulfite-reducing clostridial spores), that can provide a general indication of the microbiological quality of a food, they can't be used to predict the safety of the product and will be influenced by the storage conditions of the product.
- hygiene indicator organisms (*Enterobacteriaceae*, coliforms and *E.coli* and faecal streptococci), their presence in fully cooked RTE foods can be an indication of poor hygiene and sanitation or inadequate heat treatment.
- specific foodborne pathogens (coagulase positive staphylococci, *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella* spp.), their presence in RTE foods may be a result of undercooking, poor handling practices and cross contamination.

Determinations were carried out in triplicates and both in puff pastry and filling of SRNs formulated with different fat blends before and after baking.

A 20-g sample was aseptically weighed and transferred to a stomacher bag (Seward Model, UK), containing 180 mL of sterile 0.85% peptone solution and homogenised for 2 min in a stomacher (Lab-Blender 400, PBI, Italy) at room temperature to obtain a representative sample (homogenate). Thus, dilution 10^{-1} was prepared. The other decimal dilutions were prepared and aliquots of the appropriate dilutions were plated in triplicate on solid suitable microbiological media.

Mesophilic and psychrotrophic aerobic bacteria, *Enterobacteriaceae*, coliform, β -glucuronidase-positive *Escherichia coli*, faecal streptococci, coagulase positive staphylococci, sulfite-reducing clostridial spores, *Bacillus cereus*, moulds and yeasts, and the presence of *Listeria monocytogenes* and *Salmonella* spp. were determined according the official ISO methods.

Plate Count Agar (PCA) medium (Oxoid, Milano, Italy) was used for mesophilic and psychrotrophic aerobic counts, incubated at 30 °C for 2 days and 5 °C and 7 days, respectively by the pour plate method (ISO 4833:2006). *Enterobacteriaceae* were detected and enumerated on Violet Red Bile Glucose Agar (VRBGA) medium (Oxoid, Milano, Italy) after 48 h at 37 °C by the pour plate method (ISO 21528-2:2004); coliform and *Escherichia coli* on Violet Red Bile Lactose Agar (VRBLA) medium (Oxoid, Milano, Italy) after 48 h at 37 °C and 44 °C, respectively by the pour plate method (ISO 4832:2006 and 2009). Faecal streptococci at 44 °C in Slanetz Bartley (ISO 7899-2), coagulase positive and negative staphylococci on Baird Parker Agar (BP) (Oxoid) supplemented with Tellurite Yolk Emulsion (Oxoid) by spread plate method after 24 h at 37 °C (ISO 6888-1:1999 + A1:2003). Sulfite-reducing clostridial spores were determined according to APAT, IRSA-CNR2003) and APHA (1998) methods after pre-treatment for 10 minutes at 80 °C. Suspensions were rapidly cooled and further diluted in a sterile extraction solution. An aliquot of 1 mL of each dilution was inoculated in SPS agar plates (Merck) by the pour plate method;

then plates were incubated for 24–48 h at 37 °C in an anaerobic jar with the anaerobic atmosphere generating system Anaerogen (Oxoid). Black colonies, surrounded by opaque zone and resulting catalase-negative, were identified as sulphite-reducing clostridial spores.

Bacillus cereus was detected on MYP Agar (Oxoid) for 24-48 h at 30 °C according to ISO 7932:2004.

Moulds and yeasts were detected on dichloran rose bengal chloramphenicol (DRBC) medium (Oxoid) by spread plate and incubated for 5 days at 25±1 °C (ISO 21527-1:2008). *Listeria monocytogenes* determination was carried on 25-g of sample according to ISO 11290-1:1996 + A1:2004 BS 5763-18:1997 and the detection of *Salmonella* spp. according to ISO 6579:2002 + A1:2007 on 25-g of sample.

All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium.

Results were calculated as the means of three determinations and expressed in Log colony-forming units per gram (CFU/g).

3.5.2 Chemical–physical analysis

Temperature profile during the baking process; moisture content of puff-pastry and filling, and texture were determined as previously described (§ 3.4.1).

3.5.2.1 Color change

Color parameters were determined as previously described (§ 3.4.1), on upper and lower layer of SRN.

Readings were carried out at room temperature from four different positions on the upper and lower sample surface, and the mean value was recorded. Total colour change (ΔE) was calculated using the following equation (Bai *et al.*, 2013):

$$\Delta E = [(L^*-L_0)^2 + (a^*-a_0)^2 + (b^*-b_0)^2]^{1/2}$$

The subscript ‘0’ refers to the initial color parameter of sample at the beginning of the baking experiment. At least three replications were used for each experimental condition.

3.5.2.2 Water activity determination (a_w)

Water activity (a_w) was measured using a water activity meter AquaLab Series 4TE (Decagon Devices Inc, Pullman, WA, USA) in the AwE mode. The a_w -meter was calibrated using the verification standards (a_w 0.984 and a_w 0.760) from Decagon (NE Hopkins Ct. Pullman, WA – USA) at 25 °C. For each of the five water activity levels the values were determined within the required range of 0.003. Water activity was measured both on homogenised puff pastry and filling, about 2 g was placed in a plastic box and introduced into the moisture sensor of the equipment. a_w were measured in triplicate on 3 independent baking batches 2 h after baking at 25 °C.

3.5.3 Fat characterization

Fat of SRN was characterized to evaluate the differences that occur among different lipid matrices and baking methods. Therefore, once extracted fat, FFA, PV, TPC and FA composition were performed.

Determination of FFA, PV, FA composition were carried out following the analytical methods previously described (§ 3.1.1.1 - 3.1.1.3).

3.5.3.1 Fat extraction

Extraction of fat from the raw and baked samples was carried out according to the Schmid-Bondzynski-Ratzlaff (SBR) method of lipid extraction (International Standard 5B, 1986) with some modifications.

Briefly, Samples (10 g) were homogenized with ethanol (7 mL) and mixed using a Vortex mixer for 60 s. Then, a diethyl ether-heptane mixture (10 mL, 2:1 v/v) was added and mixed by vortexing for 60 s. Samples were then centrifuged at 8000 rpm for 10 min. The diethyl ether phase containing the extracted lipids, was transferred and the residue was extracted using the same procedure three more times. The combined filtrates were concentrated in a rotary evaporator at 36 °C. Then, extracted lipid phase was dissolved in hexane and purified using sodium chloride saturated solution (3 mL). The hexane phase containing purified lipids was dried over anhydrous sodium sulfate and under nitrogen.

The total lipid obtained was determined gravimetrically using the AOAC method (1999).

Extracted fat was stored in glass tube at -20 ± 0.2 °C until the analyses.

3.5.3.2 TPC determination

Total polar compounds were determined using silica minicolumn chromatography according the method indicated by Dobarganes *et al.* (2000) based on selective adsorption of polar and non-polar lipids on a silica gel column, followed by non-polar compounds elution with the appropriate solvents. Subsequently both fractions are gravimetrically quantified.

The polar compounds include polar substances such as monoacylglycerols, diacylglycerols, and free fatty acids which occur in unused fats, as well as polar transformation products formed during frying of foodstuffs and/or during heating. Non-polar compounds are mostly unaltered triacylglycerols.

Petroleum ether/ethyl ether (ratio 90/10) was used to elute the non-polar fraction (non-altered triglycerides) to obtain a sharper separation, and a final elution of the column was made with CH₃OH to improve recovery of the sample. Polar compounds were evaluated in triplicate using silica minicolumn (Discovery SPE DSC-Si Silica Tube, 20 mL, 5g; Supelco Analytical).

The results were expressed in percent (w/w) by the following formulas:

$$w_{NP} = (m_{NP}/m) * 100$$

$$w = (m_p/m) * 100$$

m_{NP} = weight in grams of nonpolar fraction

m_p = weight in grams of polar fraction

m = weight in grams of sample

3.5.4 Sensory evaluation

In order to test the overall acceptability of the product, a sensory analysis was conducted, using a semi-structured hedonic scale (Cocci *et al.*, 2008; Meilgaard *et al.*, 2006).

A panel 42-member of untrained consumers, with some experience on sensory evaluation of SRN, was recruited. Panelists were 18 women, between 17 and 60 years old and 24 men, between 19 and 64 years old.

The samples, formulated with different fat blends (L, P, V1 and V2) were baked according alternative and conventional method, coded and served in random order in plastic cups and immediately presented individually to panelists. Panelists rinsed their mouths with bottled spring water between samples.

The tested attributes were the appearance (related to color), smell, flavor, crispy (related to texture) and global acceptability. The effects of different fat formulation and baking process parameters on overall acceptability score of SRN were evaluated.

Each panelist received a form sheet to evaluate the mentioned attributes, using a nine-point hedonic scale anchored with “Like Extremely” and “Dislike Extremely” at either end, with a neutral point of neither “Neither Like nor Dislike” (Fig. 13).

A nine-point hedonic scale

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike extremely

Figure 13_A nine-point hedonic scale.

The tests were performed in an isolated room with good illumination and natural ventilation, in groups of 7 subjects at a time.

3.5.5 Energy costs evaluation

Energy costs of treatments were estimated in terms of energy density, according to power and oven capacity information provided by oven producers and time of treatments.

3.6 Statistical analysis

Data reported are the average values of the three repetitions. The effect of fat blends on fat quality (FFA, PV) were studied by one-way ANOVA ($p \leq 0.05$). If significant difference was found, post hoc Tukey test at 5% level of significance (α) were conducted on the data using the software XLSTAT 2007 (Addinsoft, Paris, France).

Two-way analysis of variance (ANOVA) was performed to determine whether fat formulation and baking types affected quality parameters of SRN ($p \leq 0.05$). If significant difference was found, post hoc Tukey test at 5% level of significance (α) were conducted on the data using the software XLSTAT 2007 (Addinsoft, Paris, France). Therefore, when probability p is less than α ($p \leq 0.05$), hypothesis is right.

A principal component analysis (PCA) was performed in order to display SRNs that yielded results of quality attributes more similar to those obtained for control sample.

Chapter 4 – Result and Discussion

4.1 Lipid analysis

4.1.1 FFA and PV analysis

Data regarding free fatty acid (FFA) and peroxide value (PV) of the fat blends used in the production of SRN are shown in Fig. 14.

Lipids are susceptible to hydrolytic and oxidative processes. These processes can be accelerated if the fat undergoes high temperatures, such as during baking, causing thermal oxidation with an increase in free fatty acid and polar matter contents (Shahidi & Zhong, 2005).

FFA and PV are among the most common quality indicators of fats and oils during production and storage.

With reference to FFA, no statistically significant differences ($p>0.05$) emerged between lard and vegetable fat blends.

PV is a marker of the initial stages of oxidative change. By studying the kinetic of hydroperoxide concentration, it is possible to assess whether a lipid is in the growth or decay portion of the reaction process (Shahidi & Zhong, 2005).

Calligaris *et al.* (2008) reported that PV could be considered a good chemical index to monitor the loss of sensory quality of biscuits during their shelf life, as it was well correlated to sensory consumer acceptance.

As expected, Lard (control) showed the highest PV values. Lard is a semisolid fat commonly used in the food industry, especially in the preparation of bakery products, even if contains practically no natural antioxidants (Belitz & Grosch, 1999) and oxidative degradation can occur.

Thus, recent trends in the baking industry are aimed to replace lard and other animal fats with vegetable ones (Nur Illiyin *et al.*, 2013).

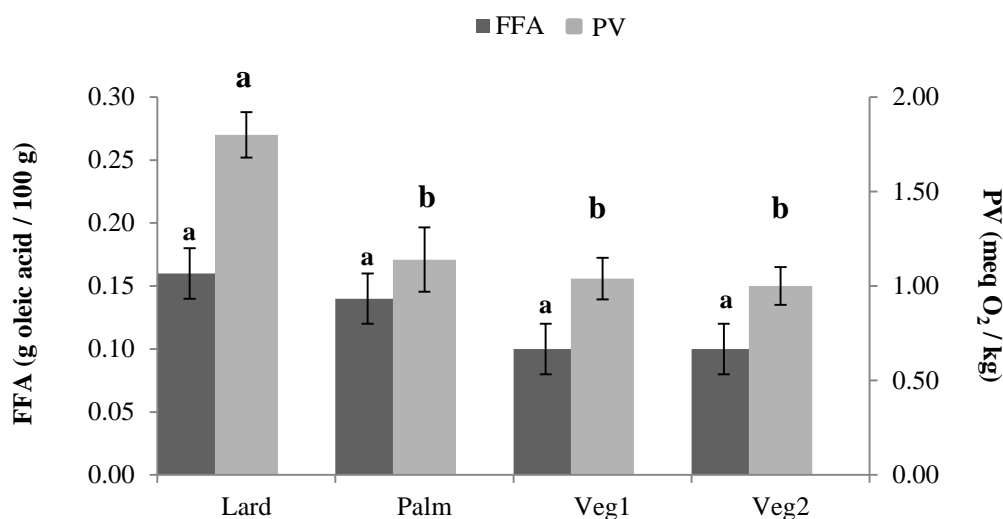


Figure 14_ Free fatty acids (g oleic acid / 100 g) and peroxides values (meq O₂ / kg) of different fat blends used in SRN production. Lard, lard; Palm, palm oil 80% and sunflower oil 20%; Veg1, sunflower oil 60%, shea butter 25% and coconut oil 15%; Veg2, sunflower oil 40%, shea butter 40% and coconut oil 20%. a-b: Different letters for each parameter correspond to statistically significant differences ($p \leq 0.05$).

4.1.2 FA composition

The mean values and standard deviations of the fatty acid composition of the fat blends utilized in SRN-making are shown in Table 4.

Veg1 contains a similar saturated fatty acid (SFA) content than lard ($40.35 \pm 0.31\%$ and $39.4 \pm 0.19\%$, respectively). Veg2 and Palm have similar SFA content ($42.43 \pm 0.23\%$ and $42.97 \pm 0.28\%$, respectively). But fat blends showed different fatty acid profiles.

The main saturated fatty acid present in Lard and Palm was palmitic acid (C16:0) with values of $24.10 \pm 0.11\%$ and $35.31 \pm 0.23\%$, respectively; while Veg1 and Veg2 showed a higher content of stearic acid (C18:0) ($20.37 \pm 0.19\%$ and $26.95 \pm 0.03\%$, respectively).

The high stearic acid content in Veg1 and Veg2 is attributed to the presence of shea butter, richer in this fatty acid (Maranz *et al.*, 2004). Thus, these fat blends, without palm oil, make the fats attractive on nutritional grounds, since stearic acid contributes to reduced plasma LDL concentrations and, hence, has no adverse effect on risk of cardiovascular disease (Mensink, 2005).

Vegetable fat blends Veg1, Veg2 and Palm differed from Lard for a higher poly-unsaturated fatty acids (PUFA) content, due to the presence of sunflower oil (Tab. 1). The high content of linoleic acid (C18:2n6c) in Veg1, Veg2 and Palm ($37.44 \pm 0.02\%$, $27.32 \pm 0.06\%$, $19.57 \pm 0.18\%$, respectively) correspond to different concentration of sunflower oil incorporated into the blends as the liquid component. Vegetable fat V1 showed the highest value in PUFA and lowest one in MUFA, due to the high percentage of sunflower oil in the mixture (60%).

Lard contained a higher level in mono-unsaturated fatty acid (MUFA) ($44.47 \pm 0.17\%$, instead $16.13 \pm 0.03\%$ of PUFA). The main MUFA was oleic acid (C18:1n9c) ($41.33 \pm 0.17\%$)

MUFA/SFA, PUFA/SFA and UFA/SFA ratios, in Table 4, well showed the different fatty acid profiles.

UFA/SFA ratio is higher in Lard. The two vegetable fats without palm oil exhibited the lowest MUFA/SFA ratio and highest PUFA/SFA one (Fig. 15 and 16).

A practically negligible content of TFAs ($<1\%$), which is in accordance with the required values (World Health Organization, 2003) in all fat blends (0.26%, 0.18% and 0.25%, in Lard, Veg1 and Veg2, respectively). They are not detected in Palm.

Table 4_ Fatty acid composition [mean±standard deviation (sd)] of the lipidic fraction of fat blends (Lard, Palm, Veg1 and Veg2) used in SRN production.

Fatty acids (%)	Lard	Palm	Veg1	Veg2
C4:0	ND	ND	0.73±0.64	0.43±0.03
C6:0	ND	ND	0.07±0.00	0.05±0.01
C8:0	ND	ND	1.05±0.03	0.74±0.03
C10:0	0.07±0.02	ND	0.81±0.05	0.54±0.01
C12:0	0.09±0.01	0.21±0.02	7.25±0.06	4.76±0.21
C14:0	1.50±0.10	1.02±0.03	2.72±0.08	1.85±0.01
C14:1	0.01±0.01	ND	ND	ND
C15:0	0.04±0.00	ND	ND	ND
C16:0	24.10±0.11	35.31±0.23	6.21±0.23	5.87±0.02
C16:1	2.63±0.14	0.17±0.01	0.03±0.02	ND
C17:0	0.27±0.00	0.06±0.01	0.04±0.00	0.06±0.01
C17:1	0.27±0.00	ND	0.01±0.00	0.01±0.00
C18:0	13.14±0.09	5.56±0.05	20.37±0.19	26.95±0.30
C18:1n9t	0.23±0.01	ND	0.09±0.14	0.18±0.01
C18:1n9c	41.33±0.17	36.84±0.2	21.73±0.04	29.59±0.03
C18:2n6t	0.03±0.01	ND	0.09±0.09	0.07±0.00
C18:2n6c	13.85±0.05	19.57±0.18	37.44±0.02	27.32±0.06
C18:3n6	ND	ND	0.22±0.03	0.35±0.02
C18:3n3	1.25±0.12	0.20±0.03	ND	ND
C20:0	0.17±0.01	0.44±0.51	0.64±0.12	0.79±0.02
C20:1	ND	0.34±0.22	ND	ND
C20:2	0.60±0.10	ND	0.02±0.01	0.03±0.01
C22:0	0.01±0.00	0.26±0.02	0.36±0.13	0.29±0.01
C20:3n6	0.07±0.01	ND	ND	ND
C20:4n6	0.32±0.00	ND	ND	ND
C23:0	ND	ND	0.01±0.01	0.01±0.00
C24:0	ND	0.02±0.01	0.10±0.00	0.09±0.00
C22:6n3	ND	ND	0.02±0.00	0.02±0.01
Σ SFA	39.40 ^b ±0.19	42.97 ^a ±0.28	40.35 ^b ±0.31	42.43 ^a ±0.23
Σ MUFA	44.47 ^a ±0.17	37.01 ^b ±0.04	21.86 ^d ±0.10	29.78 ^c ±0.04
Σ PUFA	16.13 ^d ±0.03	20.03 ^c ±0.10	37.79 ^a ±0.08	27.79 ^b ±0.02
Σ UFA	60.60 ^a ±0.19	57.04 ^d ±0.13	59.65 ^b ±0.03	57.57 ^c ±0.06
UFA/SFA	1.54 ^a ±0.01	1.33 ^c ±0.01	1.48 ^b ±0.01	1.36 ^c ±0.01
MUFA/SFA	1.13 ^a ±0.01	0.86 ^b ±0.01	0.54 ^d ±0.01	0.70 ^c ±0.00
PUFA/SFA	0.41 ^d ±0.00	0.47 ^c ±0.01	0.94 ^a ±0.00	0.65 ^b ±0.01

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids; ND = not detected.

a-d: Different letters in the same row correspond to statistically significant differences ($p \leq 0.05$).

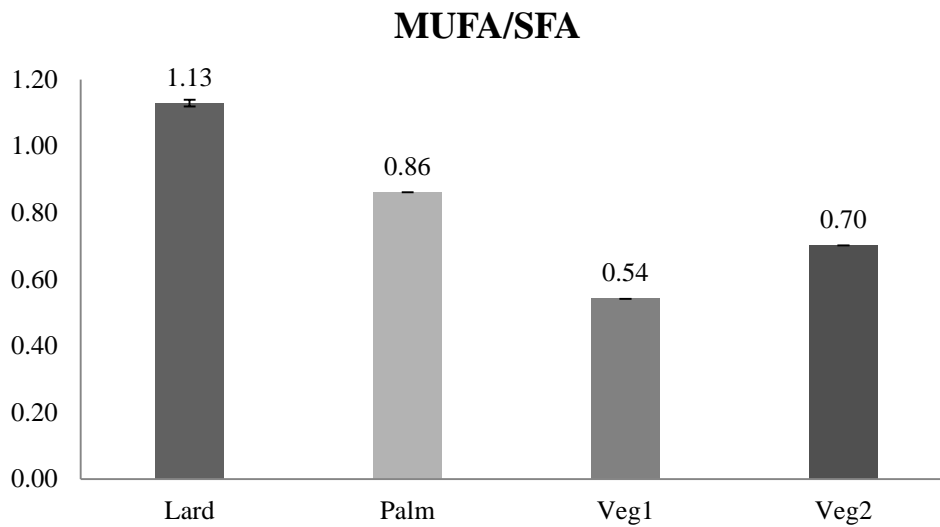


Figure 15_ MUFA/SFA ratio of fat blends (Lard, Palm, Veg1 and Veg2) (mean±sd).

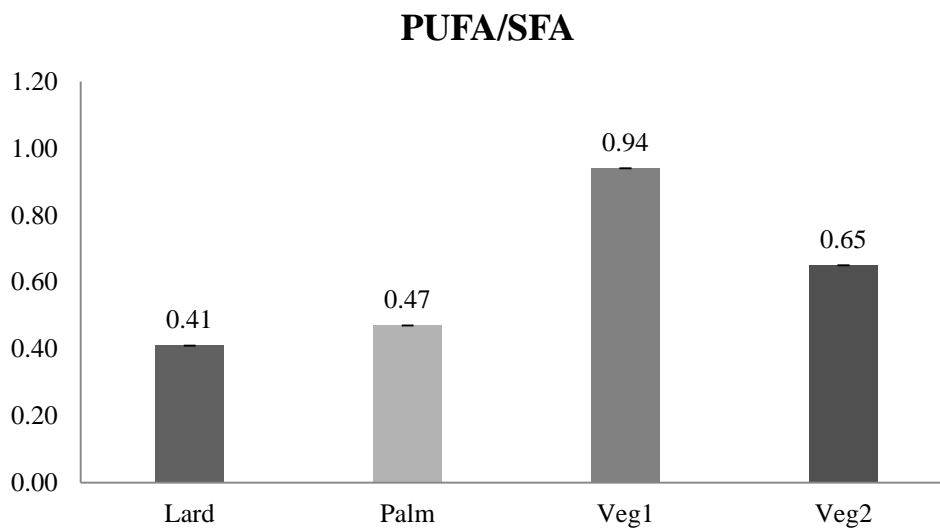


Figure 16_ PUFA/SFA ratio of fat blends (Lard, Palm, Veg1 and Veg2) (mean±sd).

4.1.3 TAG and cholesterol analysis

Fat blends consists of a mixture of a large number of TAG, as shown in Table 5.

In order for a TAG to exist in the β' crystal form, the most stable form, its carbon number should be kept as low as possible, preferably below C54 (Nor Aini & Miskandar, 2007).

Lard contains mainly C52, which is so β' tending. The C52 content is reduced in Veg1 and Veg2. On the other hand, C54 content is reduced in Lard but increased in Veg1 and Veg2.

Concurrently, Veg1 and Veg2 contain higher content of C24 to C42 due to the presence of coconut oil in the blend (Tab. 1).

It was reported in literature that at 15 °C margarines with palmitic acid (C16:0) below 11% are in β form, while those with 50% or below are in β' crystal form. β' has relatively very small crystals, which enables it to incorporate relatively large amounts of liquid oil in the crystal network. This phenomenon leads to the production of smooth, continuous and homogeneous products. Shortenings and margarines containing β' crystals appear smooth and shiny, in contrast to those containing β crystals which produce a dull and mottled product (deMan & deMan, 1994).

Because of the high content of palmitic acid ($35.31 \pm 0.23\%$), Palm is β' tending. It contains mainly C50 and C52 ($32.0 \pm 0.2\%$ and $34.4 \pm 0.4\%$, respectively).

As expected cholesterol content was found only in Lard (110 mg/100 g).

Table 5_ Mean TAG composition (%) by carbon number and cholesterol content of fat blends (Lard, Palm, Veg1 and Veg2) (mean \pm sd).

