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**MODELLING THE PERFORMANCES OF TRADITIONAL AND
INNOVATIVE FATS IN A PLUM CAKE FORMULATION**

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ABSTRACT

Butter is traditionally used in bakery formulations, as it guarantees good performances in terms of volume, softness and sensory outcomes; on the other hand, it contains saturated fats and cholesterol, the two dietary ingredients that increase low density lipoprotein (LDL) cholesterol. Saturated fats are also present in palm oil which is widely used as a cheaper alternative to butter. Therefore, finding alternatives to animal fats and saturated fats for bakery products is an attractive challenge.

The aim of this PhD thesis is to study the technological role of traditional and innovative fats in baked goods, using experimental designs to model their effects on final products and to optimize the formulation. The model system chosen is a plum cake formulation, produced following the creaming process, in which the characteristics of fats play an important role on the structure of the batter.

In a first phase, a central composite design (CCD) was applied in order to model and optimize fat quantity and composition in plum cake production. Fat used were blends of palm oil and palm olein with different slip melting points (SMP) ranging from 36.3°C to 11.3°C, in dependence of olein content. Plum cakes were produced varying both fat content in the batter (from 5.3% to 30.7% on batter) and percentage of olein in the fat blend (from 42.7% to 92.2%); plum cakes containing 18% butter or anhydrous butter were considered as references.

Models obtained demonstrated that cake texture was significantly affected ($p < 0.01$) both by fat content and percentage of olein in the fat blend, while volume was influenced ($p < 0.01$) only by the fat content. An optimized cake, corresponding to a formulation containing 19.7% fat in the batter and 92% olein in the fat blend, resulted similar to the butter reference for texture and volume characteristics, which are important quality indexes of a baked product. Furthermore, the comparison of the optimized formulation with reference cakes highlighted the role of fat SMP on cake volume, suggesting that a well aerated structure can be achieved even with a minimal solid fat content.

Fats and batters rheology was also studied, as well as cake characteristics during storage, comparing the formulations made with the optimized fat blend, butter and anhydrous butter. A clear influence of the fat type on batter structural characteristics was demonstrated, even if the rheological behaviour of batters did not always reflect differences observed among cakes. In particular, fat properties had an impact on batter viscoelastic behaviour, also affecting creaming performances. A higher content of unsaturated fatty acids, such as in the optimized formulation, resulted to be positive for baking performances, if adequately balanced in the formulation. In fact, the optimized cake attained good structural properties, revealing in the meantime the best oxidation stability, probably due to the antioxidant action of tocopherols and tocotrienols, naturally present in palm-derived oils.

In a second phase, the effects of the use of fats structured as organogels (OG) in the cake formulation were studied. An OG can be defined as an organic liquid entrapped within a thermo-reversible, three-dimensional gel network, formed by the self-assembly of gelator molecules. In this research, the gelators used were β -sitosterol and γ -oryzanol (2:3, w/w), phytosterols known for having antioxidant properties and lowering blood cholesterol. The gelators were dissolved in warm sunflower oil and OG at different concentrations were produced. A CCD was applied, with gelators concentration in OG (from 3.09% to 5.91%) and the quantity of OG in the batter (from 5.10% to 24.90%) as the two factors. Analysing the effects, models for moisture and some colour parameters resulted significant for OG quantity, while gelator concentration did not influence cake properties. Even though rheological evaluations of OG showed that all the gels considered in the CCD were solid-like materials, in which structuring is enhanced by increasing gelators content, the difficult standardization and time-dependent modifications of the material at low gelators concentrations may have interfered

with the evaluation of the final baked products. However, the comparison among CCD centre point (CC), a reference formulation (SC) produced with liquid sunflower oil, and a cake produced with liquid sunflower oil and gelators dispersed as powders (SPC), showed that CC cakes were significantly different ($p < 0.05$) from the others for texture, thus highlighting an influence of structured fat. Moreover, during SC and CC cake storage at 25°C, CC cakes appeared softer and moister than SC, at least in the first two weeks of storage. Instead, rheological evaluation of SC, SPC and CC batters showed no significant difference.

RIASSUNTO

Il burro è il grasso tradizionalmente utilizzato nei prodotti da forno, in quanto garantisce elevata sofficità, buon sviluppo di volume e apprezzate caratteristiche sensoriali al prodotto finito. Purtroppo contiene acidi grassi saturi e colesterolo, entrambi fattori che contribuiscono all'aumento dei livelli plasmatici di colesterolo LDL. Anche l'olio di palma, grasso vegetale ampiamente utilizzato in pasticceria per le sue caratteristiche tecnologiche, contiene grassi saturi. Perciò, la ricerca di efficaci alternative ai grassi animali e ai grassi saturi da utilizzare nei prodotti da forno rappresenta una concreta possibilità per il miglioramento del profilo nutrizionale di tali alimenti.

Lo scopo di questa tesi di dottorato è quello di studiare il ruolo tecnologico di grassi tradizionali ed innovativi nei prodotti da forno, applicando disegni sperimentali per modellare i loro effetti sui prodotti finali ed ottimizzare la formulazione. Il sistema modello scelto è una formulazione tipo *plum cake*, prodotta seguendo il processo di *creaming*, nel quale le caratteristiche dei grassi svolgono un importante ruolo sulla struttura dell'impasto.

In una prima fase, è stato applicato un Central Composite Design (CCD) per modellare ed ottimizzare la quantità di grasso e la sua composizione nella produzione dei *cake*. I grassi utilizzati sono stati miscele di olio di palma e oleina di palma con differenti punti di scorrimento (SMP), diversi in base al contenuto di oleina, che rappresenta la frazione più insatura. I fattori del CCD sono stati il contenuto di grasso (da 5.3% a 30.7% nell'impasto) e la percentuale di oleina nella miscela di grassi (da 42.7% a 92.2%); due formulazioni contenenti 18% di burro (BC) o 18% di burro anidro (ABC) sono state considerate come riferimenti. I modelli ottenuti hanno dimostrato che la quantità di grasso ha avuto un effetto significativo ($p < 0.01$) su consistenza e volume dei *cake*, mentre il contenuto di oleina (e quindi le caratteristiche di fusione) ha influenzato ($p < 0.01$) solo la *texture* dei prodotti. Applicando alle risposte la funzione di desiderabilità, è stata identificata una formulazione ottimizzata (OF) contenente il 19.7% di grasso di cui il 92% oleina. Tale formulazione ha permesso di ottenere un *cake* soffice, caratterizzato dallo stesso volume del riferimento burro e da buone proprietà strutturali, oltre che da una migliore stabilità all'ossidazione. Inoltre, il confronto tra OF, BC e ABC ha evidenziato il ruolo dello SMP sul volume del *cake*, suggerendo che una struttura ben aerata può essere raggiunta anche con un minimo contenuto di grasso solido. Dallo studio delle caratteristiche reologiche del grasso, e dei corrispondenti impasti, di BC, ABC e OF, è stata evidenziata l'influenza del tipo di grasso sul comportamento viscoelastico degli impasti, nonché sulle caratteristiche di *creaming*.

In una seconda fase, sono stati studiati gli effetti dell'uso di grassi strutturati come *organogel* (OG), nella formulazione del *cake*. Un OG può essere definito come un liquido organico (olio) intrappolato all'interno di una struttura tridimensionale, in cui specifiche molecole (*gelators*) si auto-assemblano formando un gel termo-reversibile. In questa ricerca, gli OG sono stati preparati in olio di girasole, utilizzando come *gelators* β -sitosterolo e γ -orizanolo (2:3, p/p), noti per avere proprietà antiossidanti e abbassare il colesterolo nel sangue. L'effetto degli OG nella formulazione del *cake* è stato studiato applicando un CCD a due fattori, rappresentati dalla concentrazione di *gelators* nell'olio (da 3.09% a 5.91%) e dalla quantità di OG nell'impasto (da 5.10% a 24.90%). Dall'analisi degli effetti, i modelli di umidità e alcuni parametri di colore sono risultati significativi per la quantità di OG, mentre la concentrazione di *gelators* non ha influenzato le caratteristiche dei prodotti. Sebbene le valutazioni reologiche degli OG abbiano mostrato che tutti i gel considerati nel CCD corrispondono a materiali *solid-like*, in cui la strutturazione aumenta al crescere del contenuto di *gelators*, la difficile standardizzazione e l'evoluzione nel tempo degli OG a basse concentrazioni di *gelators* ha probabilmente interferito con la valutazione delle caratteristiche del prodotto finito. Tuttavia, confrontando il punto centrale (CC) del CCD, una formulazione di riferimento (SC) prodotta con olio di girasole non

strutturato, e un *cake* con olio di girasole non strutturato e *gelators* aggiunti in polvere all'impasto (SPC), il *plum cake* CC è risultato significativamente differente ($p < 0.05$) dagli altri in termini di *texture*, evidenziando così un influsso del grasso strutturato. Inoltre, nelle prime due settimane di conservazione, il *cake* CC ha mostrato una maggiore umidità e morbidezza rispetto a SC. Al contrario, le valutazioni reologiche degli impasti non hanno mostrato alcuna differenza significativa tra i campioni.

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PREFACE

Bakery products, as other cereal-based foods, contribute to satisfy the nutritional needs of human beings. Fats are one of the key ingredients of this type of foods, as they perform major functions to entrap air during creaming process, to interfere with the continuity of starch and protein particles, and to emulsify the liquid components of the formulation (Sowmya et al., 2009). Thus, fats contribute to volume, texture and overall palatability of the baked products.

Vegetable fats are commonly used in bakery industry as cheaper and zero-cholesterol alternatives to butter; their crystallization and melting properties can influence not only quality and structure of the baked product but also its behaviour throughout storage. In addition to their technological role, lipids also have an impact on the nutritional profile of a food product. The current presence of saturated fatty acids (SFA) and *trans* fatty acids (TFA) in bakery fats, in fact, tends to raise the level of blood cholesterol and increases the risk of cardiovascular disease (Cercaci et al., 2006). On the other hand, the elimination or replacement of a portion of the high-melting fat with the more healthy unsaturated or polyunsaturated oils can lead to adverse consequences on the quality of the baked goods. In fact, the positive performances of fats in bakery are mainly a consequence of their melting characteristics that provide the desired solid fat content at processing temperature. Smith and Johansson (2004) observed in fact that fat melting characteristics affect texture and volume of bread, as an increase in solid fat leads to a softening of bread and a decrease in the rate of staling, suggesting an interaction between saturated triglycerides (TG) and amylopectin. Thus, raising the concentration of solid fat could improve bread quality, although increasing saturated fat content. Nowadays, unsaturated fats are preferred for their nutritional properties, even though they are more susceptible to oxidation thus impairing product quality during storage. Indeed, lipid oxidation reactions occurring during the storage of bakery products are the main deteriorative event affecting their quality (Calligaris et al., 2007). Römer et al. (2008) reported that oxidised lipids could even react with protein by hydroperoxides or secondary lipid peroxidation products, bringing to a nutritional loss in the product. In order to evaluate the degree of oxidation, peroxide value (PV) is commonly used; Calligaris et al. (2007) considered it as a representative index of the quality depletion of biscuits during their shelf life, linearly related to consumer acceptability.

In order to face these issues, many technological attempts have been made to find a suitable alternative to traditional shortenings. Recent findings describe edible organogels, a differentiated class of materials in which a network of self-assembled molecules (called gelators) immobilizes an organic liquid, i.e. an unsaturated oil, forming thermally reversible gels upon cooling (Wright & Marangoni, 2007). A system offering potential food application is the organogel based on a combination of phytosterols and γ -oryzanol as gelators.

