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TITOLO TESI DI DOTTORATO DI RICERCA
SHELF LIFE OF BAKERY PRODUCTS MADE WITH MONOCULTIVAR EXTRA VIRGIN OLIVE OIL FROM CALABRIA (SOUTH ITALY): LIPID MATRIX OXIDATION

(**s.s.d. AGR/15**)

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SHORT ABSTRACT

Calabria (South Italy) is one of the most important Italian regions for the production of olive oil. The extra virgin olive oil (EVOO) from Calabria could be used as an alternative to shortening or low-price vegetable oils at present used in the production of baked products. Thanks to EVOO's high biological properties, healthier baked products could be obtained, improving their shelf life and quality characteristics.

In the industrial sector there is a clear trend towards the development of new and healthier food products, linked to the evidence that the repeated consumption of high quantities of saturated and *trans* fats in the diet increases the risk of several diseases for human body. In fact, there is an increasing need to replace unhealthy fats with healthy replacement, preserving the physical and sensory properties of the final products.

The aim of this PhD project was to study the lipid oxidation of the fat extracted from different bakery products (*Cantuccini* and breadsticks) in relation to the kind of fat used in the recipe (shortening, olive pomace oil or EVOO). Moreover, in order to improve baked product sustainability, the potential use of a biodegradable packaging (PLA, polylactic acid) for *Taralli* made with EVOO was investigated, comparing it with other packaging materials.

EVOO used in the recipe improved the chemical characteristics of the lipid fraction of *Cantuccini* and breadsticks, slowing down the oxidation phenomena. Results also demonstrated that PLA acts in a similar way to the packaging commonly used. Thus, PLA was found to be a good alternative to traditional packaging, especially for periods of time that do not exceed three months.

Keywords (5-7): bakery products, lipid oxidation, extra virgin olive oil, olive pomace oil, shortening, biodegradable packaging, polylactic acid.

EXTENDED ABSTRACT

Although bakery products are not essential foods, they are traditionally high demanded generating billions of dollars in revenue. Nowadays, a wide variety of baked products are on sale, such as breads, unsweetened rolls, doughnuts, dessert pies, pizza, crackers, biscuits, and other products. This PhD thesis focused on the study of sweet and savoury baked products, with the aim of improving their quality characteristics through the enhancement of the resistance to oxidation of the lipid fraction.

Since the evidence that Calabria region (South Italy) is one of the mayor producer of olive oil in Italy and that Extra Virgin Olive Oil (EVOO) is a high-quality vegetable oil, it was evaluated as an interesting alternative to oils and fats commonly used in baked products formulation, owing to its high unsaturated fatty acid content, its high biological properties and its capability to reduce health diseases. In fact, one of the most important challenges that the bakery industries are facing today is the need to reduce the quantity of saturated and *trans* fats in bakery products, making them healthier and maintaining, at the same time, the properties of the final products. Thus, the aim of this PhD thesis was to study the quality characteristics and oxidative stability of bakery products (both sweet and savoury) whose recipes were modified by replacing the fat commonly used in their formulations with extra virgin olive oil from Calabria. The original and modified recipe products were studied and compared for 12 months. Moreover, a biodegradable packaging material was tested in order to evaluate its possible use as baked products packaging, comparing the effects due to its use with those of the packaging material commonly used.

The work was divided into three main researches:

- Research I was about the partial substitution of shortening in *Cantuccini*'s recipe;
- Research II was about the total substitution of olive pomace oil in breadsticks' recipe;
- Research III was about the testing of a biodegradable packaging material on *Taralli*.

The results obtained by this PhD thesis confirmed that lipid oxidation can be prevented by appropriate recipe formulations and a suitable packaging.

Research I: The EVOO's use improved the amount of monounsaturated fatty acids (MUFAs), reducing the amount of saturated ones (SFA). Moreover, EVOO used in the recipe improves the chemical characteristics of the lipid fraction of the products, slowing down the oxidation phenomena.

Research II: Thus, results demonstrated the essential role played by the type of oil on chemical and antioxidant properties of the lipid fraction of breadsticks. Finally, breadsticks

made with extra virgin olive oil, compared with those prepared with other olive pomace oil, showed to be more resistant to oxidation, probably due to the presence of natural antioxidants.
Research III: The obtained data showed that PLA acts in a similar way to PP and that PLA could be a good alternative to traditional packaging, especially for periods of time that do not exceed three months.
The data obtained showed that the evolution of the oxidation levels in the analysed samples during storage was related to the type of fat used in the production processes. Particularly, the use of extra virgin olive oil led to significantly lower values of hydroperoxides, ultraviolet absorption constants, and higher antioxidant capacity. The high biological properties of extra virgin olive oil improve the shelf life of bakery products in which it is used. Moreover, it was found that a biodegradable and compostable packaging (PLA) can be used for some baked products such as *Taralli*, increasing the sustainability the products.

I INTRODUCTION

Bakery products are worldwide consumed. They include cake, pastries, biscuits, bread, breakfast cereals, and other products, such as breadsticks. According to a Mordor Intelligence report (2017), the total market size of the bakery products is expected to reach USD 532 billion by 2022, at a 4.5% CAGR (Compound Annual Growth Rate) during the forecast period from 2017 to 2022. Europe represents the largest market, supported by the strong demand for in-store fresh baked goods. Even though bakery products are not essential foods, they are traditionally high demanded.

The food industry uses animal and vegetable fats for the formulation of different products. In particular, bakery sector requires solid fats for the particular rheological properties that they are able to give to the doughs and to the final products. These properties are found in animal fats or in vegetable fats with a high content of saturated fats, responsible, in fact, for the concreteness of the fat. Margarine, manufactured shortenings and butter are the fats commonly used in sweet baked products (*e.g.* biscuits, cookies, cakes, pastries) formulation because of their suitable plasticity properties (consistency and a high melting temperature), that allow the air incorporation during dough formation and make the dough able to withstand the high temperatures reached during baking and to hold its shape for long time (Litwinenko *et al.*, 2002; Lee *et al.*, 2008). Moreover, fats play an important role in the structure of biscuits and, consequently, in their mechanical properties (Tarancón *et al.*, 2013). However, many types of fats used for baked product manufacturing contain high levels of saturated fatty acids. In the industrial sector there is a clear trend towards the development of new and healthier food products. This is linked to the evidence that the repeated consumption of high quantities of saturated and *trans* fats in the diet increases the risk of several diseases for human body (Ruiz-Núñez, *et al.*, 2016; Nayeri, *et al.*, 2017; Sacks, *et al.*, 2017; Wang and Hu, 2017) caused by the increase of the concentration of cholesterol/low density lipoprotein (LDL) in the blood (European Food Safety Authority, 2010). Partial hydrogenation processes are one of the main causes of industrially produced trans fats. During partial hydrogenation, oil is "hardened", improving its commercial appeal by enhancing its sensory profile and texture and increasing its shelf life and tolerance to repeated heating. Also thermal processes (*e.g.* deodorisation of edible oils, frying) lead to the formation of *trans* fatty acids, but in much smaller quantities (Bhardwaj *et al.*, 2011). Thus, industrially produced trans fats are formed when fats and oils are modified using industrial processing techniques (Stender *et al.*, 2008).

Today there is an increasing need to replace hydrogenated fats that are now known to be unhealthy, with healthy replacements without affecting the physical and sensory properties of

the end products (Wang *et al.*, 2016). High quality vegetable oils with healthier fatty acid profiles are proposed as alternatives for formulating baked products characterise by improved nutritional characteristics. Extra virgin olive oil is an interesting alternative owing to its high unsaturated fatty acid content, its high biological properties and its capability to reduce health diseases (Psaltopoulou *et al.*,2004; Bendinelli *et al.*,2011; Salas-Salvadó *et al.*, 2011).

The aim of this PhD thesis was to study the quality characteristics and oxidative stability of bakery products (both sweet and savoury) whose recipes were modified by replacing the fat commonly used in their formulations with extra virgin olive oil from Calabria (South Italy). The original and modified recipe products were studied and compared for 12 months. Moreover, a biodegradable packaging material was tested in order to evaluate its possible use as baked products packaging, comparing the effects due to its use with those of the packaging material commonly used.

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Research I and II were carried out at the "Mediterranea" University of Reggio Calabria (Italy). Research III was carried out at the University of Barcelona (Spain).

II STATE OF THE ART

2.1 Bakery products

2.1.1 Classification

Foods products can be categorised into low-, intermediate-, and high-moisture foods depending on their water activity (a_w) values and overall moisture contents by weight. As reported by Knechtges (2012), foods with a_w values of 0.00–0.60 and less than 25% moisture content are defined low-moisture foods; foods having a_w values of 0.60–0.85 and 15–50% moisture content are intermediate moisture foods; foods with a_w values greater than 0.85 and more than 50% moisture content are high-moisture foods.

Bakery products could be classified on the basis of the category (Table 1) to which they belong (unsweetened goods, sweet goods or filled goods) or on the basis of their water activity (Table 2) (low-, intermediate- or high-moisture content baked goods).

Table 1 Classification of bakery products on the basis of categories

Categories of bakery products	Types within each category
Unsweetened goods	Bread: sliced, crusty, par-baked, ethnic
	Rolls: soft, crusty
	Crumpets
	English muffins
	Croissants
	Pizza base
	Raw pastry
Sweet goods	Large cakes: plain, fruited
	Pancakes
	Doughnuts
	Waffles
	Cookies
	Biscuits
	American muffins
	Buns
	Wafers
Filled goods	Tarts: fruit, jam
	Pies: meat, fruit
	Sausage rolls
	Pasties
	Cakes: cream, custard
	Pizza
	Ouiche

Table 2 Classification of bakery products on the basis of their water activity

Product	a_w
Low moisture content	
Cookies	0.2–0.3
Crackers	0.2–0.3
Intermediate moisture content	
Chocolate coated doughnuts	0.82–0.83
Danish pastries	0.82–0.83
Cream filled cake	0.78–0.81
Soft cookies	0.5–0.78
High moisture content	
Bread	0.96–0.98
Pita bread	0.9
Fruit pies	0.95–0.98
Carrot cake	0.94–0.96
Cheese cake	0.91–0.95
Pizza crust	0.94–0.95
Pizza	0.99

2.1.2 Technological functions of fats in bakery products

Fats and oils are important ingredients in a variety of foods, and the production of bakery products often involves their use. They confer looked-for characteristics on several foods, contribute to tenderness to some kind of sweet baked goods like shortened cakes, and by aerating batter, fats aid in establishing texture in cakes; they also add specific flavour to foods producing also a sensation of moistness in the mouth when they are eaten (Rios *et al.*, 2014). Moreover, fats are a good medium used to transfer heat to foods (Charley and Weaver, 1998). The nature and content of lipids in bakery products are very variable, depending on the type of product and the formulation. Many times, the use of fats and oils does not require solid fat content. In other applications, such as baked goods production, a certain amount of solid fat content is crucial. In general, the fats commonly used in bakery products are butter and lard (animal fats), and hydrogenated and/or refined vegetable oils, which imply nutritional and environment problems.

2.1.2.1 Shortening

Shortening is an edible fat solid at room temperature. The name shortening is derived from the effect that oils and fats have when they are added to baked products. Shortening refers to the aptitude of fats to lubricate, weaken, or shorten the structure of food components to provide a food product with desirable textural properties. Shortenings are a kind of fats providing specific functional properties (softness, texture, mouthfeel, structural integrity, air incorporation, heat transfer, and shelf life increase) to baked products (Ghotra *et al.*, 2002; McClements and Decker, 2007; O'Brien, 2009). Moreover, during dough mixing shortening prevents the cohesion between gluten and

starch granules by covering them (Shahidi, 2005), and hence providing the development of well aerated, tender, lubricated and processable viscoelastic dough structure.

2.1.2.2 Olive pomace oil

Olive pomace is one of the main by-products of oil olive oil production. It contains the solid residue of the olives together with the vegetation water (water naturally contained in olives and water added during the olive oil extraction). Pomace contains fragments of skin, pulp, pieces of kernels and some oil. It contains on average 5–8% of residual oil with 25–55% of vegetable water (Göğüş and Maskan, 2006). Due to the high moisture content that speeds up triacylglycerols hydrolysis, the oil contained in the olive pomace rapidly undergoes to deterioration. From olive pomace, refined olive–pomace oil can be obtained carrying out an extraction with authorized solvents (typically *n*-Hexane) and a refining process, which includes neutralization, deodorization and decolorization (Guimet *et al.,* 2005). Many times, this oil is improved with virgin olive oil to obtain the oil known as olive pomace oil (Kiritsakis, 1998). Owing to the low price of this oil, it is often used in baked goods that do not require solid fats for their preparation. However, the oils deriving from refining processes and/or submitted to hydrogenation processes might contain high amounts of both compounds deriving from triacylglycerol degradation (triacylglycerol oligopolymers, oxidised triacylglycerols and diacylglycerols) and trans isomers of unsaturated fatty acids (Gomes and Caponio, 1998; Gomes *et al.*, 2003; Caponio *et al.*, 2013a).

2.2 Healthy fat-substitute ingredients in bakery products

As it is well known, in recent years more and more attention has been given to the intake of saturated fatty acids (SFA) and trans fatty acids (TFA) with the diet. The European Food Safety Authority recommended that intakes of SFA should be as low as possible, based on the relationship between dietary SFA intake and increased blood cholesterol/low density lipoprotein (LDL) concentrations (European Food Safety Authority, 2010). Today's healthy diet vogue affects the development of baked products market. Consumers pay more attention to the quality composition of the foods that they consume (Renzyaeva, 2013). Nowadays, much attention is paid to the choice of fats for baked goods. Hence, the use of fat mimetics, shortenings, and emulsifiers with a healthier fatty acid profile is an alternative for the development of new bakery products recipes, without affecting the physical and sensory properties of the end products (Klonoff, 2007; Wang *et al.*, 2016). Unfortunately, the simple substitution of saturated

fat fraction with an unsaturated one does not allow to obtain foods corresponding to the qualitative characteristics expected by consumers. It is a fact that the final structure of the product, as well as its sensory properties, are strongly correlated with the degree of crystallization of the fat (solid/liquid ratio) (Da Pieve *et al.*, 2010). A winning strategy to design healthy fatty foods could be to replace the plastic structure of saturated fats with solidified unsaturated oils, by adding molecules that form self-assembled networks (Zetzl and Marangoni, 2011). The required consistency is often obtained by structuring the aqueous phase with hydrophilic and/or hydrocolloid gelling agents (Gabriele *et al.*, 2009) or the oily phase by fat crystallisation (Pernetti *et al.*, 2007). As for water-in-oil emulsions, the crystallization of the oil phase is a common technique. It is used to produce "solid fats", such as shortenings, margarines and other biphasic systems for the food industry in order to increase their hardness (Lupi *et al.*, 2011). The new trans-fat regulation (European Parliament, 2016) and scientific results concerning the consumption of TFA force the food industry to find alternative ways to produce structured oil phases. These new solutions are based both on the crystallisation of edible oils (rich in unsaturated compounds) by adding suitable organogel systems, such as triglycerides (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), fatty acids, fatty alcohols, waxes, etc. (Marangoni, 2009), both on the use of low and high melting point fat mixtures (Higaki *et al.*, 2003).

Maltodextrin and polydextrose are two of the most popular carbohydrate-based fat replacers. Carbohydrates-based fat replacers form a gel-like matrix in the presence of substantial levels of water, resulting in lubricant and flow properties like those of fats they replace (Sudha *et al.*, 2007).

2.3 Extra Virgin Olive oil: focus on Calabria region

Over 750 million olive trees are cultivated in the world, most of them (98%) are in the Mediterranean region (Southern Europe, Iberian Peninsula, North Africa and the Near East). In Europe, Spain, Italy, and Greece together produce about 77% of the world's olive oil (Torres *et al.*, 2017). the Mediterranean countries play a very important role considering production, consumption and trade. In 2017/2018 EU countries olive oil production was 2183000 tons. Spain (1256200 tons) is at the first place in olive oil production, followed by Italy (428900 tons) and Greece (346000 tons) (IOC, 2018).

Calabria region (Southern Italy) is one of the major producer of olive oil in Italy (ISMEA, 2018). In 2016, it was the second Italian region (755032 tons) in terms of olive oil production, preceded only by Puglia region (1060500 tons) (ISTAT, 2016). In

Italy, about 500 *Olea europaea* L. cultivars are present (Albertini *et al.,* 2011; Muzzalupo *et al.*, 2014) and several of them are still cultivated in Calabria region.

2.3.1 Extraction process

Extra Virgin Olive Oil (EVOO) is an excellent quality vegetable oil obtained by mechanical extraction from the olive (*Olea europaea* L.) fruit without any thermal or chemical treatments and it can be consumed crude.

Olive oil extraction typically consists of three working steps after defoliation and washing. The first one is the olive crushing, where fruit cells are broken, and the oil released. It is a very important step because during olive crushing the development of the main hydrophilic phenols of virgin olive oils happens thanks to the endogenous catalyser β-glucosidases from the hydrolysis of oleuropein, dimethyl-oleuropein and ligstroside (Obied *et al.,* 2008). The following phase is malaxation, where the breakdown of water-oil emulsion happens, allowing oil droplets to form larger droplets, which separate easily from the aqueous phase during the solid-liquid and liquid-liquid separation processes. Olive oil extraction can be conducted according to one of the following processes: traditional discontinuous press or by centrifugation. The centrifugation is carried out using decanters, that can be classified as follows: traditional three-phases decanters, two-phases decanters (which can operate without the addition of water and do not produce vegetation water as a by-product of the extraction oil process) and new three-phases decanters, working at low water consumption (Klen and Vodopivec*,* 2012; Servili *et al.*, 2012). The last step is the separation of oil from vegetation water, solid particles and mucilage (oily must) by using vertical centrifuges, suitable for separating solid impurities with a specific weight ranging from 1.050 to 1.150 (Servili *et al.*, 2012).

2.3.2 Antioxidant compounds of extra virgin olive oils

Traditionally, the nutritional values of EVOOs are due to the high monounsaturated fatty acid (MUFA) content. However, in recent years several studies demonstrated that EVOO's remarkable antioxidant potential derived also from its high level of phenolic compounds (Servili *et al.*, 2004; Quiles *et al.*, 2006; Servili *et al.*, 2009; Omar, 2010; Obied *et al.*, 2012). In general, virgin olive oil (VOO) is mainly constituted by glycerols that represent more than 98% of the total oil weight. Although minority constituents only represent the remaining 2% of oil weight, they include more than 230 chemical compounds such as aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants (Servili *et al.*, 2004). The main antioxidants of VOO are

carotenes and phenolic compounds that include lipophilic and hydrophilic phenols. The hydrophilic phenols, that in general are not present in other vegetable oils, are secondary plant metabolites that show peculiar sensory and healthy proprieties (Servili *et al.*, 2004). The most important phenolic compounds identified on EVOO could be divided into different groups such as phenolic acids, phenolic alcohols, secoiridoids, lignans, and flavones (Bendini *et al.*, 2007). The phenolic acids, phenyl-alcohols, hydroxy-isochromans and flavonoids are present in small quantities in EVOO (Bendini *et al.*, 2007; Saitta *et al.*, 2009;), while secoiridoids and lignans are the most concentrate EVOO's phenolic compounds. Phenolic acids, secondary aromatic plant metabolites, contain two distinguishing constitutive carbon frameworks: the hydroxycinnamic and hydroxybenzoic structures. Phenolic acids are simple phenols and were the first group of phenols observed in VOO (Montedoro, 1972; Vasquez-Roncero, 1978). They have been associated with colour and sensory qualities, as well as with the health-related and antioxidant properties of foods (Robbins, 2003; De Oliveira *et al.*, 2014; Genovese *et al.*, 2018). Phenolic acids found in VOO are: vanillic acid, syringic acid, p-coumaric acid, o-coumaric acid, gallic acid, caffeic acid, protocatechuic acid, p-hydroxybenzoic acid, ferulic acid, cinnamic acid, 4-(acetoxyethyl)-1,2-dihydroxybenzene, benzoic acid, hydroxy-isocromans (Baccouri *et al.*, 2007; Bendini *et al.*, 2007; Cerretani *et al.*, 2009; Saitta *et al.*, 2009).

Secoiridoids are the main class of phenol compounds present in olive fruit and oil. In particular, oleuropein and ligstroside are the two principal secoiridoids present in olive fruit. During olive oil production, their aglycon derivatives are produced. According to several authors (Ghanbari *et al.*, 2012), the most abundant secoiridoids of VOO are the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-dihydroxyphenyl-ethanol) or tyrosol (p-hydroxyphenyl-ethanol), respectively 3,4-DHPEA or p-HPEA, and an isomer of the oleuropein aglycon 3,4-dihydroxyphenylethanol linked to elenolic acid (3,4-DHPEA-EA). Oleuropein, ligstroside aglycon and their dialdehydic forms were also detected as minor hydrophilic phenols of VOO (Owen *et al.*, 2000; Rovellini and Cortesi, 2002). Hydroxytyrosol and Tyrosol are the main phenolic alcohols of VOO; their concentration is usually low in fresh oils but increases during oil storage (Cerretani *et al.*, 2009). In fact, as reported by Brenes *et al.* (2001) during oil storage 3,4-DHPEA and p-HPEA content increases due to the hydrolysis of VOO secoiridoids such as 3,4-DHPEA-EDA (3,4-dihydroxyphenyl-ethanol linked to dialdehydic form of elenolic acid), p-HPEA-EDA (p-

hydroxyphenylethanol linked to dialdehydic form of elenolic acid) and 3,4-DHPEA-EA into hydroxytyrosol and tyrosol.

2.4 Lipid Oxidation

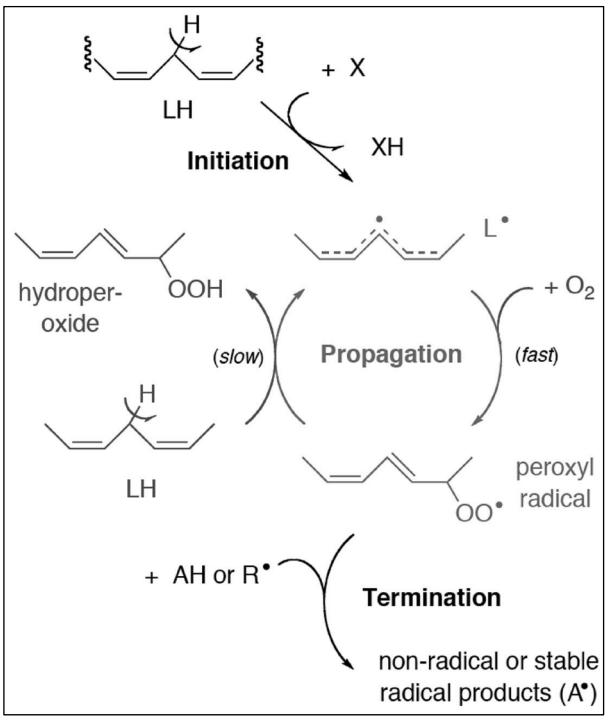
Oxidation is the most important alteration that can take place on fat, involving the production of undesirable compounds, responsible for unpleasant odours and flavours in food and the consequent decay of organoleptic characteristics. Some of these products have been accused of being toxic and constitute a potential danger to the health of consumers (Cabras and Martelli, 2004). Fats are often used in high temperature preparations, both in the industrial and domestic sectors. These conditions (temperatures of 160-180 °C) support oxidation reactions, with consequent production of oxidised species, especially peroxides, from which derive aldehydes, ketones and acids responsible for the characteristic smell of fried and rancid fats (Tirosh *et al.*, 2015).

Fat oxidation reaction consists in the oxidation of fatty acid molecules by radical or not-radical reactive oxygen species (ROS) (Choe and Min, 2006a). This oxidation leads to the formation of free radicals from fatty acids and therefore activates a chain oxidation of the remaining non-oxidized fatty acids, it is an auto-catalytic reaction. Factors that favour it are oxygen, heat, light, metals, enzymes and modifications by microorganisms (Choe and Min, 2006b). Some ROS, although not radical, are strong oxidants and this leads to the damage/attack of other molecules with the formation of free radicals.

2.4.1 Autoxidation

The most important of the processes involved in the oxidative degradation of lipids is the autoxidation of fatty acids. This process can also take place at room temperature, proceeding with a speed directly proportional to the degree of unsaturation; it is also accelerated by high temperatures, as occurs during frying. Autoxidation is a radical chain reaction and can be divided into three distinct processes: initiation, propagation and termination (Figure 1).

Figure 1 Three phases of the autoxidation process



Reproduced from Schneider, 2009.