Triglycerides (%)	Lard	Palm	Veg1	Veg2
C24-C42	11.8 \pm 0.3	0.3 \pm 0.2	20.1 \pm 0.2	20.5 \pm 0.2
C44	0.3 \pm 0.0	0.2 \pm 0.0	7.0 \pm 0.2	5.1 \pm 0.1
C46	0.8 \pm 0.0	1.0 \pm 0.0	6.0 \pm 0.2	5.0 \pm 0.1
C48	4.8 \pm 0.3	7.6 \pm 0.1	22.4 \pm 1.7	13.0 \pm 0.0
C50	19.5 \pm 0.6	32.0 \pm 0.2	ND	5.6 \pm 0.0
C52	44.1 \pm 6 \pm 1.2	34.4 \pm 0.4	10.4 \pm 0.0	11.3 \pm 0.1
C54	18.7 \pm 0.3	23.5 \pm 0.2	32.8 \pm 1.1	38.8 \pm 0.9
Others	ND	1.0 \pm 0.1	1.3 \pm 0.1	0.7 \pm 0.2
Cholesterol (%)	Lard	Palm	Veg1	Veg2
	0.11 \pm 0.3	ND	ND	ND

4.1.4 Solid fat content

The SFC is the percentage of lipid that is solid at the selected temperatures. Since SFC influences physical properties such as spreadability, hardness, mouthfeel, and stability, the SFC values are required to characterize the properties of plastic fats (Lee *et al.*, 2008). It is an important index to determine whether a certain type of fat or fat blend is suitable for a particular application (Graef *et al.*, 2012). Thus, it is an essential measurement for characterising edible oils/fats used in the bakery and confectionery.

An optimum ratio of liquid oil to solid fat, coupled with a suitable crystal size and form, is desirable. The SFC of the fat was measured by NMR procedure. The NMR technique is non-destructive, so repeatability or other measurements can be made on the same sample. Also NMR measurement time is short (typically 6 seconds).

The SFC profiles of the vegetable fat blends are compared to that of Lard, as shown in Figure 17.

The plastic range of Lard is narrow. Its solid fat content profile is rather steep and lower than the other fats.

The SFC of vegetable fats at 0 °C was found higher than SFC of Lard. Veg2 showed a value of 42.3%, similarly to Palm 43.0%; whereas Veg1 at the same temperature were 40.4% and Lard 35%.

The SFC of Lard was found to drop to 18.0% at 20 °C and tended to become almost zero at 40 °C.

Vegetable fat Veg1 and Veg2 displayed higher profile than Lard because of the high content of high melting TAG molecules ($\geq C54$) (Tab. 5). The increasing SFC values were found to correlate well with the increasing proportion of TAG molecules at high molecular weight ($\geq C54$) in the fat blends.

Of these, Veg1 was found to display closer compatibility to Lard as the SFC values of Lard fell to a level similar than Veg1 at 30 °C; while the SFC of Veg2 was similar than Lard above 30 °C.

The SFC profiles of Veg2 and Palm were found to be quite similar above 15 °C. Hence, Veg2 showed better compatibility to Palm in terms of SFC profiling.

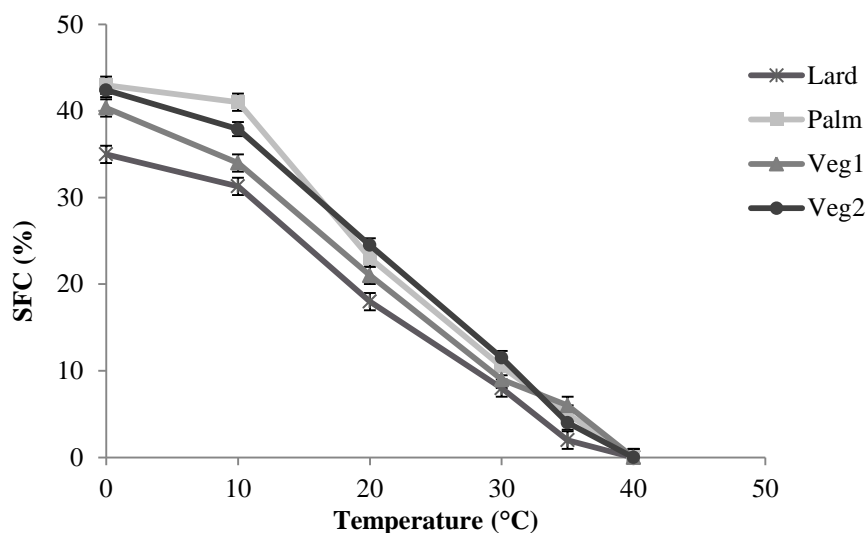


Figure 17_ Solid fat profiles of fat blends (Lard, Palm, Veg1 and Veg2).

4.2 Baking process

SRN is a distinctly layered pastry with a flaky texture and a creamy filling. There are no previous reports about the baking conditions in a microwave-IR oven of this complex type of confectionery product; for this reason, the first step in this research, was to determine the potency and baking time conditions for RTE-SRN.

Baking process optimization was made on SRN produced with Lard (coded as L, control sample).

Experimental cooking was composed of a pre-cooking phase followed by a heating phase.

The reason for selection of microwave time and electric oven time as independent variables for pre-cooking, and microwave power and microwave-IR time as independent variables for heating phase, was the significant effect on quality of final product. The levels of these independent variables were determined by preliminary experiments and previous researches.

Moisture of filling and puff pastry, color parameters of upper and lower puff pastry, texture and core temperature were chosen to be the dependent variables since they are the most effective quality attributes in terms of consumer expectation and food security.

A cubic design models were used to express the responses as a function of the independent variables for the pre-cooking phase, a quadratic model for the latter phase.

The main statistical parameters of applied response surface (RS) models for each response relative to pre-cooking and heating phases of L are reported in table 6 and 8, respectively. Coefficient of determination (R^2) is a measure of the amount of variation around the mean explained by the model, the adjusted coefficient of determination ($Adj-R^2$) is the same index adjusted for the number of terms in the model. Tables 7 and 9 show the final model equations.

For the first phase, significant models were calculated for all the response variables, except for temperature (T). In particular, significant ($p \leq 0.05$) linear models were calculated for upper L*, lower b*, filling moisture and puff pastry moisture, while complete quadratic models were found for upper a* and firmness; significant interaction model, as indicated by 2FI, was found for lower L* and lower a*, mean model only for upper b*.

It was reported by Gan *et al.* (2007) that, in a good fit model, the minimum value for R^2 should be 80%. The results showed that the models for all the response variables can be considered as good fit model due to their coefficient of determination which was $\geq 80\%$, except for lower b*, upper b* and lower L*.

The coefficient of determination $R^2 = 0.94, 0.97, 0.88$ and 0.87 for puff pastry and filling moisture, upper a* and firmness, respectively (equations indicated in Table 7) indicated that the linear and quadratic model applied showed an optimal fit to the experimental data.

The two factors interaction (2FI) model obtained for equations of lower L* and a* (Tab. 7), showed a coefficient of determination of $R^2 = 0.76$ and 0.91 , respectively, which indicate that the experimental model of fit the model present a high level of interaction between the studied parameters and lower L* and a*. For lower b*, the linear model obtained for equation, showed a coefficient of determination of $R^2 = 0.55$, which indicate that the model had a moderate fit to the experimental data.

Also, the Adj-R² was quite high for most of the quality parameters, especially for filling and puff pastry moisture (0.93% and 0.96%, respectively), which indicate that the models applied showed a good fit to the experimental data.

In order to measure the failure of a model to represent data in the experimental domain at which points were not included in the regression, the lack of fit test can be used (Varnalis *et al.* 2004).

Among the variables with significant models, upper a* and b*, filling and puff pastry moisture and firmness, the lack of fit was not significant ($p > 0.05$), thus indicating an adequate description of the true shape of the response surfaces.

On the other side, upper L* and lower L*, lower a* and lower b* showed a significant lack-of-fit ($p \leq 0.05$), which means that the models did not correctly fit the experimental data, as it is given in Table 6.

The response variables for the heating phase were fitted to a second-order polynomial model equation to correlate the response variables to the independent variables. The final model equations are shown in Table 9.

Except of lower a* and b* and filling and puff pastry moisture, the model describes all parameters were significant. In particular, significant ($p \leq 0.05$) linear models were found for upper and lower L* and upper b*, complete quadratic model for firmness (N), while cubic model was calculated for Temperature and, finally, mean model for upper a*.

R² was $\geq 80\%$ in upper L*, firmness and T, as it can see in Table 8. For upper b*, the linear model obtained showed a coefficient of determination of $R^2 = 0.54$, which indicate that the model had a moderate fit to the experimental data.

In particular, the high Adj-R² (0.99) for T, indicated that the linear model applied showed an optimal fit to the experimental data.

Table 6_ Main statistical parameters of cubic design model applied for pre-cooking phase (control sample L).

	upper L*	upper a*	upper b*	lower L*	lower a*	lower b*	filling moisture (%)	puff pastry moisture (%)	firmness (N)	T (°C)
RS Model	linear	quadratic	mean	2FI	2FI	linear	linear	linear	quadratic	cubic
Lack of fit (F)	36.8	4.5 ^{ns}	4.3 ^{ns}	4.7	144	7.7	0.7 ^{ns}	2.0 ^{ns}	4.1 ^{ns}	9.3
R²	0.78	0.88	-	0.76	0.91	0.55	0.97	0.94	0.87	0.65
Adj-R²	0.75	0.84	-	0.72	0.89	0.49	0.93	0.96	0.89	0.32
Range	34.3-64.2	0.9-15.4	12.2-33.0	40.9-57.1	1.1-15.7	12.9-31.2	37.5-54.7	6.4-24.7	4.9-16.3	96-100

L, SRN made with Lard; RS, Response Surface; Adj-R², adjusted coefficient of determination; n.s. not significant.

Table 7_ Regression equations for L baked under different processing conditions in a microwave-electric combination oven.

Quality parameter	Equation
upper L*	$Y = 83.3 - 1.8 * A - 1.8 * B$
upper a*	$Y = -40.6 + 2.0 * A + 5.2 * B - 0.0 * AB - 0.0 * A^2 - 0.1 * B^2$
upper b*	$Y = 23.4$
lower L*	$Y = 46.6 + 3.3 * A + 0.6 * B - 0.3 * AB$
lower a*	$Y = -0.9 - 1.0 * A + 0.1 * B + 0.2 * AB$
lower b*	$Y = 6.7 + 1.7 * A + 0.4 * B$
filling moisture (%)	$Y = 0.3 - 0.0 * A - 6.3E-003 * B$
puff pastry moisture (%)	$Y = 0.6 - 0.0 * A - 3.7E-003 * B$
firmness (N)	$Y = -41.6 + 1.8 * A + 4.8 * B - 0.0 * AB - 0.0 * A^2 - 0.1 * B^2$
T (°C)	$Y = 8.2 - 6.5 * A + 20.4 * B + 0.1 * AB + 1.2 * A^2 - 1.4 * B^2 - 0.0 * A^2 B + 7.5E-00 * AB^2 - 0.0 * A^3 + 0.0 * B^3$

A, microwave time; B, electric time; T, temperature.

Table 8_ Main statistical parameters of quadratic design model applied for heating phase (control sample L).

	upper L*	upper a*	upper b*	lower L*	lower a*	lower b*	filling moisture (%)	puff pastry moisture (%)	firmness (N)	T (°C)
RS Model	linear	mean	linear	linear	linear	linear	cubic	cubic	quadratic	cubic
R²	0.84	-	0.54	0.36	0.34	0.09	0.73	0.71	0.81	0.995
Adj-R²	0.82	-	0.47	0.27	0.25	n.a.	0.33	0.40	0.83	0.99
Range	41.0-52.3	7.7-12.3	15.6-30.4	45.7-57.7	2.6-12.7	17.1-30.3	8.3-39.3	5.2-19.4	7.3-18.3	50-102

L, SRN made with Lard; RS, Response Surface; Adj-R², adjusted coefficient of determination; n.a. not available.

Table 9_ Regression equations for L baked under different processing conditions in a microwave-IR combination oven.

Quality parameter	Equation
upper L*	$Y = 57.9 - 6.4E-003 * A - 1.7 * B$
upper a*	$Y = 10.1$
upper b*	$Y = 31.4 - 4.4 * A - 1.7 * B$
lower L*	$Y = 58.2 - 4.2E-003 * A - 0.8 * B$
lower a*	$Y = 1.0 + 8.1E-004 * A + 1.1 * B$
lower b*	$Y = 20.1 - 3.2E-003 * A + 0.4 * B$
filling moisture (%)	$Y = 0.2 - 1.6E-003 * A - 0.0 * B + 6.9E-005 * AB + 3.8E-006 * A^2 + 0.0 * B^2 - 2.3 * A^2 * B + 1.3 * AB^2 - 2.2E-009 * A^3 - 2.3E-003 * B^3$
puff pastry moisture (%)	$Y = 0.4 - 4.7E-003 * A + 0.2 * B + 3.7E-004 * AB + 9.2E-006 * A^2 - 0.0 * B^2 - 7.7 * A^2 * B + 3.0 * AB^2 - 4.0E-009 * A^3 - 5.6E-004 * B^3$
firmness (N)	$Y = 32.6 + 2.0 * A + 3.4 * B - 0.0 * AB - 0.0 * A^2 - 0.1 * B^2$
T (°C)	$Y = -12.1 + 0.4 * A + 20.3 * B - 0.0 * AB - 5.0E-004 * A^2 - 0.9 * B^2 + 2.8E-005 * A^2 * B + 5.8E-004 * AB^2 + 2.5 * A^3 + 0.0 * B^3$

A, microwave power; B, Microwave-IR time; T, temperature.

RSM aims especially to find the optimum conditions for a process to obtain the desired responses (Kahyaoglu & Kaya, 2006). Thus, in order to identify a baking process optimized for quality attributes, a desirability function was constructed. This function reaches a maximum value of 1 when the aims set for the chosen variables are fully achieved.

According to experimental data, to optimize the process parameters of pre-cooking phase, it was considered that following target values of the response variables: minimum temperature and puff pastry moisture, maximum moisture of filling and colour parameters to a value target equal to the colour of the control sample. For the heating phase minimum temperature for consume, and moisture of puff pastry, color parameters and filling moisture content to a value target equal to that obtained with conventional baked L (the control).

To these variables was given the same importance because they were all representative of a baked product quality. A linear desirability function (weight = 1) was chosen.

From elaborating data, the highest desirability corresponded to an optimized pre-cooking phase at power of 300 W for 4 min followed by cooking at 200 °C for 16 min in electric oven, with a predicted value of response variable illustrated in Table 10. The desirability function reached a value of 0.70 (Fig. 18a).

The optimized heating phase was obtained at microwave power of 100 W and IR power of 900 W for 2 min, with a desirability of 0.84 (Fig. 18b). Predicted values of response variables are illustrated in Table 11.

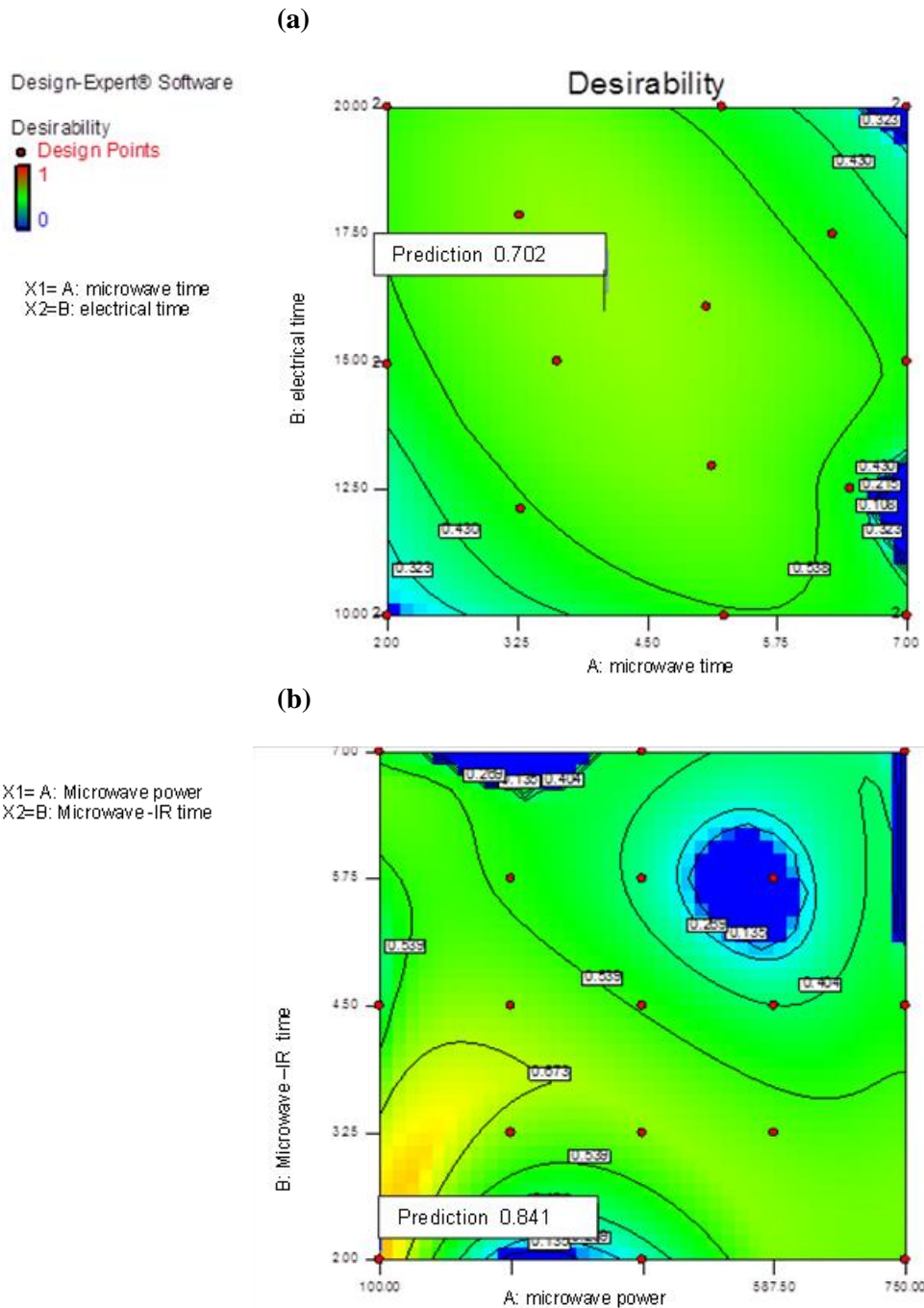


Figure 18_ Desirability function contour plot for optimization of pre-cooking (a) and heating (b) phases of L.

The optimized L was experimentally tested twice and the measured values for pre-cooking and heating phases are reported in Table 10 and 11, respectively.

The optimized values (optimized L) were similar than those predicted by the model (predicted L). The validation values were fairly consistent with those predicted, showing a full correspondence. Only temperature showed higher value ($p \leq 0.05$) in measured value, probably as temperature was not a significant model for experimental data in pre-cooking phase, as previously reported.

Table 10_ Comparison of predicted and optimized response values of L for pre-cooking phase (mean±sd).

Pre-cooking

	upper L*	upper a*	upper b*	lower L*	lower a*	lower b*	filling UR (%)	puff pastry UR (%)	firmness (N)	T (°C)
Predicted L	46.8±0.0 a	12.6±0.0 a	23.3±0.0 a	50.8±0.0 a	7.4±0.0 a	20.7±0.0 a	47.0±0.0 a	15.0±0.0 a	12.1±0.0 a	97.0±0.0 b
Optimized L	44.9±1.3 a	13.3±1.4 a	25.5±1.1 a	51.5±0.5 a	5.1±1.6 a	20.5±1.6 a	48.4±0.5 a	15.6±0.6 a	11.3±0.7 a	98.0±0.0 a

a-b: Different letters in the same column indicate significant differences ($p \leq 0.05$).

Table 11_ Comparison of predicted and optimized response values of L for heating phase (mean±sd).

Heating

	upper L*	upper a*	upper b*	lower L*	lower a*	lower b*	filling UR (%)	puff pastry UR (%)	firmness (N)	T (°C)
Predicted L	43.8±0.0 a	10.1±0.0 a	27.5±0.0 a	54.3±0.0 a	5.9±0.0 a	20.5±0.0 a	37.2±0.0 a	12.0±0.0 a	12.8±0.0 a	62.0±0.0 b
Optimized L	41.4±0.9 a	11.6±0.8 a	24.8±1.0 a	52.8±0.9 a	6.3±0.3 a	21.6±1.0 a	44.6±0.4 a	14.2±0.7 a	11.7±0.4 a	72.0±0.1 a

a-b: Different letters in the same column indicate significant differences ($p \leq 0.05$).

4.3 Alternative vs. traditional baking - Quality Measurements of SRN

Once optimized the process parameters, SRNs formulated with different vegetable fats (P, V1 and V2) were baked with the optimized treatment (alternative) and were analysed in order to compare with control sample made with Lard (L) and thus with sample baked in the conventional oven (traditional).

The quality parameters measured for vegetable SRNs (P, V1 and V2) and traditional SRN made with Lard (L) were analysed by Principal Component Analysis (PCA). The PCA biplot is reported in Fig. 19, with a total variation of the data explained up to 96.61% (67.82% by PC1 and 28.79% by PC2).

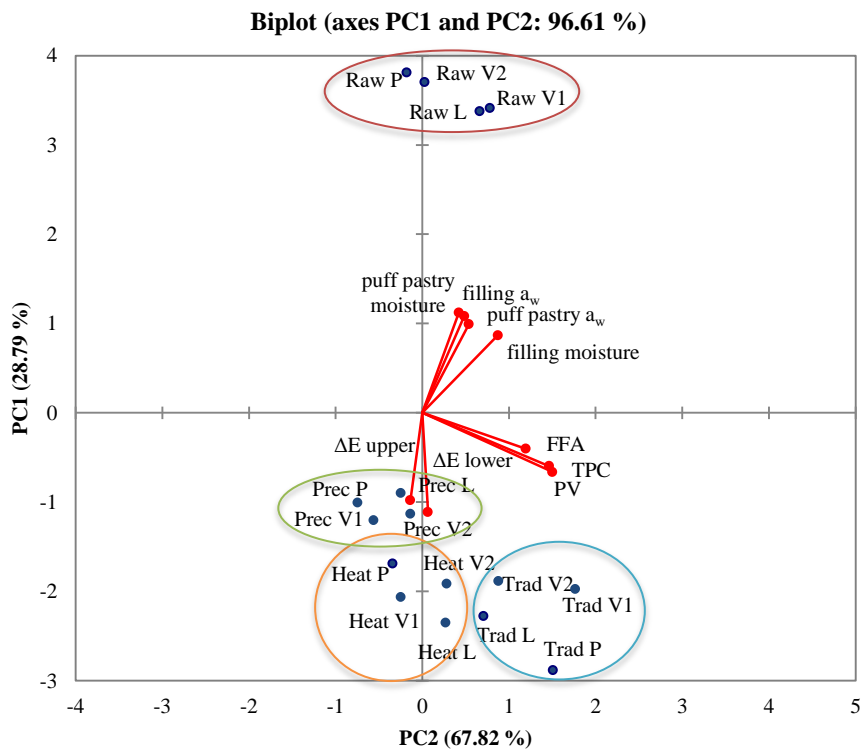


Figure 19_ PCA biplot of raw and baked (in traditional and alternative method) SRNs with different fat blends (L, P, V1 and V2)
L, SRN made with Lard; P, SRN made with Palm; V1, SRN made with Veg1; V2, SRN made with Veg2.

A different distribution of the samples can be observed. In particular, raw SRNs are collocated in opposite area respect to baked SRNs, with higher moisture and a_w contents in filling and puff pastry, as expected. ΔE and FFA, PV and TPC values are the lowest. While all alternative SRNs (prec. and heat.) are collocated in the same area of traditional ones (Trad), characterized by lower FFA, PV and TPC values than traditional SRNs.

Changes in microbial growth, chemical–physical parameters including texture, sensory attributes and fat characterization of alternative SRNs were compared to traditional ones.

Energy costs of treatments were estimated in terms of energy density, according to power and oven capacity information provided by oven producers and time of treatments.

4.3.1 Microbiological analysis

Foodborne disease outbreaks linked with RTE foods have been associated with various foodborne pathogens (Gibbons *et al.*, 2006; Gilbreth *et al.*, 2005).