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1. State of the Art

Cereal based foods are very ancient products that have been employed by humans since prehistoric times. In fact, the first traces of bread can be tracked back to 30000 years ago but only with the beginning of agriculture, in the Neolithic age, bread was produced widely. The introduction in the diet of a “cake” was a following step. At first, cakes were sweetened with honey and produced adding nuts and dried fruits. When cane sugar made its way from India to Arab countries, it became one of the main ingredients of this product. Furthermore, a key ingredient that differentiates a cake from bread is the high content of fat, making a cake a calorie rich food product.

Nowadays, in the developed and richer countries such as Europe, the act of eating has become not only essential for living but also, and in some cases mostly, a moment of indulgence. For this reason, many people suffer from pathologies related to the excess of food and calories intake deriving from sweet, fatty products and the so-called “junk food”. The understanding of how food products impact on people’s lives and the improvement of their quality and nutritional characteristics is a topic worth to be studied.

1.1. Lipid chemistry: an overview

Fats are constituted by triglycerides, esters derived by glycerol and three carbon chains, called fatty acids; the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds (Fig. 1.1).

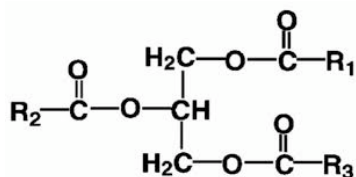


Figure 1.1. General representation of a triglyceride structure; R represents the alkyl chain.

Fatty acids (FA) are almost entirely straight chain aliphatic carboxylic acids; they can be unsaturated or saturated, depending on the presence -or absence- of double bonds between the carbons of the chain. An unsaturated fatty acid might present one (MUFA, Mono Unsaturated Fatty Acids) or several (PUFA, Poly Unsaturated Fatty Acids) double bonds, which confer to the triglyceride a less compact structure. Naturally formed double bonds are generally in the *cis* configuration, meaning that adjacent hydrogen atoms are on the same side of the double bond; this causes the chain to bend and restricts the conformational freedom of the fatty acid. On the contrary, if the double bond is *trans*, the two hydrogen atoms lay at opposite sides of the bond and the chain conformation results similar to the corresponding saturated fatty acid. *Trans* configuration often derives from hydrogenation process (see § 1.5.2). Unsaturated FA can be discriminated also by the position of the first carbon involved in a double bond, counting from the terminal methyl carbon, named the ω group. Thus the families of ω -9, ω -6 and ω -3 FA are identified, each bringing a different biological significance.

The different length (from 4 to 22 carbons) and saturation (from none up to 6 double bonds) of fatty acids determine their physical and chemical properties, e.g. melting point, susceptibility to oxidation, enzyme reactivity. These FA characteristics, as well as their position on the glycerol backbone, influence the corresponding properties of TG. In plant oils, unsaturated FA predominate in the *sn*-2 position, with more saturated acids in the *sn*-1 and *sn*-3 position; in animal fats, the type of fatty acid predominating in the *sn*-2 position is more variable (Scrimgeour, 2005).

1.2. Vegetable fats and oils

In the last decades, food industry showed a growing interest in using fat materials different from traditional animal fats (such as butter, lard, tallow), for nutritional and economic issues. Moreover, the availability of relatively inexpensive palm-oil fractions and concerns about overfishing in some of the world's seas has meant there has been a major shift in European countries to the use exclusively of vegetable oil products in bakery fats (Podmore, 2002).

Vegetable oils and fats include a wide variety of materials, obtained from seeds, pulp or kernels of plants. Fats can be obtained either by mechanical extraction, using presses or expellers, or by chemical extraction, employing organic solvents. Regardless of the extraction method used, the majority of vegetable oils is refined in order to remove undesired components (waxes, phospholipids), prolong shelf life and improve sensorial characteristics; as a major drawback, this process has the depletion of the active compounds of raw materials, such as tocopherols and polyphenols.

Vegetable fats are commonly used in the bakery industry, such as in doughs and biscuit fillings, as cheaper and zero-cholesterol alternatives to butter. They contribute to incorporation of air into the dough, enhance heat transfer during cooking and give finished products characterized by a moist mouthfeel and a softer texture (Conforti, 2006a). They are used as such or emulsified (margarines) and in some applications they present better performances than butter (e.g. in puff pastry) (Stauffer, 1996). The capability of vegetable fat blends to be used as shortenings in bakery products depends on their crystallization and melting properties (Bell et al., 2007). These ingredients are in fact mainly constituted of different families of triglycerides, which crystallize at different temperatures depending on their chemical composition. Frequently, the choice of fats and oils to be used in a blend for a shortening is a function of empirical experience more than scientific choice, as not much is known about the cumulative effect of chemical and physical properties of lipids of different sources (Dogan et al., 2007). For this reason, the control of fat physical properties has been of importance in research efforts: monitoring solid fat content (SFC), melting profile and polymorphism can clarify molecular and physical structure of TG and their crystals, which strongly influence rheological and texture properties (Koyano & Sato, 2002).

1.2.1. *Crystallization behaviour*

Polymorphism is defined as the ability of a chemical compound to form different crystalline or liquid crystalline structures (Koyano & Sato, 2002). In lipids, differences in hydrocarbon chain packing and variations in the angle of tilt of the hydrocarbon chain packing differentiate polymorphic forms (Metin & Hartel, 2005). The crystallization behaviour of TG, including crystallization rate, crystal size, morphology, and total crystallinity, is affected by polymorphism. The molecular structure of the TG and several external factors like temperature, pressure, rate of crystallization, impurities, and shear rate, also influence polymorphism (Sato, 2001).

The general scenario for fat crystallization is first nucleation and growth of crystals, followed by aggregation due to van der Waals attractions, ending up with the formation of a crystalline three dimensional network (Walstra et al., 2001). Depending on crystallization conditions, triglycerides crystallize either in α , β' or β form, as shown in Fig. 1.2. In fact, when a fat is quickly cooled, with a chilling speed over 0.25°C/min (Van Malssen et al., 1996), it will crystallize in the rather unstable α form, characterised by hexagonal subcells (Koyano & Sato, 2002). This is a waxy solid that quickly changes into long needle-like clusters of β' crystals which immobilize several times their own weight in liquid oil. This is the preferred crystal form for plastic shortenings, as clusters are readily broken when squeezed, giving an overall feeling of a very smooth and creamy solid. If this crystal phase is not stabilized by proper tempering at

the time of manufacture, the solid phase reorganizes into the most stable β form (Stauffer, 1996); these larger and coarser crystals bring to lower plasticity, making the fat feel sandy or grainy, and also oily (Podmore, 2002).

The type of crystals in a shortening is also influenced by the composition of the fat: solid triglycerides with a relatively homogeneous fatty acid composition tend to align more readily and to pack more closely together, because the similarity of their molecular configuration favours free interaction of their attractive forces. On the other side, a more heterogeneous fatty acid composition will form a more loosely arranged crystal lattice, impeding the transformation to their higher melting polymorphic forms (Pylar, 1988).

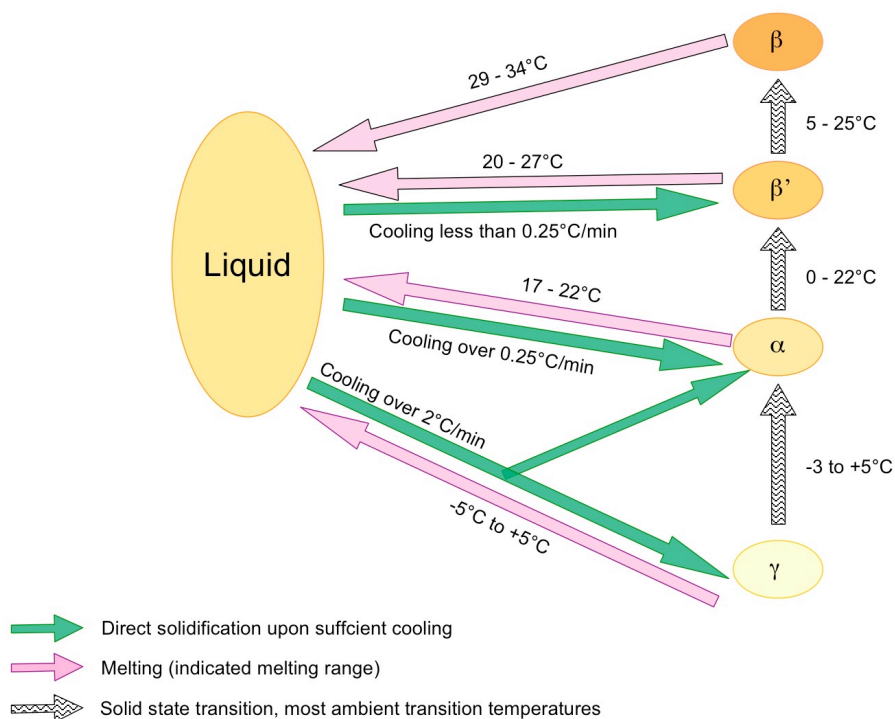


Figure 1.2. Schematic representation of crystal formation in a liquid fat upon cooling or heating (Van Malsen et al., 1996).

Other factors that have to be taken into consideration are the manner of texturation or plasticization, the temperature and duration of tempering, the presence of additives (i.e. emulsifiers, crystal modifiers), the time and temperature of storage (Pylar, 1988). All these aspects have an impact even when the fat is included in food formulations. Fat bloom in chocolate, for instance, is a well known example of how the change of polymorph (in this case, from IV-V form to the more stable VI) damages the quality of the end product. More generally speaking, Koyano and Sato (2002) say that transformation of polymorph β' to polymorph β in food fats often causes physical deterioration of the product, mostly because of changes in the crystal morphology and network. Thus, transport and storage conditions, especially temperature fluctuations, should be accurately monitored for high fat food.

1.2.2. *Palm oil*

Palm oil is one of the most common fats used in the food industry, especially in confectionery and bakery products, because it is a low cost material, stable towards oxidation and versatile. *Trans* free uses of palm oil are shortenings, margarine, puff pastry margarine, frying oil, and vanaspati (<http://www.americanpalmoil.com>).

It is obtained from the mesocarp (pulp) of the fruit of the oil palm whose inner nut additionally yields palm kernel oil. Each palm tree produces approximately one fruit bunch of 4-20 kg, containing 200-2000 individual fruits that furnish palm oil (20-24%) and palm-kernel oil (2-4%) (Gunstone, 2005). In addition, each palm tree continues producing fruit economically for up to 25 years. This ensures a constant stable supply, as compared with other annual crops (<http://www.andrew.cmu.edu/user/jitkangl/Index.htm>). Palm oil production is still increasing rapidly and is likely to become the world's most produced oil within 10 years (Timms, 2005).

Palm oil contains almost equal proportions of saturated (48% palmitic and 4% stearic) and unsaturated (37% oleic and 10% linoleic) fatty acids (Gunstone, 2005). It is essentially composed of three types of triglycerides: trisaturated (mainly tripalmitin), disaturated (mainly 2-oleodipalmitin, 30-40%) and monosaturated (mainly 1-palmito-2-3-diolein, 20-30%). At room temperature, palm oil is a semi-solid material in which texture, hardness and spreadability depend on the amount, size and tri-dimensional organization of the fat crystal network; it melts typically in a range of 33-41°C (Tarabukina et al., 2009). Unrefined palm oil presents a bright orange colour and a strong taste, mainly due to the presence of carotene, tocopherols and tocotrienols (vitamin E), valuable by-products of the refining process (Gunstone, 2005). In fact, palm oil is usually refined, bleached, deodorized and eventually fractioned, obtaining different blends with different melting points, on the basis of the triglyceride composition (Fig. 1.3).

Fractionation is undoubtedly the oldest fat modification process and was the foundation of the modern edible-oil and fat-processing industry. It is a purely physical process, carried out in two stages. At first, a crystallisation stage allows the formation of crystals by cooling the oil; their separation from the liquid phase is easier in the presence of a solvent, which dilutes the oil and lowers the viscosity (Timms, 2005).