During the first phase, a radical $L^{\bullet}$ is formed by tearing off a hydrogen atom from the chain of a lipid substrate (LH) (Lee *et al.*, 2004). During the propagation phase, the chain reaction between fatty acid radicals and molecular oxygen leads to the formation and accumulation of the primary hydroperoxide products (LOOH): oxygen and the fatty acid are fed into the cycle to give the hydroperoxide as product (Keller *et al.*, 2015). Termination of the process occurs when either two radicals react giving non-radical products, or when an antioxidant (AH), such as polyphenol or tocopherol, reduces the peroxyl to a hydroperoxide while being transformed into a stable radical ($A^{\bullet}$) (Schneider, 2009). Schematically, the reaction takes place as follows:

- Initiation: $LH^{\bullet} \rightarrow L^{\bullet} + H^{\bullet}$
- Propagation: $L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$
- $LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$
- Termination: $LOO^{\bullet} + LOO^{\bullet} \rightarrow$ non-radical product
- $LOO^{\bullet} + L^{\bullet} \rightarrow$ non-radical product
- $L^{\bullet} + L^{\bullet} \rightarrow$ non-radical product

The energy required to remove hydrogen from lipid molecules depends on its position in the molecules. Allylic hydrogen, especially that attached to the carbon between two double bonds, is easily removed (Min and Boff, 2002).

2.4.2 Photo-oxidation

Exposure to light accelerates the formation of hydroperoxides from unsaturated fatty acids; this can happen in two ways: either by direct photo-oxidation (photochemical oxidation) or by photosensitive oxidation of fatty acids.

In direct photo-oxidation there is the formation of radicals from the exposure to light of fatty acids, even if this reaction is active only when the oils are exposed directly to light.

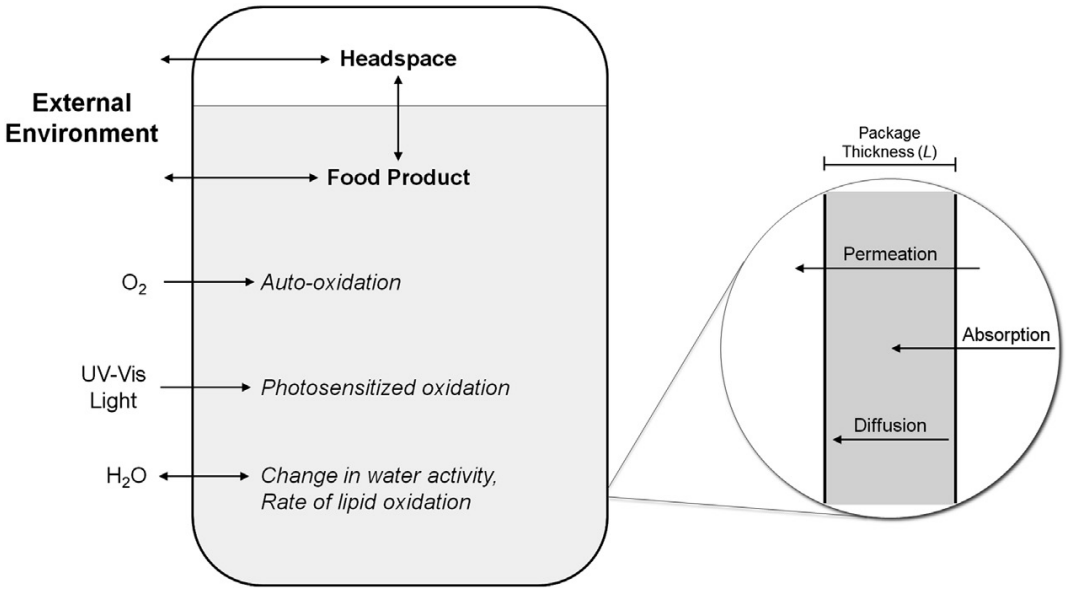
2.5 Methods to prolong the shelf life of bakery products

2.5.1 Packaging of baked goods

Food packaging is often defined as a passive barrier that protects foods from the adverse effects of the external environment, such as microorganisms, water vapour, gases, odours, light, dust, and mechanical forces. Although the functions of functions of packaging materials include convenience and the role in marketing and communication (Aguirre *et al.*, 2013; Majeed *et al.*, 2013; Restuccia *et al.*, 2010), the prevention of lipid oxidation, and its deleterious effects on food quality, is one of the main goals of food packaging. In fact, as well known, lipid oxidation processes are strictly related to the development of off-flavours, altered texture and colour, and reduced nutritional value. Moreover, the oxidation of food products' lipid fraction causes the degradation of fatty acids in addition to the production of toxic compounds for the human body and oxidised polymers. In fact, it is well known in the literature that the intake of lipid oxidation products with the diet can promote the development of several diseases in the human body (Kanazawa *et al.*, 2002; Riemersma, 2002; Kanner, 2007; Dhaka *et al.*, 2011; Vieira *et al.*, 2017).

When a food is contained in a packaging material, many interactions occur: the food product interacts with the headspace and with the external environment, as well as the headspace interacts with the external environment too (Figure 2). Indeed, despite the use of packaging, some diffusing substances can penetrate the packaging through 3 main phases: the permeate dissolves in the film matrix at the higher concentration side, diffuses through the film, driven by a concentration gradient, and evaporates from the other surface (Siracusa, 2012).

Figure 2 Interactions occurring in foods contained in polymer packaging



The passive barrier properties of packaging polymers are of paramount importance as they determine the resistance to the absorption and diffusion of permeant, such as O_2 and water vapour, and consequently the shelf life of foods they contain (Koontz, 2016). Thus, since the evidence that the permeability to some reagents may be the limiting factor in the shelf life of foods, such as baked goods, the choice of packaging material is closely related to this property and to the characteristics of the food to be packaged (Galić *et al.*, 2009; Sancho-Madriz, 2003). The permeability of the packaging material was found to be even more important than leakage sites in the seals (Chung *et al.*, 2003).

Bakery products, like many processed foods, are exposed to physical, chemical and microbiological deterioration. While microbiological deterioration by bacteria, yeasts and molds is the concern in high moisture products, i.e. products with water activity a_w>0.85, physical and chemical deterioration limits the shelf life of baked goods with low and intermediate humidity (Smith *et al.*, 2004). Crackers, biscuits, wafers and savoury snacks have a much longer shelf life in comparison with bread, cakes and doughnuts owing to their low moisture content. For these types of products, the wrapping must provide an excellent moisture barrier to prevent loss of crispness and texture. Moreover, for these driest baked goods, fat rancidity caused by oxygen and moisture is the problem to target. Oxidation of fats and oils in bakery products can lead to the formation of malodorous aldehyde substances such as hexanal and heptanal, which upon the opening of the package will cause rejection of the product (Day, 2008).

Different packaging material is used for different products depending on the type and composition of the product. In Table 3 the distinct packaging materials used for cereal based food packaging are reported.

Table 3 Packaging materials used for cereal based food packaging

Food application	**Packaging materials**
Fresh bread, sandwich	Waxed paper Nitrocellulose coated cellophane (MS) Low density polyethylene (PE-LD) Polypropylene (PP)
Bread bags, sandwich bags, frozen food bags, crusty bread, pies, bread crumbs, biscuits	Linear low-density polyethylene Cellulose/Polyethylene/Cellulose Polyethylene/Polypropylene Paper/Polyvinilydene chloride/Polyethylene Paper/Polyethylene/Polyvinilydene chloride (PAP/PE/PVDC) Oriented polypropylene/Oriented polypropylene (OPP/OPP) Oriented polypropylene/Paper (OPP/PAP) Oriented polypropylene/Paper/Aluminium foil (OPP/PAP/Al) Oriented polypropylene/Aluminium foil/Hotmelt (OPP/Al/Hotmelt) Coextruded oriented polypropylene/Coextruded oriented Polypropylene(OPPcoex/OPPcoex) Coextruded oriented polypropylene/Coextruded metallized oriented polypropylene(OPPcoex/OPPcoexmet) Polyvinylidene chloride coated cellophane (MXXT) Polypropylene (PP)
Cakes, biscuits, crisps, snack foods, biscuits	Aluminium foil/Paper Polyvinilydene chloride coated polypropylene/Polyvinilydene chloride coated polypropylene (PVDC-PP/PVDC-PP) Polypropylene (PP)
Cereal meals, baked products	Paper/Polyethylene Polyethylene terephthalate/Polyethylene (PET/PE) Polyamide (Nylon)/Low density polyethylene (PA/PE-LD)
MAP - Baked products	Polypropylene/Ethylene vinyl acetate (PP/EVAC) Metallized polyethylene terephthalate/Polyethylene (PETmet/PE) Polypropylene/Low density polyethylene/Ethylene vinyl Acetate (PP/PE-LD/EVAC) Oriented poly(ethylene terephthalate)/Polyvinilydene chloride/Polyethylene- Polyvinyl chloride/Polyethylene (OPET/PVDC/PE-PVC/PE) Oriented metalized poly(ethylene terephthalate)/Polyethylene (OPETmet/PE)

	Oriented polyethylene terephthalate/Polyvinilydene chloride/Polyethylene (OPET/PVDC/PE) Polyamide/ Polyethylene (PA/PE)

Polypropylene (PP) is one of the most used packaging materials for baked goods because it has moderate permeability to gases and odours and a higher barrier to water vapour, which is not affected by changes in humidity. These properties make it particularly suitable and widely used to pack biscuits, snack-foods and dried foods (Hirsch, 1991).

2.5.1.1 Active packaging

In recent years, a new concept of packaging has been created: the active packaging. It is a type of packaging that goes beyond the habitual role of packaging, by imparting specific and intentional functionalities to the packaging system. In fact, active packaging is designed in order to extend the shelf life of foods, to improve their preservation and or to increase their safety and quality (Ozdemir and Floros, 2004). Robertson (2006) defined active packaging as "packaging in which subsidiary constituents have been deliberately included in or on either the packaging material or the package headspace to enhance the performance of the package system".

Active packaging was also defined by European Regulation (EC) No 450/2009 as follow: "*'active materials and articles' means materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food".*

Active packaging systems can be divided into active scavenging systems (absorbers) and active-releasing systems (emitters). Table 4 provides an overview of the active packaging technologies, their mechanisms of action and their food applications.

Active packaging systems commonly used to prolong the shelf life of low moisture baked goods such as biscuits and snacks are oxygen scavengers, preservative releasers, moisture absorbers and flavour/odour absorbers.

Table 4 Examples of active packaging systems

Active packaging system	Mechanisms	Food applications
Oxygen scavengers	Iron based Metal/acid Nylon MXD6 Metal (e.g. platinum) catalyst Ascorbate/metallic salts Enzyme based	Bread, cakes, cooked rice, biscuits, pizza, pasta, cheese, cured meats and fish, coffee, snack foods, dried foods and beverages
Carbon dioxide scavengers/emitters	Iron oxide/calcium hydroxide Ferrous carbonate/metal halide Calcium oxide/activated charcoal Ascorbate/sodium bicarbonate	Coffee, fresh meats and fish, nuts and other snack food products and sponge cakes
Ethylene scavengers	Potassium permanganate Activated carbon Activated clays/zeolites	Fruit, vegetables and other horticultural products
Preservative releasers	Organic acids Silver zeolite Spice and herb extracts BHA/BHT antioxidants Vitamin E antioxidant Chlorine dioxide/sulphur dioxide	Cereals, meats, fish, bread, cheese, snack foods, fruit and vegetables
Ethanol emitters	Encapsulated ethanol	Pizza crusts, cakes, bread, biscuits, fish and bakery products
Moisture absorbers	PVA blanket Activated clays and minerals Silica gel	Fish, meats, poultry, snack foods, cereals, dried foods, sandwiches, fruit and vegetables
Flavour/odour absorbers	Cellulose triacetate Acetylated paper Citric acid Ferrous salt/ascorbate Activated carbon/clays/zeolites	Fruit juices, fried snack foods, fish, cereals, poultry, dairy products and fruit
Temperature control packaging	Non-woven plastics Double-walled containers Hydrofluorocarbon gas Quicklime/water Ammonium nitrate/water Calcium chloride/water Super corroding alloys/salt water Potassium permanganate/glycerine	Ready meals, meats, fish, poultry and beverages
Temperature compensating films	Side chain crystallisable polymers	Fruit, vegetables and other horticultural products

Reproduced from Day, 2008

Oxygen scavengers

It is well-known that oxygen in packaging negatively affects the quality and shelf life of several foods because it is involved in the oxidation processes that occur on food products (Choe and Min, 2006a). Oxygen also promotes the growth of aerobic microorganisms and the consequent microbial spoilage (Lee, 2010; Hogan and Kerry, 2008), colour modifications and sensory changes (Rooney, 2005; Gibis and Rieblinger, 2011; Li *et al.,* 2013; Hutter *et al.,* 2016), or nutritional losses (Chung *et al.,* 2004; Lopez-Gomez and Ros-Chumillas, 2010; Van Bree *et al.,* 2012). Oxygen absorbing systems represent good alternatives to vacuum and gas flushing packaging, improving product quality and shelf life. Moreover, they are economically worthwhile in reducing packaging costs and increasing profitability (Ozdemir and Floros, 2004). Most of the

time, these chemical systems react with water supplied by the food to generate a reactive hydrated metallic reducing agent that scavenges oxygen within the food package, converting it to a stable oxide. Commonly the iron powder is contained in a high permeable sachet labelled 'Do not eat'.

Preservative releasers

In recent times, the potential use of antimicrobial and antioxidant packaging films, that have preservative features for prolonging the shelf-life of a wide range of food products, has been investigated (Tian *et al.,* 2013). Even if many patents exist, and some antimicrobial and antioxidant films have been commercialised and used, in the past the greater number of this kind of packaging materials have so far failed to make marketable because of worries about their effectiveness, adverse secondary effects, narrow spectrum of activity, economic factors and/or regulative limitations (Rooney, 1995; Day, 2003; Brody, 2005). Antioxidant and antimicrobial agents may be added to packaging in different forms such as:

- sachets or mats with volatile antimicrobial compounds;
- active substances embedded in the polymer structure;
- active substances applied to the polymer surface;
- active substances immobilized on the polymer using ionic and covalent bonds;
- packaging films, which have antimicrobial properties (e.g. films based on chitosan);
- edible food coatings.

Antioxidants have been incorporated into plastic films (particularly polyolefins) to stabilise the polymer and control oxidative deterioration in food systems (Robertson, 2013). For many years, the research was focused on the potential for evaporative migration of antioxidants from packaging material into foods. The challenge is to find the match between the rate of diffusion and the needs of the food. Butylated Hydroxyanisole (BHA) and the related compound Butylated Hydroxytoluene (BHT) were used by the USA cereal industry as preservatives from waxed paper liners into breakfast cereals and snack products (Labuza and Breene, 1989), and today are widely used in food packaging to prevent lipid oxidation (Torres-Arreola *et al.,* 2007). In recent years there is a growing interest in the addition of natural antioxidants such as plant extracts, essential oils, polyphenols and tocopherols to active packaging materials (Day, 2003; Nerín *et al.,* 2006; Park *et al.,* 2012; Barbosa-Pereira *et al.,* 2014; Marcos *et al.,* 2014). Vitamin E was found to be useful and effective as a replacer of BHA and BHT

since there have been raised doubts regarding BHT and BHA's safety (Van Aardt *et al.*, 2007; Day, 2008). Antioxidant packaging systems containing volatile extracts, essential oils or active components of plants or spices have been developed to improve quality and to extend the shelf life of various food products (Camo *et al.,* 2008; Camo *et al.*, 2011; Busolo and Lagaron, 2015; Carrizo *et al.,* 2016).

Antimicrobial food packaging is characterised by the ability to inhibit the growth of spoilage and pathogenic microorganisms. The most studied and used antimicrobial food packaging systems are: essential oils, enzymes and bacteriocins, antimicrobial polymers and organic acids, their derivatives and other organic compounds. Many synthetic and natural preservatives have been proposed and/or tested for antimicrobial activity in plastic and edible films (Llana-Ruiz-Cabello *et al.,* 2015; Kaya *et al.,* 2015; Soysal *et al.,* 2015; Wen *et al.,* 2016; Yildirim *et al.,* 2018). The major potential food applications for antimicrobial films include meats, fish, bread, cheese, fruit and vegetables.

Moisture absorbers

According to Brody *et al.* (2001), active moisture scavengers can be separated into 2 main categories: relative humidity (RH) controllers that scavenge humidity in the headspace, such as desiccants, and moisture removers that absorb liquids. In baked products packaging desiccants are used to control humidity in the packaging headspace. Examples of desiccants are: silica gel, clays, molecular sieves (synthetic crystalline version, such as from zeolite, sodium, potassium, calcium aluminosilicate), humectant salts (such as sodium chloride, magnesium chloride, calcium sulphate), and other humectant compounds (such as sorbitol); as well as calcium oxide (Cohen, 2003). Each desiccant has its water vapor sorption isotherm and the absorption capacity depends on it (Sängerlaub *et al.,* 2013). Moisture absorbers are commonly contained in sachet and used to maintain low levels of moisture in dried food packages, such as chips, nuts, spices, biscuits, crackers, milk powder, and instant coffee.

Flavour/odour absorbers

Active packaging can also be used to remove undesirable taints or odours from packaged food and several such absorbers were released last century to absorb volatile amines from the breakdown of fish muscle, as well as aldehydes from the autoxidation of fats and oils (Robertson, 2013). Another kind packaging technology for this specific problem represents aldehyde scavengers incorporated into polymers. Examples of these scavengers are nylon, d-sorbitol and alpha-cyclodextrin that can be incorporated into PET and demonstrated selective aldehyde scalping (López-Rubio *et al.,* 2008).

2.5.1.2 Bio-based and biodegradable packaging materials

Except for paper-based products, food packaging materials have traditionally been based on non-renewable materials. Nowadays, an increasing attention is given to sustainability and the replacement of non-renewable resources with those from renewable sources, essentially plant-derived products and by-products from their fermentation. While it could be argued that fossil resources are bio-based and renewable, it takes more than a million years for biomass to be converted into oil that is used as feedstocks to produce plastics (Robertson, 2013). If we consider that the world plastic production has reached more than 250 million tons·year^{-1} during the last years (thermoplastics and polyurethanes), combined with the evidence that packaging products are usually short-term applications, they represent a very big source of plastic wastes (Plastic Europe, 2016). These facts have led to an increasing concern on the use of more sustainable polymers for food packaging purposes known as biopolymers, including bio-based and biodegradable polymers (Avérous, 2004).

The complete biodegradation (sometimes called mineralization) occurs when a material is degraded to carbon dioxide, water, mineral salts and, possibly, other molecules of low molecular weight, by microorganisms or in any case by the action of a biological agent; such degradation must occur, or at least complete, in conditions of aerobiosis. According to EN 13432 (2000), materials that "biodegrade" in specific tests for at least 90% in 6 months are considered compostable.

III MATERIALS AND METHODS

3.1 Research 1: Oxidative stability of Italian sweet products. A comparison between the traditional recipe and an EVOO-modified recipe.

3.1.1 Samples

Figure 3 Italian Cantuccini biscuits

Cantuccini or *Cantucci* are typical Italian sweet products. They are one of the biggest confectioners of the Tuscan tradition but are produced and sold throughout Italy. They are dry biscuits with almonds, obtained by slicing the dough in slices when it is still hot. The producer has declared on the label a 12 months shelf-life. Shortening is the fat commonly used in *Cantuccini*'s recipe. However, given the considerable amount of SFA in this type of fat, it was decided to study the oxidative stability of *Cantuccini* obtained with the original recipe and to compare it with the same type of products obtained replacing part of the shortening with extra virgin olive oil from Calabria region. The extra virgin olive oil used in this research was from Ottobratica cultivar, i.e. an autochthonous cultivar grown in the province of Reggio Calabria. It was obtained by a three-phase extraction system during the harvest year 2015.

Original recipe (OR) and EVOO recipe (ER) *Cantuccini* were prepared in an industrial bakery located in the province of Reggio Calabria (Italy). In brief, wheat flour, sugar, shortening, almonds, baking powder, eggs, flavouring and honey have been mixed in a kneading machine at about 20 °C until a homogeneous dough was obtained. Then, the dough was transferred to a work surface in order to give it a rectangular elongated shape, convex in the upper part. Therefore, the dough was placed in the oven at a temperature of 200 °C for 20 min. the dough, therefore, was placed in the oven at a temperature of 200 °C for 20 min. After this time, it has been extracted from the oven and, consequently, cut into slices to give to *Cantuccini* their characteristic shape and, subsequently, it was placed in the oven at the same temperature for 10 min. Afterwards, *Cantuccini* were extracted from the oven and cooled, before being packaged. The ER was obtained replacing the 75% of shortening with the same amount of extra virgin olive oil.

3.1.2 Physical characterisation of *Cantuccini*

Cantuccini, both OR and ER, were analysed the day of production (*t0*) and then after 2, 4, 6, 8, 10, 12 months (*t2*, *t4*, *t6*, *t8*, *t10* and *t12*, respectively) to obtain information about water activity, moisture content, texture and colour. During each sampling, the lipid fraction was

extracted in order to perform further analyses on it. To perform some analyses, the samples were ground. In these cases, the almonds present in the mixture were removed from the samples before grinding.

3.1.2.1 Moisture content determination

Thermogravimetric analysis is a method of thermal analysis used to determine the loss of weight of a sample exposed to heat over time. In this type of analysis, the sample is weighed before and after exposure to heat. Through the difference between the initial and final weight, the loss of mass of the sample is measured. Moisture content (%) was determined placing the ground sample (about 10 grams) in an oven at 105 °C until constant weight was reached. The analysis was performed in triplicate on different samples.

3.1.2.2 Water activity measurement

Water activity (a_w) is the ratio of the partial vapour pressure of water in a substance (p) with the partial vapour pressure of pure water (p_0) at the same conditions:

$$a_w = \frac{p}{p_0}$$

Water activity values range from 0 (water completely bound) to 1 (pure water). It is one of the most important parameters related to food safety. Water activity represents the amount of water free of bounds in a food. Since the evidence that free water plays a key role in supporting microbial growth, spoilage processes, chemical and enzymatic reactions, it is important to know the water activity of a food system in order to predict and control its shelf-life. Water activity was measured on ground samples using LabMaster-a_w Novasina instrument (Lachen, Switzerland).

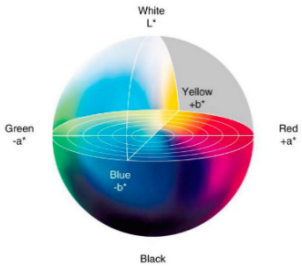
3.1.2.3 Textural Profile Analysis

Textural properties (hardness, g) of *Cantuccini* were evaluated by a Three-point bending test (TPB) using a TA-XT plus Texture Analyser (Stable Micro Systems, Godalming, Surrey, UK). TPB is a destructive test based on the application of a vertical force to obtain texturometric parameters. Each sample was placed on the two strands of the adaptor and the cutting probe was moved vertically until it encounters the sample. Thus, the probe acts as a third contact point, exerting an increasing pressure until the structure of the product breaks. The maximum peak force was used to the calculate hardness value. The experiment was carried out following these conditions: Pre-test speed: 1 mm·s^{-1}, Post-test speed: 15 mm·s^{-1}, Distance: 10 mm, Test speed: 3 mm·s^{-1}.

3.1.2.4 Colour determination

Colour was measured using a reflection colorimeter (Minolta CR 300, Osaka, Japan), previously calibrated, based on CIE *L*a*b** coordinates. The CIE *L*a*b** system (Figure 3) measurement parameters are:

Figure 4 CIELAB colour space



- The *L** coordinate (brightness, perpendicular to the plane of the figure) included, by convention, between 0 (zero brightness, black) and 100 (maximum brightness, is a white element chosen as a reference);
- Component *a** = from green to red (range $-60 \leq a^* \leq +60$):

  $-a^*$ (the intensity of the green colour);
  $+a^*$ (the intensity of the red colour);

- Component *b** = from blue to yellow (range $-60 \leq b^* \leq +60$):

  $-b^*$ (the intensity of the blue colour);
  $+b^*$ (the intensity of the yellow colour);

- The chroma indicates the fullness of an area in proportion to the brightness of a similarly lit object that appears white (or very transmitting, in the case of transparent objects). In brief, Chroma (*C**) represents the degree of saturation or fullness of colour, and it was calculated with the following formula:

$$C^* = \sqrt{(a^{*2} + b^{*2})}$$

On *Cantuccini*, colour was measured both on the inner surface and on the external surface.

3.1.3 Oil extraction

Lipid fraction was cold extracted from the samples according to the method proposed by Folch *et al.* (1957), with some modifications. Lipid fraction was extracted at each sampling time.