The microbiology of RTE foods during preparation in factories, in domestic kitchens, in canteens and on street corners by street vendors has previously been investigated (Aycicek, *et al.*, 2004; Bas *et al.*, 2006; Beumer & Kusumaningrum, 2003; Cogan *et al.*, 2002; von Holy & Makhoane, 2006).

SRN is a susceptible product for proliferation of microorganisms. This phenomenon is attributable to the rich filling and other parameter such as water activity, propitious for microorganisms proliferation.

Unbaked SRN (coded as raw), alternative baked SRN (composed of pre-cooking and heating) and traditional baked SRN microbial counts are made on puff pastry and filling.

Following the cooking process the number of surviving bacteria was significantly reduced, both in alternative and traditional baking, ranging between 2.2 and 2.3 Log CFU/g in L puff pastry, and 3.1 and 3.7 in L filling, respectively (Tab. 12).

As can be seen, a microbiological stability was ensured already in pre-cooking phase, with a reduction of mesophilic aerobic count of about 2 Log, ranging similar value than traditional baked SRN. It may be due to an explosion of internal pressure generated within the core by microwave, as reported by Puligundla *et al.* (2013).

Therefore, the difference between the treatments was not statistically significant, pre-cooking and traditional methods caused a similar antimicrobial effect, although the baking time was about 2-folds shorter during pre-cooking phase.

Fat formulation didn't influence the microbiological counts. Microbial counts of L, P, V1 and V2 showed similar values.

It is known that fat content may influence heat tolerance of bacteria (Juneja & Eblen, 2000; Shachar & Yaron, 2006), but there are no studies about the influence of fat type on heat tolerance of bacteria.

Table 12_ Microbiological parameters of L in puff pastry (a) and filling (b) (mean±sd).

Parameters (Log CFU/g)	a) Puff pastry			b) Filling		
	Raw	Pre-cooking	Traditional	Raw	Pre-cooking	Traditional
Mesophilic aerobic count	4.0 ^a ±0.1	2.2 ^b ±0.0	2.3 ^b ±0.0	5.8 ^a ±0.1	3.1 ^c ±0.1	3.7 ^b ±0.1
Psychrotrophic aerobic count	2.5±0.1	<1	<1	4.3±0.1	<1	<1
<i>Enterobacteriaceae</i>	2.3±0.1	<1	<1	3.5±0.0	<1	<1
Coliform	2.3±0.0	<1	<1	3.7±0.0	<1	<1
β-glucuronidase-positive <i>Escherichia coli</i>	<1	<1	<1	<1	<1	<1
Faecal streptococci	<1	<1	<1	<1	<1	<1
Staphylococci	<1	<1	<1	<1	<1	<1
Sulfite-reducing clostridial spores	<1	<1	<1	<1	<1	<1
<i>Bacillus cereus</i>	<1	<1	<1	<1	<1	<1
Yeasts	2.6±0.0	<1	<1	2.0±0.0	<1	<1
Moulds	2.5±0.0	<1	<1	<1	<1	<1
<i>Listeria monocytogenes</i> (25g)	undetectable			undetectable		
<i>Salmonella spp.</i> (25g)	undetectable			undetectable		

a-c: Different letters within row for puff pastry and filling correspond to statistically significant differences ($p \leq 0.05$).

4.3.2 Chemical-physical analysis

4.3.2.1 Heat profile

Temperature is an important parameter for limiting the growth of pathogens. During baking process the temperature of the pastry must be carefully controlled to achieve the food security and for the structure throughout the final product to be adequately rigid.

During baking, the temperature in the center of the crumb can reach 100 °C for a few minutes (Smith *et al.*, 2004). According to Bryan *et al.* (1997) vegetative pathogenic microorganisms should be readily destroyed during baking, due to their low thermal resistance (D values). The minimal cooking temperature usually recommended by government agencies to enhance safety of products in various countries is ~75 °C for few minutes. A core temperature of 75 °C (or equivalent time-temperature combination), is considered effective to achieve a 6-D reduction in the number of

Listeria monocytogenes cells. *L. monocytogenes* is regarded as the most heat resistant non-spore-forming foodborne pathogen. Alternative cooking time/temperature combinations may be used so long as to achieve the same lethal effect as 75 °C instantaneously. Scientifically accepted alternative time/temperature combinations include: 70 °C for 2 minutes, 67 °C for 5 minutes and 64 °C for 12 minutes and 37 seconds.

Indeed, while 11.5 min of cooking was needed to reach 75 °C in the middle of pastry cooked by convection, alternative baking required only 2.5 min (Fig. 20). The different SRNs showed similar temperature profile. The main benefit of microwave is the ability to uniformly heat all the mater within a shorter time. Thus, the increase of the core temperature of the microwave baked sample in the first phase of alternative baking was very sharp, while the temperature profile of convection baked pastry was very mild.

The surface of SRN baked in only microwave oven, which touches the cold air and chamber walls, was cooled down, probably due to temperature gradient and cooling effect through water evaporation or melting of fat. Combined heating, by both microwave and convection, kept the temperatures relatively uniform in different internal and external parts of the products, and increased the heating rates. So the heating was slightly accelerated in the combined treatment.

Examination of time–temperature profiles for SRNs baked in convective and microwave ovens revealed an almost linear temperature increase for those baked in the convective oven, while the microwave-baked samples (in alternative method) showed a sudden initial rise in temperature, as reported in other studies, such as in Sumnu *et al.* (1999).

During conventional baking, the temperature increased rapidly to reach a maximum temperature of approximately 100 °C, after which a temperature plateau was observed. This behaviour has been found by other researchers (Lambert *et al.*, 1992; Thorvaldsson & Skjoldebrand, 1998; Lostie *et al.*, 2002). The temperature profile of the alternative-baked products resulted in a shortening of the overall baking time, as reported also by Baik *et al.* (2000).

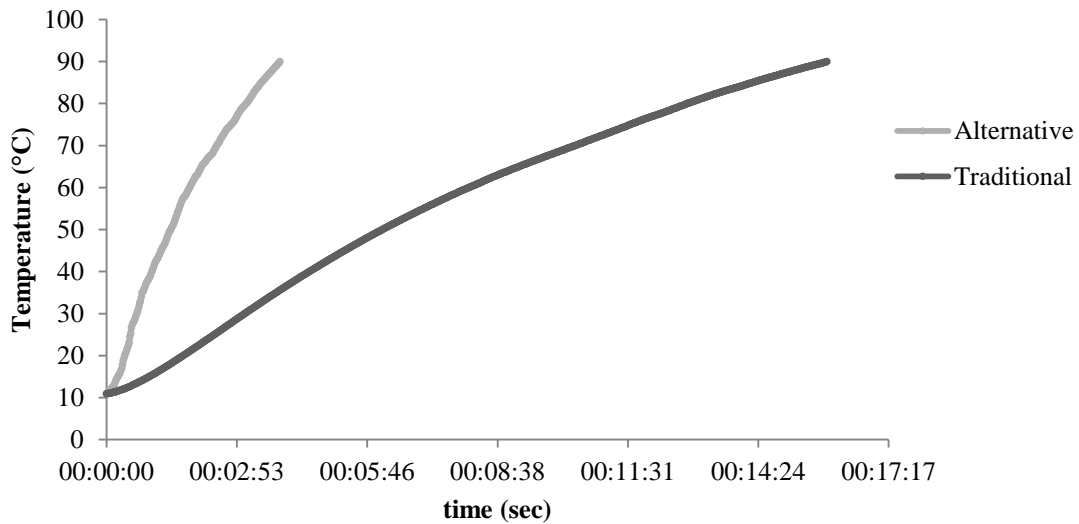


Figure 20_ Typical temperature core profile in alternative (microwave oven) and traditional (electric oven) SRN.

4.3.2.2 Colour change - ΔE

Colour is an important quality attribute in the food industries, it influences consumer's choice and preferences (Pathare *et al.*, 2013).

In Figure 21 is reported alternative (a) and traditional (b) L (SRN made with Lard).

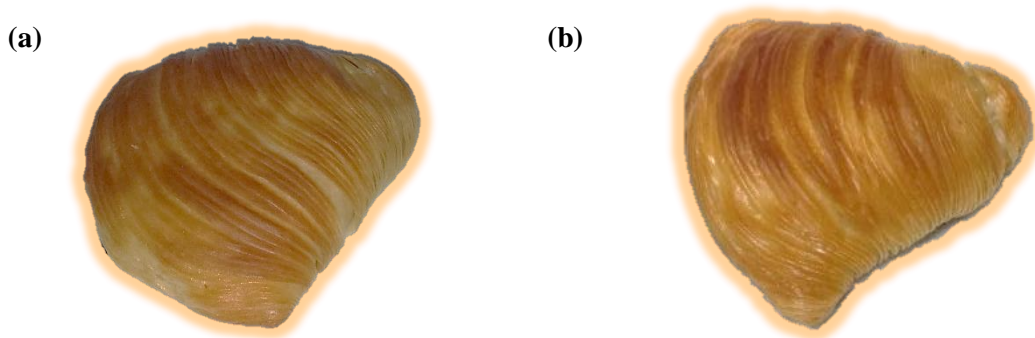


Figure 21_ Comparison of alternative (a) and traditional (b) L.

As expected, it was not possible to observe a significant colour change during microwave baking of SRN. In fact, microwave baked SRN had the lowest ΔE values (ranged between 1.3 ± 0.1 and 3.4 ± 0.1), which corresponded to a similar colour value to that of the unbaked SRN, very close to zero, as also reported in Keskin *et al.* (2004). There was no significant difference between ΔE values of SRN made with different fat blends. The variation of fat did not have effect on the color of cake samples baked with alternative and traditional methods.

Thus, the microwave power had no significant effect on the ΔE value of SRN. This can be explained by the short baking times and low temperatures in microwave processing. Microwave power doesn't have any effect on the ambient air temperature (Zuckerman & Miltz, 1997) and accordingly on the surface temperature of the product, so cannot reach the required high values for browning reactions on the crust

color formation in according to Sevimli *et al.* (2005). Maillard and caramelization reactions require temperatures above 150 °C (Turpin, 1989).

Instead, microwave combined with other heat sources allowed to increase surface temperature of sample, which may affect the crust colour formation and reach the required values for browning, in agreement with Sakiyan *et al.* (2007) and Keskin *et al.* (2004). In conventional ovens, the air surrounding the product is hot. Therefore, in conventionally heated products, the surface is the hottest part and the surface temperature reaches the required values for browning.

According to ANOVA analysis for the ΔE values (Fig. 22 and 23), it was possible to obtain a similar colour with conventionally baked SRN by the optimal treatment for all fats. P showed the lowest ΔE , but no statistically significant differences emerged compared between alternative and traditional treatment. These differences in ΔE values may be attribute to different fat origin.

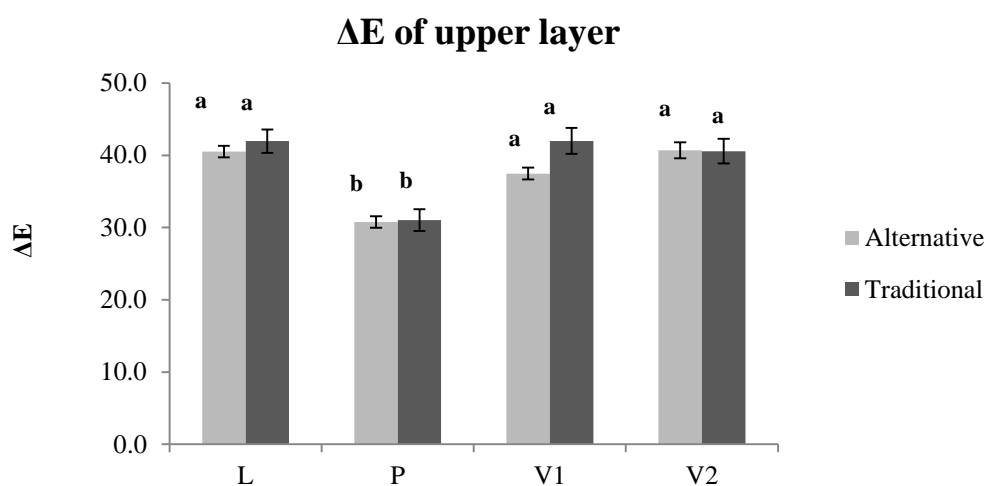


Figure 22_ ΔE values of upper layer in SRNs (L, P, V1 and V2) baked with alternative and traditional treatments.

a-b: Different letters indicate statistically significant differences ($p \leq 0.05$).

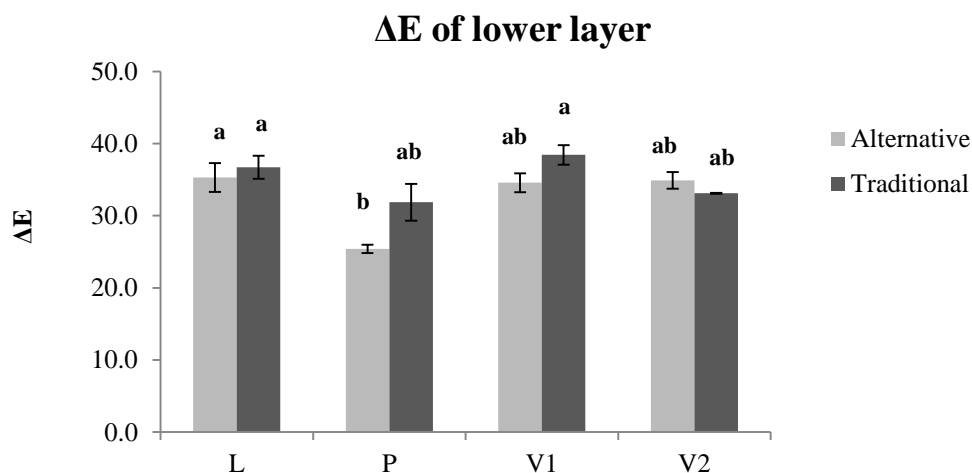


Figure 23_ ΔE values of lower layer in SRNs (L, P, V1 and V2) baked with alternative and traditional treatments.

a-b: Different letters indicate statistically significant differences ($p \leq 0.05$).

4.3.2.3 Moisture content determination

As expected, moisture content in filling (Fig. 24a) was higher than in puff pastry (Fig. 24b). Filling moisture ranged between 31.4% and 54.0%, instead, puff pastry showed a moisture content between 8.9% and 15.2%.

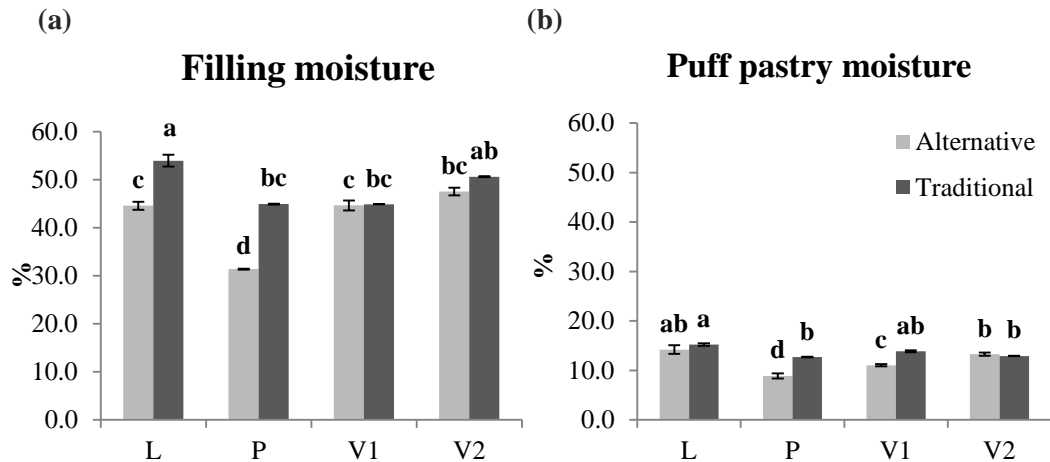


Figure 24_ Moisture content (%) of filling (a) and puff pastry (b) in SRNs (L, P, V1 and V2) baked with alternative and traditional treatments.

a-d: Different letters indicate statistically significant differences ($p \leq 0.05$).

Two-way ANOVA results, showed that there was a significant difference of moisture content in filling between the alternative and traditional, except for V1 (Fig. 24a).

Moisture content of filling in alternative treatment was lower than traditional one. This behaviour agrees to the data published by Sánchez-Pardo *et al.* (2012), who evaluated the pound cake baked by microwaves and conventionally. The authors reported a higher moisture loss when microwave was applied.

Moisture content of L was 17% lower in alternative product than traditional, these results are in agreement with those of Megahey *et al.* (2005), who reported a 18.1% moisture loss in cake baked at 250 W for 140 s. They reported also that the rate of moisture loss for cake baked at 250 W and 440 W was similar.

Sánchez-Pardo *et al.* (2007) reported 35% lower moisture in a pound cake baked in a single cycle microwave oven compared to conventionally bake. Breads baked at higher heating rates, in general, had a lower moisture content compared to breads baked at lower heating rates.

These differences in moisture content could be attributed partly to the modes of heating and the initial dough size (Patel *et al.*, 2005). The high moisture loss in microwave baked products has also been reported by other researchers (Sahin *et al.*, 2002; Seyhun *et al.*, 2003; Sumnu *et al.*, 1999).

Experimental temperature and moisture loss profiles can provide useful insights into the mechanisms of heat and mass transfer during microwave heating and thus, be used to improve the quality of microwave-baked products (Sumnu *et al.*, 1999).

For the microwave baking of cakes, Lambert *et al.* (1992) reported that the drying rate increased slowly during baking until the temperature of the cake batter reached at least 70 °C, after which it increased more rapidly towards a constant rate period, which was reached just before the conclusion of heating. In contrast, during the convective baking of bread, Hasatani *et al.* (1992) reported that the drying rate initially increased until a maximum rate (coinciding with the onset of the temperature plateau) was achieved, then gradually decreased until the end of baking. The time to reach the maximum drying rate decreased with an increase in oven temperature (Hasatani *et al.*, 1992; Baik & Marcotte, 2002).

However, it was possible to reduce the weight loss of microwave baked cakes by combining microwaves with conventional heating, as in alternative method herein proposed.

Alternative V2 showed lower moisture content than conventional one, but the difference wasn't significant.

Within the baking method, V2 was similar than the reference L, instead, P showed the lowest moisture content both in alternative and traditional samples.

In puff pastry, there were no statistically differences between traditional and alternative L and V2. P and V1 showed lower values in alternative than traditional samples (Fig. 24b).

Within alternative samples, V2 was the most similar than L; no showing statistically difference from L.

In traditional samples, all vegetable SRNs exhibited lower moisture content than L. Animal fat seem to coat more efficiently the layer surface and limit the moisture migration during baking. Only V1 exhibited the same behaviour of traditional L, no statistically difference from L emerged.

P and V1 reported the higher moisture loss of puff pastry when baked with alternative treatment. This can be due to higher moisture loss in IR-microwave heating because of high pressure and moisture gradients in microwave baked products as compared to conventional baking, in according as reported by Keskin *et al.* (2004) and Sumnu *et al.* (2001). The increase in the microwave power and the halogen lamp power has also been shown to increase the moisture loss of breads (Keskin *et al.*, 2004a; Demirekler *et al.*, 2004).

4.3.2.4 Water activity determination - a_w

Water activity (a_w) describes the contribution of “free” water to the support of bacteria and fungi. Quantifying a_w is the key to research on food preservation and safety.

Water activity values of filling were high (ranged from 0.92 to 0.96), showing lower values in alternative treatment (Fig. 25a).

Within the treatment, V2 exhibited the highest a_w and more similar than L both in alternative and traditional treatment; P and V1 showed more similar behaviour in both treatments.

Water activity values of puff pastry (Fig. 25b) were lower respect those of filling, ranging between 0.78 and 0.83.

No significant differences were observed in L baked following the two treatments for puff pastry a_w , while alternative a_w of puff pastry of vegetable fats were 1%, 4%, 5% (P, V1 and V2, respectively) lower than traditional ones.

Puff pastry V1 showed the lowest value of a_w in both traditional and alternative samples.

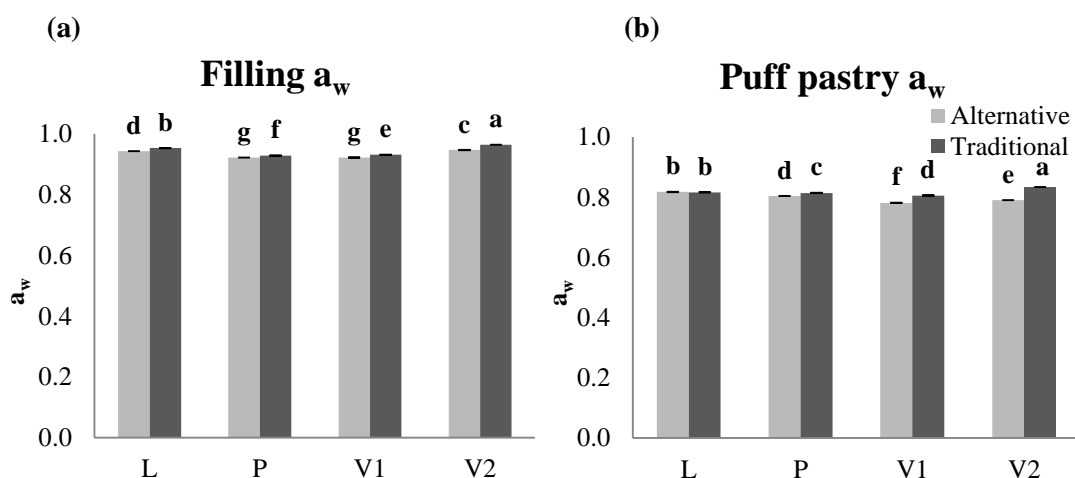


Figure 25_ a_w value of filling (a) and puff pastry (b) in SRNs (L, P, V1 and V2) baked with alternative and traditional treatments.

a-e: Different letters within fat, indicate statistically significant differences ($p \leq 0.05$).

4.3.3 Fat characterization

The effects of baking treatments on the thermoxidative degradation of fat extracted from SRNs were determined. A comparative study was carried out on the deterioration of fat as a result of alternative and traditional baking. Degradations were quantified by determination of FFA, PV, TPC and FA composition.

4.3.3.1 FFA, PV and TPC determination

Chemical characteristics of the initial untreated (Raw) and treated (Alternative and Traditional) SRN made with different fat blends (L, P, V1 and V2) are shown in Table 13. Alternative baking is composed of Pre-cooking and Heating treatments.

For all samples, each value is the mean of four data corresponding to two measurements from two replicates.

Table 13_ Effects of alternative (pre-cooking and heating) and traditional baking on free fatty acidity, peroxide value, and TPC of SRNs with different fat blends (L, P, V1 and V2).

Samples	FFA (% oleic acid)	PV(meq O ₂ /kg)	TPC (%)
Raw L	0.58±0.0	3.53±0.0	8.84±0.2
Pre-cooking L	0.66±1.3	6.06±0.1	9.61±0.1
Heating L	0.66±0.0	6.67±0.2	8.76±0.2
Traditional L	0.72±0.0	15.75±0.3	14.55±0.1
Raw P	0.20±0.0	3.23±0.0	9.17±0.0
Pre-cooking P	0.23±0.0	4.58±0.1	8.83±0.1
Heating P	0.30±0.0	5.35±0.2	9.04±0.1
Traditional P	0.35±0.0	9.13±0.0	15.22±0.1
Raw V1	0.58±0.0	3.03±0.1	9.65±0.1
Pre-cooking V1	0.66±0.0	3.07±0.1	8.44±0.1
Heating V1	0.66±0.0	3.12±0.2	9.20±0.1
Traditional V1	0.83±0.0	7.25±0.2	16.44±0.1
Raw V2	0.11±0.0	2.88±0.0	9.19±0.1
Pre-cooking V2	0.13±0.0	3.28±0.0	8.22±0.1
Heating V2	0.16±0.0	4.03±0.0	8.98±0.1
Traditional V2	0.18±0.0	5.35±0.16	13.39±0.0

Formation of free fatty acids might be an important measure of rancidity of foods. FFAs are formed due to hydrolysis of triglycerides (Frega *et al.*, 1999).

The acidity value ranged from 0.11% to 0.58% in raw SRN.