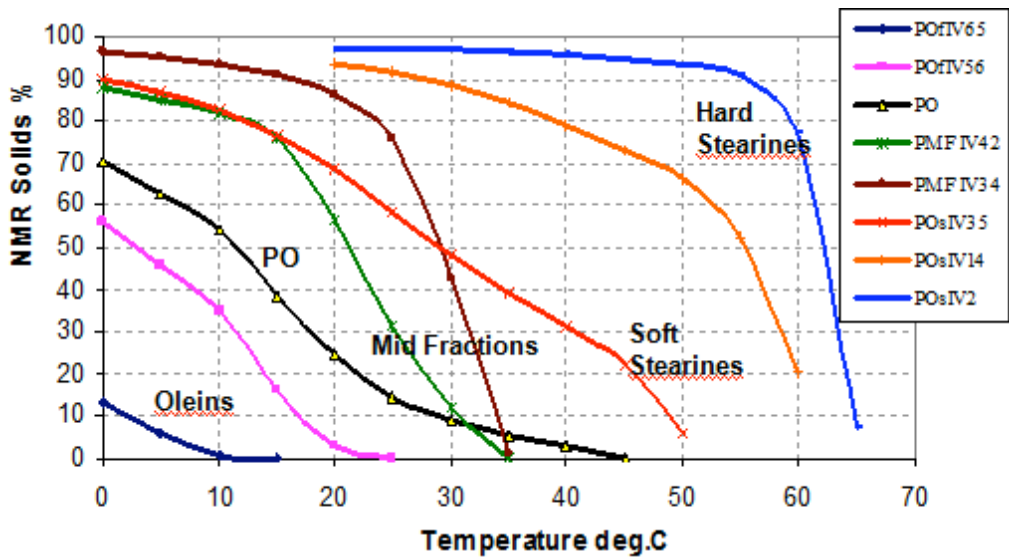


Figure 1.3. Solid fat content of palm oil fractions with different iodine values. PO: palm oil; POF: palm oil fractionated; PMF: palm oil mid-fractions; POs: palm oil stearin; IV: iodine values (<http://lipidlibrary.aocs.org>, courtesy of IOI Edible Oils).

The second stage consists in the separation of the solid crystals, after which two components are obtained: palm olein, a liquid oil composed by monosaturated triglycerides, and palm stearin, a solid fat composed by the trisaturated ones (<http://www.americanpalmoil.com>) (Fig. 1.4). Olein, a high-quality and highly stable frying oil, has a cloud point of 7–10°C and can be fractionated further to give even more unsaturated oleins and palm mid fractions (Gunstone, 2005).

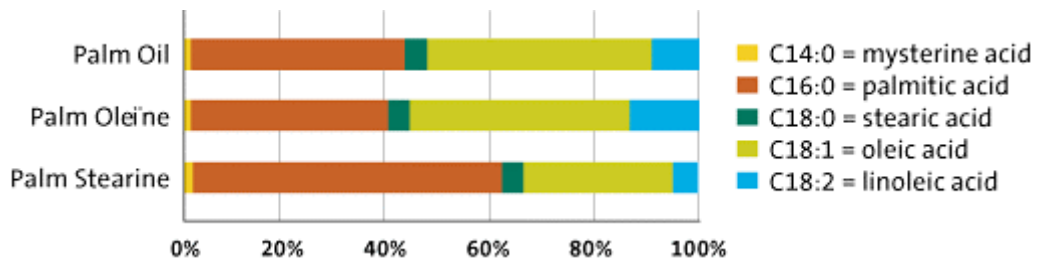


Figure 1.4. Typical fatty acid composition of palm oil fractions (www.vettefeiten.nl).

On the nutritional side, some studies have compared palm oil with other oils or fats, in order to understand the effect of its consumption on blood cholesterol level. Choudhury et al. (1995) compared the effects of palm olein and olive oil on plasma lipids on a group of normo-cholesterolemic young adults (both men and women). The total cholesterol and low-density lipoprotein (LDL) cholesterol had comparable levels, both in palm olein and olive oil. These results confirmed what had already been found by Ng et al. (1992), who provided either a palm olein-rich diet or an olive oil-rich diet to 33 normo-cholesterolemic subjects, previously fed with a coconut oil-rich diet for 4 weeks. Also in this study, results showed identical cholesterol (LDL, high-density lipoprotein - HDL and total) and triglyceride levels, confirming the beneficial effects of palm olein fatty acid profile. Substitution of the usual saturated fat (animal fats and hydrogenated oils) consumption of Dutch males with palm oil resulted in a 11% increase in HDL cholesterol compared to the control group, still showing the same levels of

total cholesterol (Sundram et al., 1992). In conclusion, there is evidence that palm olein has similar effects to olive oil on total cholesterol, LDL cholesterol and HDL cholesterol, and that palm oil can be considered a valid substitute for saturated fats.

1.2.3. *Sunflower oil*

Sunflower oil is a vegetable oil expressed from the seeds of Sunflower (*Helianthus annuus*). A typical fatty acid composition is reported below (British Pharmacopoeia, 2005), having in mind that variation in fatty acids profile is strongly influenced by both genetics and climate

Palmitic acid: 4 - 9%

Stearic acid: 1 - 7%

Oleic acid: 14 - 10%

Linoleic acid: 48 - 74%

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

In addition to traditional sunflower oil, other kinds are available on the market, as reported by the National Sunflower Association (www.sunflowernsa.com):

- high oleic sunflower oil, which has at least 82% oleic acid
- NuSun® oil, the 'new' mid-oleic sunflower oil, lower in saturated fat (less than 10%) than linoleic sunflower oil and with higher oleic levels (55-75%) with the remainder being linoleic (15-35%)
- high stearic sunflower oil, developed in Spain in the last decades to avoid the use of partially hydrogenated vegetable oils in the food industry.

All types mentioned have a very low level of *trans* fatty acids. Due to the high content of unsaturated FA (Fig. 1.5), sunflower oil is liquid at room temperature. It is widespread in food industry for multiple purposes, in particular for frying processes, thanks to its high smoke point (around 230°C). It is characterised by a clean taste and a light colour, which allowed its diffusion also in the domestic environment.

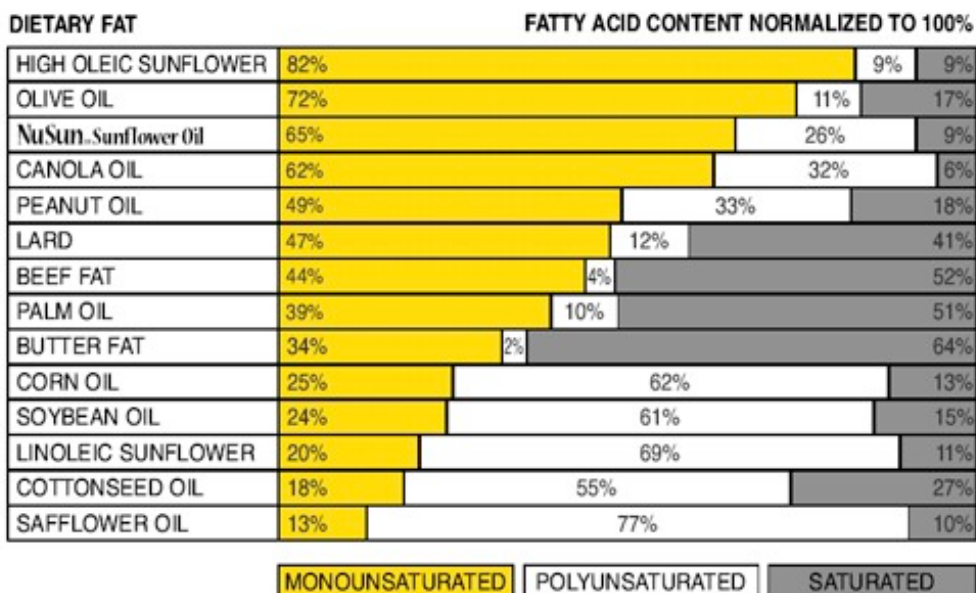


Figure 1.5. Average fatty acid content for various vegetable and animal oils/fats (www.sunflowernsa.com).

Besides its technological applications, the oil composition itself has an important nutritional value, containing high quantities of linoleic acid, an essential fatty acid (see § 1.5). Sunflower oil also contains lecithin, tocopherols (vitamin E, an essential vitamin and anti-oxidant compound), carotenoids and waxes. Chandrashekar et al. (2010) reported that sunflower oil showed good hypocholesterolemic effect thanks to its unsaturated fatty acid content. In an American study (Binkoski et al., 2005), the NuSun sunflower oil diet decreased both total and low-density lipoprotein cholesterol levels compared with the average American diet and the olive oil diet. Total cholesterol decreased of 4.7% and LDL cholesterol decreased of 5.8%. The higher PUFA content appeared to account for the greater total and LDL cholesterol lowering and reduction in lag time of the NuSun sunflower oil diet. Sunflower oil appeared to have a positive effect also on insulin responses. Pedersen et al. (1999) found that glucose and insulin responses after 30 minutes were significantly lower after meals containing PUFA than after meals containing MUFA; in particular, differences in insulin responses to rapeseed and sunflower oil were detected for meals containing only 15 g oil.

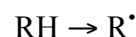
1.3. Fat oxidation

Lipid oxidation is one of the most fundamental reactions in lipid chemistry. Lipids are susceptible to oxidative processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, giving rise to the development of off-flavours and loss of essential amino acids, fat-soluble vitamins, and other bioactive compounds. This process involves the continuous formation of hydroperoxides as primary oxidation products that may break down to a variety of non-volatile and volatile secondary products (Shahidi & Zhong, 2005).

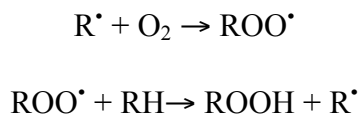
Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids. The process can be accelerated if the oil undergoes high temperatures, such as during deep-fat frying, causing thermal oxidation

with an increase in free fatty acid and polar matter contents (Shahidi & Zhong, 2005). The reaction mechanism consists in three steps:

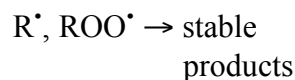
- **initiation**: the chain reaction is initiated by abstraction of an allylic hydrogen from the fatty acid chain, to give an allylic radical. At this stage, oxidation proceeds very slowly and the concentration of free radicals increases until the autocatalytic propagation steps become dominant. This stage is also called the induction phase.



- **propagation**: the first step of the propagation sequence is the reaction of the allylic radical with molecular oxygen, producing a peroxy radical (Scrimgeour, 2005). Oxidation products increase suddenly; the propagation rate is regulated by the velocity of the subsequent abstraction of another allylic hydrogen by the peroxy radical, producing both an allylic hydroperoxide and a new allylic radical, that continues the chain reaction (Scrimgeour, 2005).



- **termination**: the combination of two radicals (either alkyl or hydroperoxy) leads to non-radical products and molecular oxygen, or the reaction of a free radical with an anti-oxidant (free-radical scavenger) generates a more stable radical.



Fatty acid composition (and thus the degree of unsaturation) affects the rate of auto-oxidation, which accelerates when more unsaturated bonds are present in a single fatty acid (Pareyt et al., 2011).

Lipid hydroperoxides have been identified as primary products of autoxidation; decomposition of hydroperoxides yields aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids and epoxy compounds, known as secondary oxidation products. These compounds, together with free radicals, constitute the basis for measurement of oxidative deterioration of food lipids (Shahidi & Zhong, 2005) and are responsible of rancidity and decreased quality of edible oils and fat-containing food (Gómez-Alonso et al., 2004).