In brief, a 250 g aliquot of ground sample was weighed and homogenised with 1125 mL of Chloroform/Methanol (2/1, v/v) at 40°C for 20 min. Thus, it was filtered with filter paper. The filtrate was mixed with 750 ml of a 1 M Potassium Chloride (KCl) solution and was left overnight at 4 °C to achieve phases separation. The lower phase, containing the lipid fraction, was collected, filtered on filter paper and Anhydrous Sodium Sulphate (Na_2SO_4), and subsequently evaporated to dryness with a rotary vacuum evaporator.

3.1.4 Characterisation of fat extracted from *Cantuccini*

3.1.4.1 Acidity Value determination

Acidity values were determined according to European Commission (2015). A 2 g aliquot of oil was placed in a conical flask and dissolved in 50 mL Diethyl Ether/Ethanol (1/1, v/v) mixture neutralised at the moment of use with the 0.1 N Sodium Hydroxide (NaOH) solution with the addition of 0.3 mL of the Phenolphthalein solution (1% in Ethanol) per 100 mL of mixture. The determination was carried out by titration while stirring with the 0.1 N NaOH solution until the indicator changes. Results are expressed as percentage of oleic acid by weight of oil (% oleic acid), and they are equal to:

$$AV = \frac{V \cdot c \cdot M}{10 \cdot m}$$

where:

V is the volume (mL) of titrated NaOH solution used;

c is the concentration in moles per litre of the titrated NaOH solution used;

M is the molar mass in $g \cdot mol^{-1}$ of oleic acid (282 $g \cdot mol^{-1}$);

m is the mass of the sample, expressed in grams.

3.1.4.2 Determination of Fatty Acid Methyl Esters (FAMEs) by Gas Chromatography

Fatty acid methyl esters were prepared according to the European Commission (2015). A 0.1 g aliquot of extracted fat was weighed in a 5 mL screw-top test tube and 2 mL of n-Hexane were added. After shaking, 200 µL of 0.2 N methanolic Potassium Hydroxide solution (KOH) were added and the tube was capped. After vigorous shaking for 30 seconds, the solution was left to stratify until the upper solution become clear. The n-Hexane solution (containing the FAMEs) was placed in 2 mL GC vial crimp top with septum and was ready for gas chromatographic determination. FAMEs were analysed by gas chromatography (Thermo Trace 1300) fitted with a flame-ionization detector (FID) and split injector. The temperature of the split injector was 250 °C, with a split ratio of 35, and the detector temperature was 280 °C. The oven temperature was 100 °C then increased up to 160 °C, at 2 $°C \cdot min^{-1}$, then isothermal at 160 °C for 5 min, and increased until 230 °C at 4 $°C \cdot min^{-1}$ and finally held at 230 °C for10 min. Helium, Air and Hydrogen flows were respectively: 2.7 $mL \cdot min^{-1}$, 350 $mL \cdot min^{-1}$ and 35 $mL \cdot min^{-1}$, kept constant overtime. FAMEs were identified by comparing their retention times with those of pure standards previously injected and with literature data.

3.1.4.3 Spectrophotometric investigation in the ultraviolet

Spectrophotometric investigation in the ultraviolet was carried out to obtain information about the oxidation state of the oil at each sampling time. The determinations of extinction

coefficients (K_{232} and K_{270}) were carried out according to European Commission (2015). The detected absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems resulting from oxidation processes.

A 0.25 g aliquot of the sample was weighed in a 25 mL graduated flask, made up to the mark with the Isooctane and then dissolved. The sample so prepared was placed in rectangular quartz cuvette, having an optical path-length of 10 mm, necessary to perform the reading of the absorbance in a double-beam ultraviolet-visible spectrophotometer. An UV/Vis Spectrometer λ2, Perkin Elmer (Waltham, MA, USA), was used. After the baseline correction with Isooctane in both quartz cells (sample and reference), the absorbances at λ 232 and 270 nm were detected. The following formula was used to calculate the extinction coefficients (Kλ) at the specific wavelengths:

$$K\lambda = \frac{E\lambda}{c \cdot s}$$

where:

$E\lambda$ is the extinction measured at wavelength λ;

c is the concentration (g·100 mL^{-1}) of the solution;

s is the path length (cm) of the quartz cell.

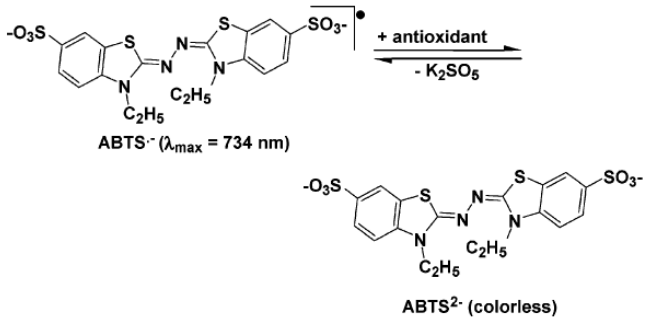
3.1.5 Antioxidant activity of the extracted fat

Antioxidant activity was studied both on and Hydrophilic Antioxidant Extract (HAE) and on the fat as it was. HAE was obtained following the method proposed by Goldsmith *et al.* (2014) with some modification. A 2.5 g aliquot of each sample was mixed for extraction with a 5 mL of Methanol/Water solution (80/20, v/v). After shaking with a Vortex for 1 min, the mixture was centrifuged at 5000 rpm for 7 min. The supernatant containing the antioxidants was kept. The operation was repeated one more time adding 5 mL of Methanol/Water mixture and the two extracts were mixed together to obtain the first HAE. After this, two more HAE were prepared to obtain three different AE from the same oil.

3.1.5.1 ABTS assay on Hydrophilic Antioxidant Extract

The ABTS assay is a spectrophotometric assay based on interaction between antioxidants and ABTS radical cation ($ABTS^{\bullet+}$) generated by reacting a strong oxidizing agent (Potassium Persulfate $K_2S_2O_8$) with the ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt). The blue–green $ABTS^{\bullet+}$ chromophore is a stable radical in absence of antioxidant compounds, but it reacts energetically with a hydrogen-donating antioxidant as a result of which it is converted back to its colourless neutral form (MacDonald-Wicks *et al.,* 2006).

Figure 5 Reaction between ABTS radical and antioxidant



Reported from: Huang *et al.*, 2005

For the determination of the scavenging activity of HAE, the method proposed by Re *et al.* (1999) was used, with some modifications. A 0.025 mL aliquot of HAE was added to 2.475 mL of a 7mM ABTS ethanolic solution and the absorbance was immediately measured (*abs t0*) at 734 nm using an Agilent 8453 spectrophotometer (Santa Clara, CA, USA). After measuring, the mixture was vigorously shaken in the dark for 6 min. Therefore, the absorbance was again measured (*abs t6*). The radical scavenging activity was calculated as % of inhibition using the following formula:

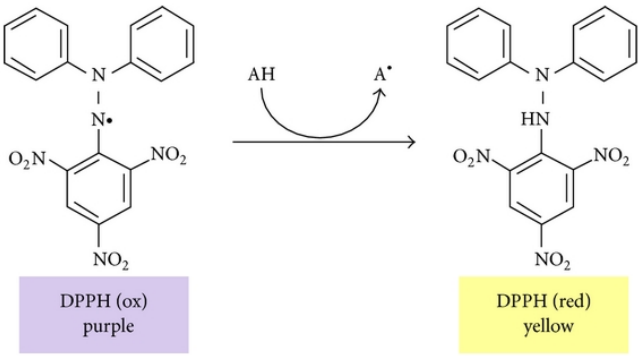
$$\text{ABTS \% inhibition} = \left[\left(\frac{abs\ t0 - abs\ t6}{abs\ t0}\right)\right] \cdot 100$$

The radical scavenging activity (% of inhibition) was plotted against a Trolox calibration curve and results were then expressed as TEAC values (μmol TE·100g^{-1} of fat extracted).

3.1.5.2 DPPH assay on Hydrophilic Antioxidant Extract

DPPH assay is a decolourisation assay first reported by Brand-Williams *et al.* (1995). It is based on measurement of the change of colour at 515 nm that occur when a solution (methanolic solution) of the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) is reducing by antioxidant compounds forming the correspondent yellow hydrazine. The decolourisation is proportional to the concentration of free radical scavenging added to DPPH solution.

Figure 6 Principle of DPPH radical scavenging capacity assay



Reported from: Teixeira *et al.*, 2013

Kalantzakis *et al.* (2006) proposed an application of DPPH assay method to olive oil, and it was used for this work with some modifications. A 0.10 mL aliquot of HAE was added to 2.40 mL of a 60 µM DPPH methanolic solution and the absorbance was immediately measured (*abs t0*) at 515 nm using an Agilent 8453 spectrophotometer (Santa Clara, CA, USA). After measuring, the mixture was vigorously shaken in the dark for 5 min. Thus, the absorbance was again measured (*abs t5*). The radical scavenging activity was calculated as % of inhibition using the following formula:

$$\text{DPPH \% inhibition} = \left[\left(\frac{abs\ t0 - abs\ t5}{abs\ t0}\right)\right] \cdot 100$$

The radical scavenging activity (% of inhibition) was plotted against a Trolox calibration curve and results were then expressed as TEAC values (µmol TE·100g^{-1} of fat extracted).

3.1.5.3 DPPH assay on extracted fat

The DPPH assay on the extracted fat was performed without extracting antioxidant compounds from the fat. It was conducted in an UV/Vis Spectrometer λ2, Perkin Elmer (Waltham, MA, USA), using the method proposed by Kalantzakis *et al.* (2006), modified as follows. First of all, the oil was diluted with Ethyl Acetate (1/10, v/v). Then, 0.25 mL of diluted oil were added to 2.25 mL of a 10^{-4} M DPPH• solution, previously prepared with ethyl acetate. Thus, the absorbance of the mixture was immediately measured at 515 nm (*abs t0*) and after 30 minutes of shaking and incubation in the dark (*abs t30*). The % of inhibition was calculated as follows:

$$\text{DPPH \% inhibition} = \left[\left(\frac{abs\ t0 - abs\ t30}{abs\ t0}\right)\right] \cdot 100$$

The radical scavenging activity (% of inhibition) was compared with a Trolox calibration curve and results were then expressed as TEAC values (µmol TE·100g^{-1} of fat extracted).

3.1.6 Statistics

Data were subjected to analysis of variance (one-way ANOVA, post-hoc Tuckey test $p < 0.05$ using IBM SPSS Statistics 25.0 Software (IBM, Armonk, NY, USA). Analyses were carried out on two batches for each *Cantuccini* recipe (three or five replicates for each batch, as specified in each caption of tables). Values are expressed as means. In rows, different capital letters indicate significant differences between the different recipes. In columns, different small letters indicate significant differences between the different times of storage (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$).

Two-way ANOVA, followed by Tukey HSD test for multiple comparisons, was conducted to examine the effect of time of storage and the recipe modification on the characteristics of *Cantuccini* and on the oxidation of their lipid matrix.

3.2 Research 2: Oxidative stability of breadsticks in relation to the quality of the oil used in the recipe.

3.2.1 Samples

Figure 7 Italian Breadstick snacks

Breadsticks are Italian savoury products. This kind of snack, known in Italy as *Treccine*, are produced and sold throughout Italy. They are dry snacks that can be flavoured in different ways. The producer has declared on the label a 12 months shelf-life. In general, the vegetable oil used in their recipe is a low-quality oil, known as olive pomace oil (OPO). For this study, breadsticks recipe was modified by replacing totally olive pomace oil with EVOO from Calabria. The extra virgin olive oil used was from Ottobratica cultivar which is autochthonous in the province of Reggio Calabria. EVOO was obtained by a three-phase extraction system during the harvest year 2015. Breadsticks' oxidative stability was studied for 12 months, with a sampling every two months, both for OPO and for EVOO recipe.

Four types of breadsticks differently flavoured were used for this study: with garlic and chilli pepper (GP), with onions and olives (OO), with wild fennel seeds (F) (*Foeniculum vulgare* Mill), with potatoes and rosemary (PR). Both OPO and EVOO product typologies were made in a food establishment located in the province of Reggio Calabria (Italy). Manufacturing conditions were the same for each product type, except for the vegetable oils used.

This kind of bakery products are about 20 cm long and characterised by a plaited shape. All formulations were packaged (400 g per package) in a transparent polypropylene film (PP) and the internal atmosphere was air.

3.2.2 Physical characterisation of breadsticks

Breadsticks, both OPO and EVOO, were analysed the day of production (*t0*) and then after 2, 4, 6, 8, 10, 12 months (*t2*, *t4*, *t6*, *t8*, *t10* and *t12*, respectively) to obtain information about water activity, moisture content, texture and colour. During each sampling, the lipid fraction was extracted in order to perform further analyses on it. To perform some analyses, the samples were ground.

3.2.2.1 Moisture content determination

Moisture content (%) was determined as reported in the paragraph 3.1.2.1, placing the ground sample (about 10 grams) in an oven at 105 °C until constant weight was reached. The analysis was performed in triplicate on different samples.

3.2.2.2 Water activity measurement

Water activity was measured on ground samples using LabMaster-a_w Novasina instrument (Lachen, Switzerland), as reported in the paragraph 3.1.2.2 of this thesis.

3.2.2.3 Textural Profile Analysis

Textural properties (hardness, g) of breadsticks were evaluated by a Three-point bending test (TPB) using a TA-XT plus Texture Analyser (Stable Micro Systems, Godalming, Surrey, UK), following the same conditions reported in the paragraph 3.1.1.3.

3.2.2.4 Colour determination

Colour determination regarded the measurement of L^*, a^*, b^*, and C^* parameters. On breadsticks, colour was measured as reported in the paragraph 3.1.2.4, but only on the external surface.

3.2.3 Oil extraction

Lipid fraction was cold extracted from the samples according to the method proposed by Folch *et al.* (1957), with some modifications. Lipid fraction was extracted at each sampling time, following the conditions reported in the paragraph 3.1.3.

3.2.4 Characterisation of fat extracted from breadsticks

3.2.4.1 Acidity Value determination

Acidity values were determined according to European Commission (2015), following the conditions reported in the paragraph 3.1.4.1. Results are expressed as percentage of oleic acid by weight of oil, and they are equal to:

$$AV = \frac{V \cdot c \cdot M}{10 \cdot m}$$

where:

V is the volume (mL) of titrated NaOH solution used;

c is the concentration in moles per litre of the titrated NaOH solution used;

M is the molar mass in g·mol^{-1} of oleic acid (282 g·mol^{-1});

m is the mass of the sample, expressed in grams.

3.2.4.2 Determination of Fatty Acid Methyl Esters (FAMEs) by Gas Chromatography

Fatty acid methyl esters were prepared according to the European Commission (2015), following the conditions describe in the paragraph 3.1.4.2. FAMEs were analysed by gas chromatography (Thermo Trace 1300) fitted with a flame-ionization detector (FID) and split injector. The temperature of the split injector was 250 °C, with a split ratio of 35, and the detector temperature was 280 °C. The oven temperature was 100 °C then increased up to 160 °C, at 2 °C·min^{-1}, then isothermal at 160 °C for 5 min, and increased until 230 °C at 4 °C·min^{-1} and finally held at 230 °C for10 min. Helium, Air and Hydrogen flows were respectively: 2.7 mL·min^{-1}, 350 mL·min^{-1} and 35 mL·min^{-1}, kept constant overtime. FAMEs were identified by comparing their retention times with those of pure standards previously injected and with literature data.

3.2.4.3 Peroxide Value determination

The determinations of peroxide values (PV) were carried out according to European Commission (2015). A 3 g aliquot of oil was weighed in a flask, with ground neck and stopper, and 10 mL of Chloroform were used to dissolve the test portion rapidly by stirring. 15 mL of Acetic Acid were added, and inert gas (N_2) was insufflated through the mixture. Then, 1 mL of Potassium Iodide (KI) saturated solution was added, and the stopper was quickly inserted. After shaking for one minute, the sample was left for exactly five minutes in the dark. About 75 mL of distilled water were added and the determination was carried out titrating the liberated iodine with the 0.01 N Sodium Thiosulphate ($Na_2S_2O_3$) solution, shaking vigorously and using a 1% starch solution as indicator.

The peroxide value (PV), expressed in milliequivalents of active oxygen per kilogram of oil (meq O_2·kg^{-1}), is given by the following formula:

$$PV = \frac{V \cdot T \cdot 1000}{m}$$

where:

V is the number of mL of the standardised $Na_2S_2O_3$ solution used for the test;

T is the exact molarity (mol·L^{-1}) of the $Na_2S_2O_3$ solution used;

m is the mass of the sample, expressed in grams.

3.2.4.4 Spectrophotometric investigation in the ultraviolet

Spectrophotometric investigation in the ultraviolet was carried out to obtain information about the oxidation state of the oil at each sampling time. The determinations of extinction coefficients (K_{232} and K_{270}) were carried out according to European Commission (2015), and in accordance with what described in the paragraph 3.1.4.3.

3.2.5 Antioxidant activity of the extracted fat

Antioxidant activity was studied both on and Hydrophilic Antioxidant Extract (HAE) and on the fat as it was. HAE was obtained following the method proposed by Goldsmith *et al.* (2014) with some modifications, according to the conditions described in the paragraph 3.1.5.

3.2.5.1 ABTS assay on Hydrophilic Antioxidant Extract

For the determination of the scavenging activity of HAE, the method proposed by Re *et al.* (1999) was used, with some modifications. A 0.050 mL aliquot of HAE was added to 2.450 mL of a 7mM ABTS ethanolic solution and the absorbance was immediately measured (*abs t0*) at 734 nm using an Agilent 8453 spectrophotometer (Santa Clara, CA, USA). After measuring, the mixture was vigorously shaken in the dark for 6 min. Therefore, the absorbance was again measured (*abs t6*). The radical scavenging activity was calculated as % of inhibition using the following formula:

$$\text{ABTS \% inhibition} = \left[\left(\frac{abs\ t0 - abs\ t6}{abs\ t0}\right)\right] \cdot 100$$

The radical scavenging activity (% of inhibition) was plotted against a Trolox calibration curve and results were then expressed as TEAC values (μmol TE·100g^{-1} of fat extracted).

3.2.5.2 DPPH assay on Hydrophilic Antioxidant Extract

The antioxidant activity was determined following the method proposed by Kalantzakis *et al.* (2006) and modified as reported in the paragraph 3.1.5.2.

3.2.5.3 DPPH assay on extracted fat

The DPPH assay on the extracted fat was performed without extracting antioxidant compounds from the fat. It was conducted in an UV/Vis Spectrometer λ2, Perkin Elmer (Waltham, MA, USA), using the method proposed by Kalantzakis *et al.* (2006), modified as follows. First of all, the oil was diluted with Ethyl Acetate (1/10, v/v). Then, 0.5 mL of diluted oil were added to 2 mL of a 10^{-4} M DPPH$^{\bullet}$ solution, previously prepared with ethyl acetate. Thus, the absorbance of the mixture was immediately measured at 515 nm (*abs t0*) and after 30 minutes of shaking and incubation in the dark (*abs t30*). The % of inhibition was calculated as follows:

$$\text{DPPH \% inhibition} = \left[\left(\frac{abs\ t0 - abs\ t30}{abs\ t0}\right)\right] \cdot 100$$

The radical scavenging activity (% of inhibition) was compared with a Trolox calibration curve and results were then expressed as TEAC values (μmol TE·100g^{-1} of fat extracted).

3.2.6 Statistics

Data were subjected to analysis of variance (one-way ANOVA, post-hoc Tuckey test $p < 0.05$ using IBM SPSS Statistics 25.0 Software (IBM, Armonk, NY, USA). Analyses were carried out on two batches for each *breadsticks* recipe (three or five replicates for each batch, as specified in each caption of tables). Values are expressed as means. In rows, different capital letters indicate significant differences between the different recipes. In columns, different small letters indicate significant differences among the different times of storage (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$).

Two-way ANOVA, followed by Tukey HSD test for multiple comparisons, was conducted to examine the effect of time of storage and the recipe modification on the characteristics of breadsticks and on the oxidation of their lipid matrix.

3.3 Research 3: Oxidative stability of bakery products stored in different packaging films at room temperature

3.3.1 Samples

Taralli, typical south Italy bakery products, were prepared in an artisan bakery located in the province of Reggio Calabria (Italy). *Taralli* are artisanal products and their shelf life is of 6 months. The traditional recipe was modified using extra virgin olive oil. The extra virgin olive oil used was from *Grossa di Gerace* (autochthonous in Reggio Calabria province) and produced during the harvest year 2017. In brief, wheat flour, water, extra virgin olive oil (20%) and salt have been mixed in a kneading machine at about 20 °C for 20 min and a homogeneous dough was obtained. Then, the dough was left to rest for 30 min and, after this time, it was transferred to a work surface to give the characteristic shape to *Taralli*. They were baked on a baking tray at 230 °C for 20 min.

Figure 8 Italian Taralli snacks

After baking, *Taralli* were packaged (150 g per package) in four different ways: polypropylene (PP), poly-lactic acid (PLA) as biodegradable polymer, PLA with moisture absorbent and a metallic film (Doypack®Clicky Clear) with lowest permeability to water vapour and oxygen and opaque to light. Samples were named as the packaging films used:

- PP (sample packaged with polypropylene film);
- PLA (sample packaged with poly-lactic acid);

- PLA+ABS (sample packaged with poly-lactic acid and a moisture absorber);
- BARR (sample packaged with an opaque film).

3.3.2 Packaging Data Sheets or Packaging characteristics

Oxygen transmission rate (OTR) and water vapour transmission rate (WVTR) are two key material specification properties which determine the shelf life of food contained in the packaging. OTR is the steady state rate at which oxygen gas permeates through a film at specified conditions of temperature and relative humidity. WVTR is the standard indicator of how easily moisture can permeate a packaging film.

The characteristics of the packaging used for this research are reported in Table 5.

Table 5 OTR and WVTR of the packaging material used

Material	Code	*OTR* *($cm^3 \cdot m^{-2} \cdot 24\ hr^{-1}$)*	*WVTR* *($g \cdot m^{-2} \cdot 24\ hr^{-1}$)*
Polypropylene	PP	160	0.25
Polylactic Acid	PLA	730	270
Doypack®Clicky Clear	PET12/ALU8/PE100	0.1	0.01

Doypack®Clicky Clear has very low OTR and WVTR. Polypropylene has poor oxygen barrier but moderate moisture barrier properties, while PLA is characterised by high OTR and WVTR. In order to reduce PLA's WVTR, it was tested also in association with a moisture absorber. The moisture absorber was Silicon Dioxide (SiO_2), also known as Silica Gel. It was contained in small paper bags with a "do not eat" warning. This moisture absorber is often included in dry food packages to absorb any humidity that might cause spoilage of the food. Silicon Dioxide pellets can absorb the 10%, 20% or 35% of moisture at 20%, 40% or 80% of Relative Humidity (RH), respectively.

For the present work, these packaging materials were studied in order to compare them in relation to the shelf life of the products they contain (*Taralli*).

3.3.3 Physico-chemical characterisation of *Taralli*

Taralli were analysed the day of production (*t0*) and then after 15, 45, 70, 90 and 180 days (*t15*, *t45*, *t70*, *t90* and *t180*, respectively) to obtain information about moisture content, water activity and texture; after 45, 90 and 180 days to investigate induction time, volatile organic compounds and to extract the oil in order to perform further analyses on it.

3.3.3.1 Moisture content determination

Thermogravimetric analysis is a method of thermal analysis used to determine the loss of weight of a sample exposed to heat over time. In this type of analysis, the sample is weighed before and after exposure to heat. Through the difference between the initial and final weight, the loss of mass of the sample is measured. Moisture content (%) was determined by the MA160 Infrared Moisture Analyser (Sartorius, Germany). This analyser uses infrared rays that penetrate the samples without being impeded and heat the samples at 105 °C. Samples were grounded and 5 g were placed in disposable sample pans for the measurement. Analyses were conducted in duplicate.

3.3.3.2 Water activity measurement

Water activity (a_w) is the ratio of the partial vapour pressure of water in a substance (p) with the partial vapour pressure of pure water (p_0) at the same conditions:

$$a_w = \frac{p}{p_0}$$

Water activity values range from 0 (water completely bound) to 1 (pure water). It is one of the most important parameters related to food safety. Water activity represents the amount of water free of bounds in a food. Since the evidence that free water plays a key role in supporting microbial growth, spoilage processes, chemical and enzymatic reactions, it is important to know the water activity of a food system in order to predict and control its shelf-life. Water activity was measured using LabMaster-a_w Novasina instrument (Lachen, Switzerland).