An appreciable change in acidity was observed in the samples after both traditional and alternative baking ($p \leq 0.05$). But the effect of alternative baking was greater when compared with conventional one (Fig. 26).

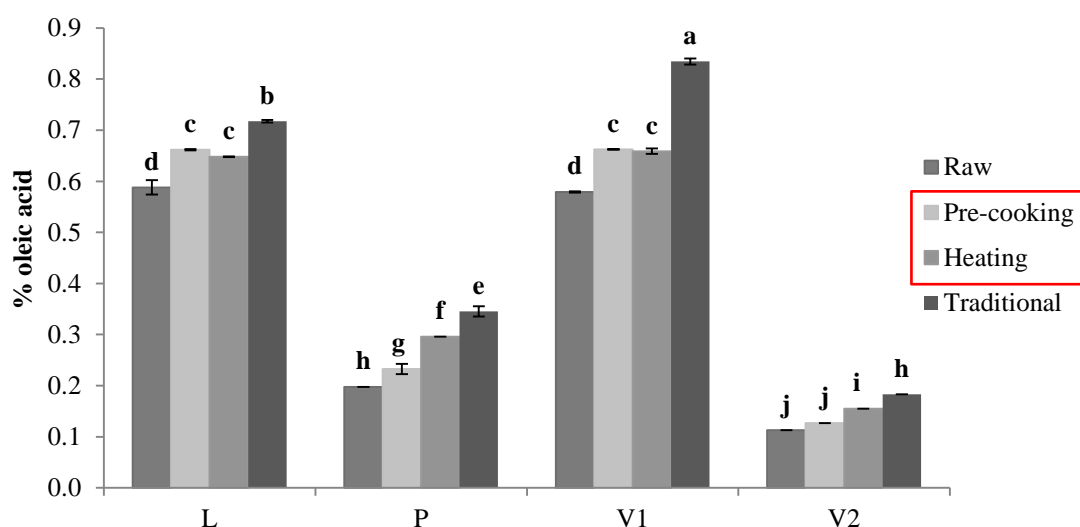


Figure 26_ Free fatty acids values of fat extracted by raw and baked (in alternative and traditional treatments) SRNs with different fat blends (L, P, V1 and V2).
a-j: Different letters correspond to statistically significant differences ($p \leq 0.05$).

For instance, the acidity of raw L was 0.58%; in traditional L was 0.72%, whereas it was lower, 0.66% and 0.65%, in pre-cooking and heating samples, respectively.

V1 showed similar values than the control sample (L), for which the highest values were indicated. In alternative method, heating treatment didn't influence the free fatty acidity content.

V2 exhibited the lowest value of acidity, in comparison with others fats.

A 12% and 24% increasing in alternative and traditional L, respectively, as it can be seen in Fig. 27. Greater increasing was shown for the other fats (50% and 75% in alternative and traditional P, and 46% and 64% in V2), but the FFA content were always lower than L and V1 (Fig. 26).

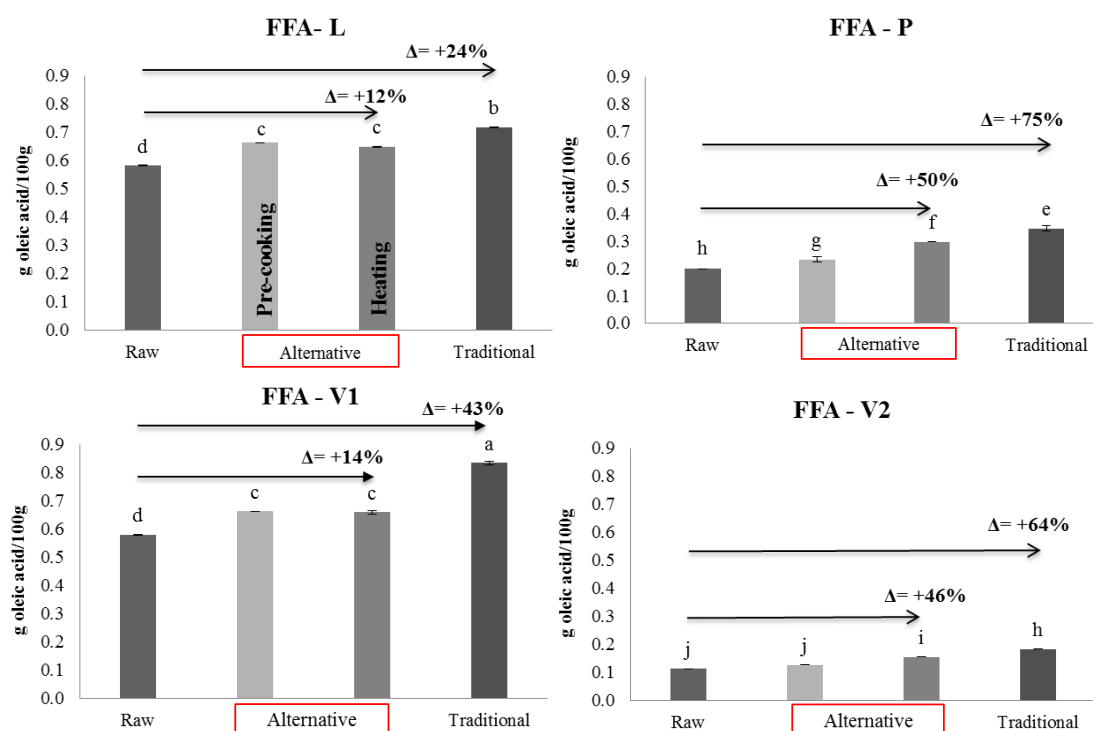


Figure 27_ Effects of alternative and traditional baking on Free Fatty Acidity of SRNs with different fat blends (L, P, V1 and V2)

a-j: Different letters correspond to statistically significant differences ($p \leq 0.05$).

The analysis of peroxide value measures hydroperoxides which are transient products of lipid oxidation. As the lipid oxidation progresses, hydroperoxide formation increases and the peroxide value increases (Kim *et al.*, 2007).

Peroxide values were illustrated in Figure 28. Raw samples exhibited PV between 2.88 and 3.53 meq O₂/kg. PV content went on increasing with the traditional baking for all the samples, but no same behaviour was observed after alternative method.

L exhibited the highest values, while vegetable SRNs the least. PV of 15.75 meq O₂/kg fat in traditional L sample, 6.06 meq O₂/kg and 6.67 meq O₂/kg in pre-cooking and heating ones, respectively ($p \leq 0.05$). There was no increase in PV after pre-cooking in V1 and V2.

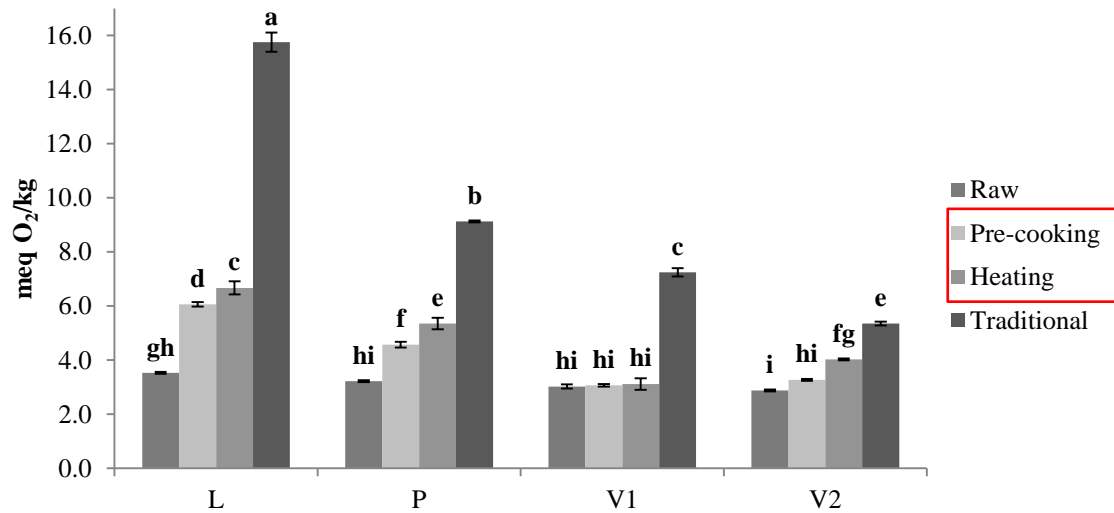


Figure 28_ Peroxide values of fat extracted by raw and baked (in alternative and traditional treatments) SRNs with different fat blends (L, P, V1 and V2).
a-i: Different letters correspond to statistically significant differences ($p \leq 0.05$).

The results showed that PV was gradually increased from 3.53 meq O₂/kg in unbaked L to 15.75 meq O₂/kg after conventional heating, with an increase of 347% (Fig. 29). Alternative L showed a higher thermoxidative stability.

Traditional V2 showed the lowest percentage increase (only $\Delta +86\%$). Peroxide content for alternative V2 sample also increased but this increase was about half ($\Delta +48\%$).

Microwave-electric oven seem not significantly influences on PV in palm oil-free SRNs (V1 and V2).

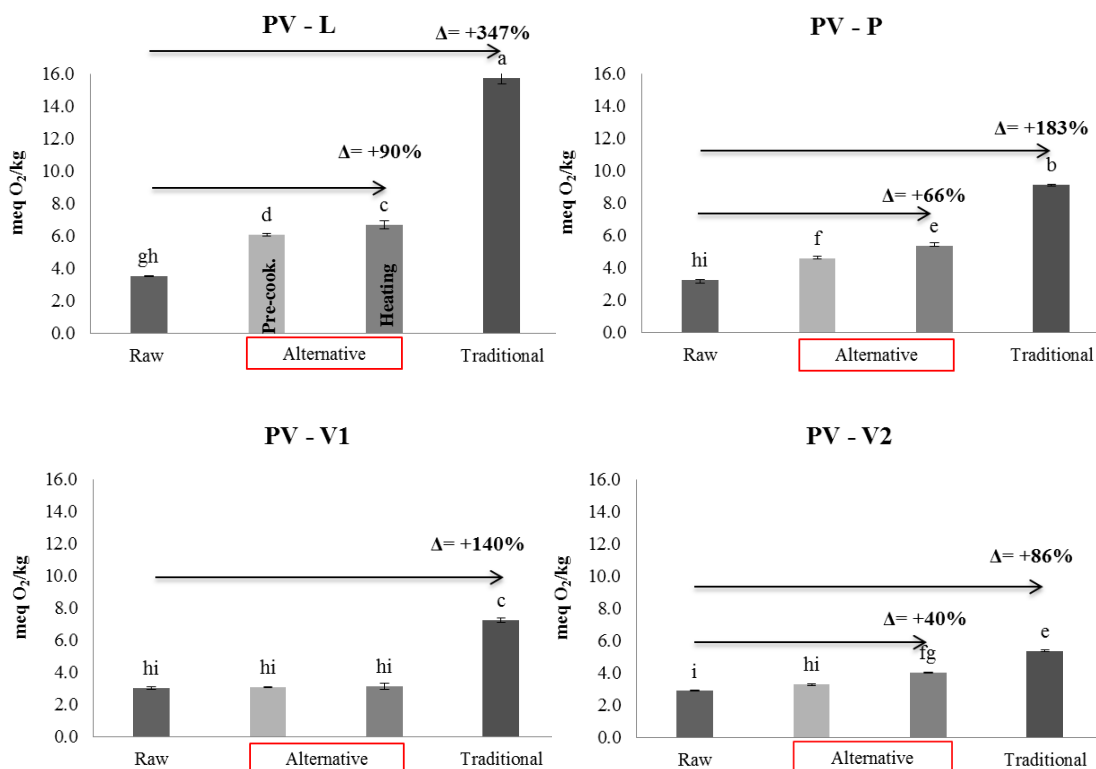


Figure 29_ Effects of alternative and traditional baking on peroxide value of SRNs with different fat blends (L, P, V1 and V2).
a-i: Different letters correspond to statistically significant differences ($p \leq 0.05$).

The level of polar compounds is a good indicator of the quality of used heating fats and oils, giving information of the total amount of newly formed compounds having higher polarity than that of triacylglycerols and being the most common method of evaluation of the alteration of fats, due to its accuracy and reproducibility (Waltking & Wessels, 1981). TPC of heated fats provides the most reliable measure of the extent of oxidative degradation (Fritsch, 1981).

This analysis is indicated as the official analysis of oils by the IUPAC (International Union of Pure and Applied Chemists) (Waltking & Wessels, 1981).

Total polar compounds were in the range 8.22–16.44% (Fig. 30).

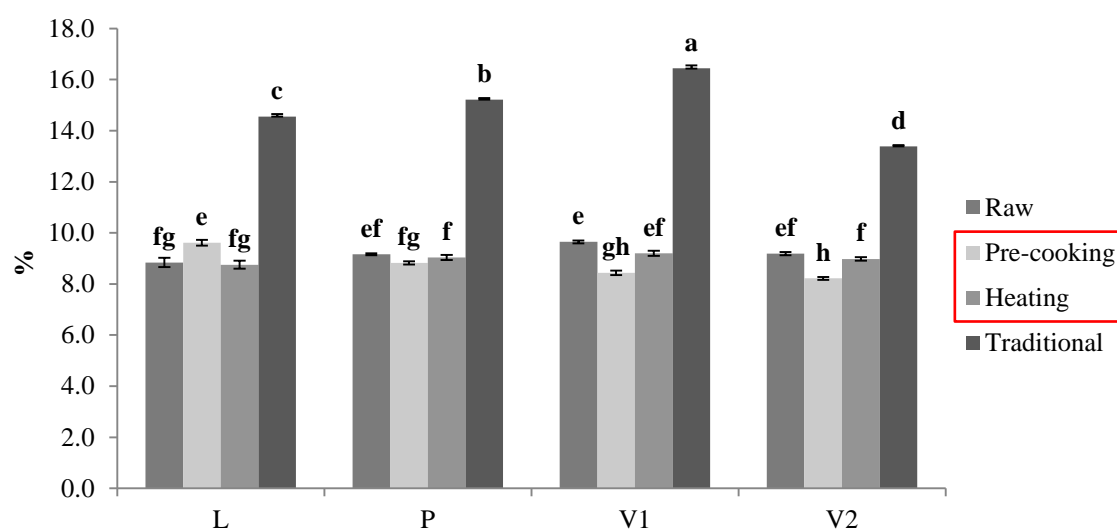


Figure 30_ Total polar compounds of fat extracted by raw and baked (in alternative and traditional treatments) SRNs with different fat blends (L, P, V1 and V2).
a-h: Different letters correspond to statistically significant differences ($p \leq 0.05$).

The results showed that the contents of TPC increased, as well as FFA and PV, with conventional oven baking. The initial TPC content of fats extracted from raw L, P, V1 and V2 was 8.84, 9.17, 9.65 and 9.19 %, respectively, which was significantly increased to 14.55, 15.22, 16.44 and 13.39 % after traditional heating.

Maximum TPC content was for V1 (16.44%), followed by P and L. V2 showed the minimum value (13.39%).

Alternative SRNs, at all fat blends, controlled TPC appreciably; revealing good thermoxidative stability. In fact, from ANOVA results no statistically significant differences ($p > 0.05$) emerged between raw and alternative SRNs for all fats and no percentage increases (Fig. 31).

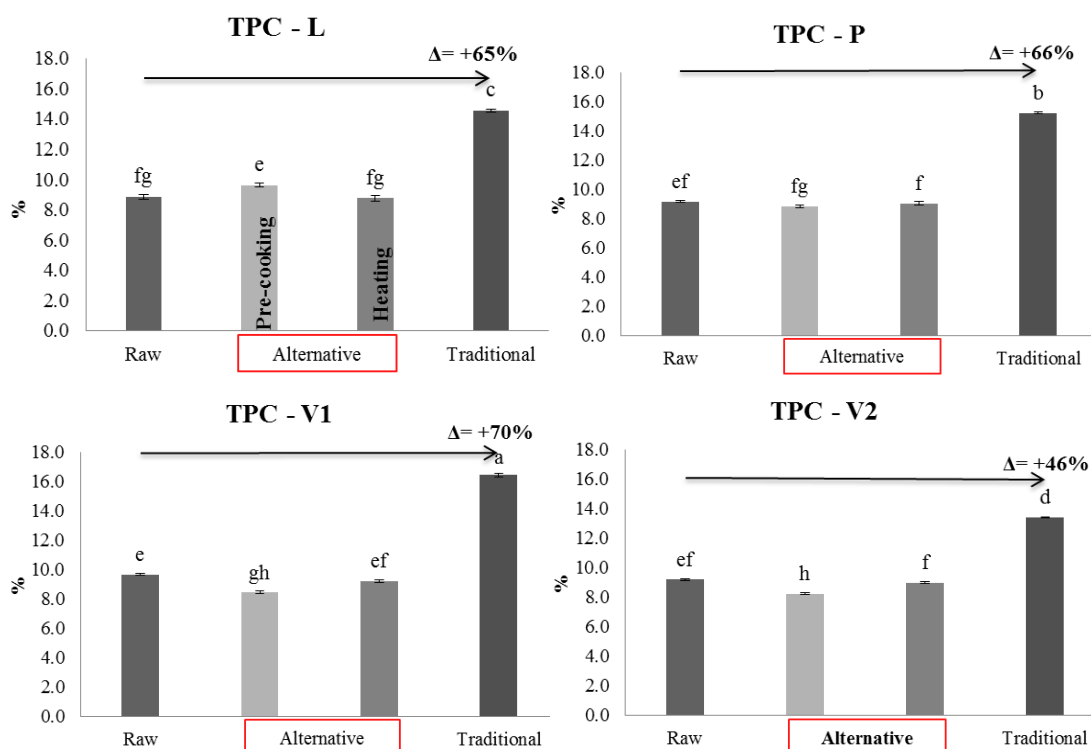


Figure 31_ Effects of alternative and traditional baking on total polar compounds of SRNs with different fat blends (L, P, V1 and V2).

a-h: Different letters correspond to statistically significant differences ($p \leq 0.05$).

Results indicate that hydrolytic and oxidative degradations took place during baking, but less significantly in microwave combination method respect to conventional oven heating. These data are in accordance with those of Manzo *et al.* (2017), Yoshida *et al.* (1993) and Cossignani *et al.* (1998) that showed that microwave heating did not cause some acceleration in the oxidation of the oils. Other studies, instead, highlighted severe hydrolytic and thermoxidative changes during microwave heating (Daglioglu *et al.*, 2000; Yoshida *et al.*, 1992).

Alternative SRNs showed the lowest FFA and PV increasing during the baking, for all fat formulations. TPC did not show statistically significant differences between the raw SRN and alternative samples ($p > 0.05$).

Within the fats, L corresponded to very poor quality of fat, instead V2 showed better thermo-oxidative stability, with the lowest FFA, PV and TPC values and the lowest increasing in PV (Fig. 29) and in TPC (Fig. 31).

4.3.3.2 FA composition

Table 14 presents the fatty acid composition of unbaked SRNs.

Table 14_ FA composition of raw SRNs with different fat blends (L, P, V1 and V2). (means±sd).

Fatty acids (%)	Raw SRN			
	L	P	V1	V2
C4:0	0.23±0.02	0.65±0.04	0.14±0.02	0.74±0.13
C6:0	0.16±0.02	0.33±0.02	0.13±0.01	0.42±0.06
C8:0	0.10±0.02	0.25±0.02	0.73±0.08	0.96±0.16
C10:0	0.28±0.02	0.50±0.02	0.66±0.04	1.52±0.11
C12:0	0.32±0.01	0.33±0.07	4.21±0.29	4.45±0.38
C14:0	2.31±0.07	1.75±0.13	2.68±0.13	3.55±0.23
C14:1	0.11±0.00	0.23±0.00	nd	0.26±0.01
C15:0	0.15±0.01	0.23±0.01	nd	0.22±0.02
C16:0	28.21±1.00	40.88±2.30	17.06±0.55	12.59±0.80
C16:1	2.63±0.08	0.68±0.04	1.27±0.05	0.69±0.04
C17:0	0.35±0.02	0.15±0.00	0.19±0.01	0.16±0.01
C17:1	0.37±0.01	nd	0.16±0.01	nd
C18:0	12.54±0.63	3.46±0.59	13.78±0.53	19.85±1.11
C18:1n9t	0.42±0.02	0.26±0.05	0.23±0.01	0.25±0.03
C18:1n9c	34.85±0.03	32.77±1.43	31.07±0.36	29.64±0.44
C18:2n6c	13.79±0.24	17.37±1.53	25.54±0.13	23.68±0.19
C18:3n6	0.22±0.02	nd	0.39±0.02	nd
C18:3n3	0.80±0.02	0.16±0.06	0.52±0.01	0.44±0.02
C21:0	1.00±0.11	nd	0.49±0.02	0.30±0.04
C22:0	1.16±0.13	nd	0.49±0.04	nd
C22:1n9	nd	nd	0.10±0.01	nd
C20:4n6	nd	nd	0.16±0.01	0.28±0.03
Σ SFA	46.81 ^a ±0.28	48.53 ^a ±2.04	40.56 ^b ±0.52	44.76 ^{ab} ±0.73
Σ MUFA	38.38 ^a ±0.06	33.94 ^b ±1.43	32.83 ^b ±0.33	30.284 ^b ±0.49
Σ PUFA	14.81 ^b ±0.31	17.53 ^b ±1.61	26.61 ^a ±0.19	24.40 ^a ±0.24
UFA/SFA	1.14 ^a ±0.00	1.06 ^a ±0.11	1.47 ^a ±0.03	1.23 ^a ±0.04
TFA	0.42 ^a ±0.03	0.26 ^{ab} ±0.03	0.23 ^b ±0.00	0.25 ^b ±0.02
Σ omega 3	0.80 ^a ±0.14	0.16 ^c ±0.06	0.52 ^{ab} ±0.02	0.44 ^{bc} ±0.01
Σ omega 6	14.01 ^c ±0.33	17.37 ^b ±1.54	26.09 ^a ±0.17	23.96 ^a ±0.22

Each value is as wt% of total fatty acid methyl esters; nd= non detected

a-c: values in each row followed by the same letter are not significantly different ($p>0.05$).

The major fatty acids in the samples were oleic (C18:1n9c), palmitic (C16:0), stearic (C18:0), and linoleic (C18:2) acids, while the remaining fatty acids, except lauric (C12:0), myristic (C14:0), palmitoleic (C16:1) acids, were around 1% or less in the samples.

The ratio of saturated to unsaturated fatty acids (UFA/SFA) did not change significantly for different fats, but the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) contents were different.

Total saturated fatty acid (SFA) contents ranged from 40.56 to 48.53% of the total fatty acids, but the profiles were different. In L, P and V1 the major SFA was palmitic, in V2 stearic acid. It is reported that the palmitic acid content above 17% tends in β' form, desirable for plastic fats, and below 11% in β form (D'Souza & deMan, 1991). Thus, the fat crystals were in stabilized β' form in all SRNs.

Total unsaturated fatty acid (UFA) contents ranged from 51.47 to 59.44% and the major proportion of them was composed of monounsaturated fatty acids (MUFA). On the other hand, V1 and V2 contained less MUFA and a higher percentage of linoleic acid than L and P.

The majority of SRNs contained trans fatty acids (TFA), but always less than 0.5%. The level ranged from 0.23 to 0.42% of the total fatty acids. The highest amount of TFA was determined in L (0.42%). The predominant trans isomer found in all samples was C18:1n9t monounsaturated fatty acid (Tab. 14). Ronald *et al.* (1993) also reported that trans monounsaturated fatty acids with 18 carbon atoms (C18:1n9t) are typically abundant in cereal-based foods. As trans polyunsaturated fatty acids, C18:2n6t was detected much less than 0.1%. Trans fatty acids (TFA) were lower in vegetable fats without palm oil.

Significant differences in total fat and TFA levels among the analysed samples can be attributed to the composition of the fat used in SRN production.

ANOVA showed that SFA (such as stearic and palmitic acid) and the oleic acid contents of L, as well as the ratio of saturated to unsaturated fatty acids did not change significantly ($p>0.05$) during microwave baking or conventional baking.

When comparing the FA composition for two different heating procedures, while the significant change was observed in the FA composition for traditional baking, it was not always observed for alternative baking.