Lipid oxidation can occur both in the raw material, during storage of the oil, or in the finished product. A food process involving heat and light (such as frying, baking, roasting) triggers the reaction, especially if the product does not contain anti-oxidants compounds (naturally present in the food or added during production), such as tocopherols, ascorbic acid, carotenoids, phenols. Moreover, Da Pieve et al. (2011) highlighted the lack of information on the effect of food structure on oxidation rate. In fact, in complex systems such as food products, unexpected behaviours of oxidation kinetics are frequently observed; this situation could be possibly explained by the different viscosity and/or the compartmentalisation of the food, that modulate diffusion limiting constraints, as others have reported (Calligaris et al., 2006; Manzocco et al., 2006; Relkin et al., 2009).

1.3.1. *Methods for measuring lipid oxidation*

Considering its particular kinetic and the different products it generates, lipid oxidation can be monitored by several analytical approaches. Dobarganes and Velasco (2002) report that methods can be classified in four groups, on the basis of what they measure: the absorption of oxygen, the loss of initial substrate, the formation of hydroperoxides (as primary oxidation products) or the formation of secondary oxidation products (originated from hydroperoxide decomposition). Only the last two methods will be here described, since they were employed in the experimental part.

Peroxide value (PV) is one of the most common quality indicators of fats and oils during production and storage, as it is a marker of the initial stages of oxidative change. In fact, the formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. By studying the kinetic of hydroperoxide concentration, it is possible to assess whether a lipid is in the growth or decay portion of the reaction process (Shahidi & Zhong, 2005). Analytical methods for measuring hydroperoxides in fats and oils can be classified as those determining the total amount of hydroperoxides and those based on chromatographic techniques, giving detailed information on the structure and the amount of specific hydroperoxides present in a certain oil sample. Calligaris et al. (2008) reported that PV could be considered a good chemical index to monitor the loss of sensory quality of the product during storage, as it was well correlated to sensory consumer acceptance. Carbonyl compounds are the secondary oxidation products generated from degradation of hydroperoxides; they include aldehydes and ketones, which are believed to be the major contributors to off-flavours associated with the rancidity of many food products. Due to the enormous variety of molecules that can be generated during the secondary oxidation, standard compounds are chosen as indicators of reaction entity, depending on the type of fat studied. The choice of the marker is essential to assess the oxidative deterioration of different food systems (Shahidi & Zhong, 2005). Hexanal serves as a reliable indicator of lipid oxidation in foods rich in ω -6 fatty acids, such as linoleic acid, or more generally in seed oils (Gómez-Alonso et al., 2004); it can be quantified by chromatography or as the intensity of the carbonyl band by NIR spectroscopy. Other carbonyl compounds, including propanal, pentanal, decadienal, are also used for evaluating lipid oxidation in foods. For instance, propanal is a recommended indicator for lipid oxidation in foods that are high in ω -3 fatty acids, such as marine oils, while 2,4-decadienal was found to be the major degradation compound for sunflower oil (Guillén & Uriarte, 2012) and thus used as indicator. These compounds can be monitored by head space HPLC (Da Pieve et al., 2011) or by spectrophotometry, on the basis of the reaction between carbonyl compound and 2,4-dinitrophenylhydrazine, forming the corresponding 2,4-dinitrophenylhydrazone derivatives, which are turned into quinoidals, coloured wine-red in alkaline environment (Endo et al., 2003).

1.4. Cakes

The term 'cake' includes a broad range of food products, differing for ingredients and process conditions. Generally speaking, cake is formed by an internal structure consisting of gas cells surrounded by an external gel-like material, resulting from the heat setting of cake ingredients (Mizukoshi, 1985). It is obtained from a batter, containing higher levels of water, sugar and, for some formulations, fat, compared to bread dough; moreover, soft flour is used, characterized by weak proteins that allow a higher batter viscosity (Rogers, 2004) and a broader expansion in the later stages of baking, allowing cell walls to rupture, thus resulting in a labyrinthine structure (Street, 1991).

Formulation, process conditions and ingredient quality influence the final outcomes in terms of quality characteristics; in particular, the role of fat is a key aspect in cakes production.

1.4.1. Cake formulation

Cake ingredients can be grouped considering their function: tougheners, tenderizers, moisturizers, driers (Lai & Lin, 2006). In the first category can be ascribed flour and eggs, which contain the proteins that contribute to structure the cake; they are counterbalanced by fats, sugar and leaveners, that confer a crumbly, soft and aerated texture to the product. In particular, sugar raises gelatinization temperature for starch, during baking: this allows a longer time for gluten to stretch, resulting in a higher volume on the final product (Conforti, 2006b).

Regarding fats, Pyler (1988) stated that tenderness of cake crumb increases progressively with the fat content up to an optimum level, after which additional fat inclusions causes lesser improvements in cake tenderness, until the practical limit of fat addition is reached. Liquid ingredients like eggs, milk, liquid sugars and obviously water, provide moisture and have the opposite effect of driers, such as flours and starches. Finally, it is important to mention that flour, containing protein and carbohydrates, and sugar, being a carbohydrate itself, are responsible for the Maillard's reaction, which produces the desired brown colour on cake crust. In order to obtain an adequate cake formulation, ingredient groups should be balanced: tougheners should compensate tenderizers, moisturizers should compensate driers (Lai & Lin, 2006).

On the basis of their formulation and mixing method, cakes are frequently classified in three groups: foam type, chiffon type and batter type (Lai & Lin, 2006).

Foam type cakes depend on eggs for their structure and volume; they include angel food and sponge cakes (Conforti, 2006b). For their preparation, the general rules to apply are to equal egg whites and sugar in weight and to have the weight of flour to be 1/3 of that of sugar. Angel food cake differs from sponge cake just in the use of egg whites instead of whole eggs (Pyler, 1988). Foam cakes results from a light, airy batter that produces a baked cake with a coarse texture and moderately large cells (Lai & Lin, 2006).

Chiffon cakes are a cross between a foam-style cake and a shortened cake, as they are prepared using beaten egg whites that help with the leavening -along with chemical leavening- but also liquid oil (Conforti, 2006b).

Both chiffon and foam cakes contain no or little shortening, thus resulting with a springy texture and a tougher structure, allowing the preparation of desserts that require more handling.

Finally, batter type cakes contain a high quantity of fat or shortening and their structure depends on flour, eggs and milk; volume of the cake depends mainly on baking powder. The proportions among ingredients follow the general rule that fat should not exceed eggs and sugar, and that sugar should not exceed liquid; so, flour, sugar, fat and eggs should be almost in the same quantity (Conforti, 2006b).

1.4.2. *Processing conditions*

Cake quality is largely affected by processing conditions, as they can -for instance- enhance the cohesiveness of the dough or weaken the gluten network, thus changing the baked product characteristics. The mixing procedure will differ in the order of ingredient incorporation, the duration and rate of mixing action during the different stages (in multi-stage methods), the temperature of the ingredients and other factors (Pyler, 1988).

The simplest method for cake preparation is the single-stage method, which consists in blending all the ingredients at once and mixing afterwards. The air is entrapped in the water phase rather than in the shortening, thus an emulsifier is needed to retain the air-in-water emulsion throughout the baking time (Conforti, 2006b). This method is commonly used at household level because it is easy and quick.

The creaming method, also called the conventional method, is characterized by a first thorough blending of shortening (fat or oil) and granulated sugar, at slow or medium speed, until the mixture becomes aerated (8-10 minutes). This stage is followed by the incorporation of eggs, while the creaming action is continued (5 minutes). Finally, milk and dry ingredients, such as flour, baking powder, salt, are added in alternate small portions, reaching a total mixing time of 15-20 minutes (Pyler, 1988). The batter thus obtained is largely aerated, with the air bubbles ideally uniform, small, and surrounded by fat (Lai & Lin, 2006); furthermore, it shows the near absence of gluten development. As highlighted by Conforti (2006a), mixing time is important: overmixing will cause loss of air and a heavy cake; on the other hand, a quick blending will bring to a curdled and not homogeneous batter.

If the preparation follows the flour batter method, shortening and flour are creamed to a fluffy mass, while simultaneously sugar and eggs are whipped; the two mixtures are then combined (Conforti, 2006b). This type of blending achieves a thorough dispersion of flour and allows higher levels of sugar than the creaming method; however, aeration is less pronounced, resulting in lower cake volume and higher gluten development (Conforti, 2006b).

1.4.3. *Bakery Fats*

In every baked product, from bread to crackers or cakes, fats play an important role during the process and influence various properties of the final product.

Considering sweet baked products such as cakes, biscuits, pastries and croissants, fats impart the desired tenderness and shortness, due to their ability to lubricate the structure of the product by being dispersed in films and globules in the dough/batter during mixing, thus physically preventing the strengthening of the gluten network and the interaction of starchy components (Pyler, 1988).

Physical and chemical characteristics of fats used in baking are essential to determine the quality of the end product. Pareyt et al. (2011) report that there are three parameters on which the suitability and application of a shortening in bakery products depend. First is the solid/liquid phase ratio at a given temperature, which determines the plasticity of the fat. The second parameter is the crystal structure of the solid lipid. Lastly, oxidative stability of the shortening, which is affected *inter-alia* by FA composition and degree of unsaturation.

Plasticity of fat is of major importance for fat performances in baking. Pyler (1988) reports the conditions necessary to have a fat apt to baking applications. In the material, both a solid and liquid phase must be present; the solid phase has to be in a fine dispersion, in order that the particles are held together by internal cohesive forces. The two phases must be in adequate proportions to each other: solid particles should be enough to prevent the flow of the mass, without forming a rigidly-interlocked structure. This particular equilibrium between the two phases is essential as it ensures one of the major functions of fats in baked products, which is aeration. It has been shown that cakes are highly dependent on fat proper aeration (Podmore, 2002): the shortening must be solid so that air bubbles do not escape from the batter, but also plastic so that it can fold around each air pocket. This is due to the plastic character of the shortening, as it assures its dispersion in the form of films or clumps rather than in globules, and this creates a much larger fat surface than is possible with liquid oils.

From a physical point of view, this can be explained by the fact that the crystalline structure of the fat is the characteristic able to influence its properties during baking and thus final outcomes in the product. NorAini et al. (1996) stated that β' crystalline form is necessary for good baking performances. In fact, any given mass of solid shortening, when present as a larger number of smaller crystals, results in a larger interface at the surface of the expanding bubbles. Thus, shortenings containing small crystals (β') are more effective in producing high quality bread than those containing larger (β) crystals (Smith & Johansson, 2004), even though the latter are more stable due to their higher melting points. Pareyt et al. (2011) explains this further, saying that given the same amount of small crystals (β' polymorph), those with higher melting temperatures (with a corresponding fatty acid composition and substitution pattern) may have a more pronounced positive impact on bread making than the same amount of small crystals with lower melting temperatures (with a different fatty acid composition and substitution pattern).

Monitoring the crystalline structure can be done by NMR or electronic microscopy. Thus, the analysis is not always possible, especially considering an industrial environment

The polymorphic phase of the solid portion of a shortening and its structural characteristics determine the melting behaviour of the lipid (Bell et al., 2007; Ghotra et al., 2002). Short FA chains, the presence of unsaturated FA and the *cis* configuration, all decrease the melting point

(Manley, 2000), as the packing of the molecules becomes more difficult due to steric hindrance. Instead, FA chain length increase raises the melting point, as well as the presence of *trans* FA, as they show melting points closer to those of the corresponding saturates (Scrimgeour, 2005). Crystalline state is strictly related to the physical state of the fat and thus to its solid fat content (SFC). During batter mixing, fat enrobes the air included with the mixing; in this stage, the air is actually in the liquid oil so if SFC is too high there is not enough oil for adequate aeration; on the other hand, if SFC is too low the air is not trapped and escapes before the mixing of dough is complete (Stauffer, 1996). Podmore (2002) reported that, for cake production, the proportion of crystalline triglycerides at the working temperature must be above 5%. A typical European standard for cake and pastry shortening is represented by blends that have slip melting point (SMP) between 35 and 38°C and SFC of 20-35% at 20°C (Klimes, 1989). For example, palm oil has a SFC of 22-25% at 20°C, and is a valuable ingredients for shortening formulation (NorAini et al., 1996).