3.3.3.3 Textural Profile Analysis

Textural properties (hardness, g) of *Taralli* were evaluated by a Three-point bending test (TPB) using a TA-XT plus Texture Analyser (Stable Micro Systems, Godalming, Surrey, UK). TPB is a destructive test based on the application of a vertical force to obtain texturometric parameters. Each sample was placed on the two strands of the adaptor and the cutting probe was moved vertically until it encounters the sample. Thus, the probe acts as a third contact point, exerting an increasing pressure until the structure of the product breaks. The experiment was carried out following these conditions: Pre-test speed: 1 mm·s^{-1}, Post-test speed: 15 mm·s^{-1}, Distance: 10 mm, Test speed: 3 mm·s^{-1}.

3.3.3.4 Induction time measurement

Oxidation of lipids and the generation of free radicals are natural phenomena in biological and food systems, especially in food containing high amount of lipids (Nanditha and Prabhasankar, 2009). Oxidative stability was measured on 0.5 g of ground *Taralli* following

the Rancimat method (Application Bulletin 204/2 e), according to AOCS official method (Cd 12b−92), by using the 892 Professional Rancimat instrument (Metrohm, Switzerland). The heating block temperature was set at 120 °C and the air flow was 20 $L \cdot h^{-1}$. Effluent air containing volatile organic acids from the sample were collected in a measuring vessel containing milli-Q water (80 mL). The conductivity of the water was measured continuously. From the conductivity curve, the induction time (IT) in hours was calculated by means of the second derivate of this curve, setting the sensitivity at 1.

The induction time (IT) is the time needed for the sample to start oxidising rapidly and coincides with the time required until secondary reaction products are detected by the instrument.

3.3.4 Volatile Profile

Volatile Organic Compounds (VOCs) are a group of aliphatic and aromatic compounds characterised by low molecular weights and low boiling points (Fleming-Jones and Smith, 2003). VOCs can have different origins, including combustions or industrial emissions (Biedermann *et al.*, 1996; Barabad *et al.*, 2018), and are widely diffused in air, water and soil. For this reason, and for their lipophilic properties, they are present and characterise food products. VOCs contribute to the formation of an aroma of each kind of food. During food storage many changes occurs on flavour depending on numerous reactions. Although for some food products, such as cheeses or wines, these changes could be positive, in general they are undesirable.

Baking process involves the formation of a considerable number of volatile compounds that play a key role in the aroma development of the product.

Analysis of VOCs was performed following the Headspace – Solid Phase Micro Extraction (HS-SPME) method proposed by Vichi *et al.* (2003) with some modifications.

HS-SPME Sampling of Volatile Compounds

A 2 g aliquot of ground sample were placed in a 20 mL vial fitted with a silicone septum. The SPME sampling was performed by exposing the divinylbenzene/Carboxen/polydimethylsiloxane fiber (50/30 µm, 2 cm long from Supelco Ltd., Bellefonte, PA) for 30 min in the headspace of the sample maintained in a water bath at 40 °C under magnetic stirring.

The fiber was then desorbed for 5 min at 260 °C in the gas chromatograph injection port.

GC-MS Analysis

Volatile compounds were identified by gas chromatography coupled to quadrupole mass selective spectrometry using an Agilent 5973 Network detector (Agilent Technologies, Palo

Alto, CA, USA). The injections were performed in an Agilent Technologies 6890N Network GC System equipped with a split-splitless injection port, set in splitless mode. Analytes were separated on a SUPELCOWAX® 10 Capillary GC Column (60 m·0.25 mm, d_f 0.25 μm). Helium was the carrier gas with a total flow of 1.5 mL·min^{-1}. For the analysis of the volatile profile, column temperature was held at 40 °C for 10 min, increased to 150 °C at 3 °C·min^{-1} and 250 °C at 15 °C·min^{-1}. Volatile components were identified by comparing their mass spectra with the mass spectra of Wiley 6 and Wiley 138 libraries.

3.3.5 Oil extraction

Lipid fraction was cold extracted from the samples according to the method proposed by Folch *et al.* (1957), with some modifications. Lipid fraction was extracted at *t0* (the day of production of *Taralli*, after baking) and at *t45*, *t90* and *t180* (after 45, 90 and 180 days, respectively).

A 70 g aliquot of ground sample was weighed and transferred to a specific KINEMATICA Polytron™ PT 3100D Homogenizer vessel where 350 mL of Chloroform/Methanol (2/1, v/v) were added and the whole was homogenized at 20000 rpm for 40 seconds. The mixture was decanted and then filtered with Whatman nº1 filter, under vacuum. The residue was extracted twice more: the first one with 250 mL of Chloroform/Methanol (2/1, v/v) and the second with 100 mL of Chloroform. The extract was then divided into centrifuge tubes and an 8% sodium chloride (NaCl) solution was added, with a 4:1 extraction ratio. After centrifugation for 20 min at 2200 rpm, the lipophilic fraction was separated, and 100 g of Anhydrous Sodium Sulphate were added. After 30 minutes, the lipophilic fraction was filtered with the Whatman nº1 filter and the solvent was removed at 35 °C by a rotary vacuum evaporator. The oil extracted was kept in Nitrogen stream for the whole night. The lipid fraction thus obtained was divided into vials and frozen at -20 ºC until the time of analysis.

3.3.6 Characterisation of oil extracted from *Taralli*

3.3.6.1 Acidity Value determination

Acidity values were determined according to European Commission (2016). A 2 g aliquot of oil was placed in a conical flask and dissolved in 25 mL Diethyl Ether/Ethanol (1/1, v/v) mixture neutralised at the moment of use with the 0.1 N Potassium Hydroxide (KOH) solution with the addition of 0.3 mL of the Phenolphthalein solution (1% in Ethanol) per 100 mL of mixture. The determination was carried out by titration while stirring with the 0.1 N KOH solution until the indicator changes. Results are expressed as percentage of oleic acid by weight of oil, and they are equal to:

$$AV = \frac{V \cdot c \cdot M}{10 \cdot m}$$

where:

V is the volume (mL) of titrated KOH solution used;

c is the concentration in moles per litre of the titrated KOH solution used;

M is the molar mass in $g \cdot mol^{-1}$ of oleic acid (282 $g \cdot mol^{-1}$);

m is the mass of the sample, expressed in grams.

3.3.6.2 Peroxide Value determination

The determinations of peroxide values (PV) were carried out according to European Commission (2016). A 2 g aliquot of oil was weighed in a flask, with ground neck and stopper, and 10 mL of Chloroform were used to dissolve the test portion rapidly by stirring. 15 mL of Acetic Acid were added, and inert gas (N_2) was insufflated through the mixture. Then, 1 mL of Potassium Iodide (KI) saturated solution was added, and the stopper was quickly inserted. After shaking for one minute, the sample was left for exactly five minutes in the dark. About 75 mL of distilled water were added and the determination was carried out titrating the liberated iodine with the 0.01 N Sodium Thiosulphate ($Na_2S_2O_3$) solution, shaking vigorously and using a 1% starch solution as indicator.

The peroxide value (PV), expressed in milliequivalents of active oxygen per kilogram of oil (meq $O_2 \cdot kg^{-1}$), is given by the following formula:

$$PV = \frac{V \cdot T \cdot 1000}{m}$$

where:

V is the number of mL of the standardised $Na_2S_2O_3$ solution used for the test;

T is the exact molarity ($mol \cdot L^{-1}$) of the $Na_2S_2O_3$ solution used;

m is the mass of the sample, expressed in grams.

3.3.6.3 Spectrophotometric investigation in the ultraviolet

Spectrophotometric investigation in the ultraviolet was carried out to obtain information about the oxidation state of the oil at each sampling time. The determinations of extinction coefficients (K_{232} and K_{268}) were carried out according to European Commission (2016). The detected absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems resulting from oxidation processes.

A 0.25 g aliquot of the sample was weighed in a 25 mL graduated flask, made up to the mark with the Isooctane and then homogenised. The sample so prepared was placed in rectangular quartz cuvette, having an optical path-length of 10 mm, necessary to perform the reading of the absorbance in a double-beam ultraviolet-visible spectrophotometer (UV-VIS-NIR

SHIMADZU UV-3600). After the baseline correction with Isooctane in both quartz cells (sample and reference), the absorbances at 232 and 268 nm were detected. The following formula was used to calculate the extinction coefficients (Kλ) at the specific wavelengths:

$$K\lambda = \frac{E\lambda}{c \cdot s}$$

where:

$E\lambda$ is the extinction measured at wavelength λ;

c is the concentration ($g \cdot 100\ mL^{-1}$) of the solution;

s is the path length (cm) of the quartz cell.

3.3.6.4 α-Tocopherol analysis by High Pressure Liquid Chromatography

α-Tocopherol content ($mg \cdot kg^{-1}$) was determined by HPLC using an AOCS official method (2009).

In a 5 mL volumetric flask, 0.75 g of oil were weighed and dissolved with n-Hexane HPLC grade. Then, it was made up until volume with the same solvent. The sample so prepared was filtered with a PTFE filter (25mm · 0.45 μm) and kept away from light. Chromatographic determination of α-Tocopherol was performed using a liquid chromatograph Series 1100 (Hewlett-Packard, Waldbronn, Germany) with a 20 μL volume loop and a Luna column (4.6 mm i.d. × 150 mm; Phenomenex, USA) packed with 3 μm–100Å silica and with pre-column (Phenomenex security Guard Cartridge silica 4 × 3.0 μm). α-Tocopherol was isocratically eluted with n-Hexane/1,4-Dioxane (95/5, v/v) and detected using a Hewlett-Packard 1046A spectrofluorometric detector.

3.3.6.5 Total phenols content determination by High Pressure Liquid Chromatography

Phenolic compounds ($mg \cdot kg^{-1}$) were determined by HPLC using the method proposed by Pirisi *et al.* (1997), with few modifications. An aliquot of 1.5 g of oil was dissolved in a centrifuge tube with 0.5 mL of *n*-Hexane HPLC grade, and 1.5 mL of Methanol/Water (60/40, v/v) HPLC grade were added. After shaking with a Vortex, it was centrifuged at 5000 rpm for 5 min, and the separation between hydrophilic and lipophilic phases was obtained. The first one was collected and placed in another centrifuge tube; 1 mL of Methanol/Water (60/40, v/v) was added to the lipophilic phase, that was shaken and centrifuged at 5000 rpm for 5 min, twice more. The hydrophilic phase was recollected, every time, in the other centrifugation tube, where 1.5 mL of *n*-Hexane were added. After shaking and centrifugation, the hydrophilic phase was collected in a round-bottom flask and the solvent was removed under vacuum using a rotary evaporator. The sample was re-dissolved in 500 μL of Methanol/Water (1/1, v/v) HPLC grade, and it was ready to be analysed. HPLC analysis were performed using

a system equipped with a DAD detector, two pumps and a 20 µl injection loop; the HPLC was controlled by a PC running Clarity software. A reversed phase C18 column TSK gel ODS-100Z 5 µm (25 cm · 4.6 mm ID, 5 µm), was used and the column was kept at 30 °C using a column thermostat.

Mobile phase A was Ultrapure Water adjusted to pH 3 with Formic Acid, while mobile phase B was Acetonitrile acidified with Formic Acid up to pH 3. The flow rate was 1 $mL \cdot min^{-1}$ and the separation was obtained using the gradient reported in Table 6:

Table 6 Flow gradient during HPLC analyses

Time (min)	%A	%B
0 – 20	95	0
20 – 50	60	40
50 – 60	5	95
60 – 70	95	5

The wavelengths used were: a) 280 nm for Hydroxytyrosol, Protocatechuic Acid, Catechin and Epicatechin; b) 254 nm for Vanillic Acid; c) 305 nm for Ferulic Acid. All compounds were quantified with external calibration curves.

Phenols were quantified by HPLC-DAD method and were expressed as Total Phenolic Content. The colorimetric method was not applied because it could be subjected to interferences due to the incapability of Folin-Ciocalteu reagent to measure lipophilic antioxidants. This is due to the high affinity of the Folin-Ciocalteu chromophore, that is, multivalent-charged phospho-tungsto-molybdate, toward water (Cerretani *et al.*, 2009; Berker *et al.*, 2013; Bastola *et al.*, 2017).

3.3.7 Statistics

Data were subjected to analysis of variance (one-way ANOVA, post-hoc Tuckey test $p < 0.05$ using IBM SPSS Statistics 25.0 Software (IBM, Armonk, NY, USA). Values are expressed as means of two replicates. In columns, different capital letters indicate significant differences among the different packaging films. In rows, different small letters indicate significant differences among the different times of storage (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$).

Two-way ANOVA, followed by Tukey HSD test for multiple comparisons, was conducted to examine the effect of time of storage and packaging on the characteristics of *Taralli* and on the oxidation of their lipid matrix.

IV RESULTS

4.1 Research 1

4.1.1 Effect of storage time and recipe modification on physical characteristics of *Cantuccini*

Water activity and moisture content of *Cantuccini* are reported in Table 7. Both parameters significantly increased during storage duration. a_w significantly increased ($p < 0.001$) both for OR and for ER. This is in accordance with Cauvain and Young (2000), who stated that in a given product, as the moisture content increases or decreases, the water activity increases or decreases accordingly. Although at the end of the storage (*t10* and *t12*) no differences were found between OR and ER samples, significant differences were found at the beginning of the storage, until *t8*. In particular, samples with EVOO in their recipe (ER) showed lower a_w values. Water activity gives us important information since it accounts for the availability of water for degradation reactions (Mathlouthi, 2001). a_w data indicate that both recipes led to results within the critical a_w limit of 0.430 for biscuits and crackers identified by Labuza and Contreras-Medellin (1981). The same trend was found for moisture content (%) that significantly increased for both recipes, and was significantly higher in OR samples, with the exception for *t4*. According to Bertagnolli *et al.* (2014), cookies with low moisture content will have longer shelf life if they are stored with appropriate packaging and proper storage condition. Thus, substitution of the 75% of shortening for EVOO in the preparation of cookies will produce more shelf-stable product due its lower moisture content.

In Table 8, hardness (g) values are reported. Although no statistical differences were found at *t0* between OR and ER samples, it is possible to notice that the ER sample, in which the shortening has been partially substituted with EVOO, has a higher hardness. This agreed with findings of Jacob and Leelavathi (2007) about cookies containing liquid oil: in fact, it was evidenced that they had a relatively harder texture compared to the ones containing bakery and hydrogenated fat. *Cantuccini*'s hardness increased up to 6 months of storage, but then decreased, and the mean values were similar to the initial ones. The changes undergone by food hardness are directly linked to structural changes undergone during its storage and water is one of the main elements responsible for these changes (De Morais *et al.*, 2018).

Table 9 shows the values of the colour parameters L^*, a^*, b^* and Chroma of the external surface. Almost all the samples stand in the clear zone, with values of brightness (L^*) above 50. However, if OR samples maintained more or less constant values, L^* significantly increased for ER samples with storage duration. In all cases the values of a^* are positive, thus indicating the predominance of the colour red over the green. The coordinate b^* assumes also positive values, indicating a strong predominance of the yellow colouration, in disfavour of

the blue. Its values increased for ER samples during storage. Chroma also increased with storage duration, showing that ER samples had a more saturated colour in comparison with OR ones.

In general, no significant differences were found studying the changes during time of the internal surface colour, except for L^* coordinate. All the samples stand in the clear zone, with values of brightness (L^*) above 50. Coordinate a^* values are all positive, but lower than those of the same coordinate found on the external surface. No relevant differences were found comparing the samples obtained with the two different recipes.

Table 7 Effect of time and recipe on water activity and moisture content (%) of Cantuccini

	a_w			Moisture (%)		
time	OR	ER	sign.	OR	ER	sign.
t0	0.283^{aB}	0.223^{aA}	***	4.08^{aB}	3.55^{aA}	**
t2	0.234^{bA}	0.235^{abB}	***	4.18^{aB}	3.74^{aA}	**
t4	0.330^{Bb}	0.266^{bcA}	***	4.32^{a}	4.41^{b}	ns
t6	0.333^{bcB}	0.255^{abA}	***	4.88^{bB}	3.92^{aA}	**
t8	0.343^{cB}	0.295^{cA}	**	4.95^{bB}	3.92^{aA}	***
t10	0.362^{d}	0.344^{d}	ns	5.36^{cB}	4.34^{bA}	***
t12	0.366^{d}	0.378^{d}	ns	6.62^{dB}	4.56^{bA}	***
sign.	***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 8 Effect of time and recipe on Hardness (g) of Cantuccini

	Hardness (g)		
time	OR	ER	sign.
t0	4401^{a}	5619^{c}	ns
t2	3224^{aA}	5405^{cB}	**
t4	5138^{ab}	3659^{ab}	ns
t6	6918^{b}	5261^{c}	ns
t8	4114^{aA}	4568^{bcB}	**
t10	3058^{a}	3117^{a}	ns
t12	4617^{aB}	2950^{aA}	**
sign.	***	***	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 9 Effect of time and recipe on the external surface colour of Cantuccini

	external surface											
	*L**			*a**			*b**			Chroma		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	56.6^{B}	51.9^{aA}	*	13.5	13.9^{c}	ns	29.7	29.1^{a}	ns	32.6	32.2^{ab}	ns
t2	56.7^{B}	53.4^{aA}	**	13.0	12.8^{bc}	ns	30.2	29.8^{a}	ns	32.9	32.4^{ab}	ns
t4	51.7	55.0^{a}	ns	13.8^{B}	12.0^{abcA}	*	29.5	29.0^{a}	ns	32.6	31.4^{a}	ns
t6	49.5	55.4^{a}	ns	12.6	13.7^{bc}	ns	27.5	30.1^{a}	ns	30.3	33.1^{ab}	ns
t8	52.7	55.0^{a}	ns	14.6	13.1^{bc}	ns	31.9	31.8^{ab}	ns	35.1	34.5^{abc}	ns
t10	57.4^{A}	65.7^{bB}	**	12.6	11.1^{ab}	ns	31.6	34.3^{b}	ns	34.0	36.0^{bc}	ns
t12	51.1^{A}	75.5^{cB}	***	13.2^{B}	10.1^{aA}	**	27.8^{A}	35.7^{bB}	*	30.8^{A}	37.1^{cB}	*
sign.	ns	***		ns	**		ns	***		ns	**	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 10 Effect of time and recipe on the internal surface colour of Cantuccini internal surface

	*L**			*a**			*b**			Chroma		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	68.8	66.0^{ab}	ns	4.3	5.8	ns	23.0^{A}	28.5^{B}	*	23.4^{A}	29.1^{B}	*
t2	66.7^{B}	55.9^{aA}	*	4.6^{A}	6.9^{B}	**	23.6^{A}	26.7^{B}	*	24.1^{A}	27.6^{B}	*
t4	65.4	56.1^{a}	ns	5.8	6.9	ns	27.0	26.4	ns	27.6	27.3	ns
t6	69.3	69.9^{b}	ns	5.0	5.4	ns	25.0	28.7	ns	25.5	29.2	ns
t8	64.5	62.6^{ab}	ns	5.5	6.0	ns	25.2^{A}	29.7^{B}	*	25.8^{A}	30.3^{B}	*
t10	60.7^{A}	66.7^{bB}	*	6.7	5.5	ns	26.0	28.6	ns	26.9	29.1	ns
t12	67.7	68.1^{b}	ns	5.0	5.2	ns	25.8^{A}	29.6^{B}	*	26.3^{A}	30.1^{B}	*
sign.	ns	**		ns	ns		ns	ns		ns	ns	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

4.1.2 Effect of time storage and recipe modification on chemical characteristics of the fat extracted from *Cantuccini*

Table 11 shows the data regarding the chemical characterisation of the fat extracted from *Cantuccini*. Results obtained clearly show that the lipid fraction of *Cantuccini* prepared only with shortening (OR) was markedly more degraded than that prepared by substituting shortening with EVOO (75%), with significantly higher values of AV, K_{232} and K_{270}. This was probably due to the higher stability and the higher content of antioxidants (total phenols and carotenoids) that characterised EVOOs (Servili *et al.*, 2004; Quiles *et al.*, 2006; Servili *et al.*, 2009; Omar, 2010; Obied *et al.*, 2012).

Data available in the literature on DPPH and ABTS assays show that they are not always well correlated, and they often don't give the same results because they deal with two different activeness because of the use of two different radicals (Rubio-Senent *et al.*, 2013). For this reason, both assays were considered in this study. As expected, ABTS and DPPH assays, both performed on the hydrophilic antioxidant extract (HAE), gave different responses in terms of antioxidant activity (Table 12). It is interesting to note that for the hydrophilic fraction, the DPPH test gave lower results than the ABTS assay. This is probably due to the different reaction properties of the fat antioxidants extracted in the hydrophilic fraction. Results from the DPPH assay performed on fat were higher than those in the hydrophilic fraction. This was expected as the DPPH assay in total fat also assesses tocopherols which show a synergistic action with phenolics (Espin *et al.*, 2000; Minioti and Georgiou, 2010). Looking at ABTS assay results, we can notice that OR samples showed higher values of TEAC in comparison to ER ones. It could be due to a TEAC overestimation caused by the fact that $ABTS^{\bullet+}$ reacts with any hydroxylated aromatics independent of their antioxidant potential. Thus, the ABTS test is reduced to titration of aromatic OH-groups including those which do not contribute to the antioxidation (Arts *et al.*, 2003; Roginsky and Lissi, 2005). During storage OR samples' TEAC decreased, while no changes were found in ER TEAC. Regarding DPPH assay on HAE, values increased during storage for both recipes. Results about the DPPH assay performed on the fat evidenced that ER samples showed a higher antioxidant capacity, probably due to the tocopherols present in the EVOO used in the recipe.

Tables from 13 to 16 list the most important fatty acids found during the characterisation of the fat extracted from *Cantuccini* and the changes occurring during time. As reported in these tables, the fatty acids composition changed when EVOO was used in the recipe (ER). In fact, in ER samples were characterised already at the beginning of the study by higher values of Margaric Acid (C17), Heptadecenoic Acid (C17:1), Oleic Acid (C18:1ω9) and Vaccenic Acid

(C18:1ω7). Olive oils, in fact, are characterised not only by high amount of oleic acid, but also by the presence of small amounts of other fatty acids, such that found in this study.

The use of EVOO in *Cantuccini* recipe led the reduction of saturated fatty acids (SFAs) and improved the amount of unsaturated fatty acids (UFAs). In OR UFAs amount at *t0* was 61.4%, contrarily to 80.71 % found in ER one. In particular, EVOO improved the amount of monounsaturated fatty acids (MUFAs), that in OR were the 39.16%, becoming the 62.35 % in ER. As a consequence, the UFAs/SFAs ratio moved from 1.59 (OR) to 4.18 (ER).