About the reference L, the changes observed in the traditional SRNs were occurred on unsaturated fatty acids, particularly in the percentage of the linoleic acid. Its content decreased from 13.8% (in raw L) to 12.9% (in traditional L). At the same moment, a significant increase in C4:0 and C14:0 was observed after traditional heating (Fig. 32). A lower increase was shown in alternative L.

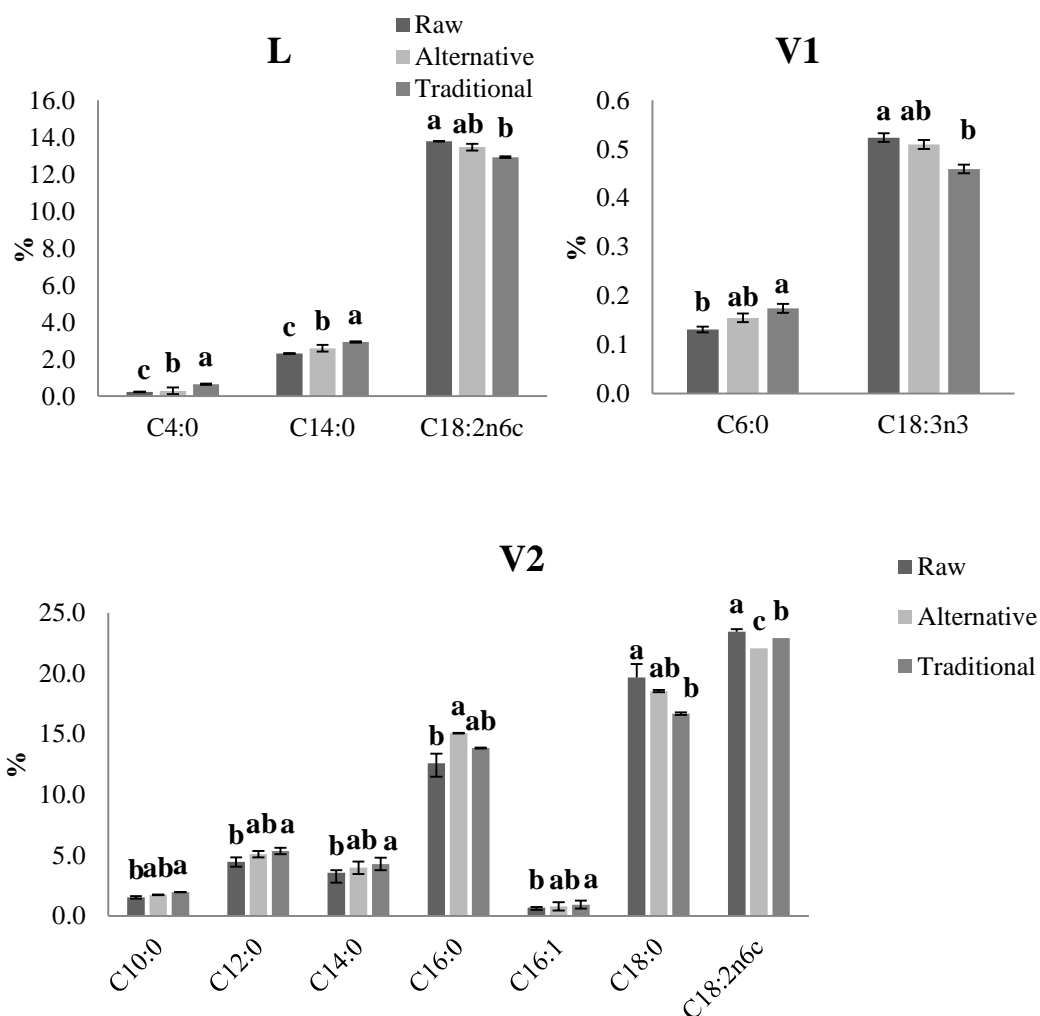


Figure 32_ Change in fatty acid contents of raw and baked (Alternative and Traditional) SRNs with different fat blends (L, P, V1 and V2).

a-c: Different letters correspond to statistically significant differences ($p \leq 0.05$).

No statistically differences emerged among all P samples.

A reduction of linolenic acid (C18:3n3) in traditional V1 and of linoleic acid (C18:2n6c) in traditional V2 were shown, with an increase of some saturated fatty acid (C10:0, C12:0, C14:0). The analysis of variance also indicated no significant decrease for linolenic acid ($p > 0.05$) between raw and alternative baking in V1. These reductions in unsaturated fatty acid result from oxidation that occurs during baking.

Published work (Yoshida *et al.*, 2002) indicates that thermal oxidative deterioration causes an increase in non-volatile compounds such as free fatty acids, as well as indicated in Figure 27. The results indicate a decrease in the oxidative stability in baked SRNs. The effect of traditional method exceeded that of the alternative baking, especially in samples formulated by Lard.

There were no significant differences ($p > 0.05$) between the TFA of traditional and alternative SRNs; however, during traditional baking the trans oleic acid content of L increased somewhat, but the difference was insignificant ($p > 0.05$). Trans linolenic acid was not present at a detectable level in all L samples.

Baking method did not substantially alter the TFA composition, in contrast with results of Topkafa & Ayyildiz (2017), who reveals a much more TFA formation in the MW heating procedure compared to the conventional heating procedure, due to the effect of the MW energy.

4.3.4 Texture measurement

The textural characteristics of baked SRN play an essential role in determining the global acceptability of the food by consumers.

A typical force–time curve of baked SRN is shown in Figure 33. The profile is characterized by an indented curve, typical of crispy layer.

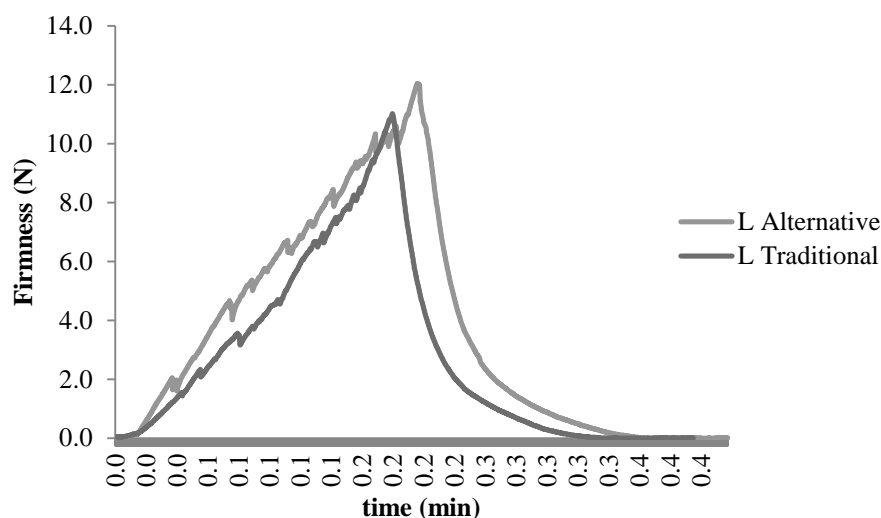


Figure 33_ Force-time curve of L baked with traditional and alternative treatments.

Change in firmness of SRNs containing different fat blends and baked in traditional and alternative method is presented in Figure 34.

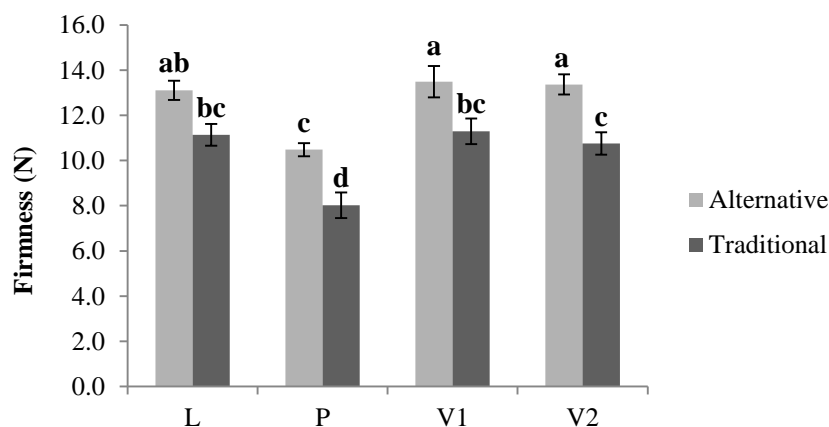


Figure 34_ Firmness of SRNs (L, P, V1 and V2) baked with traditional and alternative treatments.

a-d: Different letters correspond to statistically significant differences ($p \leq 0.05$).

For all the fat formulation and baking conditions, SRNs firmness values were in the range of 8.02 to 13.49 N.

SRN baked in microwave-electric-infrared combination oven had significantly higher firmness values than the ones baked in conventional oven, except for L ($p \leq 0.05$).

Traditional SRNs ranged from 8.02 to 11.30 N, while alternative SRNs ranged from 10.48 to 13.49 N.

Generally, firmer texture of SRNs baked in combination oven may be explained by the higher moisture loss of these products (Fig. 24) which is in agreement with previous studies (Sumnu *et al.*, 2005; Sevimli *et al.*, 2005). Instead, microwave baked pastry were found to be softer than conventionally baked ones by other researchers (Bernussi, 1998). However, it was possible to obtain pastry with desirable textural properties, similar to those obtained by conventional baking, by combining IR heating with microwaves (Keskin *et al.*, 2005). Sevimli *et al.* (2005) also found an increase of cake firmness with increasing IR power, due to major weight loss.

It is not yet clearly understood how the physiochemical mechanisms of microwave baking are fundamentally different from those of conventional baking.

Although, moisture loss rates in cakes baked in microwave ovens have, in general, been found to be greater than those baked in convection ovens (Lambert *et al.*, 1992; Capp, 1993; Pan & Castell-Perez, 1997; Sumnu, 1997). Sumnu *et al.* (1999) observed that the weight loss of cakes increased with an increase in microwave oven power. Instead, Baker *et al.* (1990) reported that conventionally baked cakes lost more water than microwave-baked cakes. Pan & Castell-Perez (1997) found that biscuit dough baked in a microwave oven had similar textural characteristics to samples baked in a conventional oven.

Oil has a plasticizing effect on the viscoelastic properties of dough since it has an ability to reduce the concentration of entanglements tending to a temporary network structure (Fu *et al.*, 1997). This plasticizing effect of fat has a strong ability on rheological properties as well as product quality.

P showed the lowest firmness values in both alternative and traditional methods.

However, replacement of Lard with palm oil-free vegetable fats didn't influenced the texture in both traditional and alternative baking.

Within the treatments, the differences in the firmness of the examined samples are probably a consequence of the differences of fat in the SFC (Fig. 17) and, probably, in the composition of triglycerides, i.e. in the distribution of fatty acids in the molecule of a triglyceride, in accordance with the data found in the references regarding the importance of the fat consistency in the forming of a characteristic multi-layered structure (Stauffer, 1996; Moustafa & Stauffer, 1997, Simovic *et al.*, 2009).

P showed a SFC farther from reference L. Veg1 and Veg2 were obtained by enzymatic interestification. They were found to display a SFC closer compatibility to that of Lard.

4.3.5 Sensory evaluation

The results of the sensorial evaluation of L baked with traditional and alternative treatments are shown in Figure 35. Each class is represented by a vertical bar whose height is equal to the mean of responses.

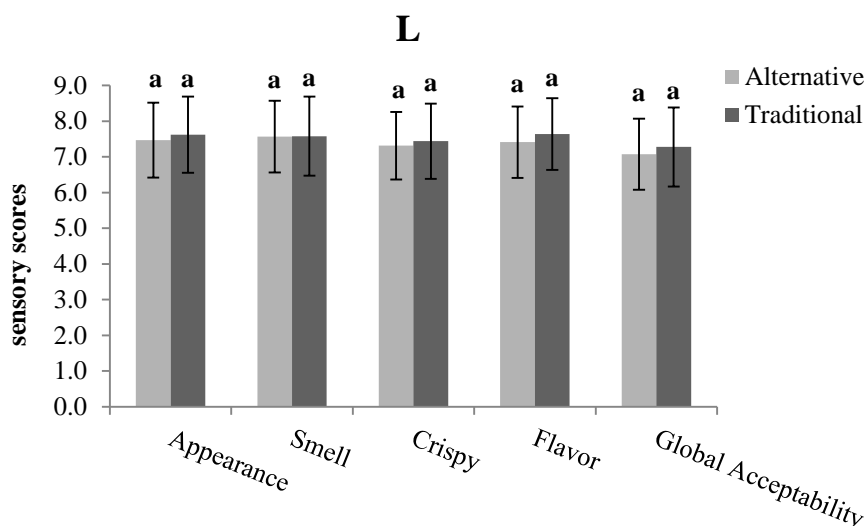


Figure 35_ Sensory analysis on acceptability of SRN made with lard (L) and baked by alternative and traditional methods.
Different letters correspond to statistically significant differences ($p < 0.05$).

All mean hedonic scores of traditional and alternative L for all sensory attributes were generally high. A two-sample comparison analysis showed that there was no significant difference in the acceptance level of both products ($p > 0.05$).

It can be observed that all sensory attributes for alternative L were high, indicating a strong consumer appeal as those of reference. In fact, a mean value of 7.4 was obtained, corresponding to between “Like very much” and “Like moderately”.

SRN with vegetable fat blends showed good sensory acceptance, although P presented lower scores than the control L for the global acceptability (Fig. 36).

In general, alternative SRNs were well accepted by the consumers, with scores about 7 for the sensory attributes studied.

No statistical difference emerged among the fat formulations ($p > 0.05$)

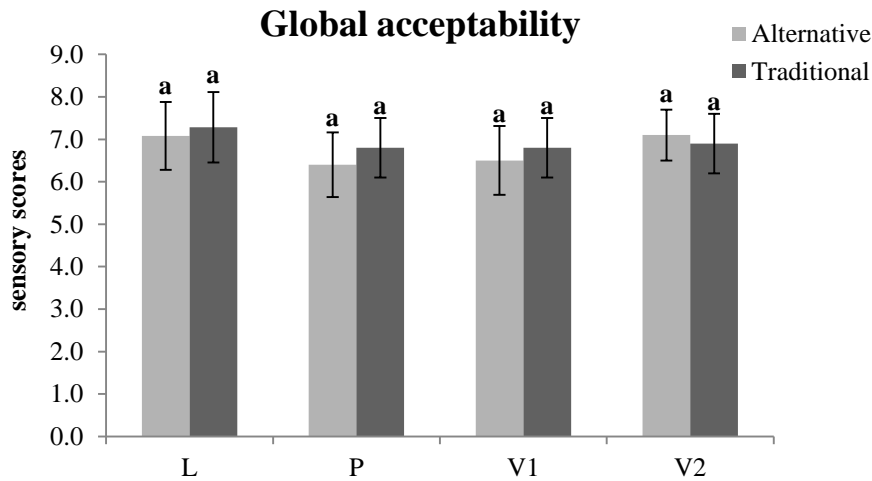


Figure 36_ Global acceptability of SRNs with different fat blends (L, P, V1 and V2) baked with traditional and alternative treatments.
Different letters correspond to statistically significant differences ($p \leq 0.05$).

4.3.6 Energy costs evaluation

Considering oven power, baking time and oven capacity (Tab. 15), traditional baking required an energy density of 107 kJ/kg. Microwave combination baking allowed reduction of the conventional energy costs by approximately 60%, in accordance with Puligundla *et al.* (2013) and Sumnu *et al.* (2001). These data show that the traditional method is at an obvious disadvantage regarding energy consumption.

Moreover, in the RTE product the cooking time dropped to 22.0 min (20 min of pre-cooking and 2 min of heating by consumer), reducing cooking time of SRN of about 51%.

It is reported that microwave application is economically viable for cases in which the cost of the raw materials is negligible compared to the final price of the product (products of great added value), such as reheating of precooked dishes in bars and restaurants, baking of donuts, etc.

In conclusion, traditional treatment is more expensive in terms of time and energy, while alternative treatment offers to the **consumer** advantages in terms of time (time saving x 23) and costs (energy saving x 21).

Table 15_ Comparison on energy consumption of baking treatments.

Treatments	Conditions of treatments	Power (W)	Energy density (kJ/kg)
Traditional	200°C - 45 min	2000	107
Alternative	Microwave 300W - 4 min	300	3
	Pre-cooking + Electric oven 200°C - 16 min	2000	38
	Heating Microwave 100W / Infrared 900W - 2 min	1000	5

4.4 Conclusion

SRNs baked with alternative method (microwave-electric-infrared heating) showed values of colour similar to control.

Proposed treatment showed the lowest FFA and PV increasing during the baking, for all fat formulations. TPC did not show statistically significant differences between the raw SRN and alternative treatment ($p>0.05$).

Indeed, SRNs baked with alternative treatment had similar acceptance from a panel than the conventional SRNs.

Microwave combination baking allowed reduction of the conventional energy costs by 60% and cooking time of SRN by about 51%.

Finally, the reformulation of recipe for the production of SRN with vegetable fat blends allowed a higher thermal-oxidative stability, in particular when V2 was used. V2 was found to display the closer compatibility to Lard about other quality parameters, such as moisture and texture.

The evaluation of microbiological stability of pre-cooked SRN during storage at room temperature and refrigeration condition may further contribute to reduce time and costs of RTE-SRN production.

Second phase_Storage evaluation

Chapter 5 - Materials and Methods

5.1 Sample preparation

Raw materials used for SRN production were the same described in § 3.2. The four SRN formulations used for the experimental procedure were L, P, V1 and V2, prepared following the recipe reported in § 3.2, using Lard, Palm, Veg1 and Veg2, respectively (composition of fat blends in § 3.1).

The product was pre-cooked according the alternative method (microwave at 300 W for 4' and electric oven at 200°C for 16 min) in the same ovens reported in § 3.3.

5.2 Freezing storage

After cooling at room temperature in open air, the pre-cooked SRNs were frozen and packaged as reported in § 3.3.3, and stored in a freezer at -22 ± 2 °C for 12 months.

5.2.1 Sampling and analysis

Each month, frozen pre-cooked SRNs were removed from the freezer and heated according the alternative treatment (microwave at 100 W - IR at 900 W for 2'), as reported in § 4.2.

The effect of frozen storage time on the quality characteristics of RTE-SRNs formulated with different fats, such as microbiological stability, color, moisture, a_w , lipid rancidity, texture and sensory properties, were investigated.

5.3 MAP packaging

Pre-cooked SRNs were cooled at room temperature in open air and packaged by using gas barrier trays (one piece for each tray) (Aerpack B5-43, Coopbox Italia, Reggio Emilia, Italy), wrapped with a barrier film ($PO_2 = 1.3 \text{ cm}^3/\text{m}^2/24 \text{ h/atm}$ at 23°C, 0% RH). A packaging machine (TSM, 105 Minipack Torre; Cava dei Tirreni [SA], Italy) was used for modified-atmosphere packaging (Fig. 37) by creating a vacuum in the tray and then flushing the gas mixture before heat sealing.

The samples were packed using (i) air, as control (AIR), (ii) 70% N₂- 30% CO₂ (MAP1) and (iii) 50% N₂-50% CO₂ (MAP2). Pre-cooked SRNs were stored in a refrigerator at a constant temperature 5 ± 1 °C and 20 ± 1 °C for 49 days.



Figure 37_ Modified atmosphere packaging machine.

5.3.1 Sampling and analysis

Pre-cooked SRNs were sampled for analysis at 0, 3, 7, 10, 14, 21, 28, 35, 42 and 49 days under each atmosphere condition. Samples were heated in microwave-infrared oven, as reported in § 3.3.2 until reaching a core temperature of 60 °C (60 sec. for refrigerated samples, 40 sec. for room temperature samples).

Changes in in-package gas composition, microbial growth, chemical–physical parameters (including lipid rancidity and texture) and sensory attributes were monitored.

5.4 Headspace gas composition

A portable PBI Dansensor A/S (Check Mate 9900 O₂/CO₂; Ringsted, Denmark) analyzer (accuracy ±0.1%) was used to monitor O₂ and CO₂ concentrations (volume/volume %) in the package headspace, by sampling 3 mL of gas from the package headspace with a syringe needle (0.8 mm by 40 mm; Thermo Europe N.V., Leuven, Belgium).

5.5 Microbiological analysis

Mesophilic and psychrotrophic aerobic bacteria, *Enterobacteriaceae*, the presence of Staphylococci, moulds and yeasts were determined, as reported in § 3.5.1.

5.6 Chemical-physical and textural analysis

Moisture content (%) and texture were performed as reported in § 3.4.1, a_w and fat extraction were determined as reported in § 3.5.2.2 and § 3.5.3.1, respectively.

Then, FFA, PV and TPC were measured as reported in § 3.1.1.1, § 3.1.1.2 and § 3.5.3.2, respectively.

5.7 Color measurement

Upper and lower color parameters (L^* , a^* and b^*) of SRNs was measured as reported in § 3.4.1. Color variation was estimated according to the following equation (Giannou *et al.*, 2005):

$$E = \sqrt{L^{*2} + a^{*2} + b^{*2}}$$

5.8 Sensory analysis

Sensory analysis involved asking forty-two untrained consumers, but with some experience on sensory evaluation of SRN, to evaluate the acceptance of the samples by using an hedonic scale from 1 to 9 (1, Dislike Extremely; 9, Like Extremely) for appearance (related to color), smell, flavor, crispy (related to texture). An overall acceptability score was also computed as the mean of the four attributes, as also reported in § 3.5.4

5.9 Statistical analysis

Two replicates and two repetitions of each analysis were carried out. XLSTAT 2007 software (Addinsoft, Paris, France) was used to perform statistical analyses. For freezing storage a two-way analysis of variance (ANOVA) was performed to determine whether storage time and fat formulation affected quality parameters of SRN ($p \leq 0.05$). In the second case, a factorial design with four factors (atmosphere, temperature, time and fat formulation) was used. If significant difference was found, post hoc Tukey test at 5% level of significance (α) were conducted on the data.

Chapter 6 – Results and Discussion

6.1 Freezing storage

6.1.1 Microbiological analysis

SRNs made with different fats (L, P, V1 and V2) exhibited the same trend (data not shown). Figure 38 reports the means of counts at different storage time of SRNs made with different fat blends (L, P, V1 and V2).

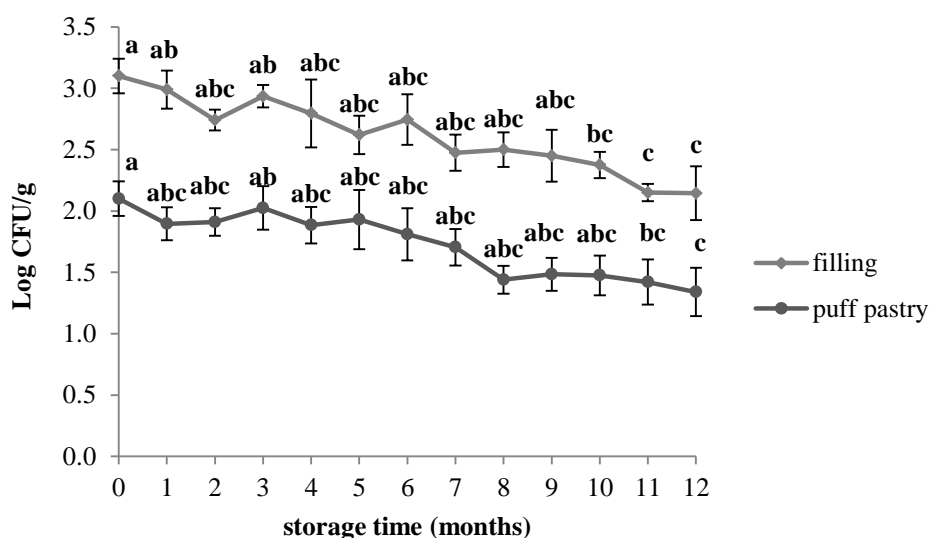


Figure 38_ Evolution of mesophilic aerobic count of SRN under prolonged frozen storage. The counts at different time are means of SRNs with different fat blends (L, P, V1 and V2).

a-c: Different letters in each parameter indicate significant differences ($p \leq 0.05$).

As expected, microorganisms did not grow at frozen temperatures. Rather, mesophilic aerobic count gradually decreased with increased storage period for all SRNs ($p \leq 0.05$).

At time 0 mesophilic bacteria count of the filling samples was about 1 Log CFU/g higher than that of puff pastry (3.10 Log CFU/g vs. 2.10 Log CFU/g) (Fig. 38). As it can be seen, the freezing process alone resulted in about 31% mesophilic count loss in filling and 36% in puff pastry after 12 months frozen storage.