1.4.4. *Batter characteristics*

Besides fat properties, the characteristics of a baked cake, such as volume and texture, can be correlated with the properties of batter (Sakiyan et al., 2004). Many foodstuffs are examples of soft solids whose microstructure (and therefore properties and behaviour) are dictated by their ingredients, formulation, and processing conditions; cake batter is a prime example of a structured, soft solid (Chesterton et al., 2011).

Batter is a wet foam generated by the aeration of a water-based mixture, typically containing wheat flour, sugar, egg, fat, leavening agents, salt, water and milk powder in varying proportions (Mizukoshi et al., 1979). The three main goals of mixing ingredients to obtain a cake batter are: to combine all ingredients into a smooth, uniform batter; to incorporate and form air cells into the fat phase of the batter; to develop the proper texture in the finished product (Lai & Lin, 2006).

The air bubble volume fraction in batter is typically 0.25-0.45 (Lai & Lin, 2006), so the foam behaves as a bubbly liquid, rather than a rigid foam. On heating, the foam sets to form a stable structured food product, the microstructure of which is directly determined by the batter from which it is baked (Chesterton et al., 2011). In fact, changes in batter viscosity can be correlated with variations in cake volume, as an increase in batter viscosity can aid air incorporation and enhance air bubbles retention during the first stages of baking (Gómez et al., 2008). Fats play an important role in this, as they contribute to the incorporation of air into the batter and enhance heat transfer during cooking, bringing to finished products a moist mouthfeel and a softer texture (Conforti, 2006a).

Studies on the role of fat in stabilisation of gas cells in cake batters and in bread doughs have emphasised the importance of the adsorption of a number of fat crystals at the surface of gas bubbles in dough. Chevallier et al. (2000) proposed a mechanism in which, due to crystals melting during baking, the fat-liquid interface of the adsorbed crystals provides a source of extra interfacial material for the bubble surface, which allows expansion without rupture of the bubble, as also shown in Fig. 1.6. The rate at which bubbles rise due to buoyancy is inversely proportional to viscosity. Thus rapidly rising bubbles in a low viscosity batter may result in a final volume loss; on the contrary, higher cake batter viscosities help to incorporate more air bubbles into the batter and keep them from escaping from the mass, giving the system more stability (Sanz et al., 2008).

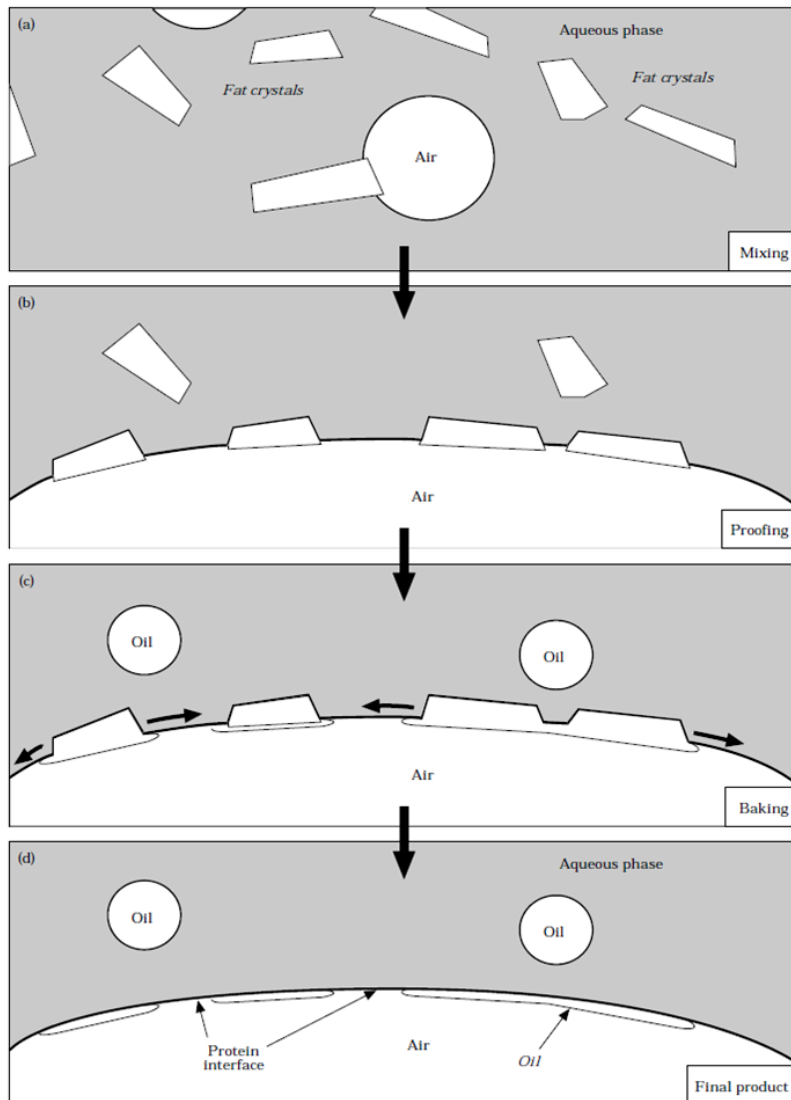


Figure 1.6. Graphical representation of oil-air interaction during different stages of bread production (Brooker, 1996).

The role that fats have in batter also depends on the process conditions used: fat may be added prior to, or following, the aeration process yielding a three-phase fluid, composed by air bubbles, suspended insoluble matter and a fat-water emulsion as the continuous phase (Chesterton et al., 2011).

1.4.5. *Quality characteristics of cakes*

Evaluation of a baked product includes many different aspects. Appearance has an important impact, as this is what the consumer perceives at first sight, judging the product; thus, colour, volume, and sensory evaluation are often performed. In addition to those, texture is another

fundamental attribute to evaluate cake quality, as it is easily perceived by consumers, mirroring processing conditions and ingredients choice. Texture can be determined by instrumental or sensory methods; instrumental methods offer some advantage over sensory analysis because they are rapid and objective (Baixauli et al., 2008).

Furthermore, it is fundamental to evaluate not only performances of fresh product but also its behaviour during storage. Shelf life of baked products can be defined as maintenance of sensory and physical characteristics associated with freshness such as crumb tenderness, compressibility and moistness, by preventing alteration associated with staling during storage (Baixauli et al., 2008). Staling is a general term used to describe the loss of attractive properties upon storage and is mainly due to moisture loss and starch retrogradation. Typical changes in sensory perceptions include crumb firming, crust toughening, and loss of flavour (Pyler et al., 2011); microbiological deterioration is often not an issue, as the product becomes not acceptable (mostly in terms of texture and/or fat oxidation) earlier than any biological contamination. Generally speaking, shelf life of cakes depends on the formulation, the packaging and the storage conditions and it is normally between 1 and 4 weeks long (Gelinis et al., 1999).

As previously said (§ 1.4.1), in the fresh baked product the tenderness of cake crumb increases progressively with the fat content. However, some authors (Rogers et al., 1988; Smith & Johansson, 2004) noted that not only initial crumb firmness, but also the firming rate of the crumb is affected by addition of shortening to a bread dough formula. In fact, fat delays water migration inside the product, forming a thin layer around the gluten matrix, thus postponing starch retrogradation which is responsible of the negative changes in product texture (along with moisture migration and loss). In particular, Smith and Johansson (2004) observed that the linear hydrocarbons of saturated fats form a complex with helical starch, slowing crystallization and subsequent staling and development of firmness; thus, a part of the fat employed in a baked product should be solid fat. This finding is confirmed in a study of sensory preferences performed by Heenan et al. (2010), in which cakes produced with butter or margarine -thus mostly saturated TG- were considered as the freshest (on the day of baking and after 15 days) from a trained panel. Concerning the role of fats, it is also worth considering that the oxidative stability of the shortening, depending on FA composition and the degree of unsaturation, influences the rate of auto-oxidation (Ghotra et al., 2002).

1.5. Fats and nutrition: a technological challenge

Lipids are essential for every living organism, as they support multiple biological functions in the body. First of all, fats yield a higher caloric power (9 kcal/g) compared with carbohydrates and proteins (4 kcal/g each). They serve as the structural building material of all membranes of cells and organelles, besides playing a fundamental role in neuronal transmission and brain formation in infants. They ease the intestinal absorption and transport of fat-soluble vitamins A, D, E, and K, and act as signalling molecules, facilitating a variety of physiological functions. Furthermore, they guarantee skin and hair health, and insulate body organs against thermal shock, maintaining body temperature. Recent literature also demonstrates a specific role of fatty acids in gene modulation and protein expression to influence risk of chronic disease (Watkins et al., 2005). In particular, among the broad range of fatty acids that can be found in nature, two are essential for humans: linoleic acid (ω -6 FA, 18:2, 9, 12) and α -linolenic acid (ω -3 FA, 18:3, 9, 12, 15). The human body lacks the ability to introduce double bonds in fatty acids beyond carbon 9 and 10, so it cannot synthesize these FA, which have to be introduced with the diet (from fish and shell fish, vegetable oils -canola, hemp, soya-, walnuts).

The biological effects of dietary lipids on human health remain a primary focus of nutrition research as consumption recommendations are continually updated in response to new

information obtained through epidemiological, clinical, and animal investigations (Watkins et al., 2005).

1.5.1. *Health related issues*

Health risks related to high fat consumption have been widely reported in literature in the last twenty years. The increase of blood lipids and LDL cholesterol concentrations has been correlated with an enhanced cardiovascular risk (Aro et al., 1997; Sundram et al., 1997); this condition is a consequence of the intake of saturated fatty acids (SFA) and trans fatty acid (TFA). Moreover, fat-rich foods are frequently part of a high calorie diet that can often lead to overweight and obesity, two other conditions that increase the chance to suffer from the previously cited pathologies.

The American Heart Association (2008) suggested that no more than 10% of daily energy should be consumed from trans and saturated fats (Rogers, 2009). As part of a strategy to decrease risk factors for coronary heart diseases in the population, Marangoni et al. (2007) suggest to achieve energy balance and a healthy weight and to limit energy intake from fats while also shifting fat consumption from saturated fats to unsaturated oils, and eliminating TFA from the diet. In fact, it has been demonstrated that consumption of polyunsaturated fatty acids lowers the total to high-density lipoprotein cholesterol ratio, perhaps the best single lipid predictor of coronary heart disease risk (Lewington et al., 2007). As additional benefits, Salmeron et al. (2001) and Summers et al. (2002) found that PUFA consumption may also improve insulin resistance, while Mozaffarian et al. (2010) hypothesized a reduction in systemic inflammation.

In this framework, the aim of food industry is to gradually substitute SFA with unsaturated oil, also considering the growing consumers demand for healthy products and, at the same time, the more strict regulations imposed by national governments and international boards (such as EFSA – European Food Safety Authority or American FDA – Food and Drug Administration).

1.5.2. *Fat replacement and structurization*

Many consumers expect particular properties from a given food product. The aroma, texture and mouth sensation experienced when consuming these foods are strongly dependent on their fat content and can eventually shape dietary choices in the long term (Drewnowski et al., 1989). Moreover, consumers may tend to conclude their decisions on the basis of their memory of a food, and not on the basis of their actual perceptions (Mojet & Koster, 2005). In this framework, knowing that fats largely contribute to mouthfeel and sensory perceptions, modifying traditional recipes to obtain a healthier and more nutritionally valuable food product is challenging, especially for some product categories.