Table 11 Effect of time and recipe on AV, K_{232} and K_{270} of Cantuccini

	AV (% oleic acid)			*K_{232}*			*K_{270}*		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	1.52^{aB}	1.02^{aA}	**	2.71^{aB}	2.05^{aA}	***	2.31^{bcB}	0.69^{cA}	***
t2	1.54^{aB}	1.08^{aA}	**	2.58^{aB}	2.27^{aA}	***	1.77^{aB}	0.68^{cA}	***
t4	1.61^{abB}	1.12^{abA}	***	2.71^{aA}	3.54^{cB}	**	1.90^{aB}	0.58^{abA}	***
t6	1.78^{bB}	1.20^{bcA}	***	2.75^{aA}	3.22^{bcB}	***	2.28^{bcB}	0.58^{abA}	***
t8	1.98^{cB}	1.19^{bcA}	***	2.80^{aA}	3.19^{bB}	**	2.13^{bB}	0.56^{aA}	***
t10	2.10^{cB}	1.26^{cA}	***	3.24^{b}	3.21^{bc}	ns	2.36^{cB}	0.64^{bcA}	***
t12	2.47^{dB}	1.39^{dA}	***	3.44^{b}	3.39^{bc}	ns	2.57^{dB}	0.67^{cA}	***
sign.	***	***		***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 12 Effect of time and recipe on antioxidant capacity of the fat extracted from Cantuccini

	ABTS assay on HAE TEAC (µmol TE·100g^{-1} fat)			*DPPH assay on HAE µmol TE·100g^{-1} fat*			*DPPH assay on fat µmol TE·100g^{-1} fat*		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	538.79^{cB}	148.95^{A}	***	92.72^{abB}	34.06^{aA}	***	141.38^{aA}	177.81^{bB}	**
t2	583.90^{cB}	142.65^{A}	***	69.73^{aB}	33.40^{aA}	***	140.58^{aA}	181.83^{bB}	***
t4	425.08^{bB}	133.11^{A}	***	73.50^{aB}	32.38^{aA}	***	149.83^{abA}	197.31^{cB}	***
t6	320.47^{aB}	144.13^{A}	***	91.40^{abB}	42.76^{bA}	**	153.97^{bcA}	203.74^{cB}	***
t8	299.35^{aB}	150.43^{A}	**	100.91^{bB}	43.22^{bA}	**	164.15^{cA}	198.97^{cB}	***
t10	294.25^{aB}	150.99^{A}	***	99.90^{bB}	42.82^{bA}	**	162.79^{c}	168.91^{a}	ns
t12	292.40^{aB}	134.38^{A}	***	106.87^{bB}	43.14^{bA}	***	161.04^{cA}	168.76^{aB}	**
sign.	***	ns		**	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 13 Effect of time and recipe on FAMEs composition (%) of the fat extracted from Cantuccini

	C4			*C6*			*C8*			*C10*			*C12*			*C14*			*C16*		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	1.54^{bA}	2.92^{eB}	***	0.17^{dB}	0.02^{A}	***	0.01^{A}	0.07^{B}	***	0.01^{A}	0.07^{cB}	***	0.14^{bA}	0.45^{eB}	***	0.70^{bB}	0.30^{cA}	***	31.34^{cB}	10.99^{cA}	***
t2	1.93^{dA}	2.46^{dB}	***	0.11^{bcB}	0.01^{A}	ns	0.01	0.07	ns	0.01	0.07^{bc}	ns	0.11^{a}	0.30^{a}	ns	0.60^{aB}	0.29^{cA}	***	28.98^{abB}	10.40^{bA}	***
t4	3.15^{fB}	0.74^{aA}	***	0.12^{cB}	0.01^{A}	***	0.01	0.04	ns	0.01^{A}	0.06^{abB}	***	0.10^{aA}	0.35^{bcB}	***	0.58^{aB}	0.25^{abA}	***	28.19^{aB}	10.41^{bA}	***
t6	2.45^{eB}	1.03^{bA}	***	0.13^{cB}	0.01^{A}	***	0.01	0.07	ns	0.01	0.06^{abc}	ns	0.11^{a}	0.36^{d}	ns	0.61^{aB}	0.24^{aA}	***	30.49^{bcB}	9.97^{aA}	***
t8	1.69^{cB}	0.78^{aA}	***	0.12^{cB}	0.01^{A}	***	0.01^{A}	0.05^{B}	***	0.01	0.05^{a}	ns	0.11^{aA}	0.34^{bB}	***	0.61^{aB}	0.28^{bcA}	***	31.11^{cB}	10.74^{cA}	***
t10	0.62^{aA}	1.42^{cB}	***	0.09^{ab}	0.01	ns	0.01	0.05	ns	0.01^{A}	0.05^{aB}	***	0.11^{aA}	0.35^{cB}	***	0.61^{aB}	0.25^{abB}	***	31.28^{cB}	9.97^{aA}	***
t12	0.59^{aA}	1.42^{cB}	***	0.08^{aB}	0.01^{A}	***	0.01^{A}	0.05^{B}	**	0.01^{A}	0.06^{abB}	***	0.10^{aA}	0.35^{cB}	***	0.58^{aB}	0.25^{abA}	***	31.36^{cB}	9.85^{aA}	***
sign.	***	***		***	ns		ns	ns		ns	***		***	***		***	***		***	***	

Table 14 Effect of time and recipe on FAMEs composition (%) of the fat extracted from Cantuccini

	C17			*C17:1*			*C18*			*C18:1ω9*			*C18:1ω7*			*C18:2*		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	0.09^{bcA}	0.11^{cdB}	**	0.04^{aA}	0.17^{bB}	***	4.12^{B}	3.88^{dA}	**	37.55^{aA}	59.02^{aB}	***	0.90^{aA}	1.95^{aB}	***	21.27^{dB}	17.89^{bA}	***
t2	0.09^{abcA}	0.12^{dB}	**	0.04^{abA}	0.18^{bB}	***	3.90^{B}	3.58^{cA}	***	40.12^{cdA}	60.51^{aB}	***	1.13^{abA}	2.03^{aB}	***	20.96^{dB}	17.96^{bcA}	***
t4	0.10^{c}	0.11^{d}	ns	0.04^{aA}	0.17^{bB}	***	5.10	2.31^{a}	ns	40.07^{cA}	63.18^{eB}	***	1.42^{bA}	2.06^{aB}	*	19.18^{a}	18.43^{d}	ns
t6	0.10^{cB}	0.08^{aA}	*	0.04^{abA}	0.15^{aB}	***	3.89^{B}	3.32^{bA}	***	38.66^{bA}	62.32^{dB}	***	1.18^{abA}	2.31^{abB}	***	20.34^{bcdB}	18.20^{cdA}	***
t8	0.08^{aA}	0.11^{bcdB}	**	0.04^{a}	0.18^{b}	ns	3.95^{B}	3.67^{cA}	**	38.89^{bA}	61.61^{cB}	***	1.07^{abA}	2.54^{bB}	**	20.41^{cdB}	17.55^{aA}	***
t10	0.08^{ab}	0.10^{abc}	ns	0.04^{a}	0.17^{b}	ns	3.86^{B}	3.23^{bA}	***	40.56^{cdA}	61.11^{bB}	***	1.38^{bA}	2.22^{abB}	**	19.42^{abcB}	19.12^{eA}	*
t12	0.09^{bc}	0.09^{ab}	ns	0.05^{bA}	0.17^{abB}	***	3.76^{B}	3.21^{bA}	**	40.65^{dA}	61.13^{bB}	***	1.46^{bA}	2.26^{abB}	***	19.32^{ab}	19.28^{e}	ns
sign.	***	***		**	***		ns	***		***	***		**	**		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 15 Effect of time and recipe on FAMEs composition (%) of the fat extracted from Cantuccini

	C18:2TT			*C18:2CT*			*C18:2TC*			*C18:3ω3*			*C18:3ω6*			*C20*		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	0.21^{aB}	0.03^{bA}	***	0.19^{bB}	0.01^{A}	***	0.01^{b}	0.01^{b}	ns	0.53^{c}	0.42^{c}	ns	0.03	0.00	ns	0.27^{cA}	0.29^{cB}	*
t2	0.19^{aB}	0.02^{bA}	***	0.18^{abB}	0.01^{A}	***	0.00^{a}	0.01^{b}	ns	0.47^{bB}	0.40^{cA}	***	0.01	0.00	ns	0.26^{bcA}	0.27^{bcB}	*
t4	0.18^{aB}	0.00^{aA}	***	0.15^{aB}	0.00^{A}	***	0.00^{a}	0.00^{a}	ns	0.43^{abB}	0.34^{abA}	*	0.02	0.00	ns	0.27^{c}	0.27^{bc}	ns
t6	0.20^{aB}	0.00^{aA}	***	0.17^{abB}	0.00^{A}	***	0.01^{b}	0.00^{a}	ns	0.45^{bB}	0.36^{bA}	**	0.03^{B}	0.00^{A}	**	0.27^{c}	0.26^{bc}	ns
t8	0.21^{aB}	0.00^{aA}	***	0.15^{aB}	0.00^{A}	***	0.00^{a}	0.00^{a}	ns	0.42^{ab}	0.40^{c}	ns	0.02	0.00	ns	0.27^{c}	0.29^{c}	ns
t10	0.27^{bB}	0.00^{aA}	***	0.20^{bcB}	0.00^{A}	***	0.00^{a}	0.00^{a}	ns	0.40^{aB}	0.35^{bA}	**	0.01	0.00	ns	0.24^{b}	0.25^{ab}	ns
t12	0.26^{bB}	0.00^{aA}	***	0.22^{cB}	0.00^{A}	***	0.00^{a}	0.00^{a}	ns	0.39^{aB}	0.32^{aA}	**	0.01^{B}	0.00^{A}	*	0.22^{a}	0.22^{a}	ns
sign.	***	***		***	ns		***	***		***	***		ns	ns		***	***	

Table 16 Effect of time and recipe on FAMEs composition (%) of the fat extracted from Cantuccini

	C20			*C20:1*			*C22*			*C22:1*			*C24*		
time	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.	OR	ER	sign.
t0	0.27^{cA}	0.29^{cB}	*	0.20^{e}	0.21^{b}	ns	0.13	0.13^{c}	ns	0.04^{abA}	0.05^{aB}	*	0.08	0.07^{bc}	ns
t2	0.26^{bcA}	0.27^{bcB}	*	0.20^{e}	0.19^{b}	ns	0.13^{B}	0.11^{abA}	**	0.06^{b}	0.07^{ab}	ns	0.08	0.07^{c}	ns
t4	0.27^{c}	0.27^{bc}	ns	0.19^{cdB}	0.17^{aA}	*	0.14	0.10^{a}	ns	0.06^{b}	0.08^{b}	ns	0.07^{B}	0.05^{abA}	**
t6	0.27^{c}	0.26^{bc}	ns	0.19^{deB}	0.17^{aA}	**	0.12^{B}	0.09^{aA}	**	0.06^{bA}	0.12^{cB}	***	0.08^{B}	0.05^{aA}	**
t8	0.27^{c}	0.29^{c}	ns	0.18^{bcA}	0.19^{bB}	*	0.12	0.12^{bc}	ns	0.04^{abA}	0.11^{cB}	*	0.07	0.06^{abc}	ns
t10	0.24^{b}	0.25^{ab}	ns	0.17^{bA}	0.20^{bB}	**	0.11	0.10^{a}	ns	0.02^{a}	0.18^{d}	ns	0.07	0.06^{abc}	ns
t12	0.22^{a}	0.22^{a}	ns	0.15^{aA}	0.20^{bB}	**	0.10	0.09^{a}	ns	0.02^{aA}	0.18^{dB}	***	0.08	0.06^{abc}	ns
sign.	***	***		***	***		ns	***		**	***		ns	**	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

4.2 Research 2

As reported in the paragraph 3.2.1, breadsticks differently flavoured were used for this study: with garlic and chilli pepper (GP), with onions and olives (OO), with wild fennel seeds (F) (*Foeniculum vulgare* Mill), with potatoes and rosemary (PR).

4.2.1 Effect of storage time and recipe modification on physical characteristics of breadsticks

4.2.1.1 Effect of time and recipe on water activity and moisture content

The study of GP breadsticks showed that both in OPO and in EVOO samples, a_w and moisture content (%) significantly increased ($p < 0.001$) during storage (Table 17). From *t0* up to *t8*, a_w values were lower in EVOO breadsticks, but at *t10* and *t12* the lowest values belonged to OPO ones. As regards moisture, at the beginning OPO sample showed lower values, but since *t6* it had a mayor increase, being significantly higher than EVOO one.

As well as for GP breadsticks, a_w and moisture (%) of OO breadsticks increased during storage. Values about a_w parameter showed differences between recipes. OPO breadsticks had a lower a_w in comparison to EVOO ones for the whole storage duration (Table 18).

A significant ($p < 0.001$) increase in a_w and moisture content (%) was detected also for F breadsticks (Table 19). Regarding a_w, both time and recipe and, also, their interaction had very highly significant influence ($p < 0.001$) on the parameter, as confirmed by Two-way ANOVA. EVOO recipe samples had and maintained during storage higher values in comparison to the OPO recipe ones. The same happened for moisture content (%), even if at the end of the storage there were no differences between recipes.

In Table 20 results about PR breadsticks are reported. As expected, also for PR breadsticks, a_w and moisture content (%) increased during time ($p < 0.001$). Starting from *t2*, a_w values were significantly higher ($p < 0.001$) in those sample prepared with EVOO. As well as for water activity, also moisture was higher in EVOO samples.

4.2.1.2 Effect of time and recipe on hardness

Hardness significantly changed during storage almost for all breadsticks. GP breadsticks (Table 21), showed different trends for OPO and EVOO samples. OPO breadsticks had a significant decrease in hardness (g) values. On the other hand, EVOO breadsticks showed an increase in hardness up to *t8*, followed by a decrease up to the last day of storage. This is in accordance with what found in crackers by Lewicki *et al.* (2004), when crackers' hardness increased and then decreased during storage and with the increasing of a_w values. However, no statistical differences were found between recipes at *t12*. In general, we can assume that hardness is not influenced by the type and the quality of the vegetable oil used.

As well as for GP OPO breadsticks, also OO OPO ones had a significant decrease in hardness (g) values (Table 22). While OPO hardness (g) decreased, EVOO one remained constant during storage. In fact, for EVOO recipe no differences were found. In this case, OO EVOO breadsticks maintained their texture properties during storage. Two-way ANOVA demonstrated that hardness of OO breadsticks is influenced by time, recipe and also by their interaction ($p < 0.05$, $p < 0.001$**, $p < 0.01$ respectively).

Two-way ANOVA results about F breadsticks, demonstrated that their hardness (g) was highly influenced by recipe ($p < 0.001$). F EVOO samples' hardness was almost always lower than OPO samples' one (Table 23). It could be related to the higher a_w and moisture content showed by EVOO samples. In this study, while increasing water activity and moisture for OPO samples, there was a consequent reduction of the snacks' hardness.

PR breadsticks' hardness (g) values, reported in Table 24, changed during storage ($p < 0.001$). In OPO samples, hardness decreased from *t0* up to *t12*. On the contrary, EVOO samples showed a first increase of hardness (from 2273 g to 6918 g), followed by a decrease (3858 g at *t12*). At 0, 2 and 12 months, EVOO had hardness values lower than OPO samples. It could be due to the highest moisture and water activity, according to Ho and Abdul Latif (2016).

4.2.1.3 Effect of time and recipe on colour of the external surface

Tables 25, 26, 27 and 28 list the colour of the external surface of breadsticks through CIELab system, that is commonly used for food quality control. Regarding OPO breadsticks, all the samples (GP, OO, F and PR), stand in the clear zone with values of brightness (L^*) above 50, with the exception for GP breadsticks that at *t12* showed a significantly lower value (30.4). Between recipes, EVOO positively influenced L^* parameter of GP, F and PR breadsticks. In fact, for these EVOO samples the brightness was higher than OPO ones.

a^* and b^* coordinates had and maintained positive values, in all the samples studied and no many differences were revealed between recipes. This leads us to think that in this case the type of vegetable oil used does not affect the colour.

No many differences were found between recipe for colour saturation (Chroma), except for few samples.

Table 17 Effect of time and recipe on water activity and moisture content (%) of GP breadsticks

	a_w			Moisture (%)		
time	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.171^{aB}	0.153^{aA}	**	0.73^{aA}	1.53^{aB}	**
t2	0.199^{bB}	0.188^{bA}	*	1.62^{b}	1.54^{a}	ns
t4	0.241^{cB}	0.203^{cA}	**	1.84^{b}	1.61^{a}	ns
t6	0.270^{dB}	0.206^{cA}	***	2.80^{cB}	1.68^{aA}	***
t8	0.291^{eB}	0.250^{dA}	***	4.34^{dB}	2.78^{bA}	***
t10	0.318^{fA}	0.349^{eB}	***	6.99^{eB}	2.74^{bA}	***
t12	0.327^{fA}	0.365^{fB}	**	7.57^{fB}	3.94^{cA}	***
sign.	***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 18 Effect of time and recipe on water activity and moisture content (%) of OO breadsticks

	a_w			Moisture (%)		
time	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.114^{aA}	0.141^{a}	**	0.86^{a}	0.98^{a}	ns
t2	0.264^{b}	0.165^{bA}	***	1.78^{b}	2.57^{b}	ns
t4	0.264^{b}	0.215^{cA}	***	1.48^{bA}	2.68^{bB}	***
t6	0.253^{bA}	0.281^{d}	***	3.79^{c}	3.52^{c}	ns
t8	0.278^{cA}	0.328^{e}	**	4.04^{cB}	3.55^{cA}	*
t10	0.319^{dA}	0.372^{f}	***	4.72^{dB}	4.19^{dA}	**
t12	0.342^{eA}	0.387^{f}	**	5.45^{eB}	5.21^{eA}	*
sign.	***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 19 Effect of time and recipe on water activity and moisture content (%) of F breadsticks

	a_w			Moisture (%)		
time	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.140^{aA}	0.220^{aB}	***	1.53^{aA}	4.65^{aB}	***
t2	0.209^{bA}	0.278^{bB}	***	1.61^{aA}	4.73^{aB}	***
t4	0.246^{cA}	0.298^{cB}	***	2.28^{bA}	4.87^{aB}	***
t6	0.251^{cA}	0.301^{cB}	***	3.78^{cA}	4.98^{aB}	***
t8	0.266^{dA}	0.311^{cB}	**	4.53^{dA}	5.12^{abB}	*
t10	0.294^{eA}	0.360^{dB}	**	5.19^{eA}	5.53^{bcB}	*
t12	0.296^{eA}	0.428^{eB}	***	5.39^{e}	5.62^{c}	ns
sign.	***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 20 Effect of time and recipe on water activity and moisture content (%) of PR breadsticks

	a_w			Moisture (%)		
time	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.194^{a}	0.199^{a}	ns	0.94^{aA}	3.45^{aB}	***
t2	0.213^{bA}	0.242^{bB}	***	1.78^{bA}	4.61^{bB}	***
t4	0.231^{cA}	0.261^{cB}	***	2.65^{cA}	4.54^{bB}	***
t6	0.250^{dA}	0.333^{dB}	***	3.20^{dA}	5.54^{cB}	***
t8	0.274^{eA}	0.349^{eB}	***	3.63^{eA}	5.58^{cB}	***
t10	0.284^{eA}	0.468^{fB}	***	4.17^{fA}	5.80^{cB}	**
t12	0.285^{eA}	0.486^{gB}	***	4.46^{fA}	5.90^{cB}	***
sign.	***	***		**	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 21 Effect of time and recipe on hardness (g) of GP breadsticks

	Hardness (g)		
time	OPO	EVOO	sign.
t0	8721^{dB}	3034^{aA}	***
t2	5802^{bc}	6102^{ab}	ns
t4	7184^{cd}	5920^{ab}	ns
t6	5570^{b}	6450^{b}	ns
t8	3376^{aA}	10725^{cB}	***
t10	5259^{b}	5062^{ab}	ns
t12	4952^{b}	5628^{ab}	ns
sign.	***	***	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 22 Effect of time and recipe on hardness (g) of OO breadsticks

	Hardness (g)		
time	OPO	EVOO	sign.
t0	8317^{cB}	4484^{A}	***
t2	6337^{abc}	4619	ns
t4	8085^{cB}	4039^{A}	***
t6	5455^{aB}	3823^{A}	*
t8	4671^{a}	4886	ns
t10	7507^{bcB}	4118^{A}	**
t12	5978^{ab}	4662	ns
sign.	***	ns	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 23 Effect of time and recipe on hardness (g) of F breadsticks

	Hardness (g)		
time	OPO	EVOO	sign.
t0	6127^{abB}	3326^{aA}	***
t2	6659^{bB}	3454^{aA}	**
t4	6584^{abB}	3914^{aA}	**
t6	5387^{abB}	3778^{aA}	***
t8	5368^{ab}	6375^{b}	ns
t10	5453^{abB}	3779^{aA}	**
t12	5207^{a}	4790^{ab}	ns
sign.	**	**	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 24 Effect of time and recipe on hardness (g) of PR breadsticks

	Hardness (g)		
time	OPO	EVOO	sign.
t0	9892^{bB}	2273^{aA}	***
t2	6320^{aB}	2594^{aA}	***
t4	5327^{a}	5385^{bc}	ns
t6	5013^{a}	3948^{ab}	ns
t8	4804^{aA}	6918^{cB}	**
t10	4762^{a}	5120^{bc}	ns
t12	5120^{a}	3858^{ab}	ns
sign.	***	***	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 25 Effect of time and recipe on external surface colour of GP breadsticks

	*L**			*a**			*b**			*Chroma*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	64.5^{b}	68.6^{bc}	ns	8.7^{ab}	10.7^{b}	ns	32.13^{b}	33.85^{ab}	ns	33.31^{b}	35.5^{ab}	ns
t2	62.3^{b}	56.8^{a}	ns	9.9^{b}	9.1^{ab}	ns	33.01^{b}	29.28^{a}	ns	34.50^{b}	30.7^{a}	ns
t4	61.2^{bA}	69.3^{bcB}	*	7.9^{abA}	10.6^{bB}	**	29.13^{bA}	34.74^{bB}	**	30.20^{bA}	36.3^{bB}	**
t6	61.0^{b}	65.6^{abc}	ns	7.5^{ab}	9.3^{ab}	ns	32.87^{b}	32.25^{ab}	ns	33.72^{b}	33.6^{ab}	ns
t8	65.2^{bA}	74.7^{cB}	***	8.6^{ab}	7.6^{a}	ns	33.01^{b}	31.53^{ab}	ns	34.12^{b}	32.4^{ab}	ns
t10	62.4^{bA}	72.3^{bcB}	**	7.6^{ab}	7.7^{a}	ns	30.59^{b}	31.04^{ab}	ns	31.55^{b}	32.0^{ab}	ns
t12	30.4^{aA}	61.2^{abB}	**	4.6^{aA}	8.3^{aB}	***	15.67^{aA}	32.53^{abB}	***	16.35^{aA}	33.6^{abB}	***
sign.	***	***		*	***		***	*		***	*	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 26 Effect of time and recipe on external surface colour of OO breadsticks

	*L**			*a**			*b**			*Chroma*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	62.5^{ab}	63.9^{ab}	ns	12.3^{c}	10.5^{b}	ns	33.6^{b}	32.2^{bc}	ns	35.7^{b}	33.9^{bc}	ns
t2	73.0^{bB}	60.2^{abA}	*	5.6^{a}	6.7^{a}	ns	28.5^{ab}	27.3^{a}	ns	29.1^{ab}	28.2^{a}	ns
t4	67.7^{ab}	66.6^{b}	ns	10.0^{bc}	9.7^{ab}	ns	33.7^{b}	31.7^{bc}	ns	35.1^{b}	33.2^{bc}	ns
t6	67.9^{abB}	58.6^{aA}	**	9.7^{bc}	9.6^{ab}	ns	33.7^{bB}	29.4^{abA}	*	35.1^{b}	31.0^{ab}	ns
t8	69.6^{ab}	65.7^{ab}	ns	8.6^{ab}	10.2^{b}	ns	32.6^{ab}	32.8^{bc}	ns	33.7^{ab}	34.4^{bc}	ns
t10	64.1^{ab}	66.9^{b}	ns	9.3^{bc}	10.2^{b}	ns	31.7^{ab}	33.7^{c}	ns	33.0^{ab}	35.2^{bc}	ns
t12	57.7^{a}	66.3^{b}	ns	7.7^{abA}	10.9^{bB}	**	27.7^{aA}	34.5^{cB}	*	28.7^{aA}	36.2^{cB}	*
sign.	*	**		***	**		**	***		**	***	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 27 Effect of time and recipe on external surface colour of F breadsticks

	*L**			*a**			*b**			*Chroma*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	65.1^{ab}	71.1^{b}	ns	10.9^{B}	6.2^{abA}	**	33.4^{B}	29.3^{bA}	*	35.1^{B}	30.0^{bA}	*
t2	59.8^{aB}	50.0^{aA}	**	10.7^{B}	3.6^{aA}	***	31.7^{B}	19.3^{aA}	***	33.5^{B}	19.7^{aA}	***
t4	69.5^{b}	71.7^{b}	ns	8.8^{B}	4.9^{abA}	*	32.2^{B}	26.7^{bA}	*	33.4^{B}	27.2^{bA}	*
t6	69.5^{b}	68.7^{b}	ns	8.7	6.4^{ab}	ns	33.2	27.9^{b}	ns	34.3	28.7^{b}	ns
t8	60.0^{a}	69.5^{b}	ns	10.4	7.1^{b}	ns	32.3	29.5^{b}	ns	33.9	30.4^{b}	ns
t10	63.2^{ab}	69.5^{b}	ns	9.8^{B}	4.4^{abA}	***	32.1^{B}	25.8^{bA}	**	33.6^{B}	26.2^{bA}	**
t12	62.2^{aA}	73.8^{bB}	***	9.6^{B}	5.6^{abA}	***	31.7^{B}	25.2^{bA}	***	33.1^{B}	25.8^{bA}	***
sign.	**	***		ns	*		ns	***		ns	***	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 28 Effect of time and recipe on external surface colour of PR breadsticks

	*L**			*a**			*b**			*Chroma*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	56.4	65.6	ns	11.0	10.6^{c}	ns	28.9^{A}	34.2^{cdB}	**	31.0^{A}	35.8^{cdB}	**
t2	69.8	67.3	ns	8.0	7.8^{abc}	ns	32.3^{B}	29.7^{abcA}	*	33.3^{B}	30.8^{abcA}	*
t4	64.6	73.7	ns	8.1	4.4^{ab}	ns	31.4	28.7^{a}	ns	32.5	29.1^{a}	ns
t6	56.2^{A}	67.1^{B}	**	12.0	8.8^{bc}	ns	34.4	33.6^{abcd}	ns	36.4	34.7^{bcd}	ns
t8	54.2^{A}	66.8^{B}	**	12.0	10.0^{c}	ns	30.6	33.9^{bcd}	ns	32.9	35.4^{cd}	ns
t10	61.1^{A}	70.6^{B}	**	7.7	3.8^{a}	ns	28.3	29.1^{ab}	ns	29.4	29.4^{ab}	ns
t12	61.3	63.7	ns	9.4	10.4^{c}	ns	33.0	35.8^{d}	ns	34.3	37.4^{d}	ns
sign.	ns	ns		ns	***		ns	***		ns	***	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

4.2.2 Effect of storage time and recipe modification on chemical characteristics of the oils extracted from breadsticks

4.2.2.1 Effect of time and recipe on AV, PV and spectrophotometric index

Table 29 and Table 30 show data regarding the chemical characteristics of the oils extracted from GP and OO breadsticks, respectively. Results obtained clearly show that the lipid fraction of OPO breadsticks was markedly more degraded than EVOO ones, with significantly higher values of K_{232} and K_{270}, but, above all, peroxide value (PV). This was due to the higher stability and the higher content of antioxidants (total phenols and carotenoids) that characterised EVOOs (Servili *et al.*, 2004; Quiles *et al.*, 2006; Servili *et al.*, 2009; Omar, 2010; Obied *et al.*, 2012) in comparison to refined vegetable oils such olive pomace oil (Giuffrè *et al.*, 2018). PV rapidly increased from *t8* to *t10* for both breadsticks types. OPO samples had lower acidity values (AV) according to Caponio *et al.* (2011) and Giuffrè *et al.* (2018), but also the highest increase during time. Despite EVOO samples showed the highest acidity values, they had also the lowest increase during storage, demonstrating a better response to degradation.