Enterobacteriaceae and yeasts and moulds were under the detection limit (<1 and <2 Log CFU/g, respectively) during the whole frozen storage time. This level was maintained in all samples (L, P, V1 and V2) during 12-month storage (data not shown).

The most important factor influencing microbiological spoilage of bakery products is water activity (a_w).

As reported in literature, freezing converts the free water in the food product into a non-active compound, and together with the low temperature, prevents the growth of

microorganisms and the development of the chemical and enzymatic reactions responsible for food deterioration (Barcenas & Rosell, 2006b). Moreover, SRNs have a much higher fat and sugar content, which retards staling and helps to reduce microbiological deterioration.

Therefore, due to the impossibility to grow of microorganism at frozen temperatures, the hygienic conditions in the bakeries should be carefully controlled, because the final baking is not always sufficient to inactivate microorganisms (Leuschner *et al.*, 1998).

6.1.2 Chemical-physical analysis

6.1.2.1 Moisture, a_w and color measurements

Pre-cooked SRN has high moisture and a_w content in filling, responsible of the short shelf life. Storage at low or sub-zero temperatures is the most common alternative for preventing mould contamination at industrial level, as observed from microbiological counts (Fig. 38). Moisture and a_w content and crust–crumb color stability are key quality factors of bakery products.

The effect of prolonged frozen storage on moisture content of filling and puff pastry SRNs is shown in Tables 16 and 17, respectively.

Table 16_ Filling moisture content of different SRNs during prolonged frozen storage.

Storage time (months)	Filling moisture content (%) of			
	L	P	V1	V2
0	44.53±0.2 ab	31.32±0.3 ab	44.54±0.3 a	47.45±0.3 a
1	44.58±0.1 a	31.59±0.1 a	44.23±0.1 a	47.10±0.1 ab
2	44.38±0.3 abc	30.05±0.3 c	44.20±0.3 a	45.32±0.4 cd
3	43.38±0.1 cde	31.06±0.2 ab	42.10±0.1 b	46.42±0.6 abcd
4	43.00±0.4 def	30.03±0.2 cd	42.21±0.6 b	46.55±0.4 abc
5	43.20±0.1 def	29.12±0.3 d	42.50±0.3 b	46.21±0.1 bcd
6	43.39±0.1 cde	29.73±0.4 cd	42.21±0.1 b	46.24±0.3 abcd
7	43.47±0.1 cd	30.07±0.1 c	42.37±0.1 b	46.20±0.1 bcd
8	43.50±0.4 bcd	30.40±0.3 bc	42.37±0.1 b	46.05±0.5 bcd
9	43.22±0.3 def	29.58±0.2 cd	42.19±0.1 b	46.14±0.1 bcd
10	43.24±0.3 def	29.48±0.1 cd	42.53±0.3 b	46.49±0.1 abcd
11	42.20±0.3 f	30.04±0.1 cd	42.35±0.1 b	45.27±0.1 d
12	42.37±0.3 ef	29.94±0.1 cd	42.23±0.2 b	45.30±0.3 d

a-f: Different letters in each column indicate significant differences ($p \leq 0.05$).

Table 17_ Puff pastry moisture content of different SRNs during prolonged frozen storage.

Storage time (months)	Puff pastry moisture content (%) of			
	L	P	V1	V2
0	14.18±0.1 bcdef	8.38±0.3 ab	11.60±0.3 b	13.24±0.2 bc
1	13.35±0.3 ef	8.59±0.4 a	12.23±0.1 ab	12.90±0.1 c
2	13.27±0.3 f	8.05±0.1 ab	12.52±0.2 ab	12.86±0.2 c
3	13.78±0.3 def	8.06±0.1 ab	12.11±0.1 ab	13.18±0.0 bc
4	13.81±0.2 def	8.03±0.4 b	12.04±0.1 ab	13.50±0.3 abc
5	15.04±0.1 abc	8.10±0.1 ab	12.41±0.1 ab	13.36±0.2 abc
6	14.08±0.2 cdef	8.73±0.1 ab	12.22±0.4 ab	13.34±0.3 abc
7	14.47±0.1 abcde	8.07±0.3 ab	12.20±0.1 ab	13.48±0.1 abc
8	15.50±0.3 a	8.40±0.1 ab	12.36±0.4 ab	13.41±0.1 abc
9	15.22±0.3 ab	8.58±0.3 ab	12.17±0.1 ab	13.22±0.3 bc
10	14.24±0.5 bcdef	8.48±0.1 ab	12.51±0.3 ab	14.03±0.1 a
11	14.20±0.3 bcdef	9.04±0.1 a	12.35±0.3 ab	13.91±0.1 ab
12	14.80±0.4 abcd	8.94±0.3 ab	12.53±0.3 a	13.93±0.2 ab

a-f: Different letters in each column indicate significant differences ($p \leq 0.05$).

At the beginning of storage the moisture content of filling, varied between 31.32% (P) and 47.45% (V2). It was observed a significant decrease, for all SRNs ($p \leq 0.05$), during the first months of storage under freezing conditions. Moisture content afterwards remained approximately constant. However the above findings indicate that freezing can provide relatively stable products.

As shown in Table 17, an increase in frozen storage period of pre-cooked SRNs steadily increased puff pastry moisture content. Moisture changes in samples were the result of water migration from filling and the potential difference in water vapour pressure between the storage atmosphere and the product.

P showed the lowest value of both filling and puff pastry moisture content. Palm oil-free vegetable SRNs (V1 and V2) exhibited similar values of moisture than L.

No significant differences ($p > 0.05$) were detected among the a_w of SRNs made with different fat blends during prolonged frozen storage. Figure 39 shows the means of a_w of filling and puff pastry of the different SRNs (L, P, V1 and V2).

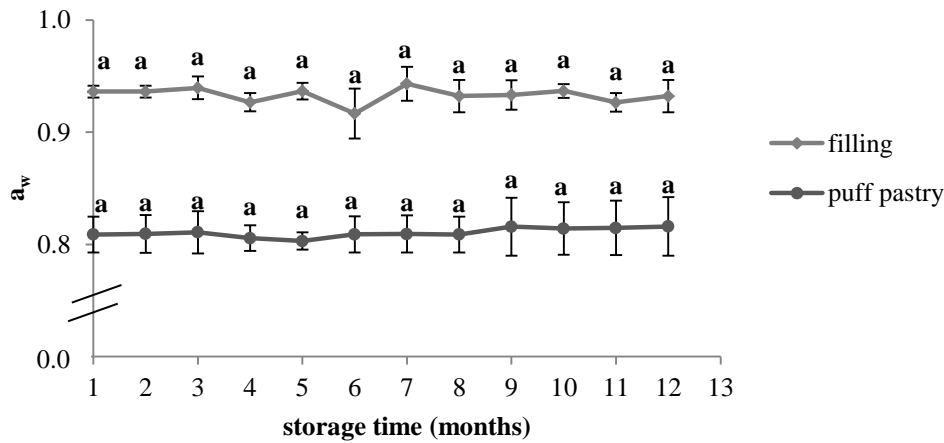


Figure 39_ Evolution of a_w content of SRN under prolonged frozen storage. The values at different time are means of SRNs with different fat blends (L, P, V1 and V2). Different letters in each parameter indicate significant differences ($p \leq 0.05$).

Among the quality characteristics of bakery products, color stability can be considered as key parameter for the performance of frozen bakery products. Upper and lower color of samples should be golden brown, and are both expected to be uniform and appealing.

Color variation, as the mean of upper and lower values of different samples, varied between 41.1 (V1) and 55.1 (V2) at time 0 (Fig. 40).

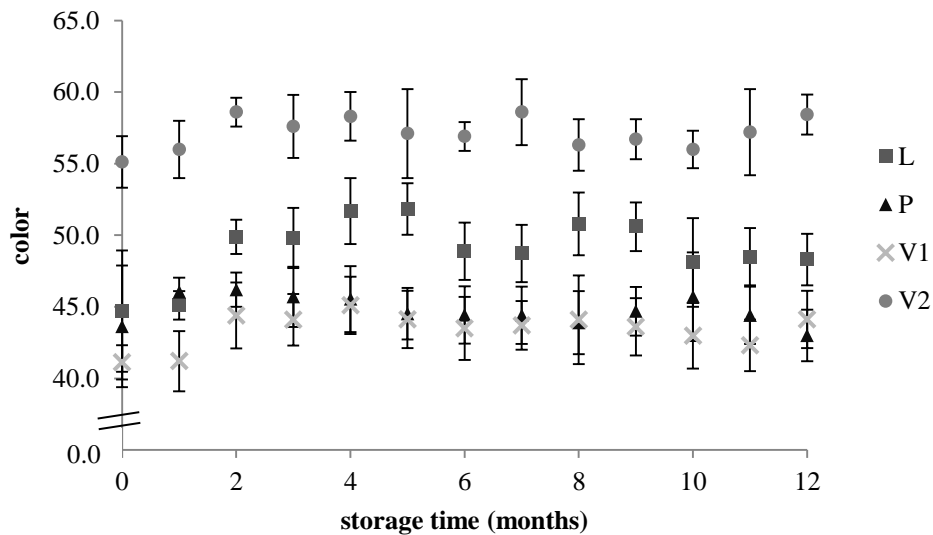


Figure 40_ Color variation of SRNs with different fat blends (L, P, V1 and V2) during prolonged frozen storage.

It appears that storage under freezing conditions causes an increase in color. Results are in accordance with data in literature, in fact several researchers have shown that one of the defects caused by frozen storage of bakery products, and especially of bread, is the appearance of discoloration that occurs after a relatively long time in the freezer, caused by the transference of moisture by sublimation and

diffusion from the highly moist center of the crumb to the low moisture content region of the crust (Pylar, 1988). After thawing this phenomena becomes weaker but does not disappear (Sluimer, 2005).

However, at the end of experiment, samples color still remained in fairly acceptable levels.

6.1.2.2 Rancidity evaluation

In general, all the SRNs analysed showed a slow rate of free fatty acids formation during frozen prolonged storage (data not shown).

FFAs of fat extracted from the SRNs after baking (at zero time) was 0.70, 0.30, 0.66 and 0.16% for L, P, V1 and V2, respectively. This level did not differ considerably from those detected at regular storage time.

The PV and TPC values are widely used as indicators of the degree of lipid oxidation and of the quality depletion of foods during their life on the shelf.

Figure 41 shows the changes in peroxide value (PV) of fat extracted by SRNs stored at -22 ± 2 °C for 12 months.

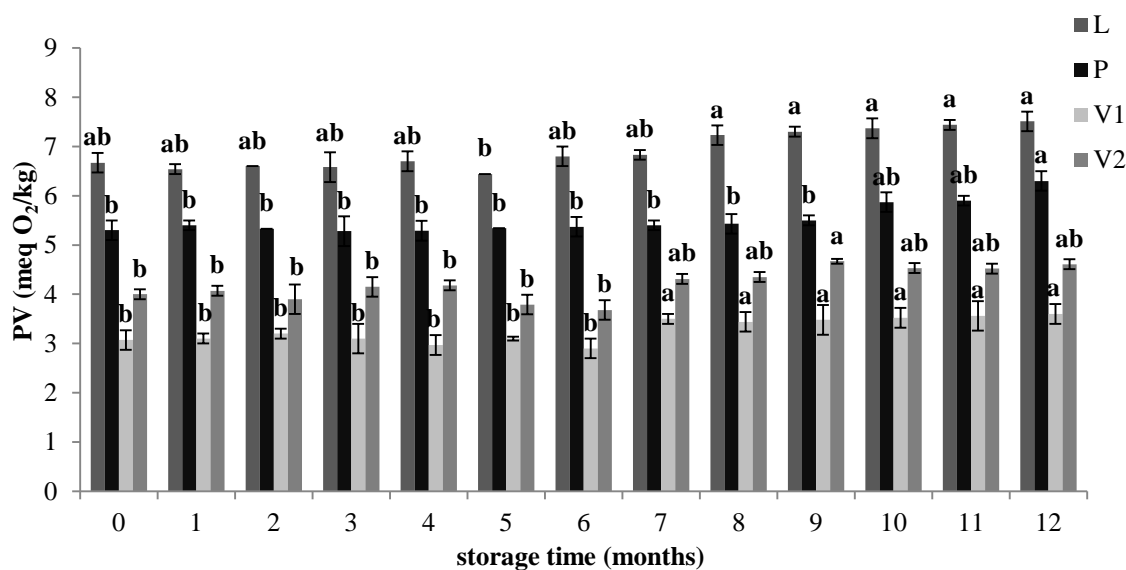


Figure 41_ PV of SRNs with different fat blends (L, P, V1 and V2) during prolonged frozen storage.

a-b: Different letters in each column indicate significant differences ($p \leq 0.05$).

As expected, prolonged frozen time influenced PV content for all SRNs. PV increased during storage, but the development of the PV was very slow in samples during the twelve month frozen storage and happened after 6-month freezing storage.

Oxidative stability of SRNs during 6 months of frozen storage was similar to freshly baked samples for all SRNs. However, L showed the highest percentage increase ($\Delta + 40\%$), V2 the lowest ($\Delta + 15\%$), probably as Lard have no natural antioxidant, instead shea butter contained in V2 at percentage of 40% (higher than 25% in V1) is reported

that have phenolic compounds (catechins) and tocopherols, with antioxidant activity although after processing of oil (Honfo *et al.*, 2014; Bail *et al.*, 2009).

Novotni *et al.* (2011) also found that the oxidative stability of pre- and fully baked bread decreased during frozen storage. Frozen pre-cooked SRNs were packaged in HD-PE film under air atmospheric. It is well known that oxygen is the promoter of oxidative rancidity (Smith *et al.*, 2004).

However, PV of RTE-SRN during frozen prolonged storage are within acceptable range, lower than PV of traditional SRN, as reported in comparison between alternative and traditional SRNs (Fig. 28).

In the conditions tested, the SRNs hydrolytic and oxidative stability remained unchanged during 12-month frozen storage ($p>0.05$) (Fig. 41). No significant differences in the FFA and TPC parameters were found among SRNs prepared with different fat blends during prolonged frozen storage ($p>0.05$) (data not shown).

6.1.2.3 Texture

Firmness is directly linked to shelf life, and flavour eating qualities (Novotni *et al.*, 2011). It is the most often measured of final product texture, because of the strong correlation between firmness and consumer perception of bread freshness (Besbes *et al.*, 2014).

Table 18 shows the changes in firmness of SRNs made with different types of fat blends over an extended frozen storage time.

Table 18_ Textural properties of SRNs with different fat blends (L, P, V1 and V2) during prolonged frozen storage. The results are expressed as mean values.

Storage time (months)	Firmness (N)			
	L	P	V1	V2
0	10.71 def, y	8.14 e, z	11.96 bc, w	11.32 de, x
1	10.75 def, w	8.71 de, x	10.85 c, w	11.61 de, w
2	10.27 f, x	8.34 de, y	11.06 bc, wx	12.05 cde, w
3	10.60 ef, wx	8.51 de, x	11.75 bc, w	11.49 de, w
4	10.95 def, wx	8.72 de, x	11.03 c, wx	12.07 cde, w
5	11.26 def, w	8.50 de, x	11.57 bc, w	10.93 e, w
6	11.34 def, w	8.82 de, x	12.61 abc, w	11.88 cde, w
7	11.51 de, w	8.75 de, x	10.93 c, wx	12.20 cde, w
8	11.72 d, x	9.90 cd, y	10.93 c, xy	13.12 bcd, w
9	11.40 de, wx	11.05 bc, x	12.30 abc, wx	12.96 bcd, w
10	13.05 c, wx	10.99 c, x	13.10 abc, w	13.60 bc, w
11	14.38 b, w	12.60 b, w	13.90 ab, w	14.28 ab, w
12	16.48 a, w	14.19 a, x	14.98 a, x	15.85 a, w

Data followed by different letters (a-f) within the same column or followed by different letters (w-z) within the same line differ significantly ($p\leq 0.05$).

In this experiment, frozen storage time had significant effect on puff pastry firmness for all SRNs ($p \leq 0.05$). The same tendency of increase in firmness during prolonged frozen storage was observed for all SRNs. The firming curve with time, as the mean value of SRNs made with different fat blends (L, P, V1 and V2), is reported in Figure 42.

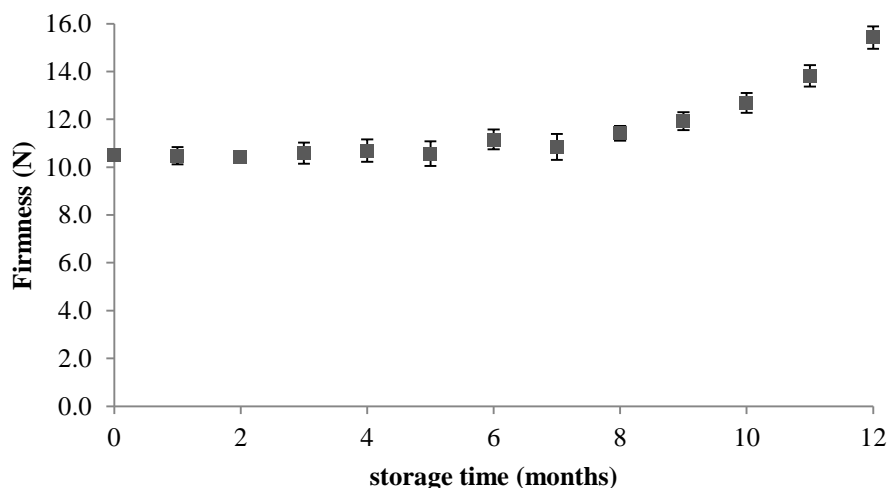


Figure 42_ Evolution of firmness during prolonged frozen storage. Values at different time are the mean values of SRNs with different fat blends (L, P, V1 and V2).

In the first months, textural changes were only slight. The more pronounced changes in texture were observed after seven months of storage.

Fik & Surowka (2002) found no statistically significant differences in crumb hardness during 11 weeks of frozen storage. In contrast, Barcenas *et al.* (2003) found that crumb hardness of the full baked bread increases with the time of frozen storage, and that crumb hardening rate during storage is determined by storage time at -25°C .

This change in firmness may be due two important physical changes occurring during storage. The first is the migration of water from the moist central filling to the dry crust, which causes some firming. The second change is due to the intrinsic firming caused by starch retrogradation.

P showed the lowest firmness value during the whole storage time; V1 and V2 had more similar firmness than L and the lower increasing in firmness; in fact L showed an 54% increase of firmness at 12-month storage, while 25% and 40% in V1 and V2.

6.1.3 Sensory analysis

For frozen samples (Tab. 19), it was found that increasing the storage time caused slight reduction in the general acceptability of SRNs samples.

Table 19_ Effect of prolonged frozen storage on global acceptability of SRNs with different fat blends (L, P, V1 and V2).

Storage time (months)	Global acceptability			
	L	P	V1	V2
0	7.71 a, w	7.14 ab, wx	6.95 ab, x	7.31 abcd, wx
1	7.75 a, w	7.21 ab, w	7.35 a, w	7.60 abc, w
2	7.27 abc, x	7.33 ab, wx	7.55 a, wx	8.04 a, w
3	7.59 ab, w	7.51 ab, w	7.24 a, w	7.98 ab, w
4	6.94 abcd, w	6.71 bc, w	7.52 a, w	7.07 abcd, w
5	6.76 abcd, w	7.00 abc, w	7.57 a, w	6.92 abcd, w
6	6.83 abcd, w	7.81 a, w	7.61 a, w	6.37 abcd, w
7	7.01 abc, w	7.24 ab, w	6.92 ab, w	7.20 abcd, w
8	6.21 abcde, w	6.90 abc, w	6.92 ab, w	6.62 abcd, w
9	5.90 cde, w	6.55 bc, w	6.30 ab, w	5.95 bcd, w
10	6.05 bcde, w	6.98 abc, w	6.09 ab, w	6.10 abcd, w
11	5.38 de, w	6.10 cd, w	5.40 ab, w	5.88 cd, w
12	4.97 e, w	5.19 d, w	4.98 b, w	5.54 d, w

Data followed by different letters (a-d) within the same column or followed by different letters (w-x) within the same line differ significantly ($p \leq 0.05$).

All mean hedonic scores of SRNs were generally high. Comparison of the values obtained for the samples at different time showed that SRNs was more acceptable for the first eight months. After that, its acceptability decreased significantly but it received scores within the acceptability. They represented an approval percentage (like moderately, like very much and like extremely scores) between 6.1 and 8.8 in all parameters.

Specifically for the appearance attribute, L had 8 of response score, 7.2 for smell, 8 for flavour and 7.1 for crispy and a total acceptability of 7.6 at time 0 (data not shown).

The process of heating, carried out after frozen storage, enabled the formation of the optimal amount of aromatic substances. For the average consumer, frozen SRNs are, after heating, virtually indistinguishable from fresh SRNs in ten months storage.

The short storage produced in itself a relatively small change in the total acceptability, reducing the score by only 1 point; moreover, from the third month of storage onwards the score remained constant until the 10th month of storage and then it decreased to 5.

This slight worsening of the sensory quality from like extremely to like was mainly due to a partial loss of the characteristic crispy and flavour of freshly baked SRN.

The lowest score in sensory quality evaluation was obtained after 12 months of storage, especially for crispy loss in L and V2.

The reason for this low quality lies in the moisture migration. As results, SRNs, during the short time of heating, cannot reach the optimal moisture content and the SRNs did not develop a puff pastry of proper crispness. These results show that crispness of this kind of confectionery product is an important attribute for consumer.

The findings are in agreement with those of Vulicevic *et al.* (2004) that also reported a significant reduction in the sensory quality of the frozen part-baked breads. Instead, Fik & Surowka (2002) found no significant correlation between the overall sensory parameters and the frozen storage time.

6.1.4 Conclusion

Nowadays, in industrial conditions, frozen storage is the most used process to preserve pre-cooked bakery products.

This technique allows the storage for long periods, because retards the bread staling process (Mandala & Sotirakoglou, 2005) and moisture equilibration between the crust and the crumb of bread. In addition it preserves the aroma of fresh bread (Sluimer, 2005).

From the aforementioned results, it can be concluded that is possible to obtain a RTE-SRN with an extensive shelf life, preserved for several months.

Frozen RTE-SRN presented a satisfactory shelf-life with rather oxidative and moisture stable behaviour during prolonged storage.

Also substituting lard with pal-oil free fat blend, it is possible to obtain a product with sensory and textural qualities close to those of fresh sample traditionally made with lard.

Acceptability test showed that SRN made with Veg2 were generally high and similar than L during the whole storage time.

The above findings can be applicable by baking industry to a large variety of confectionery bakery products.

However, it is known that freezing process demands more electrical energy than the conventional process, therefore, the effect of modified atmosphere during storage under room and refrigerated temperatures were investigated in the following section.

6.2 MAP storage

6.2.1 Headspace gas composition

The headspace O₂ concentration was less than 0.5% during the whole storage period for the pre-cooked SRNs packaged in MAP with different gas combinations.

The headspace gas composition of L is shown in Tab. 20.

O₂ concentration in MAP remained almost at the same level during storage at both 5 °C and 20 °C. Gas analysis confirm that MAP technology allows to have low O₂ concentrations, compared to the numerous studies on bakery products reported in literature (Guynot *et al.*, 2003a,b).

The level of oxygen in AIR was decreased from 21.0% to 18.7% on day 14 at 5 °C and from 21.1% to 17.6% on day 7 at 20 °C. It might be resulted from mould metabolism and were parallel with the yeasts and moulds counts as reported by Rodriguez *et al.* (2003). Similar results were obtained at earlier researches for bread (Degirmencioglu *et al.*, 2011).

The concentration of O₂ in the headspace also decreased compared with the initial gas concentration in SRNs formulated with vegetable fats (P, V1 and V2) packed in air ($p \leq 0.05$), with same percentage decrease (data not shown).

In MAP2, the headspace O₂ concentration of SRNs remained almost at the same level ($0.2 \pm 0.2\%$ for L) until the end of storage period (49 days) at 5 °C.