Fat replacement

Due to high fat and sugar content, cakes are high-calories food. Reducing or replacing fats is therefore highly desirable to lower both fat and calorie content. It has been reported that the replacement of *trans* fatty acids with unsaturated fatty acids from unhydrogenated oils is the single most effective measure for improving blood lipid profiles; even small amounts of unsaturated fatty acids have a major effect on the ratio of total to HDL cholesterol (Mensink et al., 2003). In addition, fats contribute to the appearance and aroma of the cake, other than providing a soft texture; their reduction could then worsen the quality of the final product.

Several fat replacers have been used in bakery industry and can be classified as carbohydrate-based (mimicking fats, forming gels which have a flow pattern similar to lipids), lipid-based (emulsifiers, modified TG), protein-based (micro-particulated whey proteins, protein concentrates) and their combinations (Kalinga & Mishra, 2009). Although the wide variety of

compounds available, none of them has been defined as ideal; the lack of flexibility in the applications and the possible introduction of allergens as carriers of the molecules (for example lactose, egg proteins or gluten) are two of the possible drawbacks for these compounds.

Fat structurization

Since a long time, structurization of fat has been a big challenge, as the physical state of fats has an impact on the properties of food products.

The most common way to provide texture to the liquid fatty phase of food products is by including crystalline triglycerides (TG), mainly saturated (Flöter & Bot, 2006); they have a limited solubility in the oil and, upon cooling, small crystals grow and form a network across the liquid. The actual size, shape and number of the crystals determine to a large extent the mechanical properties of the textured oil (Pernetti et al., 2007).

Hydrogenation of vegetable fat has been one of the first attempts to give a solid structure to a liquid fat. Margarines, vegetable fats in which double bonds of unsaturated FA are saturated by hydrogen molecules, have been produced since the beginning of the 20th century and are now widespread. A major issue related to this process is that hardened fats produced by partial hydrogenation contain *trans*-isomers, which are nowadays regarded as undesirable by nutritionists, and will be increasingly subject to product labelling regulations. The technologies of production and raw materials choice have thus improved over the years, aiming to merge health aspects and technological requirements. Considering marketplace surveys, however, there is an inverse relationship between the price of margarines and their content of saturated and *trans* fats (Albers et al., 2008). Thus, currently available products that are lower in saturated and *trans* fats tend to cost more, and this may be a significant barrier to their use by price-conscious, and lower income consumers (Rush et al., 2009). Consequently, there is interest in developing novel semi-solid plastic structures that are capable of encapsulating edible oil for food preparation applications without the use of saturated or *trans* fats, and that can be cost-effectively produced (Upritchard et al., 2005; Marangoni et al., 2007).

To accomplish this aim, another possibility of reorganizing fat structure, without altering its FA profile, is interesterification. This process consists in the rearrangement of the distribution of the fatty acids on the glycerol backbone, either chemically (by acidolysis and other ester exchange reactions) or enzymatically (using lipase), within and between the triglycerides (Wassel et al., 2010). It is applied to either an individual oil or a blend of oils, to produce triglycerides with different properties. The molecular species of natural triglycerides, in fact, show greater or lesser selectivity in the distribution of fatty acids between the three glycerol positions, as previously written (§ 1.1). This, as well as the overall fatty acid mixture, determines many of the technically important properties of the oil or fat, i.e. solid fat content and melting point (Scrimgeour, 2005). Liquid fats can also be hardened by interesterification with fully saturated fats (either stearin fractions or fully hydrogenated oils), raising the solid fat content without isomerizing any of the fatty acids.

In the past few years, several publications reported the use of different compounds to be added to a liquid oil in order to structure it. Generally speaking, two routes can be found in order to structure an organic liquid: dispersion of a foreign phase, such as in an emulsion, or the employment of specific molecular mechanisms, relying on particular compounds (Pernetti et al., 2007). These molecules are normally hydrophobic, easily dissolve in the oil matrix and are able to organize a structure conferring a somehow stiff texture to the oil. Bot et al. (2009b) pointed out that a successful structuring agent forms small and preferably non-spherical building blocks, so that network connections can be established on a higher specific surface area. Having in mind the gel definition by Weiss and Terech (2006), who says that a gel must be characterised by a continuous microscopic structure with macroscopic dimensions, permanent on the time scale of an analytical experiment, and by a solid-like behaviour, these structured oils have been

called organogels by several authors (Abdallah & Weiss, 2000; Wright & Marangoni, 2007; Hughes et al., 2009).

An organogel can be defined as an organic liquid entrapped within a thermo-reversible, three-dimensional gel network. This gel network is formed by the self-assembly of a relatively low concentration of organogelator molecules, which are most often low molecular weight compounds capable of gelling organic solvents upon cooling (Wright & Marangoni, 2007). The gelators tend to self-assemble through specific interactions, allowing preferential one-dimensional growth, such as fibres, strands or tapes, which are frequently crystalline (Abdallah & Weiss, 2000). The generality of thermo-reversibility of low molecular mass organic gelators is also one of the features that separate them from many polymeric gels (Abdallah & Weiss, 2000). Organogels can be formed from liquid organic solvents (like benzene, hexane, etc) or liquid oils, at organogelator concentrations as low as 0.5% wt, depending upon its chemical properties (Hughes et al., 2009).

In the literature, several types of organogels are reported and can be differentiated on the basis of the chemical characteristics of the organogelator used or the type of structure they form when assembled; here just a few examples will be reported.

Monoglycerides (MG) have been widely studied because, due to their amphiphilic nature, they can stabilize fat emulsions. Hughes et al. (2009) reported the ability of 12-hydrostearic acid (12-HSA) to stabilize water-in-oil emulsions. The mechanism of action consisted in preventing water droplets coalescence (impeding their displacement) rather than reducing their size, as surfactants do. Moreover, the interaction between 12-HSA and surfactants had a negative outcome on the structure, turning the emulsion to a lotion rather than a gel (Rush et al., 2009). Da Pieve et al. (2010) studied the effect of shear on the crystallization behaviour of MG organogels, prepared by mixing cod liver oil and saturated MG at 80°C and then crystallizing them at 20°C under shear rate. Results obtained showed that the introduction of shear during organogel formation greatly affects structure, obtaining a stronger gel network with a high oil binding capacity under static conditions. It is worth noting that the MG used in these studies are saturated MG, thus not adding nutritional value to the oil matrix.

It is known that combination of organogelators with different chemical characteristics can outperform pure hardstocks (Pernetti et al., 2007). For instance, long chain fatty acids and fatty alcohols were used by Schaink et al. (2007), who studied the textural and structural properties of organogels with stearic acid and stearyl alcohol as gelators. In their research, it has been highlighted that only particular combinations of the two gelators resulted in a gelled system.

Regarding potential food applications of organogels, Hughes et al. (2009) wrote an interesting review reporting different examples on this topic. One issue in confectionery and lipid technology is the control of oil migration in foods, as this phenomenon can adversely affect texture and sensory properties of the product. Organogelation could be a novel way to prevent and/or limit oil migration, as in theory the movement of TG out of -or through- the material could be reduced by the structured fat (Hughes et al., 2009). Experiments performed by Marty et al. (2005) using 12-HSA in a filling fatty material were not effective in reducing oil migration, although these studies are not sufficient to conclude a general behaviour. With the same organogelator, capability of modulation for carotenoids release was studied by Wright et al. (2008), showing that bioavailability of those compounds could show a gradual trend if included in an organogelled system. Rush et al. (2009) characterized a structure which encapsulates high volume fractions of liquid oil (canola oil) in a water matrix stabilized by multilamellar monostearin multilayers, called MG GEL. They set up an experimental design in which 10 volunteers consumed sandwiches of whole wheat bread with MG, unstructured MG, butter or margarine spreads. Consumption of MG GEL resulted in tempered postprandial metabolic responses (measured as serum TG, FFA and insulin) compared to those resulting

from consumption of a compositionally equivalent but unstructured oil suspension, suggesting a structure-dependent metabolic response.

1.5.3. *Sitosterol and oryzanol organogel*

Amongst the different organogels presented and studied until now, a system offering tremendous potential in food application is the organogel based on a combination of phytosterols and γ -oryzanol as gelators, the only organogel at the moment recognized to be suitable for food systems (Hughes et al., 2009).

Phytosterols

Plant sterols are cholesterol-like substances (they have the same precursor, squalene, as illustrated in Fig. 1.7) belonging to the group of isoprenoids and derived from plants; around 300 plant sterols have been identified so far.

These molecules are important structural components of plant membranes, regulating their fluidity and permeability: free phytosterols serve to stabilize phospholipid bi-layers in plant cell membranes just as cholesterol does in animal cell membranes (Moreau et al., 2002). Moreover, they act as biogenetic precursors of compounds involved in plant growth and are substrates for the synthesis of numerous secondary plant metabolites, such as glycoalkaloids and saponins (EFSA, 2008a). Sitosterol, together with stigmasterol, is the most diffused phytosterol in plants. Although stigmasterol differs from sitosterol only by the 22-double bond in the alkyl side chain, it has been demonstrated that these two sterols have markedly different effects on the permeability, ordering, and fluidity of plant phospholipid vesicles; furthermore, sitosterol- β -glucoside serves as a primer for cellulose synthase in plants (Moreau et al., 2002).

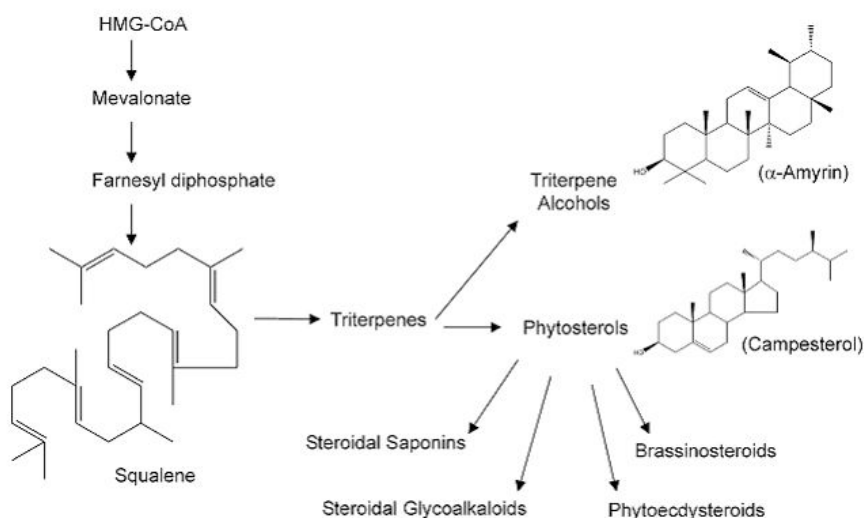


Figure 1.7. Biochemical path leading to the formation of triterpenes, which originate phytosterols and derived compounds (Moreau et al., 2002).

Sterols can be divided into phytosterols and phytostanols, the latter being the saturated form of phytosterols and serving as storage product of the cell.

Sterols are naturally supplied in an average diet as they are contained in common foods; major sources are seeds, vegetable fats and oil, nuts and cereals, especially rye. There seems to be

general agreement that the normal western diet would contribute to a daily supply of plant sterols in the range of 150-400 mg per person, vegetarian diets being closer to the upper range (EFSA, 2008a). Absorption efficiency for plant sterols in humans is considerably less than that of cholesterol, being of 2–5% for the former versus 60% for the latter (Berger et al., 2004).