The results about F breadsticks are listed in Table 31. They demonstrated that lower values of oxidative and hydrolytic degradation indices of the lipid fraction were observed in samples prepared with EVOO, except for the acidity value (AV). This suggested a lower extent of oxidative degradation in EVOO samples, compared to those made with the refined oil. In fact, they showed significantly lower values of peroxide (PV), K_{232} (an indicator of the primary oxidative degradation because of the presence of conjugated dienes) and K_{270} (mainly due to the presence of conjugated trienes and α-β unsaturated aldehydes and related to the secondary oxidative degradation). This was in accordance with what found in GP and OO breadsticks and also with Caponio *et al.* (2011; 2013a) and with Giuffrè *et al.* (2018).

The chemical characteristics of the oils extracted from PR breadsticks (Table 32) were completely different from those of GP, OO and F breadsticks. In fact, in this case the increases in AV, PV, K_{232} and K_{270} were higher in EVOO samples. It could be related to a_w and moisture content (%) (Table 19) that were significantly higher in comparison to OPO ones and also to the other types of EVOO breadsticks (GP, OO and F). It is well known, in fact, that oxidation processes are accelerated when a food contains high water amount (Smith *et al.*, 2004; Calvo *et al.*, 2010; Nogueira-de-Almeida and De Castro, 2018).

4.2.2.2 Effect of time and recipe on breadsticks' antioxidant capacity

In GP, OO and F the breadsticks, TEAC increased for both recipes with storage duration especially from *t0* to *t4*, and then started to decrease (Tables 33, 34, 35). As yet reported in

the paragraph 4.1.2, TEAC can be overestimate because $ABTS^{\bullet+}$ reacts with any hydroxylated aromatics independently of their real antioxidative potential. In fact, the ABTS test is reduced to titration of aromatic OH-groups including those which do not contribute to the antioxidation. A different trend was observed for ABTS assay compared to DPPH assay (referred to HAE). As well as happened for *Cantuccini*, the DPPH test gave lower results than the ABTS assay, probably because of the different HAEs' interaction with the radical. The differences between ABTS and DPPH hydrophilic assays performed on HAE can be due also to colour of sample. In fact, it was found that the colour of the sample can affect the absorbance values obtained with spectrophotometric assay. Arnao (2000) reported that in the DPPH the problem is more serious than ABTS assay since it does not present bands higher than 515 nm. So, at this wavelength the antioxidant activity measured is underestimated. Moreover Suarez *et al.* (2009) found also differences between DPPH and ORAC assays, confirming that DPPH assay is not suitable for the determination of antioxidant activity of matrix characterised by high complexity.

Results from the DPPH assay performed on the oils extracted were higher than those in the hydrophilic fraction. This was expected as the DPPH assay in total oil also considers tocopherols which show a synergistic action with phenolics (Espin *et al.*, 2000). The µmol $TE \cdot 100g^{-1}$ oil measured by the DPPH assay performed on oil increased from *t0* up o *t4*, and then decreased until the end of the storage.

Values about the antioxidant capacity of PR breadsticks, and the effect of time and recipe on it, are reported in Table 36. In general, the antioxidant capacity of this type of breadsticks was considerably higher than the other types studied in this work. ABTS assay and DPPH assay on the oil showed the same trend of GP, O and F breadsticks, that is to say that they initially increased and then decreased. In this case, the antioxidant activity studied by DPPH assay on HAE is very similar to the one resulted from ABTS assay on HAE. It was found that rosemary extract exhibited a great antioxidant activity detected by DPPH assay (Almela *et al.*, 2006; Dimitrios, 2006). The higher antioxidant activity found in these breadsticks could be explained by the effectiveness and the stability of particulate compounds which could migrate from aromatic plants to the product during dough mixing and storage, as well as what happened when aromatic plants macerated in oil releasing antioxidant compounds (Mockute and Bernotiene, 2001; Almela *et al.*, 2006; Politeo *et al.*, 2007; Ayadi *et al.*, 2009).

Table 29 Effect of time and recipe on AV, PV, K_{232} and K_{270} of the oil extracted from GP breadsticks

	AV (% oleic acid)			*PV (meq O_2·kg^{-1})*			*K_{232}*			*K_{270}*		
Time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.31^{aA}	0.88^{aB}	***	8.56^{aB}	5.57^{aA}	***	2.58^{aB}	1.97^{aA}	***	1.39^{cB}	0.52^{aA}	***
t2	0.35^{abA}	0.94^{abB}	**	9.65^{aB}	6.76^{bA}	***	3.59^{bB}	2.00^{aA}	***	1.13^{aB}	0.52^{aA}	***
t4	0.39^{bcA}	0.96^{abB}	***	25.09^{bB}	7.80^{cA}	***	2.46^{aB}	2.27^{bA}	***	1.29^{bB}	0.61^{bA}	***
t6	0.45^{cdA}	0.98^{abB}	***	42.18^{cB}	8.07^{cA}	***	2.64^{a}	2.37^{b}	ns	1.49^{dB}	0.56^{abA}	***
t8	0.47^{dA}	1.06^{bB}	***	57.28^{dB}	8.58^{dA}	***	3.50^{bB}	2.35^{bA}	**	1.48^{dB}	0.57^{abA}	***
t10	0.47^{dA}	1.05^{bB}	***	129.91^{eB}	9.02^{eA}	***	3.68^{bB}	2.98^{cA}	***	1.53^{dB}	0.60^{bA}	***
t12	0.56^{eA}	1.08^{bB}	***	133.53^{fB}	17.93^{fA}	***	3.69^{bB}	2.83^{cA}	***	1.55^{dB}	0.61^{bA}	***
sign.	***	**		***	***		***	***		***	**	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 30 Effect of time and recipe on AV, PV, K_{232} and K_{270} of the oil extracted from OO breadsticks

	AV (% oleic acid)			*PV (meq O_2·kg^{-1})*			*K_{232}*			*K_{270}*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.35^{aA}	0.81^{abB}	***	6.10^{aB}	4.41^{aA}	***	2.48^{aB}	1.76^{aA}	***	1.32^{bB}	0.57^{abA}	***
t2	0.38^{aA}	0.78^{aB}	***	22.53^{bB}	10.33^{bA}	***	3.27^{bcB}	2.15^{bA}	***	1.21^{aB}	0.57^{bA}	***
t4	0.47^{bA}	0.82^{abB}	***	35.17^{cB}	13.64^{bA}	***	2.43^{a}	2.49^{c}	ns	1.38^{bB}	0.64^{cA}	***
t6	0.48^{bA}	0.88^{bcB}	***	42.83^{dB}	10.94^{bA}	***	3.15^{bB}	2.57^{dA}	***	1.32^{bB}	0.68^{dA}	***
t8	0.49^{bA}	0.90^{bcdB}	***	48.55^{eB}	12.27^{bA}	***	3.55^{cdB}	2.73^{eA}	**	1.40^{bB}	0.73^{eA}	***
t10	0.50^{bA}	0.94^{cdB}	***	88.55^{fB}	13.42^{bA}	***	3.84^{dB}	2.60^{dA}	***	1.67^{cB}	0.54^{aA}	***
t12	0.56^{cA}	0.98^{dB}	***	102.89^{gB}	20.91^{cA}	***	3.70^{dB}	2.92^{fA}	***	1.58^{cB}	0.64^{cdA}	***
sign.	***	***		***	***		***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 31 Effect of time and recipe on AV, PV, K_{232} and K_{270} of the oil extracted from F breadsticks

	AV (% oleic acid)			*PV (meq O_2·kg^{-1})*			*K_{232}*			*K_{270}*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.37^{abA}	0.74^{aB}	***	6.28^{a}	5.37^{a}	ns	2.38^{aB}	1.58^{aA}	***	1.25^{abB}	0.31^{aA}	***
t2	0.35^{aA}	0.74^{aB}	***	4.92^{aA}	11.70^{bB}	***	2.91^{b}	2.89^{c}	ns	1.23^{a}	1.26^{f}	ns
t4	0.42^{bA}	0.91^{bB}	***	19.48^{bB}	15.29^{cA}	***	2.48^{aA}	2.52^{bB}	*	1.29^{bB}	0.53^{bA}	***
t6	0.49^{cA}	0.92^{bB}	***	29.77^{cB}	21.36^{dA}	***	2.98^{b}	2.88^{c}	ns	1.51^{cB}	0.61^{cdA}	***
t8	0.50^{cA}	1.12^{cB}	***	42.25^{dB}	25.09^{eA}	***	4.05^{dB}	3.14^{dA}	***	1.83^{eB}	0.65^{dA}	***
t10	0.51^{cdA}	1.14^{cB}	***	54.14^{eB}	26.01^{efA}	***	3.63^{cB}	3.07^{cdA}	**	1.58^{dB}	0.60^{cA}	***
t12	0.56^{dA}	1.22^{dB}	***	61.05^{fB}	26.38^{fA}	***	3.68^{cB}	3.03^{cdA}	***	1.77^{eB}	0.70^{eA}	***
sign.	***	***		***	***		***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 32 Effect of time and recipe on AV, PV, K_{232} and K_{270} of the oil extracted from PR breadsticks

	AV (% oleic acid)			*PV (meq O_2·kg^{-1})*			*K_{232}*			*K_{270}*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.52^{aA}	0.87^{aB}	***	5.56^{aA}	7.58^{aB}	***	2.46^{a}	2.50^{ab}	ns	1.41^{bcB}	0.86^{dA}	***
t2	0.45^{aA}	1.09^{bB}	***	7.78^{bA}	10.03^{bB}	***	3.09^{cB}	2.46^{aA}	**	1.13^{aB}	0.70^{abA}	***
t4	0.47^{aA}	1.26^{cB}	***	8.01^{bcA}	10.97^{bB}	***	2.61^{abA}	2.76^{bcB}	**	1.45^{cB}	0.68^{aA}	***
t6	0.52^{aA}	1.29^{cB}	***	8.58^{dA}	10.65^{bB}	***	2.73^{bA}	3.03^{cdB}	***	1.49^{cB}	0.79^{cA}	***
t8	0.70^{bA}	1.32^{cB}	**	8.18^{cA}	22.23^{cB}	***	3.31^{dB}	2.96^{cdA}	**	1.41^{bcB}	0.88^{dA}	***
t10	0.74^{bA}	1.65^{dB}	***	8.74^{dA}	23.80^{dB}	***	3.90^{eB}	3.04^{dA}	***	1.72^{dB}	0.73^{bA}	***
t12	0.78^{bA}	1.77^{dB}	***	9.41^{eA}	23.97^{dB}	***	3.45^{dA}	3.84^{eB}	**	1.30^{bB}	1.18^{eA}	**
sign.	***	***		***	***		***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 33 Effect of time and recipe on antioxidant capacity of the oil extracted from GP breadsticks

	ABTS assay on HAE TEAC (µmol TE·$100g^{-1}$ oil)			*DPPH assay on HAE µmol TE·$100g^{-1}$ oil*			*DPPH assay on oil µmol TE·$100g^{-1}$ oil*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	99.32^{a}	113.30^{a}	ns	12.19^{aA}	35.34^{dB}	***	87.46^{a}	87.36^{b}	ns
t2	191.75^{bB}	125.71^{abA}	***	19.76^{abA}	26.55^{cB}	*	130.30^{cB}	100.53^{cA}	**
t4	185.60^{bB}	127.99^{abA}	**	19.53^{abA}	24.34^{cB}	***	135.53^{cB}	97.98^{cA}	**
t6	231.06^{cB}	140.89^{bA}	***	23.34^{b}	21.71^{c}	ns	111.90^{bB}	65.70^{aA}	***
t8	199.38^{bB}	138.97^{bA}	**	23.7^{bB}	15.82^{bA}	*	125.95^{cB}	60.77^{aA}	***
t10	197.64^{bB}	143.94^{bA}	***	20.36^{bB}	7.12^{aA}	***	107.13^{bB}	60.66^{aA}	***
t12	193.48^{bB}	128.33^{abA}	***	22.18^{bB}	5.21^{aA}	***	103.96^{bB}	62.12^{aA}	***
sign.	***	**		**	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 34 Effect of time and recipe on antioxidant capacity of the oil extracted from OO breadsticks

	ABTS assay on HAE TEAC (µmol TE·$100g^{-1}$ oil)			*DPPH assay on HAE µmol TE·$100g^{-1}$ oil*			*DPPH assay on oil µmol TE·$100g^{-1}$ oil*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	187.37^{cB}	83.27^{aA}	***	25.31^{bc}	25.56^{b}	ns	83.02^{aB}	62.35^{bA}	**
t2	200.52^{cB}	82.54^{aA}	***	27.48^{cB}	21.28^{bA}	*	113.22^{cB}	78.16^{deA}	***
t4	222.41^{dB}	114.96^{cA}	***	26.22^{cB}	22.98^{bA}	*	133.32^{dB}	82.98^{eA}	***
t6	169.20^{bB}	148.19^{eA}	**	22.14^{bB}	14.74^{aA}	**	117.52^{cB}	66.06^{bcA}	***
t8	162.24^{bB}	131.74^{dA}	**	17.98^{aB}	14.62^{aA}	**	127.02^{dB}	71.25^{cdA}	***
t10	139.18^{aB}	119.89^{cA}	**	21.69^{abB}	13.45^{aA}	**	96.40^{bB}	57.83^{abA}	***
t12	137.19^{aB}	94.61^{bA}	**	18.38^{aB}	11.48^{aA}	**	92.27^{bB}	51.39^{aA}	***
sign.	***	***		***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 35 Effect of time and recipe on antioxidant capacity of the oil extracted from F breadsticks

	ABTS assay on HAE TEAC (μmol TE·100g^{-1} oil)			*DPPH assay on HAE μmol TE·100g^{-1} oil*			*DPPH assay on oil μmol TE·100g^{-1} oil*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	161.04^{aB}	71.93^{abA}	**	20.03^{ab}	20.89^{d}	ns	85.58^{aB}	69.40^{bcA}	*
t2	203.30^{cB}	76.03^{bA}	***	25.86^{bcB}	19.92^{dA}	***	94.94^{aB}	65.58^{abcA}	***
t4	247.36^{dB}	114.74^{dA}	***	28.71^{c}	22.09^{d}	ns	120.79^{bB}	71.14^{cA}	***
t6	195.45^{bcB}	97.73^{cdA}	**	22.30^{abcB}	18.91^{cdA}	**	142.88^{cB}	65.63^{abcA}	***
t8	188.32^{abcB}	80.51^{bcA}	***	19.89^{abB}	14.97^{bcA}	***	143.64^{cB}	63.83^{abcA}	***
t10	173.38^{abB}	61.68^{abA}	***	17.19^{aB}	11.13^{abA}	*	119.66^{bB}	59.00^{abA}	***
t12	163.44^{aB}	51.68^{aA}	***	16.17^{aB}	9.25^{aA}	***	98.37^{aB}	55.34^{aA}	**
sign.	***	***		***	***		***	**	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 36 Effect of time and recipe on antioxidant capacity of the oil extracted from PR breadsticks

	ABTS assay on HAE TEAC (μmol TE·100g^{-1} oil)			*DPPH assay on HAE μmol TE·100g^{-1} oil*			*DPPH assay on oil μmol TE·100g^{-1} oil*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	256.87^{aA}	373.11^{cB}	**	121.97^{aA}	339.51^{dB}	***	173.31^{aA}	242.67^{eB}	***
t2	405.80^{b}	408.49^{c}	ns	120.17^{aA}	315.38^{dB}	***	262.10^{cdB}	225.16^{cdA}	***
t4	408.80^{b}	383.60^{c}	ns	137.87^{bA}	257.90^{cB}	***	269.57^{dB}	223.24^{cA}	***
t6	461.47^{bB}	365.20^{cA}	**	142.84^{bA}	241.38^{bcB}	***	265.09^{dB}	230.88^{dA}	***
t8	485.47^{bB}	315.01^{bA}	**	120.24^{aA}	239.97^{abcB}	***	253.83^{cB}	160.12^{bA}	***
t10	460.07^{bB}	209.14^{aA}	**	121.40^{aA}	224.76^{abB}	***	237.67^{bB}	156.85^{bA}	***
t12	458.10^{bB}	175.08^{aA}	***	114.44^{aA}	214.85^{aB}	***	238.73^{bB}	118.60^{aA}	***
sign.	***	***		***	***		***	***	

One-way ANOVA experiment, values are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

4.2.3 Fatty acids composition of the oil extracted from breadsticks

Edible oils are made of triacylglycerol molecules, mainly formed by unsaturated and saturated fatty acids esterified to glycerol units (Barison *et al.*, 2010). Most of olive oil fatty acid chains contain 16 or 18 carbon atoms. The nutritional value and health functions of virgin olive oil (VOO) are ascribed to the presence of large amount of monounsaturated fatty acids (MUFAs) such as oleic acid (C18:1), that is the most representative, and valuable minor components (Aguilera *et al.*, 2005; Ranalli *et al.*, 2008; Khaleghi *et el.*, 2015). Fatty acid composition has strong influence on the properties of the edible oils and, in particular, on the stability to oxidation. Changes of the fatty acid percentages could be due to the chemical reactions (oxidation, hydrolysis) as a consequence of the heating treatments. In fact, the thermolability or volatility of these compounds has great effect (Carrasco-Pancorbo *et al.*, 2007).

As Tables from 37 to 48 show, the fatty acid composition was unaltered for both recipes during storage. The results of this research are similar to other studies (Caponio *et al.*, 2003; Escudero *et al.*, 2016) where the fatty acid profiles of different vegetable oils after microwave and/or conventional heating treatments were unchanged.

Table 37 Effect of time and recipe on FAMEs composition of the oil extracted from GP breadsticks

	C14			*C16*			*C16:1*			*C17*			*C17:1*			*C18*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.02	0.01	ns	12.20^{aA}	13.22^{bB}	***	0.95^{abA}	1.28^{cB}	***	0.11^{abA}	0.18^{bB}	***	0.20^{bA}	0.34^{B}	***	2.59^{d}	2.33^{d}	ns
t2	0.02	0.01	ns	12.55^{abA}	13.05^{bB}	**	0.95^{abA}	1.24^{bB}	***	0.13^{bA}	0.16^{aB}	**	0.19^{abA}	0.34^{B}	***	2.29^{cB}	2.04^{bcA}	**
t4	0.02	0.01	ns	12.62^{abA}	13.15^{bB}	***	0.93^{abA}	1.26^{bcB}	***	0.12^{abA}	0.16^{aB}	**	0.18^{abA}	0.33^{B}	***	2.25^{cB}	1.34^{aA}	***
t6	0.02	0.01	ns	12.72^{bA}	13.12^{bB}	***	0.96^{bA}	1.24^{bB}	***	0.11^{abA}	0.16^{aB}	**	0.19^{abA}	0.34^{B}	***	2.30^{cdB}	1.96^{bA}	**
t8	0.02	0.01	ns	12.35^{abA}	12.82^{aB}	***	0.91^{aA}	1.24^{bB}	***	0.09^{aA}	0.16^{aB}	***	0.17^{aA}	0.34^{B}	***	1.94^{b}	2.04^{bc}	ns
t10	0.02	0.01	ns	12.79^{b}	12.78^{a}	ns	0.93^{abA}	1.20^{aB}	***	0.11^{abA}	0.15^{aB}	*	0.18^{abA}	0.33^{B}	***	1.72^{abA}	2.07^{bcB}	*
t12	0.02	0.01	ns	12.24^{aA}	13.13^{bB}	**	0.91^{aA}	1.27^{bcB}	***	0.11^{abA}	0.16^{aB}	**	0.19^{abA}	0.34^{B}	***	1.58^{aA}	2.21^{cdB}	***
sign.	ns	ns		**	***		*	***		*	***		**	ns		***	***	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 38 Effect of time and recipe on FAMEs composition of the oil extracted from GP breadsticks

	C18:1			*C18:1ω7*			*C18:2*			*C18:3ω3*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	67.66^{aA}	69.28^{aB}	**	3.03	3.17^{ab}	ns	11.21^{cB}	8.58^{cA}	***	0.74^{dB}	0.59^{bcdA}	***
t2	68.05^{abA}	69.58^{bB}	**	2.47^{A}	3.06^{aB}	**	11.27^{cB}	8.70^{dA}	***	0.72^{cdB}	0.60^{dA}	***
t4	68.27^{bA}	70.41^{dB}	***	2.50^{A}	3.27^{bcB}	***	11.17^{cB}	8.44^{bA}	***	0.69^{bB}	0.58^{bA}	***
t6	68.18^{abA}	69.99^{cB}	***	2.37^{A}	3.18^{bB}	***	11.20^{cB}	8.38^{bA}	***	0.70^{bcB}	0.58^{bcA}	**
t8	69.14^{cA}	69.99^{cB}	**	2.60^{A}	3.15^{abB}	***	10.79^{bB}	8.58^{cA}	***	0.64^{aB}	0.60^{dA}	**
t10	69.42^{cdA}	70.41^{dB}	**	2.59^{A}	3.35^{cB}	***	10.44^{abB}	8.13^{aA}	***	0.62^{aB}	0.55^{aA}	***
t12	69.76^{d}	69.84^{bc}	ns	2.90	3.06^{a}	ns	10.42^{aB}	8.38^{bA}	***	0.63^{aB}	0.59^{cdA}	*
sign.	***	***		ns	***		***	***		***	***	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 39 Effect of time and recipe on FAMEs composition of the oil extracted from GP breadsticks

	C20			*C20:1*			*C22*			*C22:1*			*C24*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.49^{b}	0.48	ns	0.40^{cdB}	0.31^{A}	***	0.19^{abB}	0.14^{A}	***	0.02	0.01	ns	0.11^{bB}	0.08^{A}	**
t2	0.50^{b}	0.49	ns	0.41^{dB}	0.30^{A}	***	0.20^{bB}	0.15^{A}	***	0.08	0.01	ns	0.13^{bB}	0.08^{A}	***
t4	0.50^{bB}	0.48^{A}	*	0.40^{bcdB}	0.31^{A}	**	0.19^{abB}	0.14^{A}	***	0.02	0.01	ns	0.09^{a}	0.08	ns
t6	0.49^{b}	0.48	ns	0.40^{cdB}	0.31^{A}	***	0.18^{aB}	0.14^{A}	**	0.01	0.01	ns	0.09^{a}	0.08	ns
t8	0.46^{aA}	0.49^{B}	*	0.36^{aB}	0.32^{A}	*	0.18^{a}	0.15	ns	0.01	0.01	ns	0.09^{a}	0.09	ns
t10	0.49^{b}	0.49	ns	0.38^{bB}	0.30^{A}	**	0.17^{aB}	0.14^{A}	**	0.01	0.02	ns	0.09^{a}	0.08	ns
t12	0.48^{ab}	0.48	ns	0.38^{bcB}	0.31^{A}	***	0.19^{abB}	0.14^{A}	***	0.03^{B}	0.01^{A}	*	0.09^{a}	0.08	ns
sign.	**	ns		***	ns		**	ns		ns	ns		***	ns	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 40 Effect of time and recipe on FAMEs composition of the oil extracted from OO breadsticks