There was a very slight decrease, not exceeding 3.4%, in the percentages of carbon dioxide during the cold storage, probably due to a light absorption from the product itself; whereas was very low ($\leq 0.5\%$) in air packaged trays. In fact, the headspace gas composition can change during storage owing to the microbial metabolism, gas solubility and film permeability (Jakobsen & Bertelsen, 2002; Simpson *et al.*, 2009; Limbo *et al.*, 2010). These findings are consistent with the result of present research.

Changes in gas concentrations in MAP were not significant over time of analysis. Gas readings have not been carried out when mould growth on samples was visible.

Also in SRNs formulated with vegetable fats (P, V1 and V2) analysed, both O₂ and CO₂ concentrations remained constant during the entire period of storage under MAP at different gas combinations.

Table 20_ Headspace gas composition of the packed L during storage at 5 and 20 °C.

Storage time (days)	Headspace composition under conditions of:											
	AIR				MAP1				MAP2			
	% O ₂		% CO ₂		% O ₂		% CO ₂		% O ₂		% CO ₂	
	5 °C	20 °C	5 °C	20 °C	5 °C	20 °C	5 °C	20 °C	5 °C	20 °C	5 °C	20 °C
0	21.0±0.1 ^a	21.1±0.1 ^a	0.5±0.2	0.0±0.2	0.1±0.3	0.1±0.3	31.4±0.3	31.2±0.3	0.1±0.3	0.1±0.1	51.3±0.3	51.6±0.3
3	20.1±0.2 ^b	20.1±0.4 ^b	0.3±0.2	0.1±0.4	0.1±0.3	0.1±0.4	31.1±0.3	30.1±0.6	0.2±0.3	0.1±0.2	51.4±0.3	50.1±0.3
7	20.3±0.2 ^b	17.6±0.2 ^c	0.4±0.6	0.1±0.1	0.2±0.4	0.2±0.2	30.2±0.5	30.4±1.3	0.3±0.1	0.1±0.2	50.5±1.1	50.2±0.3
10	19.2±0.3 ^c	-*	0.3±0.1	-	0.2±0.2	0.3±0.4	30.4±0.7	29.8±0.7	0.2±0.2	0.4±0.3	50.2±2.0	49.8±0.3
14	18.7±0.3 ^c	-	0.4±0.3	-	0.3±0.3	0.3±0.1	29.4±1.0	30.0±0.3	0.2±0.3	0.3±0.2	49.6±0.5	49.4±0.3
21	-	-	-	-	0.3±0.1	0.2±0.8	29.5±0.8	29.1±1.0	0.2±0.1	0.3±0.1	49.2±0.4	48.4±2.0
28	-	-	-	-	0.2±0.3	0.4±0.1	29.0±0.5	28.7±0.3	0.3±0.2	0.3±0.2	50.1±0.9	50.4±0.3
35	-	-	-	-	0.4±0.2	-	30.3±0.6	-	0.3±0.3	0.4±0.5	49.1±0.3	49.5±0.6
42	-	-	-	-	-	-	-	-	0.2±0.4	0.4±0.3	49.4±0.3	48.2±0.4
49	-	-	-	-	-	-	-	-	0.3±0.2	-	48.6±0.3	-

*Gas readings have not been carried out as mould visually observed on sample.

a-c: Different letters correspond to statistically significant differences ($p \leq 0.05$).

6.2.2 Microbiological analysis

The pre-cooked SRNs have relatively high a_w values: 0.92 to 0.95 for filling and 0.79 to 0.82 for puff pastry. Therefore, they are very susceptible to microbial spoilage.

The results of the viable counts of the targeted microbial groups of sample L under the different storage conditions are reported in Tab. 21 (storage at 5 °C) and Tab. 22 (storage at 20 °C).

Table 21_ Viable counts of different spoilage-related microbial groups detected in L during storage under AIR, MAP1 and MAP2 conditions at 5°C for 49 days (mean±sd).

batches	Storage time (days)	Log CFU/g ± SD							
		Psychrotrophic bacteria		Enterobacteriaceae		Staphylococci		Yeast and Moulds	
		filling	puff pastry	filling	puff pastry	filling	puff pastry	filling	puff pastry
AIR	0	2.1±0.1	1.9±0.1	<1	<1	<1	<1	<1	<1
	3	2.7±0.0	2.1±0.4	<1	<1	<1	<1	<1	<1
	7	3.6±0.1	2.0±0.1	1.0±0.1	<1	<1	<1	<1	<1
	10	5.1±0.1	2.4±0.1	1.3±0.1	<1	<1	<1	<1	<1
	14	6.2±0.3	2.8±0.1	1.6±0.1	<1	<1	<1	<1	2.3±0.1
	21	-*	-	-	-	-	-	-	-
	28	-	-	-	-	-	-	-	-
	35	-	-	-	-	-	-	-	-
	42	-	-	-	-	-	-	-	-
	49	-	-	-	-	-	-	-	-
MAP1	0	2.0±0.1	1.1±0.1	<1	<1	<1	<1	<1	<1
	3	1.8±0.1	1.2±0.1	<1	<1	<1	<1	<1	<1
	7	1.6±0.1	1.6±0.1	<1	<1	<1	<1	<1	<1
	10	1.9±0.1	2.0±0.1	<1	<1	<1	<1	<1	<1
	14	2.3±0.1	2.1±0.1	<1	<1	<1	<1	<1	<1
	21	3.2±0.1	2.5±0.1	1.1±0.1	<1	<1	<1	<1	<1
	28	3.6±0.1	3.1±0.1	1.4±0.1	<1	<1	<1	<1	<1
	35	5.8±0.1	4.0±0.1	2.0±0.1	<1	<1	<1	<1	2.1±0.1
	42	-	-	-	-	-	-	-	-
	49	-	-	-	-	-	-	-	-
MAP2	0	2.2±0.1	1.7±0.1	<1	<1	<1	<1	<1	<1
	3	2.4±0.1	1.6±0.1	<1	<1	<1	<1	<1	<1
	7	2.0±0.1	1.9±0.1	<1	<1	<1	<1	<1	<1
	10	1.7±0.1	2.0±0.1	<1	<1	<1	<1	<1	<1
	14	2.3±0.1	1.8±0.1	<1	<1	<1	<1	<1	<1
	21	2.8±0.1	2.1±0.1	<1	<1	<1	<1	<1	<1
	28	2.1±0.1	2.4±0.1	<1	<1	<1	<1	<1	<1
	35	2.0±0.1	2.7±0.1	<1	<1	<1	<1	<1	<1
	42	2.6±0.1	2.3±0.1	<1	<1	<1	<1	<1	<1
	49	3.1±0.1	2.9±0.1	<1	<1	<1	<1	<1	<1

*ND not determined as mould visually observed on sample

Table 22_ Viable counts of different spoilage-related microbial groups detected in L during storage under AIR, MAP1 and MAP2 conditions at 20°C for 49 days(mean±sd).

batches	Storage time (days)	Log CFU/g ± SD							
		Mesophilic bacteria		Enterobacteriaceae		Staphylococci		Yeast and Moulds	
		filling	puff pastry	filling	puff pastry	filling	puff pastry	filling	puff pastry
AIR	0	2.1±0.1	1.9±0.1	<1	<1	<1	<1	<1	<1
	3	2.5±0.0	2.4±0.3	<1	<1	<1	<1	<1	<1
	7	5.6±0.2	3.5±0.2	0.8±0.1	<1	<1	<1	<1	2.2±0.3
	10	-*	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-
	21	-	-	-	-	-	-	-	-
	28	-	-	-	-	-	-	-	-
	35	-	-	-	-	-	-	-	-
	42	-	-	-	-	-	-	-	-
	49	-	-	-	-	-	-	-	-
MAP1	0	2.0±0.1	1.1±0.1	<1	<1	<1	<1	<1	<1
	3	2.3±0.1	1.2±0.1	<1	<1	<1	<1	<1	<1
	7	2.9±0.2	1.0±0.2	<1	<1	<1	<1	<1	<1
	10	1.9±0.2	2.1±0.1	<1	<1	<1	<1	<1	<1
	14	2.8±0.1	2.8±0.2	<1	<1	<1	<1	<1	<1
	21	4.3±0.0	3.2 ±0.1	1.2±0.1	<1	<1	<1	<1	2.6±0.3
	28	5.1±0.1	4.0±0.1	1.0±0.3	<1	<1	<1	<1	2.3±0.1
	35	-	-	-	-	-	-	-	-
	42	-	-	-	-	-	-	-	-
	49	-	-	-	-	-	-	-	-
MAP2	0	2.2±0.1	1.7±0.1	<1	<1	<1	<1	<1	<1
	3	2.5±0.1	2.0±0.1	<1	<1	<1	<1	<1	<1
	7	1.9±0.1	1.9±0.2	<1	<1	<1	<1	<1	<1
	10	2.7±0.2	2.3±0.1	<1	<1	<1	<1	<1	<1
	14	3.2±0.1	1.8±0.2	<1	<1	<1	<1	<1	<1
	21	4.1±0.1	2.1±0.1	<1	<1	<1	<1	<1	<1
	28	4.6±0.2	2.8±0.1	<1	<1	<1	<1	<1	<1
	35	5.0±0.1	3.3±0.1	<1	<1	<1	<1	<1	1.2±0.1
	42	5.2±0.1	4.1±0.3	<1	<1	<1	<1	<1	2.5±0.2
	49	-	-	-	-	-	-	-	-

*ND not determined as mould visually observed on sample

Filling microbial counts were higher than puff pastry during the whole storage time, due to higher moisture and a_w contents. At time zero, colony count on PCA medium evidenced a low number of 2.1±0.1 Log CFU/g in filling and 1.9±0.1 Log CFU/g in puff pastry, whereas viable cells were not shown in other media.

It can be seen by data, microbial growth was delayed by cold storage. After 14 days of storage at 5 °C, samples packaged in air presented objective signs of spoilage,

evidencing mycelia on puff pastry firstly on the plate and then, after 21 days, on the surface of the product. AIR samples at 20 °C highlighted already after seven days a microbial spoilage, with a 5.6 Log CFU/g of mesophilic microbial count in filling and a 2.2 Log CFU/g of mould count in puff pastry.

These results are in accordance with data in literature. Karaoglu *et al.* (2005) reported a microbial count increase during the storage of bread at room temperature, without the addition of anti-microbial agents. In fact, after about five days, moulds are visible on the crust, marking the end of the product shelf-life. Similarly, the effect of part baked cake storage at refrigerator temperature (4 °C) for 30, 60 and 90 days was reported (Karaoglu & Kotancilar, 2009).

MAP resulted in a delayed mould growth. The trays packaged with 30% CO₂ did not show visible mould colonies on DRBC media before 35 days of storage at 5 °C (Tab. 21), and 28 days of storage at 20 °C (Tab. 22).

After 35 days of refrigerated storage and 28 days at 20 °C under MAP1 conditions, the loads of psychrotrophic and mesophilic groups of filling L, increased to above 3 Log CFU/g. *Enterobacteriaceae* displayed values of 1.1 and 1.2 Log CFU/g after 21 days under MAP1 at 5 and 20 °C, respectively. By contrast, filling L stored under MAP2 condition showed different trend: mesophilic and psychrotrophic aerobic bacteria increased their initial number to about 1 Log CFU/g in 49 and 42 days at 5 and 20 °C, respectively; while the *Enterobacteriaceae* were not detected in whole duration of the experimentation.

All the colonies that grew on DRBC were visibly moulds, whereas yeast were not detected at all. No *Staphylococci* colonies were detected throughout the storage. *Enterobacteriaceae* were observed only in filling packaged in air after seven days.

Moulds are aerobic spoilage micro-organisms that can withstand CO₂ concentrations of 60% inside MAP (Seiler, 1989; Ooraikul, 1991). When CO₂ concentration is very high (above 80%), moulds cannot grow in bakery products, even if the residual oxygen is equal or higher than 1%, but when CO₂ levels decrease ($\leq 60\%$), even low concentrations of oxygen may result in mould development (Bogadtke, 1979; Abellana *et al.*, 2000). Guynot *et al.* (2003b) reported a significant increase in the lag phase of seven distinct fungal species isolated from bakery products when CO₂ was augmented from 0% to 100% and a_w values were higher than 0.85. Smith *et al.* (1986) reported a 0,4% O₂ concentrations for growth of *Penicillium* spp., with 60% CO₂ concentration. Accordingly to the above cited papers, it is possible that the residual oxygen content allowed the mould growth after a long lag phase, despite of high CO₂ concentrations inside packages.

The results of the viable counts of the targeted microbial groups of SRNs made with vegetable fat blends (P, V1 and V2) under the different storage conditions exhibited the same trend the control L (data not shown).

Therefore, SRNs stored under MAP2 condition showed lower counts after 49 days, when sampling of MAP1 was stopped at 35 and 28 days at 5 and 20 °C, respectively, when mould growth was visible.

Fat formulation didn't influence microbiological counts during storage under different atmospheric conditions.

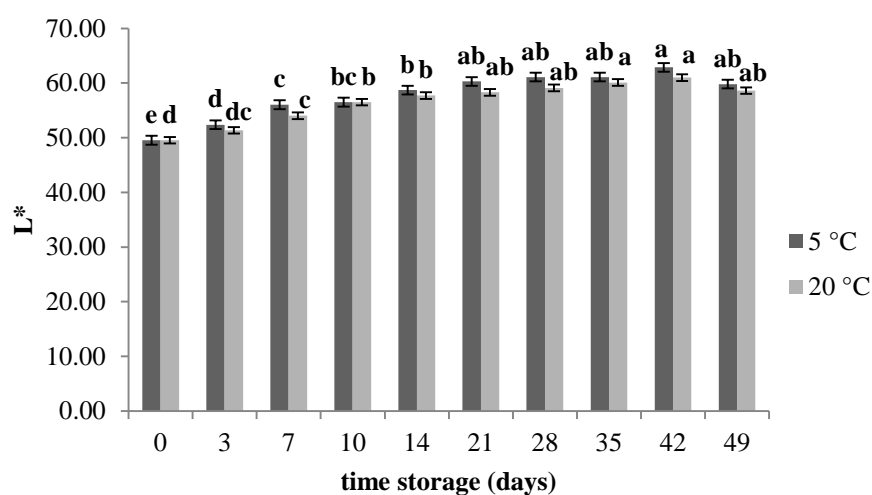
6.2.3 Chemical-physical and textural analysis

6.2.3.1 Color measurements

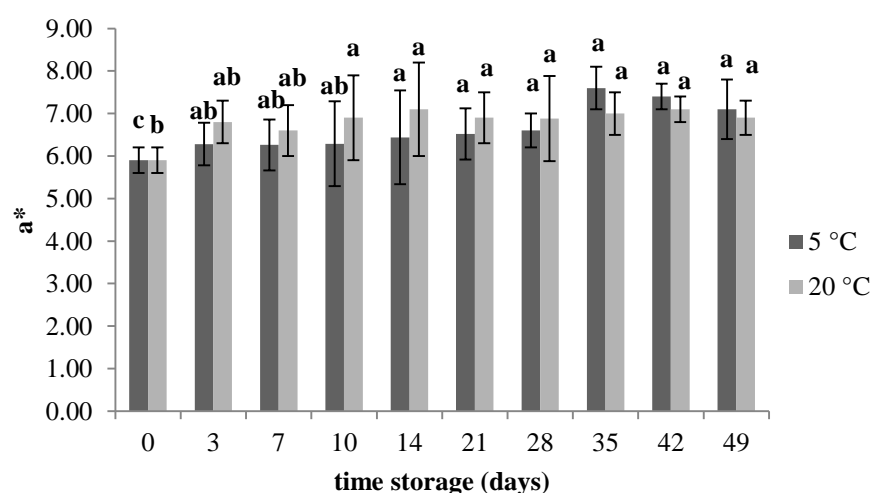
The color variation of the RTE-SRNs samples stored at 5 and 20 °C under AIR, MAP1 and MAP2 conditions is well described by color parameters. Changes in color of the samples were dependent on storage time and temperature. But, there was no difference in color changes of samples packaged under different atmospheres (data not shown).

The mean values of upper and lower L*, a* and b* color parameters of L under MAP2 at 5 and 20 °C are shown in Figure 43.

(a)



(b)



(c)

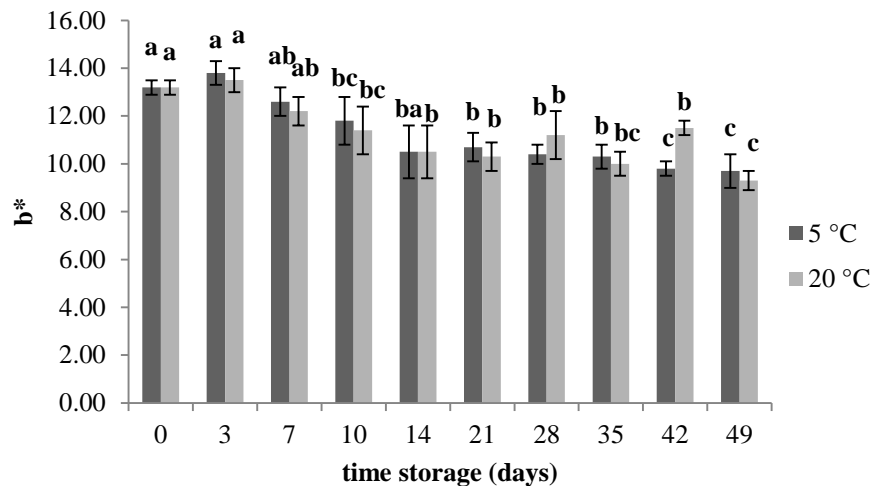


Figure 43_ Effect of storage time and temperature on color parameters [(a) L* value, (b) a*-value and (c) b*-value] of L.

a-d: Different letters in each parameter indicate significant differences ($p \leq 0.05$).

L* value increased in all the cases, and the greatest variability was observed for the sample stored under refrigerated condition. The L* values and thus, lightness of RTE-SRN increased with time of storage (Fig. 43a). This can be due to the moisture loss of samples during storage, because it has been reported that the medium with higher moisture content is darker than the drier one (Popov-Raljic *et al.*, 2009). However, it seems that staling has a more important role in increasing the brightness of samples, due to negligible changes in moisture content of SRN during storage (Fig. 44). Similar observations were also reported by Popov-Raljic *et al.* (2009) who obtained a high positive correlation between brightness of different kinds of bread and storage time.

The highest rate of increase in brightness occurred in the early days of storage, with a fast increment at 5°C, then, there was no significant difference (Fig. 43a). This could be due to the decrease in the staling rate with storage time (He & Hoseney, 1990; Ribotta *et al.*, 2004) and cold temperature.

Changes in the a* and b* parameters of L are given in Figs. 43b and 43c, respectively. Increase in the a* value and decrease in the b* value were slow with time. This may be related to the moisture migration from the filling to the puff pastry. These results are in agreement with those of Majzoobi *et al.* (2011).

In the case of the samples packed under MAP1 conditions, L* also increased linearly during the first 7 days, and it then approached a constant value during storage. Fat formulation didn't have any influence on color change.

However, the lightness (L*) under MAP2 conditions remained acceptable until 49 days of storage, with value similar than traditional baked L (58.5 ± 0.8) (data not shown).

6.2.3.2 Moisture and a_w determination

One of the major changes that occur after baking, during storage is moisture redistribution, that can result in textural changes and may even promote chemical and microbiological spoilage in products (Quail, 1996).

Figure 44 shows the evolution of moisture content in filling (a) and puff pastry (b) of L samples under AIR, MAP1 and MAP2 at 5 and 20 °C, respectively.

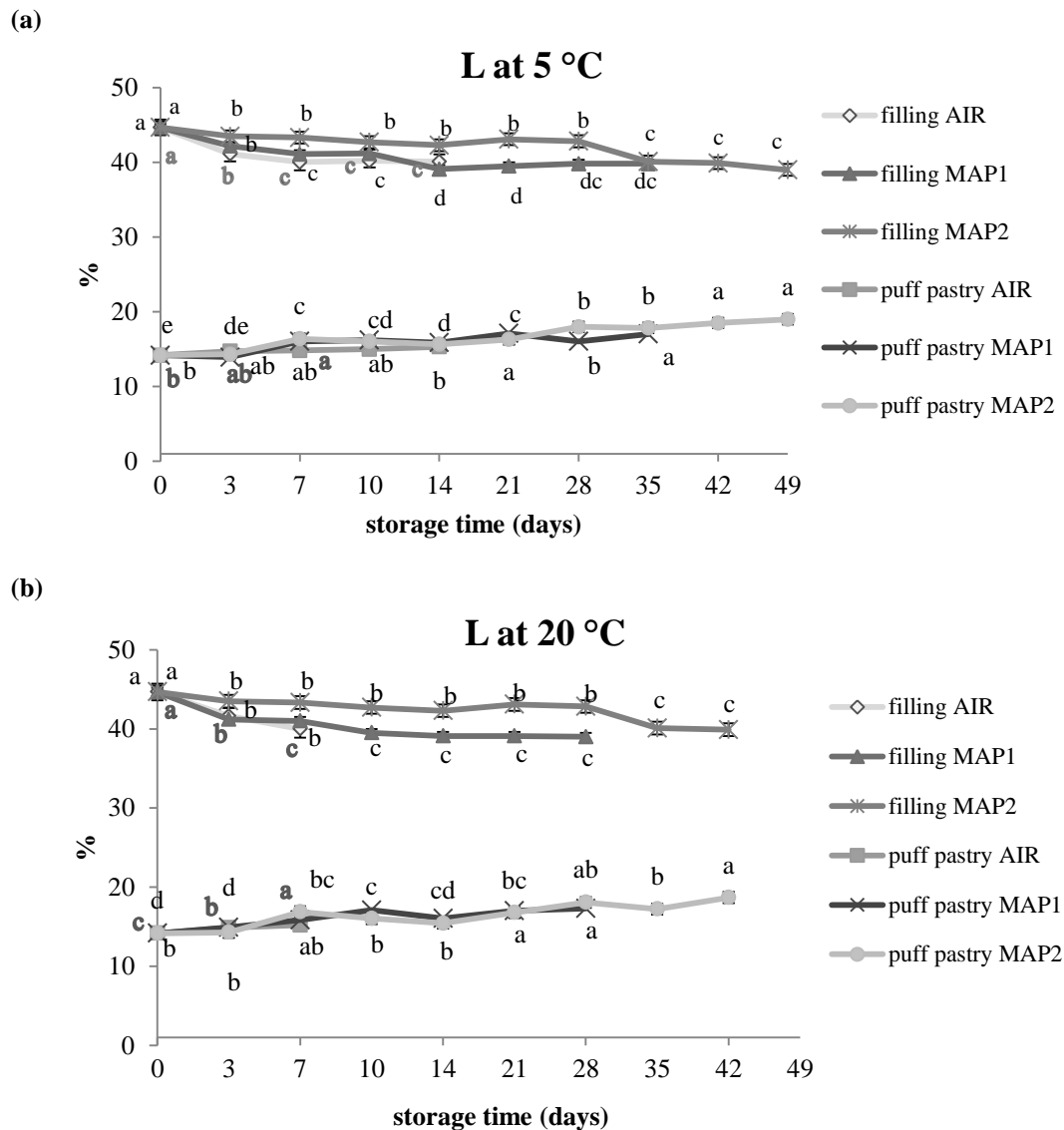


Figure 44_ Effect of storage time at 5 °C (a) and 20 °C (b) on the moisture content of the filling and puff pastry of L under different atmospheric condition (AIR, MAP1 and MAP2).

a-e: Different letters in each parameter indicate significant differences ($p \leq 0.05$).

Filling had higher moisture content than puff pastry, 44.68% vs. 14.18% in L, because a larger amount of water is evaporated during baking.

As expected for a bakery product with difference in moisture content, filling SRN went towards a light moisture loss during storage, while the moisture content of puff

pastry increased during storage ($p \leq 0.05$). Storage temperature had no effect on moisture content. Puff pastry absorbed humidity during storage until equilibrium was reached.

This can be attributed to the difference in moisture between the puff pastry and the filling, thus, moisture migration occurs from the filling to the puff pastry and finally, to the surrounding area. This behaviour was also reported by Piazaa & Masi (1995) in other bakery products with differences of moisture content between crust and crumb. The increase in the relative equilibrium humidity of the headspace, which drew off water both from the filling and the short to the surrounding atmosphere, should be responsible for the decrease in moisture content (Esse & Saari, 2004). During storage, the difference in moisture content between crust and crumb tends to balance out, making the crust become softer and the crumb more crumbly, and the fresh bread aroma mostly disappears (Sluimer, 2005).