Due to their structural similarity to cholesterol, plant sterols were first and foremost studied for their capacity to inhibit cholesterol absorption in the small intestine (Acuff et al., 2007). Since the mid 1990s, there has been considerable interest and commercial marketing of phytosterol products for this purpose, even if the first attempts to use these compounds for healthy purposes were done in the 1950s. At that time, sitosterol was used as a supplement and as a drug to lower serum cholesterol levels in hypercholesterolemic individuals. Due to poor solubility and bioavailability of the free phytosterols, the effects were not always consistent, and very high doses (up to 25-50 g/day) were sometimes required for efficacy (Moreau et al., 2002). Therefore, the physical forms, carriers and solubilisation of the phytosterols are important characteristics to determine the efficacy of phytosterols on cholesterol lowering. Also, the food matrix may affect their cholesterol-lowering efficacy significantly; for example, Clifton et al. (2004) concluded, from a randomised incomplete cross-over single-blind study, that plant sterol esters (1.6 g/day) in low-fat milk were almost three times more effective than in white bread and cereals (muesli style).

Bioavailability and efficacy of phytosterols in food products to be introduced in a balanced diet has been widely investigated in the past. In the review by Berger et al. (2004), several studies were mentioned, in which is reported that intakes of 0.8-1 g of plant sterols per day have shown a minimum of 5% reduction in LDL cholesterol levels, relative to control, or at least that those quantities can decrease the absorption of cholesterol, which is indicative, but not necessarily predictive, of actual LDL lowering. This reduction in LDL cholesterol will correlate with an approximate 6–10% reduction in coronary heart disease risk at age 70 (Berger et al., 2004). The review by Katan et al. (2003) shows that the daily intake of 2 to 2.4 g of plants sterols or stanols added to margarine (or to mayonnaise, olive oil or butter) reduced on average low-density lipoprotein (LDL) blood cholesterol levels by 8.9%. The dose-response relationship seems to level off at doses higher than 2.5 g/day and the average maximum effect was estimated to be 11.3%. The effect on cholesterol level appeared to be present either by consuming phytosterol enriched food once per day at lunch, or dividing it over three portions during the day (Plat et al., 2000); this was later confirmed by Quilez et al. (2003), who stated that ingestion of phytosterols, in order to be effective on lowering cholesterol blood levels, can happen in a different time to that of cholesterol rich foods. Moreover, both Berger et al. (2004) and EFSA (2008b) showed that the beneficial effects, identified within 2-3 weeks from the beginning of supplementation, were able to remain stable for at least one year (still consuming phytosterols). The only side effect reported in stanol supplementation in food could be a reduction in levels of the β -carotene/LDL cholesterol ratio and in absorption of other fat soluble micronutrients, such as vitamin A, K, D and lycopene (Berger et al., 2004). Katan et al. (2003) reported a significant reduction only for β -carotene. However, this can be counterbalanced by increasing the intake of fruits and vegetables, especially those rich in carotenoids; also, it is worth mentioning that many food product are nowadays supplemented with those nutrients.

In a scientific report of 2008 (EFSA, 2008a), the EFSA concluded that plant stanols are clinically safe, in the range of use that causes desirable reduction in blood levels of total cholesterol and LDL-cholesterol. In fact, if a person consumes 1.5 to 2.4 grams of plant sterols and stanols every day, this can result in cholesterol reduction by 7 to 10.5%. In order to avoid any possible depletion in carotenoids levels, the authority advises that the daily consumption of phytosterols should not exceed 3 grams; moreover, the labelling should advise individuals who are likely to be more susceptible to reduced vitamin status (pregnant or nursing women, children) to avoid consuming these products.

Concerning food applications, it is important to consider that stability of phytosterols (and their esters) during processing strongly depends on the molecule structure and the oil matrix present in the food product (Rudzinska et al., 2010). Those compounds, having a chemical structure similar to cholesterol, are in fact prone to oxidation, particularly at elevated temperatures as those used during frying; their oxidative degradation leads to the formation of oxidized sterol derivatives, volatile flavour components and oligomers. Cantrill (2008) stated that phytosterol are quite stable and undergo only limited degradation during oil processing, thanks to the steric hindrance by the ring structure. Only under harsh conditions, such as temperatures over 100°C, in the presence of oxygen, a slow oxidation of the phytosterol moiety may occur, in the same way as for cholesterol. Hypothesizing that consumers could fry a product using temperatures below 200°C for 5-10 minutes, the level of oxidation of sitosterol esters remains below 1.3% when the matrix consists of liquid oil or liquid margarine. Using free sterols, these levels are somewhat higher; also the composition of the lipid matrix affects the susceptibility to oxidation.

Oryzanol

Gamma oryzanol (Fig. 1.8), a group of ferulic acid esters of phytosterols, is a major phytochemical in rice bran oil (Khuwijtjaru et al., 2009). This compound has been recognized as a major antioxidant and scavenger, as it guarantees stabilizing properties to rice bran oil, besides reducing total plasma cholesterol and triglyceride concentrations, and increasing the HDL cholesterol level (Cicero & Gaddi, 2001). Moreover, no losses of gamma-oryzanol during deep frying were observed (Lerma-García et al., 2009), prospecting potential application of this compound in processed foods.

Molecular structure	R	Compound
		24-Methylen-cycloartanyferulate
		Cycloartenylferulate
		β -Sitosteryferulate
		Campesterylferulate

Figure 1.8. Chemical structures of the four main components of γ -oryzanol (Lerma-García et al., 2009).

Wilson et al. (2007) studied the effect of oryzanol, ferulic acid and rice bran oil in hypercholesterolemic hamsters and they found that at equal dietary levels, γ -oryzanol has a greater effect on lowering plasma non-HDL-cholesterol levels and raising plasma HDL-cholesterol compared to ferulic acid, possibly through a greater extent to increase excretion of cholesterol and its metabolites. Oryzanol and ferulic acid may have similar antiatherogenic

properties, as shown by reductions in aortic cholesterol accumulation, although their anti-atherosclerotic potentials are through different mechanisms of action.

Oryzanol and sitosterol organogel

Developed as a healthier alternative to conventional spreads that are structured with saturated and *trans*-fats, a product employing a mixture of sitosterol and oryzanol (2%, or preferably 4%, w/w in oil) to solidify edible oil was patented in 1997 (Ritter et al., 1997). This new product is considered to be an appetite suppressant due to its extended survival in the human digestive tract; reaching the ileum unabsorbed, it triggers the ileal break and leads to feelings of satiety (Hughes et al., 2009). Furthermore, the inclusion of this system in a food product could improve the antioxidant (due to γ -oryzanol) and anti-cholesterol (due to phytosterols and unsaturated oil) potentials of food, while performing the desired technological properties (Bot et al., 2009b). It is worth highlighting that this is the only example of oil structuring, based on food grade ingredients, that is not based on the ordering of fatty acid chains (Bot et al., 2009b).

Recently, this system has been widely studied by several authors that investigated its characteristics from different points of view.

The organogel forms after cooling a warm solution of oil (in most of the studies, sunflower oil) containing the two gelators. The gel formed appears transparent, implying that the building blocks of the gel are considerably smaller than the wavelength of visible light (Bot et al., 2009b). The transparency of the gel, however, depends also on the proportion between oryzanol and sitosterol, being the gels with higher percentage of the latter compound more turbid and hazy (Bot et al., 2008). On a molecular scale, Perneti et al. (2007) supposed that β -sitosterol units dock on top of each other, having the ferulic acid moiety of the γ -oryzanol sticking out (Fig. 1.9).

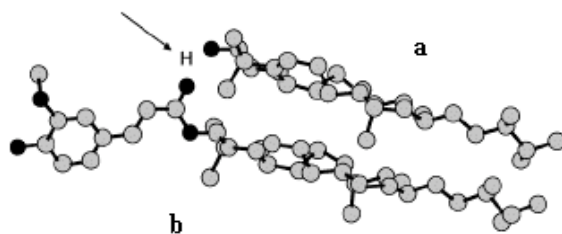


Figure 1.9. Proposed stacking of sitosterol (a) and oryzanol (b) molecules, based on a crude energy minimisation calculation (Hermant RM, Unilever R&D, unpublished data). An H-bridge can be formed near to the arrow, resulting in slightly wedge shaped stack (Perneti et al., 2007).

Bot et al. (2008) proposed that these units self assemble forming a helical ribbon; these tubules can aggregate and structure a network in which sunflower oil phase is present both outside and inside the tubule (Fig. 1.10). From small-angle X-ray scattering observations, Bot et al. (2009a) confirmed this hypothesis and measured tubules diameter and wall thickness, being 7.0 nm and 0.8 nm respectively. The wall thickness is comparable in size to the length of the long axis of the rigid ring system characteristic for sterol molecules. It seems likely, therefore, that the tubule wall is defined by the stacked rigid four-ring systems of the sterol (ester) molecule, whereas the more flexible alkyl chain and ferulic acid moiety branch outside, blending in with the surrounding TG oil (Bot et al., 2009a). Moreover, the phytosterols molecules are thought to be tilted relative to the axis and radial plane of the tubule, in such a way that their wedged stacking leads to the formation of a helical ribbon (Bot et al., 2009a).

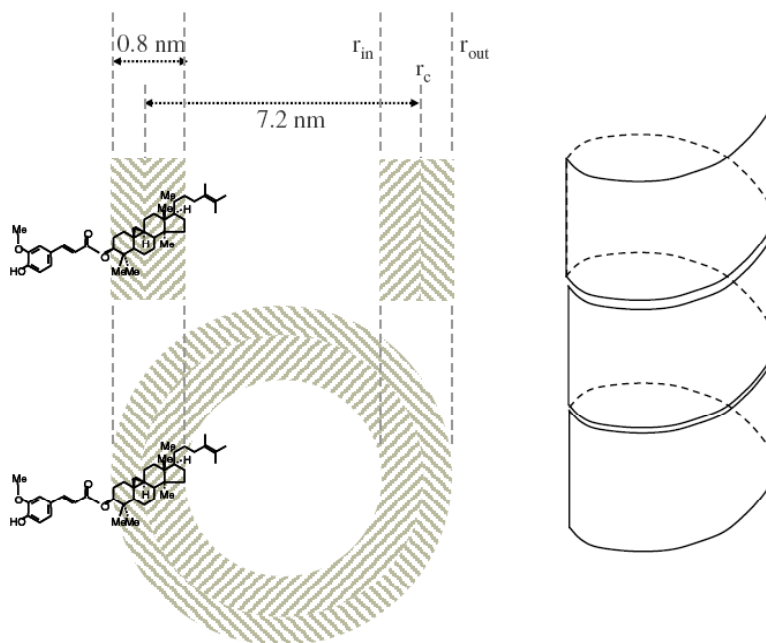


Figure 1.10. Schematic representation of the sitosterol + oryzanol tubule (left) and helical ribbon (right). The upward hatched and downward hatched area in the ring have the same size; r_{in} : inner radius, r_c : centre radius and r_{out} : outer radius (Bot et al., 2009a).

The proposed structure is not influenced neither by total gelators concentration in the oil nor by their reciprocal ratio (Bot et al., 2009a); this finding is also supported by the evidence of higher turbidity in sitosterol-rich gels, as the ferulic acid moieties of oryzanol could work as spacers and keep the nanotubes apart (Bot et al., 2008), enhancing the transparency of the gel. Moreover, the most likely dynamic of organogel formation would be via a helical ribbon, in which the wall spirals along the central axis (Bot et al., 2008). In fact, the loss of structuring potential of the organogel at a typical melting temperature (Bot et al., 2006) and the concurrent X-ray scattering curves of the organogel measured at the same melting temperature (Bot et al., 2009a), indicate that the nanotube does not persist above the melting point of the organogel. These results show that the formation of the tubules does not require an intermediate stage involving a tubular micelle (as previously proposed by Perneti et al., 2007), but they rather 'crystallize' directly from the solution (Bot et al., 2009a).