	C14			*C16*			*C16:1*			*C17*			*C17:1*			*C18*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.02^{b}	0.01	ns	11.93^{aA}	13.09^{bcB}	***	0.77^{abA}	1.22^{bB}	***	0.09^{bA}	0.15^{aB}	***	0.14^{aA}	0.32^{abB}	***	2.56^{c}	2.38^{d}	ns
t2	0.02^{b}	0.01	ns	11.96^{aA}	13.27^{cdB}	***	0.76^{abA}	1.27^{cB}	***	0.09^{abA}	0.15^{aB}	***	0.12^{aA}	0.34^{bB}	***	2.23^{b}	2.00^{bc}	ns
t4	0.02^{b}	0.01	ns	12.25^{bcA}	13.04^{bcB}	***	0.76^{aA}	1.23^{bB}	***	0.09^{bA}	0.15^{aB}	**	0.12^{aA}	0.33^{abB}	***	2.15^{bB}	1.76^{abA}	**
t6	0.02^{b}	0.01	ns	12.41^{cA}	13.36^{dB}	***	0.78^{bA}	1.18^{aB}	***	0.09^{bcA}	0.18^{bB}	***	0.13^{aA}	0.31^{aB}	***	2.24^{bB}	1.70^{aA}	***
t8	0.01^{a}	0.01	ns	12.23^{bcA}	12.76^{aB}	**	0.76^{aA}	1.22^{bB}	***	0.07^{aA}	0.16^{abB}	***	0.12^{aA}	0.34^{bB}	***	1.47^{aA}	2.24^{cdB}	**
t10	0.02^{b}	0.01	ns	12.08^{abA}	12.93^{abB}	***	0.77^{abA}	1.21^{bB}	***	0.09^{bA}	0.15^{aB}	***	0.13^{aA}	0.32^{abB}	***	1.45^{aA}	2.02^{bcB}	***
t12	0.02^{b}	0.01	ns	12.22^{bcA}	13.17^{bcdB}	***	0.90^{cA}	1.26^{cB}	***	0.11^{cA}	0.17^{abB}	**	0.18^{bA}	0.34^{bB}	***	1.41^{aA}	2.39^{dB}	***
sign.	*	ns		***	***		***	***		***	**		***	**		***	***	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 41 Effect of time and recipe on FAMEs composition of the oil extracted from OO breadsticks

	C18:1			*C18:1ω7*			*C18:2*			*C18:3ω3*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	68.34^{aA}	69.35^{aB}	**	2.24^{aA}	3.24^{B}	**	11.79^{dB}	8.61^{dA}	***	0.77^{cB}	0.58^{A}	***
t2	68.57^{abA}	69.28^{aB}	**	2.22^{aA}	3.17^{B}	**	11.89^{dB}	8.91^{eA}	**	0.76^{cB}	0.60^{A}	***
t4	69.03^{cA}	70.19^{bB}	***	2.09^{aA}	3.23^{B}	***	11.51^{cB}	8.47^{bcdA}	***	0.68^{bB}	0.59^{A}	***
t6	68.83^{bcA}	70.28^{bB}	***	2.10^{aA}	3.37^{B}	***	11.40^{cB}	8.07^{aA}	***	0.66^{bB}	0.57^{A}	**
t8	70.02^{e}	69.72^{ab}	ns	2.40^{ab}	3.34	ns	11.03^{bB}	8.56^{cdA}	***	0.63^{aB}	0.60^{A}	*
t10	70.69^{fB}	70.12^{bA}	***	2.29^{abA}	3.32^{B}	***	10.62^{aB}	8.33^{bA}	***	0.61^{aB}	0.57^{A}	*
t12	69.68^{d}	69.67^{ab}	ns	2.60^{bA}	2.97^{B}	**	11.02^{bB}	8.41^{bcA}	***	0.62^{aB}	0.58^{A}	*
sign.	***	***		**	ns		***	***		***	ns	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time are reported. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 42 Effect of time and recipe on FAMEs composition of the oil extracted from OO breadsticks

	C20			*C20:1*			*C22*			*C22:1*			*C24*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.49^{ab}	0.48^{ab}	ns	0.43^{bB}	0.32^{A}	**	0.20	0.14^{abA}	***	0.01	0.02^{b}	ns	0.12^{cB}	0.08^{A}	***
t2	0.49^{ab}	0.48^{ab}	ns	0.41^{abB}	0.29^{A}	**	0.21	0.14^{abA}	**	0.01	0.01^{a}	ns	0.11^{bcB}	0.08^{A}	**
t4	0.48^{ab}	0.46^{ab}	ns	0.40^{abB}	0.30^{A}	***	0.19	0.14^{abA}	***	0.01	0.01^{a}	ns	0.09^{a}	0.08	ns
t6	0.47^{a}	0.45^{a}	ns	0.41^{abB}	0.29^{A}	***	0.19	0.14^{aA}	***	0.01^{A}	0.02^{bB}	*	0.09^{a}	0.08	ns
t8	0.47^{a}	0.48^{b}	ns	0.40^{abB}	0.33^{A}	**	0.19	0.14^{abA}	**	0.01	0.01^{a}	ns	0.08^{a}	0.08	ns
t10	0.50^{bB}	0.47^{abA}	**	0.40^{abB}	0.31^{A}	**	0.19	0.14^{aA}	***	0.02	0.02^{b}	ns	0.09^{a}	0.08	ns
t12	0.48^{ab}	0.48^{ab}	ns	0.39^{aB}	0.31^{A}	**	0.19^{A}	0.15^{bB}	***	0.02	0.02^{b}	ns	0.09^{ab}	0.08	ns
sign.	*	*		*	ns		ns	*		ns	***		***	ns	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 43 Effect of time and recipe on FAMEs composition of the oil extracted from F breadsticks

	C14			*C16*			*C16:1*			*C17*			*C17:1*			*C18*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.02	0.01	ns	12.11^{aA}	13.12^{B}	***	0.92^{aA}	1.24^{bcB}	***	0.11^{ab}	0.16^{ab}	ns	0.18	0.33^{ab}	ns	2.62^{eB}	2.43^{dA}	**
t2	0.08	0.01	ns	12.36^{abA}	13.15^{B}	***	0.92^{aA}	1.25^{bcB}	***	0.11^{abA}	0.14^{aB}	**	0.17^{A}	0.34^{bB}	***	2.15^{d}	2.14^{c}	ns
t4	0.02	0.01	ns	12.35^{abA}	13.04^{B}	**	0.91^{aA}	1.24^{bcB}	***	0.11^{abA}	0.17^{abB}	**	0.17^{A}	0.34^{bB}	***	2.23^{dB}	1.76^{aA}	*
t6	0.02	0.01	ns	12.64^{c}	12.87	ns	0.94^{bA}	1.19^{aB}	***	0.10^{aA}	0.17^{bB}	**	0.17^{A}	0.34^{bB}	***	2.04^{cd}	1.85^{ab}	ns
t8	0.02	0.01	ns	12.49^{bcA}	12.82^{B}	**	0.93^{abA}	1.23^{bB}	***	0.10^{aA}	0.17^{abB}	***	0.17	0.35^{b}	ns	1.22^{aA}	2.04^{bcB}	**
t10	0.02	0.01	ns	12.28^{ab}	13.24	ns	0.91^{aA}	1.20^{aB}	***	0.11^{bA}	0.15^{abB}	*	0.17^{A}	0.31^{aB}	***	1.62^{bA}	2.14^{cB}	***
t12	0.01	0.01	ns	14.71^{dB}	13.06^{A}	***	0.91^{aB}	1.26^{cA}	***	0.15^{cA}	0.16^{abB}	*	0.17^{A}	0.34^{bB}	*	1.73^{bcA}	2.70^{eB}	***
sign.	ns	ns		***	ns		*	***		***	*		ns	**		***	***	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 44 Effect of time and recipe on FAMEs composition of the oil extracted from F breadsticks

	C18:1			*C18:1ω7*			*C18:2*			*C18:3ω3*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	67.18^{aA}	69.23^{aB}	***	3.08^{bc}	3.20^{bc}	ns	11.75^{deB}	8.63^{cdA}	***	0.74^{eB}	0.59^{cA}	***
t2	67.88^{bA}	69.50^{aB}	***	2.33^{aA}	3.16^{bcB}	***	11.99^{eB}	8.69^{dA}	***	0.71^{dB}	0.59^{cA}	***
t4	68.00^{bA}	70.20^{cdB}	***	2.72^{ab}	3.29^{cd}	ns	11.55^{cdB}	8.40^{bA}	***	0.66^{cB}	0.58^{bcA}	**
t6	67.95^{bA}	70.59^{dB}	***	2.41^{abA}	3.38^{dB}	**	11.80^{deB}	8.03^{aA}	***	0.67^{cB}	0.56^{abA}	***
t8	69.25^{dA}	70.21^{cdB}	**	2.50^{abA}	3.08^{bB}	***	11.38^{cB}	8.47^{bcA}	***	0.66^{cB}	0.58^{bcA}	***
t10	69.50^{d}	70.03^{bc}	ns	2.53^{abA}	3.31^{cdB}	***	10.99^{bB}	8.05^{aA}	***	0.63^{bB}	0.54^{aA}	**
t12	68.67^{cA}	69.62^{abB}	***	2.51^{abB}	2.89^{aA}	***	9.50^{aA}	8.33^{bB}	***	0.55^{aA}	0.57^{bcB}	**
sign.	***	***		***	***		***	***		***	***	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$)*

Table 45 Effect of time and recipe on FAMEs composition of the oil extracted from F breadsticks

	C20			*C20:1*			*C22*			*C22:1*			*C24*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.48^{ab}	0.49	ns	0.41^{bB}	0.33^{A}	***	0.20^{cB}	0.14^{abA}	***	0.01^{ab}	0.02	ns	0.12^{bB}	0.08^{aA}	*
t2	0.49^{bc}	0.48	ns	0.41^{bB}	0.31^{A}	***	0.20^{cB}	0.14^{abA}	***	0.01^{ab}	0.01	ns	0.09^{aB}	0.08^{aA}	*
t4	0.48^{bc}	0.47	ns	0.41^{bB}	0.29^{A}	***	0.18^{b}	0.14^{ab}	ns	0.01^{ab}	0.01	ns	0.09^{aB}	0.08^{aA}	*
t6	0.46^{a}	0.47	ns	0.40^{bB}	0.30^{A}	***	0.18^{bB}	0.14^{abA}	**	0.01^{aA}	0.02^{B}	*	0.09^{aB}	0.08^{aA}	*
t8	0.47^{ab}	0.50	ns	0.40^{bB}	0.32^{A}	*	0.18^{bB}	0.13^{aA}	***	0.01^{a}	0.02	ns	0.09^{a}	0.08^{a}	ns
t10	0.50^{c}	0.48	ns	0.39^{bB}	0.30^{A}	**	0.19^{bB}	0.13^{aA}	**	0.02^{bc}	0.01	ns	0.09^{a}	0.09^{b}	ns
t12	0.52^{d}	0.49	ns	0.31^{a}	0.32	ns	0.14^{a}	0.15^{b}	ns	0.03^{cB}	0.02^{A}	*	0.09^{aB}	0.08^{aA}	*
sign.	***	ns		***	ns		***	*		***	ns		**	***	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 46 Effect of time and recipe on FAMEs composition of the oil extracted from PR breadsticks

	C14			*C16*			*C16:1*			*C17*			*C17:1*			*C18*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.02	0.01	ns	12.09^{aA}	13.15^{abB}	***	0.93^{abA}	1.28^{bB}	***	0.11^{abA}	0.17^{B}	*	0.19^{A}	0.33^{B}	***	2.68^{eB}	2.32^{bA}	***
t2	0.02	0.01	ns	12.37^{bcA}	13.35^{bB}	***	0.94^{bcA}	1.23^{abB}	***	0.12^{bA}	0.17^{B}	**	0.20^{A}	0.33^{B}	***	2.43^{dB}	2.11^{abA}	**
t4	0.01	0.01	ns	12.50^{cA}	13.01^{abB}	***	0.93^{abA}	1.24^{abB}	***	0.10^{aA}	0.17^{B}	**	0.18^{A}	0.34^{B}	***	2.28^{dB}	1.86^{aA}	***
t6	0.02	0.01	ns	12.81^{d}	13.02^{ab}	ns	0.96^{cA}	1.22^{aB}	**	0.09^{aA}	0.17^{B}	***	0.18^{A}	0.33^{B}	***	1.75^{bcA}	2.19^{bB}	*
t8	0.02	0.01	ns	12.28^{abA}	12.79^{aB}	***	0.91^{aA}	1.23^{aB}	***	0.09^{aA}	0.17^{B}	***	0.18^{A}	0.35^{B}	***	1.88^{c}	1.85^{a}	ns
t10	0.02	0.01	ns	12.21^{abA}	12.90^{aB}	**	0.92^{aA}	1.23^{aB}	***	0.10^{aA}	0.19^{B}	***	0.19^{A}	0.34^{B}	***	1.44^{aA}	2.21^{bB}	***
t12	0.02	0.01	ns	12.22^{abA}	13.19^{abB}	***	0.92^{abA}	1.26^{abB}	***	0.11^{abA}	0.16^{B}	***	0.19^{A}	0.34^{B}	***	1.57^{abA}	2.31^{bB}	**
sign.	ns	ns		***	**		**	*		**	ns		ns	ns		***	***	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 47 Effect of time and recipe on FAMEs composition of the oil extracted from PR breadsticks

	C18:1			*C18:1ω7*			*C18:2*			*C18:3ω3*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	67.15^{aA}	69.54^{abB}	***	4.07^{dB}	3.10^{a}	***	10.79^{bB}	8.46^{abA}	***	0.72^{bB}	0.61^{abA}	***
t2	67.82^{bA}	69.13^{aB}	***	2.91^{cA}	3.43^{bB}	**	11.14^{cdB}	8.61^{bA}	***	0.71^{abB}	0.62^{bA}	***
t4	68.59^{cdA}	70.05^{cdB}	***	2.41^{abA}	3.28^{abB}	**	11.19^{dB}	8.42^{abA}	***	0.69^{abB}	0.61^{abA}	**
t6	68.47^{cA}	69.98^{cdB}	**	2.35^{aA}	3.18^{abB}	**	11.43^{eB}	8.29^{aA}	***	0.73^{bB}	0.61^{abA}	***
t8	68.97^{dA}	70.19^{dB}	***	2.60^{bA}	3.17^{abB}	**	11.03^{cB}	8.57^{bA}	***	0.69^{abB}	0.63^{bA}	**
t10	70.11^{e}	69.75^{bc}	ns	2.62^{bA}	3.46^{bB}	***	10.53^{aB}	8.27^{aA}	***	0.68^{aB}	0.59^{aA}	***
t12	69.68^{e}	69.62^{bc}	ns	2.47^{abA}	3.07^{aB}	***	10.88^{bB}	8.39^{abA}	***	0.69^{abB}	0.60^{abA}	**
sign.	***	***		***	**		***	**		**	*	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 48 Effect of time and recipe on FAMEs composition of the oil extracted from PR breadsticks

	C20			*C20:1*			*C22*			*C22:1*			*C24*		
time	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.	OPO	EVOO	sign.
t0	0.48^{ab}	0.48^{abc}	ns	0.39^{B}	0.31^{abA}	***	0.19^{B}	0.14^{A}	***	0.01	0.02^{a}	ns	0.11^{bcB}	0.08^{aA}	*
t2	0.50^{bB}	0.47^{abA}	**	0.40^{B}	0.29^{aA}	**	0.21^{B}	0.13^{A}	**	0.04	0.04^{b}	ns	0.12^{cB}	0.08^{abA}	***
t4	0.47^{ab}	0.48^{abc}	ns	0.37^{B}	0.30^{aA}	*	0.18^{B}	0.14^{A}	**	0.01	0.01^{a}	ns	0.05^{aA}	0.08^{abB}	**
t6	0.48^{ab}	0.46^{a}	ns	0.39^{B}	0.30^{aA}	***	0.18^{B}	0.14^{A}	*	0.00^{A}	0.02^{aB}	**	0.08^{b}	0.08^{a}	ns
t8	0.46^{aA}	0.50^{cB}	**	0.38^{B}	0.30^{aA}	***	0.18^{B}	0.15^{A}	**	0.01	0.02^{a}	ns	0.10^{bc}	0.08^{ab}	ns
t10	0.49^{b}	0.47^{ab}	ns	0.36	0.33^{b}	ns	0.19^{B}	0.14^{A}	*	0.01	0.02^{a}	ns	0.08^{b}	0.09^{b}	ns
t12	0.48^{ab}	0.48^{bc}	ns	0.38^{B}	0.31^{abA}	*	0.19^{B}	0.14^{A}	***	0.02	0.01^{a}	ns	0.09^{bc}	0.08^{ab}	ns
sign.	*	**		ns	**		ns	ns		ns	**		***	*	

One-way ANOVA experiment, values (%) are expressed as means of three replicates. In columns, according to Tukey's test, different small letters indicate significant differences among the different storage times. In rows, different capital letters indicate significant differences between the two recipes for each storage time. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

4.3 Research 3

As reported in the paragraph 3.3.1, samples were named as the packaging films used:

- PP (sample packaged with polypropylene film);
- PLA (sample packaged with poly-lactic acid);
- PLA+ABS (sample packaged with poly-lactic acid and a moisture absorber);
- BARR (sample packaged with an opaque film).

4.3.1 Effect of time storage and packaging on physico-chemical characteristics of *Taralli*

Moisture content (%) was influenced both by time and by packaging. Increasing values were measured for PP and BARR from *t0* to *t15*, and from *t0* to *t45* for PLA and PLA+ABS (Table 49). The trend was discontinuous over time but, in general, all the samples showed an increase in moisture (%) from the beginning to the last day of storage. At *t180*, the samples with the lowest moisture contents were PP and BARR, whose packaging had the lowest WVTR. Significant differences were found among times of storage and packaging.

Two-way ANOVA demonstrated that both time and type of packaging have a significant influence ($p < 0.001$) on water activity. One-way ANOVA among the different times of storage showed very highly significant differences ($p < 0.001$). In fact, water activity (Table 50) increased for all the samples during storage from *t0* to *t45*, when started to decrease until *t90* except for BARR sample, where a_w slightly increased until *t70* before decreasing to *t90*. PLA and PLA+ABS samples showed the highest increases from *t0* to *t45* and maintained a very similar trend over time. All values increased from *t90* to *t180*.

Among packaging, BARR always showed the lowest a_w values and reflected the same trend shown for moisture content (Table 49).

As regards hardness (g), PLA and PLA+ABS showed an increase from *t0* to *t45*, followed by a slight decrease and a consequent increase to *t180* (Table 51). These two samples reached the highest values of hardness. It could be related to the moisture absorption from the environment, as reported by Monteiro *et al.* (2016) that evaluated snacks texture throughout their shelf life, realising that with increasing moisture, the necessary force to break the snacks increased. Also Alamprese *et al.* (2017) found that in breadsticks (snacks whose recipe is similar to *Taralli*'s one) increasing values of hardness were related to moisture increase, because moisture absorption makes them less crispy and more deformable. In fact, PLA and PLA+ABS have higher WVTR in relation to the other packaging tested. Many authors studied different types of crisp foods finding that as the water activity increases the crispness decreases with simultaneous hardening of the material. Both Harris and Peleg (1996), and

Duizer and Campanella (1998) defined hardness as the force required to bite the sample. The increase of water activity in the samples and especially in PLA sample was accompanied by an increase of hardness (g). Adsorption of water added some strength to the investigated materials and reduced their brittleness (Lewicki *et al.*, 2004). Harris and Peleg (1996) explained this fact as a result of partial plasticisation of air cell wall material which increases the cohesion and hence the toughness of a product structure. PP and BARR showed the highest values of hardness at *t15* and *t70*, respectively.

The Rancimat method is an accelerated aging test in which the samples are subjected to an air flow and elevated temperature. In this process, fatty acids are oxidised and at the end of the test, volatile secondary reaction products are formed. The time until secondary reaction products are detected by the instrument is called induction time (IT). IT gives us information respectively about the secondary oxidation products of the samples. As reported in Table 52, induction time decreased significantly for all the samples during storage ($p < 0.001$). This means that lipid oxidation occurred during the storage time (Daglioglu *et al.*, 2004). As shown in Table 52, IT decreased in a slower way for the samples PP and BARR, whose packaging are characterised by lower OTR and WVTR.

No significant differences among packaging were found, except for *t180* when the lowest value was measured in PP.

Table 49 Effect of time and packaging on moisture content (%) of Taralli

Moisture content (%)

	t0	*t15*	*t45*	*t70*	*t90*	*t180*	sign.
PP	6.5^{a}	7.7 ^{cB}	7.0^{bB}	7.5^{cB}	6.6 ^{aA}	7.4^{bcA}	***
PLA	6.5^{a}	7.1^{bcAB}	7.3^{cB}	7.3^{cAB}	6.8^{abAB}	8.0 ^{dB}	***
PLA+ABS	6.5^{a}	7.2 ^{bB}	7.8^{cC}	7.4^{bB}	7.2 ^{bB}	7.9 ^{cB}	***
BARR	6.5^{ab}	6.6 ^{bA}	6.4^{aA}	6.8^{cA}	6.5^{abA}	7.3 ^{dA}	***
sign.		**	***	*	**	**	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 50 Effect of time and packaging on water activity of Taralli

a_w

	t0	*t15*	*t45*	*t70*	*t90*	*t180*	sign.
PP	0.39^{a}	0.44^{bcB}	0.46^{cB}	0.43^{bcA}	0.42^{abB}	0.53^{dB}	***
PLA	0.39^{a}	0.43^{aB}	0.55^{cC}	0.48^{bB}	0.43^{aB}	0.55^{cB}	***
PLA+ABS	0.39^{a}	0.43^{aB}	0.55^{cC}	0.48^{bB}	0.43^{aB}	0.54^{cB}	***
BARR	0.39^{ab}	0.41^{abA}	0.41^{abA}	0.42^{bA}	0.37^{aA}	0.48^{cA}	***
sign.		**	**	**	***	**	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 51 Effect of time and packaging on hardness (g) of Taralli

Hardness (g)

	t0	*t15*	*t45*	*t70*	*t90*	*t180*	sign.
PP	11548^{a}	17921 ^{cB}	13111^{abA}	11159^{a}	11791^{abA}	14495 ^{bAB}	**
PLA	11548^{a}	15533^{abAB}	19477 ^{bB}	16899^{ab}	12307^{abA}	19594 ^{bA}	*
PLA+ABS	11548^{a}	12801 ^{aA}	13250^{abA}	12623^{a}	16498 ^{bB}	14212^{abAB}	*
BARR	11548^{a}	11080 ^{aA}	10666 ^{aA}	15553^{b}	11026 ^{aA}	12264 ^{aA}	**
sign.		*	*	ns	*	*	

One-way ANOVA experiment, values are expressed as means of five replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 52 Effect of time and packaging on induction time (h) of the oil extracted from Taralli

IT (h)

	t0	*t45*	*t90*	*t180*	sign.
PP	5.89^{c}	3.93^{b}	3.05^{b}	2.07^{aA}	***
PLA	5.89^{d}	3.90^{c}	3.18^{b}	2.50^{aB}	***
PLA+ABS	5.89^{d}	3.80^{c}	3.31^{b}	2.65^{aB}	***
BARR	5.89^{d}	3.98^{c}	3.13^{b}	2.41^{aAB}	***
sign.		ns	ns	*	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

4.3.2 Effect of time storage and packaging on Volatile Profile

The volatile profile of olive oil was widely studied (Vichi *et al.*, 2003; Temime *et al.*, 2006; Kalua *et al.*, 2007; Hachicha Hbaieb *et al.*, 2016) as well as that of *Taralli* and baked products in general (Cho and Peterson, 2010; Caponio *et al.*, 2013b; Maire *et al.*, 2013; Pasqualone *et al.*, 2014) for their characterisation and because the volatile profile evolution can be useful to understand the oxidative state of the products.

Baking leads to a huge quantity of volatile compounds of neoformation which play a major role in developing the flavour of the final products. The volatile profile of baked products is mainly the result of the Maillard reaction, but both caramelization and lipid oxidation contribute to the production and the loss of volatile compounds.