Different fat formulation had no significant effect on the moisture content of samples (data not shown), while there were significant differences ($p \leq 0.05$) between air- and MA-packaged samples. In fact, samples packaged in air showed a faster moisture decrease of filling and increase of puff pastry respect to MA-packaged samples.

Samples packaged in air showed a faster decreasing of filling moisture content after seven days.

Under MAP the greater migration of water showed in the first seven days. In the following days of storage time, the rate of moisture loss in filling was reduced significantly, probably due to reach of equilibrium in moisture between the crust and the crumb (He & Hosene, 1990) then, water migration was decreased with time.

Moisture content of filling L under MAP2 decreased sharply during the whole storage time.

Filling of MAP2 has evidenced a significant higher moisture content after 28 days, compared with MAP1.

Temperature storage at 20 °C accelerated moisture lost, especially in AIR samples; although the evolution in moisture content was similar to that at 5 °C.

The moisture content of puff pastry increased during storage at 5 and 20 °C for all samples ($p \leq 0.05$). During the whole experiment, moisture of puff pastry increased from 14.18 to above 18.70 % in MAP2 at 5 and 20 °C.

The water activity is a very important parameter to evaluate the microbiological stability of the product (Huchet *et al.*, 2013).

Figure 45 shows the evolution of a_w of filling (a) and puff pastry (b) of L under the different atmospheric conditions at 5 °C. During storage a_w values of filling decreased significantly ($p \leq 0.05$).

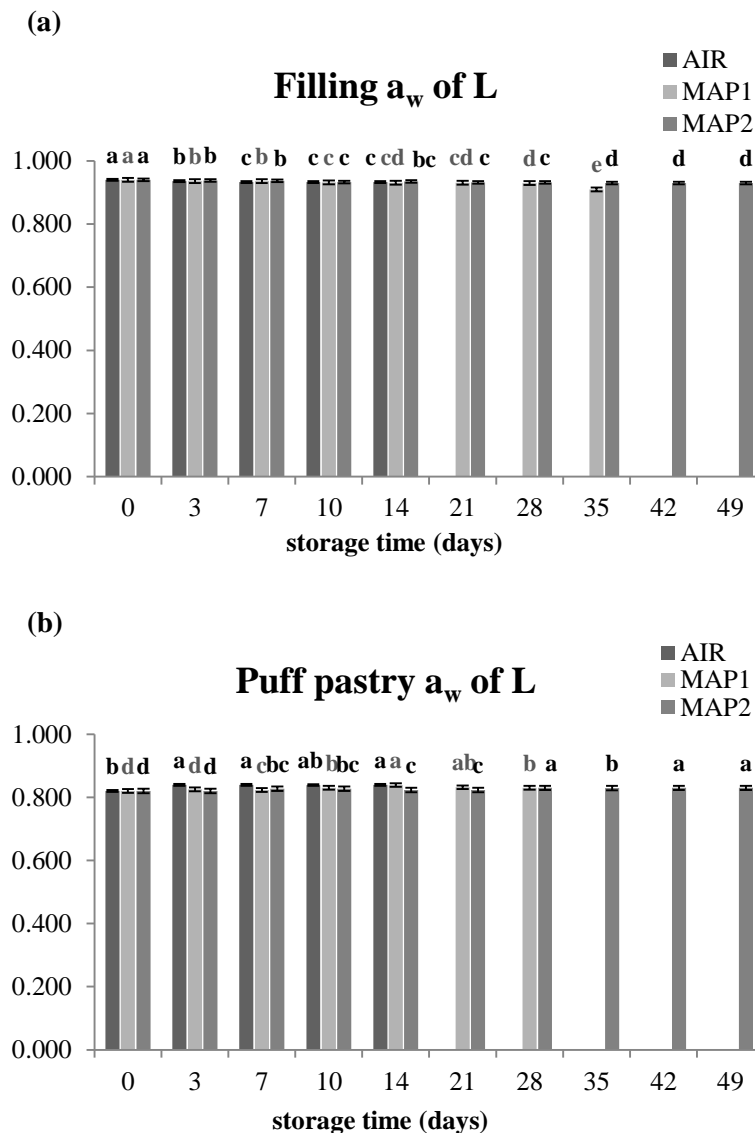


Figure 45_ Effect of storage time at 5 °C on the moisture content of the filling (a) and puff pastry (b) of L under different atmospheric condition (AIR, MAP1 and MAP2). a-e: Different letters in each parameter indicate significant differences ($p \leq 0.05$).

As expected, water activity of L at time 0 were higher in the filling than in the short (0.940 vs. 0.82).

AIR samples in filling ranged from 0.940 to 0.933 a_w content during 14 days of storage, MAP1 reached 0.931 a_w value and MAP2 0.935.

Puff pastry absorbed humidity from filling during storage, and, consequently, their water activity increased ($p \leq 0.05$).

At time 0, a_w content in puff pastry was 0.820; after 14 days of storage it increased to 0.84 in AIR, and 0.83 in MAP1 and MAP2.

a_w content in MAP increased slowly during storage. Compared to MAP1, samples stored under MAP2 showed higher a_w value. At the end of experimentation filling a_w in MAP2 was 0.93 and puff pastry 0.83.

No significant differences were found between SRNs prepared with different fat blends during the experiment ($p>0.05$).

6.2.3.3 Rancidity evaluation

SRNs can be subjected to rancidity, a lipid degradation resulting in off-odors and off-flavors, which render products unpalatable and decrease shelf life. Two types of rancidity problems can occur - oxidative and hydrolytic.

The extent of lipid hydrolysis and oxidation of the fats extracted from SRNs was determined by the free acidity, peroxide and total polar compounds content.

The peroxide value (PV) was employed for determining the formation of primary lipid oxidation products during the storage period of SRNs. Total polar compounds (TPC) was employed for determining the second stage auto-oxidation during which peroxides are oxidized to aldehyde and ketone.

The effects of different atmospheric conditions (AIR, MAP1 and MAP2) during storage at different temperatures on changes in FFA, PV and TPC of L were showed in Tables 23 and 24.

In the case of FFA values, there were no significant difference ($p>0.05$) among the samples during the storage period at both 5 and 20 °C.

Initial PV value of L samples was 6.67 ± 0.2 meq O₂/kg. TPC was 8.76 ± 0.1 at time 0. During storage PV and TPC did not change in both AIR and MAP packages ($p>0.05$). This behaviour also showed in the other SRNs made with different fat formulations, that observed lower value than L (data not shown).

It is well known that oxygen is the promoter of oxidative rancidity (Smith *et al.*, 2004). The constancy of thermo-oxidative values in AIR packages is probably due to the few days of storage (14 and 7 days at 5 and 20 °C in AIR, respectively).

However, using oxygen free MAP packages resulted in low PV and TPC values until 35-49 days of storage. Our findings are consistent with literature data.

Table 23_ Effects of different atmospheric conditions (AIR, MAP1 and MAP2) during storage at 5 °C on changes in FFA, PV and TPC of L.

Storage time (days)	L stored at 5 °C								
	AIR			MAP1			MAP2		
	FFA (%oleic acid)	PV (meq O ₂ /kg)	TPC (%)	FFA (%oleic acid)	PV (meq O ₂ /kg)	TPC (%)	FFA (%oleic acid)	PV (meq O ₂ /kg)	TPC (%)
0	0.62±0.0	6.67±0.2	8.76±0.1	0.62±0.0	6.67±0.2	8.76±0.1	0.62±0.0	6.67±0.2	8.76±0.1
3	0.64±0.0	6.65±0.1	9.53±0.4	0.61±0.1	6.66±0.1	9.21±0.1	0.62±0.0	6.66±0.1	8.80±0.1
7	0.68±0.0	6.68±0.0	8.82±0.3	0.66±0.0	6.70±0.0	9.10±0.1	0.65±0.0	6.65±0.4	8.81±0.1
10	0.70±0.0	6.71±0.1	8.89±0.1	0.66±0.1	6.69±0.2	8.81±0.3	0.67±0.0	6.68±0.2	9.19±0.2
14	0.71±0.0	6.75±0.2	9.30±0.1	0.68±0.0	6.65±0.1	9.42±0.1	0.69±0.0	6.70±0.1	8.83±0.1
21	-	-	-	0.71±0.0	6.68±0.0	8.90±0.4	0.72±0.0	6.71±0.0	8.78±0.1
28	-	-	-	0.72±0.0	6.67±0.2	9.03±0.2	0.68±0.0	6.69±0.2	9.22±0.2
35	-	-	-	0.70±0.0	6.71±0.1	9.51±0.1	0.67±0.0	6.70±0.1	8.81±0.2
42	-	-	-	-	-	-	0.71±0.0	6.72±0.0	9.03±0.1
49	-	-	-	-	-	-	0.69±0.0	6.73±0.1	8.82±0.2

*ND not determined as mould visually observed on sample.

Table 24_ Effects of different atmospheric conditions (AIR, MAP1 and MAP2) during storage at 20 °C on changes in FFA, PV and TPC of L.

Storage time (days)	L stored at 20 °C								
	AIR			MAP1			MAP2		
	FFA (%oleic acid)	PV (meq O ₂ /kg)	TPC (%)	FFA (%oleic acid)	PV (meq O ₂ /kg)	TPC (%)	FFA (%oleic acid)	PV (meq O ₂ /kg)	TPC (%)
0	0.62±0.0	6.67±0.2	8.76±0.1	0.62±0.0	6.67±0.2	8.76±0.1	0.62±0.0	6.67±0.2	8.76±0.1
3	0.66±0.0	6.67±0.1	8.89±0.1	0.63±0.0	6.65±0.1	8.80±0.0	0.60±0.0	6.67±0.1	8.79±0.0
7	0.67±0.0	6.69±0.0	9.33±0.0	0.65±0.0	6.68±0.0	9.11±0.1	0.64±0.0	6.70±0.0	8.76±0.1
10	-*	-	-	0.68±0.0	6.67±0.2	8.77±0.0	0.65±0.0	6.69±0.3	8.78±0.0
14	-	-	-	0.70±0.0	6.65±0.1	8.69±0.2	0.68±0.0	6.71±0.1	9.39±0.1
21	-	-	-	0.69±0.0	6.68±0.0	8.82±0.1	0.67±0.0	6.72±0.1	8.80±0.1
28	-	-	-	0.73±0.0	6.67±0.2	9.15±0.2	0.71±0.0	6.70±0.2	8.84±0.2
35	-	-	-	-	-	-	0.70±0.0	6.71±0.1	9.41±0.2
42	-	-	-	-	-	-	0.72±0.0	6.72±0.0	8.84±0.0
49	-	-	-	-	-	-	-	-	-

*ND not determined as mould visually observed on sample.

6.2.3.4 Texture analysis

Firmness is a texture property, which has attracted most attention in bakery products assessment because of its close association with human perception of freshness. It can be characterised by the force required to compress a given area of a product.

SRNs firmness increased as the storage time increased and the firming curve with time is flatter than the control curve (freshly baked product) (Fig. 46). This may be caused by the migration of water from the moist filling to the dry puff pastry, causing the loss of crunchiness.

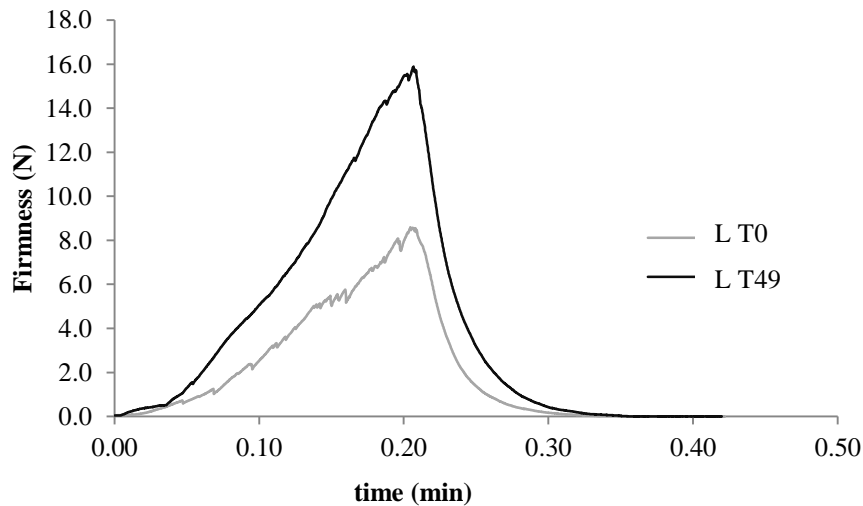


Figure 46_ Comparison of firmness curve of L freshly baked (L T0) and L after 28 days of storage (L T49).

The effect of storage times on firmness of L, under different storage conditions, is shown in Fig. 47.

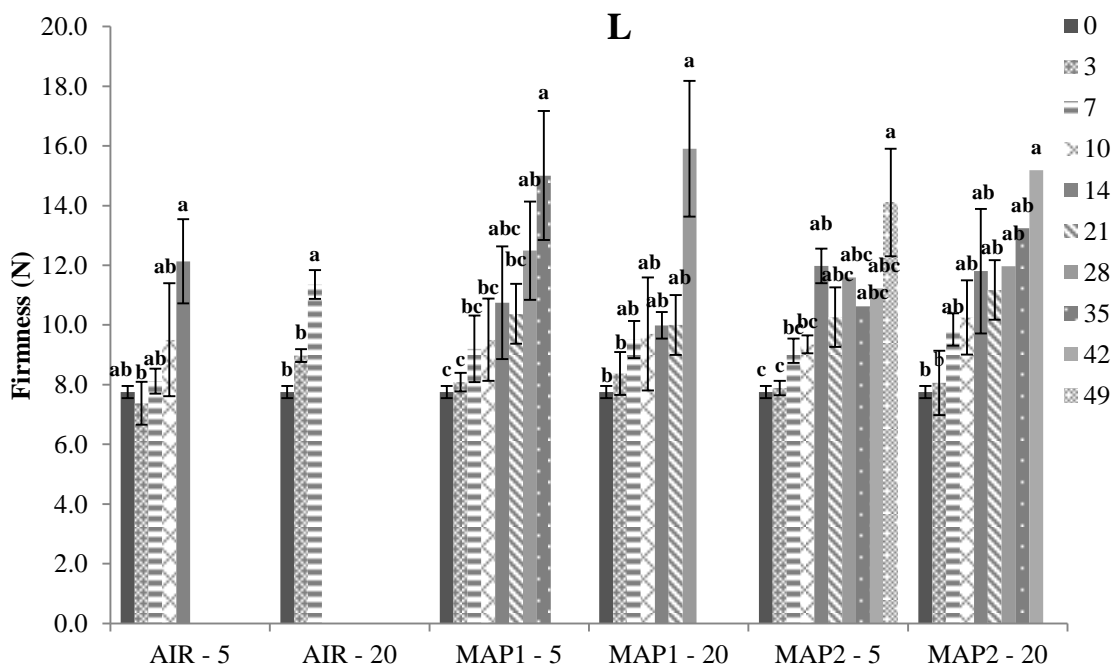


Figure 47_ Evolution of firmness of L under different atmospheric conditions (AIR, MAP1 and MAP2) and different storage temperature (5 and 20 °C).
a-c: Different letters correspond to statistically significant differences ($p \leq 0.05$).

As shown in Figure 47, the increase of storage period of SRNs increased linearly firmness of samples at both 5 °C and 20 °C ($p \leq 0.05$).

Hardening of bakery products is a complex phenomenon, widely studied (Baik *et al.*, 2000; Gomez *et al.*, 2007, 2010; Sumnu *et al.*, 2005), but is still not completely understood.

The increase of firmness can be ascribed to different chemical-physical changes, such as the amylose and amylopectin recrystallisation, the formation of complexes between starch and proteins, the water redistribution among the constituents during storage (Seyhun *et al.*, 2003). It was reported that, a temperature of 60 °C was required to overcome staling of bakery products and to resolubilise crystalline amylopectin molecules (Leuschner *et al.*, 1997). Heating of pre-cooked SRNs allowed to reach the temperature of 60 °C, required to resolubilise crystalline amylose molecules.

After 7 days storage at 5 °C, firming rate was increased from 7.75 N in freshly baked to 8.12 N, 9.20 N and 9.14 N in L samples under AIR, MAP1 and MAP2, respectively. While AIR, MAP1 and MAP2 -L samples stored at 20 °C showed firmness values of 11.35 N, 9.51 N and 10.25 N, respectively. Firmness increased to 11.35 N at higher storage temperature (20 °C) under AIR.

The texture measurement revealed that air packaged SRNs hardened already after 7 days at 20 °C and 14 days at 5 °C. In fact, the significant increase of the maximum force accounts for the increased hardness of the surface, whereas the increase of area explains crispness loss. Samples packaged with an atmosphere containing 30% CO₂ showed a significant change only after 28 days of storage at 5 and 20 °C. A greater increase in maximum force was observed at 20 °C ($\Delta +105\%$) respect to low storage temperature ($\Delta +61\%$).

Samples packaged with 50% CO₂ showed the same behaviour of the previous samples, since it followed the same trend as the 30% CO₂ samples, but with lower increases.

Results revealed that the air packaged samples were the hardest, whereas MAP2 packaged samples showed lower firmness than those of 30% CO₂ packaged SRNs after 28 days. Hardening should be attributed to moisture loss from the filling and then from the puff pastry, and only to a lesser extent to staling of the surface. In fact, filling lost a considerable amount of water and this was transferred to puff pastry and to headspace of tray. This migration of water may account for hardening, while the fat content of the puff pastry has surely prevented or slowed down the staling process.

SRNs prepared with different fat blends showed significant differences in the firmness during storage ($p \leq 0.05$), displayed the lowest increase of firmness SRNs prepared with Veg1 and Veg2 during storage.

Figure 48 shows the changes in firmness of SRNs made from different types of fats over an extended storage time under MAP2 at 5 °C.

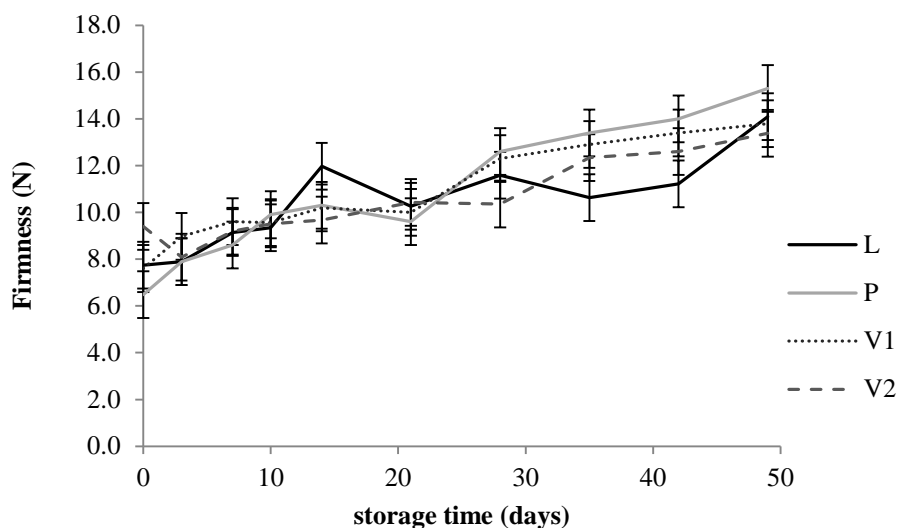


Figure 48_ Firmness evolution of SRNs with different fat blends (L, P, V1 and V2) during storage under MAP2 at 5 °C.

On the first days after baking, P showed no significant differences in firmness from those SRNs, but they showed significant differences later, after 35 days storage ($p \leq 0.05$).

Vegetable SRNs, V1 and V2, had similar firmness than the samples made with animal fats ($p \leq 0.05$), the firmness change was much less rapid than L. In vegetable fat blends production, β -tending hard fats are used to serve as quick-forming nuclei that cause solids in the base oil to precipitate in small enough crystals to ensure pourability and prevent separation (O'Brien, 2009). It should be stored between 18 and 35 °C (O'Brien, 2009). Vegetable SRNs stored at 5 °C were not significantly firmer than SRNs stored at 20 °C ($p > 0.05$).

6.2.4 Sensory analysis

Scores for appearance, smell, flavor and crispy of all RTE-SRN samples showed similar trends in terms of decreasing sensorial quality up to the final day of refrigerated and room temperature storage, especially for the experimental batch that withstood prolonged storage. Table 25 reports the mean of scores for each sensory parameter of SRNs formulated with different fat blends (L, P, V1 and V2).

However, the overall acceptability was always above the threshold for all the storage period, although flavor and crispy fell down this limit after 42 days on RTE-SRN packaged with the MAP2, as shown in Tab. 25.

Seems that the variability in the results of moisture and texture of the samples negatively affected sensory perception in prolonged storage.

No significant differences were detected among batches, except for MAP1 samples, which showed worse crispy than those packaged with 50% CO₂ at 28 day of sampling. Water absorption was probably the main limiting factor of SRN shelf life.

Crispiness is a complex attribute resulting from multiple sensations and influenced by numerous physical parameters (moisture and processing condition).

It can be concluded that in terms of overall quality, MAP2 samples were the most preferred for SRNs samples during the storage period.

Table 25_ Changes in sensory attributes of RTE-SRN packaged under different conditions during 48 days of storage.

batches	Storage time (days)	Storage at 20 °C				
		Quality attribute				
		appearance	smell	flavor	crispy	global acceptability
AIR	0	6.30 a,w	6.30 a,w	6.01 a,w	5.91 a,w	6.14 a,w
	3	6.10 a,w	6.40 a,w	5.33 a,x	5.23 a,x	5.77 a,x
	7	6.13 a,w	6.01 a,w	5.31 a,x	5.28 a,x	5.68 a,x
	10	-*	-	-	-	-
MAP1	0	6.30 a,w	6.30 a,w	6.03 a,w	5.91 a,w	6.14 a,w
	3	5.86 a,w	5.50 a,x	5.43 a,x	5.36 a,x	5.54 a,xy
	7	5.90 a,w	5.35 a,x	5.42 a,x	5.43 a,x	5.56 a,xy
	10	5.86 a,w	5.85 a,wx	5.70 a,wx	5.40 a,wx	5.70 a,x
	14	5.80 a,w	5.83 a,wx	5.73 a,wx	5.42 a,wx	5.69 a,x
	21	5.93 a,w	5.52 a,wx	5.42 a,x	5.48 a,wx	5.59 a,x
	28	6.13 a,w	5.10 a,y	5.48 a,x	4.91 b,y	5.41 a,y
	35	-	-	-	-	-
MAP2	0	6.30 a,w	6.30 a,w	6.03 a,w	5.91 a,w	6.14 a,w
	3	5.84 a,w	5.44 a,wx	5.48 a,wx	5.51 a,wx	5.57 a,xy
	7	5.87 a,w	5.51 a,wx	5.46 a,wx	5.53 a,wx	5.59 a,xy
	10	5.78 a,w	5.31 a,x	5.20 a,x	5.30 a,x	5.40 a,y
	14	5.80 a,w	5.30 a,x	5.23 a,x	5.27 a,x	5.40 a,y
	21	5.68 a,wx	5.12 a,y	5.46 a,wx	5.38 a,wx	5.41 a,y
	28	5.70 a,wx	5.50 a,wx	5.50 a,wx	5.39 a,wx	5.52 a,xy
	35	5.86 w	5.31 x	5.46 wx	5.40 wx	5.51 xy
	42	5.70 wx	5.01 y	4.89 z	4.88 y	5.12 z
	49	-	-	-	-	-

*Gas readings have not been carried out as mould visually observed on sample.

Data followed by different letters (w-z) within the same column and batch or followed by different letters (a-b) within the same column and sampling day differ significantly ($p \leq 0.05$).

6.2.5 Conclusion

Mould growth is the major problem in the shelf life of bakery products. MAP could extend the refrigerated storage of pre-cooked SRNs up to three times compared to air packaging.

The result of present study showed that the best preservation for pre-cooked SRNs was in MAP2 (50% CO₂:50% N₂) gas composition at 5 °C, which ensured acceptable sensory, physical and chemical analyses until the end of storage period of analysis of 49 days.

Refrigeration is more effective than room temperature storage from a microbiological point of view.

In conclusion, MAP seems to be a very promising technique to extend the shelf life of intermediate moisture bakery products, providing an effective microbiological control and for keeping original texture and sensory attributes of the product.

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