The GC/MS analysis allowed the identification of 41 volatile compounds, most of which produced by thermally induced reactions occurring during baking process. Their prevailing origins were: Maillard reaction and/or sugar degradation, lipid oxidation, sulfur amino acids degradation, and raw materials. In Tables 53, 54, 55 and 56, only few of them are reported. Data were reported as Peak Area $\cdot 10^{-6}$. Aldehydes were the most abundant class of compounds detected and those related to lipid oxidation are reported in Tables 53, 54 and 55. Almost all compounds tend to increase with storage duration. Hexanal, coming from linoleic and arachidonic acids oxidation, is often used as a marker of lipid oxidation (Maire *et al.*, 2013), and it was the most abundant volatile compound since *t0* for all samples. Hexanal amount significantly increased during storage especially for PLA and PLA+ABS, even if no statistical differences were found at *t180* among samples. This was in accordance with what found by Giarnetti *et al.* (2012) studying the evolution of the volatile profile of *Taralli* as a function of both the kind of oil used in the dough and the storage time. Pentanal, n-Heptanal, 2-Heptenal, (E)-, 2,4-Heptadienal, (E,E)-, 2-Octenal, Furfural, 2-Furanmethanol and 2-Acetylfuran content tended to increase with storage duration, but not in all samples. After 180 days of storage, no many differences were found among packaging material, except for PLA+ABS that showed different values of some compounds (n-Heptanal, Octanal, Nonanal) in relation to the other packaging materials. It was probably due to the influence of the moisture absorber, that especially after 180 days probably lost its effectiveness. In general, PLA maintained a trend similar to PP and BARR, with only few exceptions.

Also Furans were found and they are probably consequence of MR (Limacher *et al.*, 2008; Cho and Lee, 2014).

Table 53 Effect of time and packaging on some VOCs of Taralli

	Pentanal					*Hexanal*					*n-Heptanal*					*2-Heptenal, (E)-*				
	t0	*t45*	*t90*	*t180*	*sign.*	*t0*	*t45*	*t90*	*t180*	*sign.*	*t0*	*t45*	*t90*	*t180*	*sign.*	*t0*	*t45*	*t90*	*t180*	*sign.*
PP	8.09	14.89	14.59 ^{A}	20.31	ns	21.43^{a}	41.19^{ab}	42.87^{abA}	74.22 ^{b}	*	2.41	3.45	3.00 ^{A}	3.52 ^{A}	ns	2.98^{a}	6.01^{ab}	4.83 ^{abA}	9.28 ^{b}	*
PLA	8.09^{a}	19.39^{ab}	31.37^{b} ^{B}	27.36^{b}	*	21.43^{a}	41.77^{b}	65.50^{c} ^{B}	90.11 ^{d}	***	2.41^{a}	2.68^{a}	3.96^{bA}	2.35 ^{aA}	*	2.98^{a}	5.84^{a}	10.73^{b} ^{B}	12.33^{b}	**
PLA+ABS	8.09^{a}	13.04^{a}	20.30^{abA}	34.19^{b}	**	21.43^{a}	28.61^{ab}	46.35^{b} ^{A}	107.17^{c}	***	2.41^{a}	3.06^{a}	8.02^{aB}	25.25^{bB}	**	2.98	3.86	3.60 ^{A}	9.34	ns
BARR	8.09^{a}	16.50^{b}	17.37^{b} ^{A}	18.96^{b}	**	21.43^{a}	37.01^{b}	49.87^{b} ^{AB}	80.31 ^{c}	***	2.41	2.91	3.07 ^{A}	3.92 ^{A}	ns	2.98^{a}	4.95^{ab}	4.41 ^{abA}	6.54 ^{b}	*
sign.		ns	**	ns			ns	*	ns			ns	**	**			ns	*	ns	

One-way ANOVA experiment, values (Peak Areas) are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 54 Effect of time and packaging on some VOCs of Taralli

	2,4-Heptadienal, (E,E)-					*Octanal*					*2-Octenal, (E)-*					*Nonanal*				
	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.
PP	0.00^{a}	0.00^{a}	0.96^{abB}	1.93^{b}	**	2.05	2.10	1.72 ^{A}	2.62 ^{A}	ns	2.24^{a}	3.99^{a}	3.24^{aAB}	5.93^{b}	**	10.94	10.31	6.46	7.50 ^{A}	ns
PLA	0.00^{a}	0.00^{a}	1.56^{c} ^{C}	1.41^{b}	***	2.05	1.57	2.21 ^{A}	2.12 ^{A}	ns	2.24^{a}	3.61^{b}	5.59^{cB}	5.71^{c}	**	10.94^{b}	7.83^{ab}	8.67^{ab}	5.77^{b} ^{A}	*
PLA+ABS	0.00^{a}	0.00^{a}	0.00^{a} ^{A}	1.12^{b}	***	2.05^{a}	4.18^{a}	10.29 ^{abB}	21.34 ^{bB}	*	2.24^{a}	2.66^{a}	2.14^{aA}	6.21^{b}	**	10.94	9.02	7.57	13.79 ^{B}	ns
BARR	0.00^{a}	0.00^{a}	0.72^{b} ^{B}	1.54^{c}	**	2.05	1.93	1.76 ^{A}	2.00 ^{aA}	ns	2.24^{a}	3.54^{ab}	2.80^{aA}	4.87^{b}	*	10.94^{b}	10.51^{ab}	5.78^{a}	6.79^{abA}	*
sign.		ns	***	ns			ns	***	**			ns	*	ns			ns	ns	**	

One-way ANOVA experiment, values (Peak Areas) are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 55 Effect of time and packaging on some VOCs of Taralli

	2-Decenal, (E)-					*2,4-Decadienal, (E,E)-*					*Benzaldehyde*					*Furfural*				
	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.
PP	1.11	1.78	1.09	1.05	ns	0.91	1.35	0.87	0.98	ns	17.38^{b}	12.70^{ab}	7.91^{a}	11.18^{aA}	*	7.24^{a}	8.55^{aBC}	8.55^{aA}	17.21^{b}	**
PLA	1.11	1.48	1.65	1.00	ns	0.91	1.13	1.24	0.84	ns	17.38	10.60	12.40	15.97 ^{B}	ns	7.24	10.11 ^{C}	15.63 ^{B}	20.20	ns
PLA+ABS	1.11	1.65	1.19	1.22	ns	0.91	1.30	0.93	1.01	ns	17.38^{b}	7.89^{a}	6.92^{a}	16.52^{bB}	*	7.24^{a}	6.06^{aA}	6.98^{aA}	21.25^{b}	**
BARR	1.11	1.70	0.81	0.95	ns	0.91	1.34	0.80	1.01	ns	17.38^{b}	12.82^{ab}	7.67^{a}	9.59^{aA}	*	7.24^{a}	7.83^{aAB}	7.27^{aA}	11.90^{b}	**
sign.		ns	ns	ns			ns	ns	ns			ns	ns	**			**	*	ns	

One-way ANOVA experiment, values (Peak Areas) are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 56 Effect of time and packaging on some VOCs of Taralli

	2-Penthylfuran					*2-Furanmethanol*					*2-Acetylfuran*				
	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.	t0	t45	t90	t180	sign.
PP	0.43	0.31 ^{A}	0.58	0.50 ^{A}	ns	6.20	5.95	5.14	9.90	ns	0.00^{a}	0.00^{a}	0.95 ^{bA}	13.04 ^{cB}	***
PLA	0.43	0.19 ^{A}	0.60	0.57 ^{A}	ns	6.20^{a}	6.04^{a}	7.66^{a}	11.23^{b}	**	0.00^{a}	0.00^{a}	1.64 ^{bB}	7.37 ^{cAB}	***
PLA+ABS	0.43	0.40 ^{A}	0.36	0.71 ^{A}	ns	6.20	5.72	5.42	9.04	ns	0.00^{a}	0.00^{a}	0.65 ^{bA}	5.43 ^{cA}	***
BARR	0.43^{a}	1.48^{abB}	1.37^{ab}	3.06^{bB}	*	6.20^{bc}	4.99^{b}	3.70^{c}	6.64^{c}	**	0.00	0.00	0.64 ^{A}	3.16 ^{A}	ns
sign.		**	ns	**			ns	ns	ns			ns	**	*	

One-way ANOVA experiment, values (Peak Areas) are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

4.3.3 Effect of time storage and packaging on the characteristics of the oil extracted from *Taralli*

Acidity value (AV) significantly increased for all samples, especially for PLA and PLA+ABS (Table 57). In fact, among the different packaging materials, the highest increase in AV was observed in these two samples. Nevertheless, at *t180* differences among packaging materials were found and the lowest values belonged to PP and BARR.

As regards PV, a significant increase was observed for all kind of packaging overtime ($p < 0.001$). After 180 days of storage, BARR sample showed the lowest PV and, consequently, the best response among packaging films (Table 58). Moreover, for this sample the increase in PV was slower than others. These peroxide values could be attributed to the high amount of poly-unsaturated fatty acids (linoleic and linolenic acid) present in EVOO. Moreover, is well known that if an oil is exposed to heating (e.g. frying, baking) it is more susceptible to oxidation (Choe and Min, 2007; Kaleem *et al.*, 2015; Giuffrè *et al.*, 2018). The effect of packaging can be noted only after 180 days, when significant differences were found.

Extinction coefficients K_{232} and K_{268}, listed in Table 59, are related to conjugate dienes and trienes which are consequence of primary and secondary oxidation, respectively, and refining processes in a fat. As well as what found for PV, also K_{232} and K_{286} values increased with storage duration, except for K_{268} parameter whose PLA sample did not increase significantly. About K_{268}, significant differences were found among samples at *t45* when PP and PLA showed higher values.

Tocopherols are methylated phenols characterised by their Vitamin E activity, of who antioxidant interactions have been discussed and demonstrated in vitro (Buettner, 1993) and later in vivo (Bruno *et al.*, 2006). In nature, Vitamin E exists as at least eight naturally occurring compounds, including α-, β-, δ- and γ-tocopherol and α-, β-, δ- and γ-tocotrienol (Cayuela and García, 2017). As we can see in Table 60, and as confirmed by Deiana *et al.* (2002), α-tocopherol content decreases during storage. This happens because α-tocopherol is one of the molecules easily oxidable (Rastrelli *et al.*, 2002). As reported in Table 61, statistical differences over time were found. Among packaging, no statistical differences were found at every time of sampling. Tukey HSD test evidenced the influence of time, and of its interaction with packaging on α-tocopherol.

The phenolic compounds found on the oil extracted from *Taralli* are the typical compounds that are generally present in virgin and extra virgin olive oils, and also those that is possible to find in wheat. Phenolic compounds in cereals exist in free, soluble conjugated and bound forms, where the bound form represents the major proportion of phenolic acids (Ragaee *et al.*, 2011; Dziki *et al.*, 2014).

In Table 62 data about total phenol content are reported. Except for BARR sample, total phenolic content significantly increased during storage, especially between 90 and 180 days after production. This increase can be due to the fact that the concentration of many phenolic compounds in olive oils, especially in VOO and EVOO, is generally low in fresh oils but increases with storage duration (Montedoro *et al.*, 1992; Servili *et al.*, 2004). It is due to the hydrolysis of complex phenolic compounds, such as secoiridoids, into low molecular weight phenolics, such as Hydroxytyrosol and Tyrsol (Cerretani *et al.*, 2009). Moreover, Han and Koh (2011) and Dziki *et al.* (2014) found in bread preparation that antioxidant activity and free phenolic acid levels were reduced during mixing process, and then recovered after fermentation and baking. These authors explained those increase by the fact that bonds with antioxidants are hydrolysed during fermentation, releasing antioxidants. At the same time, we have to take into account that baking process involves thermal and moisture conditions that facilitate the Maillard reaction (MR) and, contemporarily, the destruction of natural-labile antioxidant compounds and the formation thermally-induced ones. MR can be activated by severe heat-treatment during food processing, especially temperatures range from 100 to 250 ºC (baking, grilling, frying, extruding and roasting) and/or during long periods of storage at room temperature. These facts could explain the reasons why total phenol content increased between 90 and 180 days of storage.

Table 57 Effect of time and packaging on acidity value (AV) of the oil extracted from Taralli

	AV (% oleic acid)				
	t0	*t45*	*t90*	*t180*	sign.
PP	0.48^{a}	0.56^{abB}	0.53^{ab}	0.63^{bA}	*
PLA	0.48^{a}	0.55^{aB}	0.53^{a}	0.71^{bB}	**
PLA+ABS	0.48^{a}	0.47^{aA}	0.52^{a}	0.71^{bB}	**
BARR	0.48^{a}	0.50^{aAB}	0.51^{ab}	0.59^{bA}	*
sign.		*	ns	**	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 58 Effect of time and packaging on peroxide value (PV) of the oil extracted from Taralli

	PV (meq $O_2 \cdot kg^{-1}$)				
	t0	*t45*	*t90*	*t180*	
PP	4.6^{a}	14.0^{b}	27.4^{c}	50.3^{dB}	***
PLA	4.6^{a}	14.4^{b}	24.3^{c}	45.5^{dB}	***
PLA+ABS	4.6^{a}	15.7^{b}	24.9^{b}	41.8^{cAB}	***
BARR	4.6^{a}	12.4^{b}	21.0^{c}	31.8^{dAB}	***
		ns	ns	*	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 59 Effect of time and packaging on spectrophotometric index of the oil extracted from Taralli

	K_{232}					K_{268}				
	t0	*t45*	*t90*	*t180*	*sign.*	*t0*	*t45*	*t90*	*t180*	*sign.*
PP	2.14^{a}	3.48^{bC}	4.69^{b}	6.42^{cB}	**	0.49^{a}	0.58^{bB}	0.48^{a}	0.60^{b}	**
PLA	2.14^{a}	3.27^{bB}	4.09^{c}	5.29^{dA}	***	0.49	0.53^{AB}	0.49	0.66	ns
PLA+ABS	2.14^{a}	3.05^{bA}	4.12^{c}	5.31^{dA}	***	0.49^{b}	0.44^{aA}	0.48^{ab}	0.60^{c}	***
BARR	2.14^{a}	3.12^{bA}	4.22^{c}	5.79^{dAB}	***	0.49^{ab}	0.45^{aA}	0.46^{ab}	0.55^{b}	*
sign.		***	ns	*			*	ns	ns	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 60 Effect of time and packaging on a-Tocopherol content of the oil extracted from Taralli

α-Tocopherol (mg·kg^{-1})

	t0	*t45*	*t90*	*t180*	sign.
PP	238.7^{c}	252.5^{c}	171.4^{b}	97.48^{a}	**
PLA	238.7^{b}	204.8^{b}	178.1^{ab}	115.9^{a}	**
PLA+ABS	238.7^{b}	208.6^{b}	149.6^{a}	119.5^{a}	**
BARR	238.7^{b}	219.8^{b}	154.9^{a}	148.1^{a}	***
sign.		ns	ns	ns	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

Table 61 Effect of time and packaging on total phenol content of the oil extracted from Taralli

Total Phenols (mg·kg^{-1})

	t0	*t45*	*t90*	*t180*	sign.
PP	8.63^{b}	6.00^{aA}	7.54^{abAB}	17.78 ^{cA}	***
PLA	8.63^{a}	10.70^{aB}	9.88^{a} ^{B}	32.52 ^{bB}	***
PLA+ABS	8.63^{a}	7.58^{aAB}	9.77^{a} ^{B}	27.05 ^{bB}	**
BARR	8.63	6.26 ^{A}	6.83 ^{A}	9.15 ^{A}	ns
sign.		*	*	**	

One-way ANOVA experiment, values are expressed as means of two replicates. In columns, according to Tukey's test, different capital letters indicate significant differences among the different packaging materials. In rows, different small letters indicate significant differences among the different storage times. (, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, p > 0.05)*

V CONCLUSIONS AND FUTURE PERSPECTIVES

The use of EVOO in fat-rich baked products instead of refined oils and shortening has been studied, assessing the evolution of oxidative and hydrolytic degradation during storage. The results obtained show that the use of EVOO in baked goods is really a valid alternative in terms of health, since the oxidation parameters are significantly lower. The results obtained by this PhD thesis confirmed that lipid oxidation can be prevented by appropriate recipe formulations and a suitable packaging.

The obtained data showed that the evolution of the oxidation levels in the analysed samples during storage was related to the type of fat used in the production processes. Particularly, the use of EVOO led to significantly lower values of hydroperoxides, ultraviolet absorption constants, and higher antioxidant capacity. The high biological properties of extra virgin olive oil improve the shelf life of bakery products in which it is used.

The use of Calabrian EVOO in the studied products would lead not only to a qualitative improvement of the products themselves, but also to a greater territorial valorisation. In addition, having overcome the health aspect, and based on sustainability, EVOO has an advantage over other oils used in this case too. In the case of the breadsticks, in fact, the pomace olive oil is the lipid used for production. Pomace olive oil is a direct competitor of VOOs, however it requires an energy expenditure ten times higher than that required for the mechanical extraction of oil from olives, in addition to generating toxic wastes and to use chemical compounds harmful to the environment and to human health.

Moreover, it was found that a biodegradable and compostable packaging (PLA) can be used for some baked products such as *Taralli*, increasing the sustainability the products.

The obtained results are summarised in the following paragraphs.

5.1 Research 1:

As reported in the paragraph 3.1.1, *Cantuccini*, typical Italian sweet products, are produced using shortening as fat ingredient. However, shortening contains considerable amount of SFA. The aim of the research was to study the oxidative stability of *Cantuccini* obtained with the original recipe (OR) and to compare it with the same type of products obtained replacing part of the shortening with extra virgin olive oil from Calabria region (ER). The ER was obtained replacing the 75% of shortening with the same amount of extra virgin olive oil.

As reguards physical characteristics, samples with EVOO in their recipe (ER) showed lower a_w and moisture content values. Since the evidence that water activity gives us important information since it accounts for the availability of water for degradation reactions

(Mathlouthi, 2001), ER samples are better preserved because of their lower a_w values. Moreover, ER samples had lower moisture content and, consequently, longer shelf life if they are stored with appropriate packaging and proper storage condition. Thus, substitution of the 75% of shortening for EVOO in the preparation of cookies will produce more shelf-stable product due its lower moisture content. However, ER samples, in which the shortening has been partially substituted with EVOO, had a higher hardness. The liquid oil, in fact, made the biscuits harder in comparison to the ones containing bakery and hydrogenated fat.

According to data about the chemical characterisation of the fat extracted from *Cantuccini*, the lipid fraction of *Cantuccini* prepared only with shortening (OR) was markedly more degraded than that prepared by substituting shortening with EVOO (75%), with significantly higher values of AV, K_{232} and K_{270}. ABTS and DPPH assays, both performed on the hydrophilic antioxidant extract (HAE), gave different responses in terms of antioxidant activity. It is interesting to note that for the hydrophilic fraction, the DPPH test gave lower results than the ABTS assay. OR samples showed higher values of TEAC in comparison to ER ones. During OR samples' storage TEAC decreased, while no changes were found in ER TEAC. Regarding DPPH assay on HAE, values increased during storage for both recipes. Results about the DPPH assay performed on the fat evidenced that ER samples showed a higher antioxidant capacity.

Fatty acid composition changed when EVOO was used in the recipe (ER). In fact, in ER samples were characterised already at the beginning of the study by higher values of Margaric Acid (C17), Heptadecenoic Acid (C17:1), Oleic Acid (C18:1ω9) and Vaccenic Acid (C18:1ω7). Olive oils, in fact, are characterised not only by high amount of oleic acid, but also by the presence of small amounts of other fatty acids, such that found in this study.

Thus, the use of EVOO in *Cantuccini* recipe led the reduction of saturated fatty acids (SFAs) and improved the amount of unsaturated fatty acids (UFAs). In particular, EVOO increased the amount of monounsaturated fatty acids (MUFAs) and, as a consequence, the UFAs/SFAs ratio. Moreover, EVOO used in the recipe improved the chemical characteristics of the lipid fraction of the products, slowing down the oxidation phenomena. In conclusion, the use of EVOO in *Cantuccini* recipe is highly recommended, to improve quality characterstics and healthy properties of *Cantuccini*.

5.2 Research 2:

As reported in the paragraph 3.2.1, four types of breadsticks differently flavoured were used for this study: with garlic and chilli pepper (GP), with onions and olives (OO), with wild fennel seeds (F) (*Foeniculum vulgare* Mill), with potatoes and rosemary (PR). These types of snacks are normally produced using olive pomace oil, a low-quality olive oil. Thus, the aim of the research was to study the breadsticks physical characteristics, as well as the oxidative stability of the oil extracted from the products in order to compare OPO recipe and EVOO recipe breadsticks during 12 months of storage.

The study of the four types of breadsticks showed that both in OPO and in EVOO samples, a_w and moisture content (%) significantly increased ($p < 0.001$) during storage, and that in general, the kind of oil used did not influence *Taralli*'s physical properties

Hardness significantly changed during storage almost for all breadsticks. But, in general, we can assume that hardness was not influenced by the type and the quality of the vegetable oil used.

If the colour of breadsticks external surface is considered, no many differences were revealed between recipes, except for few samples and for brightness that was higher in EVOO than OPO samples.

As regards results about the quality characteristics of the extracted oils, they clearly show that the lipid fractions of GP, OO and F OPO breadsticks were markedly more degraded than EVOO ones, with significantly higher values of K_{232} and K_{270}, but, above all, peroxide value (PV). This is due to the higher stability and the higher content of antioxidants (total phenols and carotenoids) that characterised EVOOs in comparison to refined vegetable oils such olive pomace oil.

Thus, results demonstrated the essential role played by the type of oil on chemical and antioxidant properties of the lipid fraction of breadsticks. Finally, breadsticks made with extra virgin olive oil, compared with those prepared with other olive pomace oil, showed to be more resistant to oxidation, probably due to the presence of natural antioxidants.

5.3 Research 3:

The aim of the third research was to study of oxidative stability of *Taralli* prepared with EVOO and packaged in different packaging films: PP (sample packaged with polypropylene film); PLA (sample packaged with poly-lactic acid); PLA+ABS (sample packaged with poly-lactic acid and a moisture absorber); BARR (sample packaged with an opaque film). The main research question was: is an alternative packaging (PLA) able to offer similar oxidative stability and the maintenance of quality attributes in comparison to traditional packaging (PP) of low moisture baked products?

Moisture content (%) was influenced both by time and by packaging. At *t180*, the samples with the lowest moisture contents were PP and BARR, whose packaging had the lowest WVTR. Significant differences were found among times of storage and packaging.

Two-way ANOVA demonstrated that both time and type of packaging have a significant influence ($p < 0.001$) on water activity. One-way ANOVA among the different times of storage showed very highly significant differences ($p < 0.001$). Between packaging, BARR always showed the lowest a_w values and reflected the same trend shown for moisture content.

As regards hardness (g), PLA and PLA+ABS reached the highest values of hardness. It could be related to the moisture absorption from the environment, as reported by several authors that evaluated snacks texture throughout their shelf life, realising that with increasing moisture and water activity, the necessary force to break the snacks increased. In fact, PLA and PLA+ABS have higher WVTR in relation to the other packaging tested.

Induction time decreased significantly for all the samples during storage ($p < 0.001$). This means that lipid oxidation occurred during the storage time (Daglioglu *et al.*, 2004). IT decreased in a slower way for the samples PP and BARR, whose packaging are characterised by lower OTR and WVTR, but no significant differences among packaging were found, except for *t180* when the lowest value was measured in PP.

GC-MS analysis showed that Aldehydes were the most abundant class of compounds. After 180 days of storage, no many differences were found among packaging material, except for PLA+ABS that showed different values of some compounds (n-Heptanal, Octanal, Nonanal) in relation to the other packaging materials. It was probably due to the influence of the moisture absorber, that especially after 180 days probably lost its effectiveness.

Acidity value (AV) significantly increased for all samples, especially for PLA and PLA+ABS that were the two samples in which the highest increase in AV was observed.

As regards PV, the effect of packaging can be observed only after 180 days, when significant differences were found.

As well as what found for PV, also K_{232} and K_{286} values increased with storage duration, except for K_{268} parameter whose PLA sample did not increase significantly.

α-tocopherol content decreased during storage and statistical differences over time were found. Among packaging, no statistical differences were found at every time of sampling. Tukey HSD test evidenced the influence of time, and of its interaction with packaging on α-tocopherol.

Total phenols content significantly increased during storage, especially between 90 and 180 days after production, for all samples except for BARR one.

In general, the biodegradable packaging (PLA) demonstrated to be a good alternative to PP up to 90 days especially when associated with moisture absorber. However, the moisture absorber demonstrated to lose its effectiveness after 180 days. It is true that PLA has poor gas and moisture barrier properties that affected PV and hardness, but it is also true that *Taralli* packaged in this packaging material showed good response for IT and K_{268} as well as for α-tocopherol content, when no differences were found among packaging at the end of the storage. Moreover, total phenols and VOCs analysis demonstrated that PLA acts in a similar way to PP. For these reasons we can assume that PLA could be a good alternative to traditional packaging, especially for periods of time that do not exceed three months.

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