The proportion between the two gelators, even if not affecting the microstructure of the organogel, has an impact on gel properties. Sawalha et al. (2011), by measuring scattered light intensity as a function of decreasing temperature, detected that aggregation peaks took place at different temperatures depending on total gelator concentration and their ratio. Structurization of the tubules was observed by the same authors by rheological tests, in which storage modulus values (during cooling) showed a dramatic increase in correspondence to aggregation temperatures previously measured, confirming that higher concentrations of gelators correspond to higher aggregation temperatures. Bot et al. (2008) noted that the gap between the temperature of complete melting obtained during heating and the onset temperature of crystallisation obtained during cooling, measured by DSC, increases with increasing γ -oryzanol content. For all the concentrations and ratios used in the study, however, absolute transition temperature during cooling and heating were not exactly the same.

The optimal oryzanol:sitosterol molar ratio, with higher aggregation temperature, higher consistency and transparency, was found to be 2:1, corresponding to 3:2 w/w ratio; with this proportion, the gel resulted stable and thermo-reversible from concentrations as low as 2% (Ritter et al., 1997), appearing transparent and stiff at concentrations higher than 8% (Bot et al., 2008).

Studying the rheological behaviour of a 3:2 oryzanol:sitosterol ratio (named gel from here onwards), Bot et al. (2009a) noticed an increase in consistency of the gels as long as gelators concentrations increased. This behaviour can be linked to what was found by Sawalha et al. (2011), who studied cooling profiles of the gels by light scattering: sudden high peaks of intensity, reasonably due to the aggregation of tubules, were observed, corresponding to higher temperatures, as concentrations increased (from 15°C at 8% gelators concentration, to 45°C at 16%). For a 8% gelators concentration gel, 70°C is the temperature at which there is a full melting of the gel and no tubules are found above this temperature (Bot et al., 2009a). Regarding rheological properties, Bot et al. (2006) observed that controlled low shear conditions promotes gel formation, while the effect of oscillatory shear parameters (amplitude, frequency) is small. The mechanical characteristics of gels formed by rheological testing was in accordance with those of gels formed in quiescent conditions (bulk gels), the latter needing a longer time and lower temperatures to be structured (Bot et al., 2006). Large-deformation properties of the gels highlighted that these system are much firmer under compression than under shear; also, their strength is proportional to the amount of structuring material, suggesting that the gels break at the building blocks and not at the cross links of the tubules (Bot et al., 2006).

As a further step, some researchers (Bot et al., 2009b; Duffy et al., 2009; Bot et al., 2011) have investigated the possibility to include the 3:2 oryzanol:sitosterol organogel into an emulsion, as many food products are based on emulsion technology. Bot et al. (2009b) proved that the two compounds are able to structure a water-in-oil emulsion, being oil-continuous up to 0.8 water fraction. However, the structured emulsion resulted in a lower firmness compared to the corresponding pure oil organogel; this is probably due to the formation of large crystalline fibers instead of nanotubules, which make the structure weaker but, at the same time, adsorbed at the water droplet interface, playing a role in stabilizing the emulsion (Bot et al., 2011). Moreover, it was found that the emulsions, besides being firmer as gelator concentration increases, are more sensitive to sitosterol:oryzanol ratio compared to pure organogels (Bot et al., 2009b). Duffy et al. (2009) investigated the possibility to create oil-in-water emulsion, using pre-formed organogel and also adding surfactants; these organogel-based emulsion systems formed fibrils visible by light microscopy and showed retarded TG hydrolysis by pancreatic lipase compared to standard emulsions.

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2. Aim of the study

This research project aims to study the effect of the substitution of saturated animal fats with healthier vegetable fats/oils using a response surface methodology. The model system chosen is a plum cake formulation, in which lipids play an important role on final product characteristics. As reference cake, a formulation containing butter is considered, since this fat is traditionally employed for its outstanding technological performances.

In a first phase, the effects of fat melting properties and fat content are modelled. Different blends of palm oil – palm olein are crystallized. The two components used come from the same raw material but have different physical characteristics, depending on their fatty acid profile. The final scope of the modelling process is to obtain an optimized formulation, resembling as much as possible to the butter cake reference. In order to better understand the relationships among fat and batter properties and the characteristics of the final baked products, rheological properties of fats and batters are as well investigated, also simulating baking conditions.

In a second phase, the use of innovative ingredients is considered, for enhancing the nutritional profile of the plum cake without compromising final quality. Considering recent studies on structured fats, an organogel formed by a blend of γ -oryzanol and β -sitosterol, dispersed in sunflower oil, is chosen. This material shows a solid like structure, thus potentially influencing cake structure; moreover, it is characterized by a fatty acid profile typical of unsaturated vegetable oils, with the additional benefit of antioxidant and anti-cholesterol properties of the two phytosterols used as gelators. Cake formulation is studied by the Design of Experiment technique, in order to understand the effect of organogel quantity and gelators concentration on the characteristics of cakes. Properties of organogels are evaluated through rheology and by monitoring the oxidative status of cakes during storage.

3. Modelling and optimization of fat quantity and composition in a plum cake

In the literature, few studies have been addressed to investigate how the interaction of fat quantity and composition affects the characteristics of a sweet, chemically leavened baked product. Kalinga and Mishra (2009) studied physical properties of low fat cakes, in which the traditional recipe with 100% margarine was compared to experimental formulations where fat was partially replaced by β -glucan concentrates: volume and consistency were evaluated, finding out that a lower fat content brought to a less developed and harder cake. Dogan et al. (2007) produced layer cakes with interesterified and non interesterified blends of palm oil and cottonseed oil: the incorporation of palm oil brought to good aeration and excellent creaming properties, while an increase of cottonseed oil led to a higher batter density. The same effect was found by Sowmya et al. (2009), who studied cakes produced with the creaming process, in which shortening was replaced by different percentages of sesame oil: increasing oil content, batter viscosity and cake volume decreased, while cake hardness and chewiness increased. Sudha et al. (2007), studying the possibility to reduce fat content in soft dough biscuits, found an increase in hardness and cohesiveness of the dough and the finished product.

The aim of this work was to model the effect of fat quantity and composition on the characteristics of a plum cake and to optimize the formulation on the basis of texture and volume performances. In order to study simultaneously the effects of the two considered factors and of their interactions, a Design of Experiment (DoE) technique was applied, combined with response surface methodology. A plum cake formulation was chosen because the high fat percentage of the recipe could help in highlighting the effects of fat on the final product. Fats used were Refined Bleached and Deodorized (RBD) palm oil and bi-fractionated palm olein, blended in different proportions in order to modulate the melting characteristics of fat blends without altering palm oil triglyceride and fatty acid composition by the addition of liquid oil from a different vegetable source. Butter was considered as the reference fat, as it is the raw material traditionally used in cakes, because of its performances in terms of volume, softness and sensory quality.

3.1. Materials and methods

3.1.1. *Materials*

The reference butter cake (named BC) was produced using soft wheat flour type '00' (Molino Quaglia SpA, Vighizzolo d'Este, Italy), pasteurized liquid whole egg (Cascina Italia, Spirano, Italy), white sugar (Südzucker AG, Mannheim, Germany), butter (F.lli Galbusera, Casatenovo, Italy), baking powder (PaneAngeli, Desenzano del Garda, Italy).

In experimental formulations, butter was replaced by blends of RBD palm oil (PO) and bi-fractionated palm olein (superolein, SO) (IGOR SpA, Orzinuovi, Italy).

A formulation with anhydrous butter (named ABC) was also prepared to estimate the cake performances in the absence of the water fraction contained in butter (about 18%). Anhydrous butter was prepared melting butter up to 60°C and centrifuging it at 5000 rpm for 5 min at 30°C. The oily phase was immediately separated from the aqueous phase and then filtered through a rough filter paper (60 g/m³, 0.14 mm thickness and capillary rise > 79 mm; ALBET-Hahnemuehle, Barcelona, Spain) and crystallized as described below for fat blends preparation (§ 3.1.2). Crystallized anhydrous butter was stored at 20 °C in a closed container until use.

3.1.2. Fat blend preparation

For the preparation of fat blends, PO and SO were weighted to obtain a batch (700 g) with the desired fat composition and heated up to 48°C. The melted blend was then poured into a scraped surface chilling unit (Simac 5000, Treviso, Italy) for crystallization. The chilling unit provided a cooling speed of 2.5°C/min, favouring the nucleation of fat crystals in the unstable α form (Van Malssen et al., 1996). The product was extracted from the chilling unit when the actual temperature of the mass was 15°C below the previously measured SMP of the blend. The crystallized fat was then gently stirred (low speed, flat beater) in a N-50G Hobart mixer (Hobart, Troy, USA) for 5 min at 20°C, in order to allow the conversion of α form crystals in the more stable β' form (Van Malssen et al., 1996). Crystallized fats were stored at 20°C in a closed container until use.

3.1.3. Experimental design

A two-factor, five-level Central Composite Design (CCD) was used to plan the experiments. The two factors considered were the quantity of fat blend in the cake batter and the percentage of SO in the fat blend. The factor levels in the coded form were: $-\sqrt{2}$, -1 , 0 , $+1$, $+\sqrt{2}$. The design consists of thirteen experimental points (runs): four square points, that combine the -1 and $+1$ levels of the two factors; four star points (specific of this type of experimental design) which cross the 0 level of one factor with the $\pm\sqrt{2}$ levels of the other; five central points that are replications of the experiment which crosses the 0 levels of both factors. The star points are calculated so that their distance from the centre is the same as the distance of the square points; as a result, all square and star points are located on the same circle, thus carrying information of equal weight (Fig. 3.1).

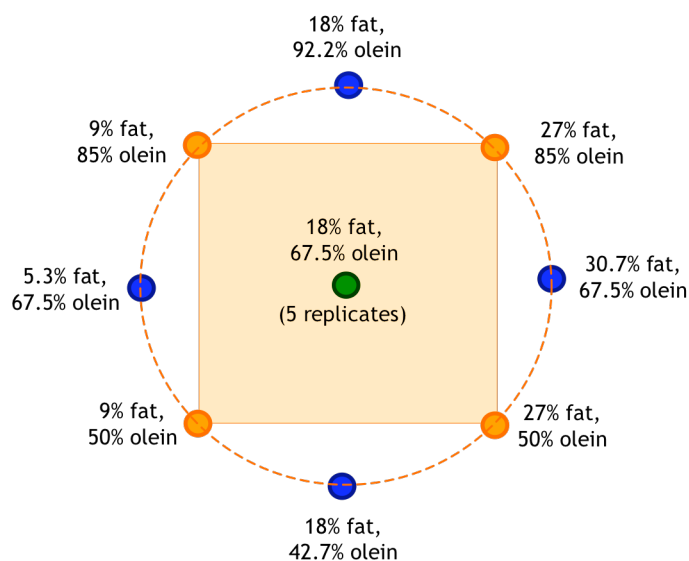


Figure 3.1. Graphical representation of CCD runs; ● : star points; ● : cube points; ● : centre points.

The 0 levels of the two considered factors were chosen on the basis of traditional butter cake recipes: 18% fat content in the batter and a fat blend containing 67.5% olein having melting characteristics similar to those of butter. The ± 1 fat content levels (9 and 27%) were selected on

the basis of different cake recipes reported in the literature (Khalil et al., 1998; Dogan et al., 2007; Kocer et al., 2007; Kalinga et al., 2009; Sowmya et al., 2009). The ± 1 levels of SO content (50-85%) were chosen in the steepest region of the SMP curve built with various PO-SO blends containing 0–100% SO (Fig. 3.2).

The order of experiments was fully randomized to avoid systematic biases and to minimize the effects of uncontrollable nuisance factors. Run order, coded and uncoded factor levels are reported in Table 3.1.

3.1.4. *Statistical analyses*

Response variables were analyzed by response surface regression for a second-order polynomial model, which contained linear, quadratic and interaction terms for the two factors. The response surfaces were generated from Eq. (1) (Montgomery, 2001):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \quad (1)$$

where Y is the predicted value of the considered response variable; x1 and x2 are the fat content (%) in the batter and the SO level in the fat blend (%), respectively; β_0 is a constant value; β_1 and β_2 are the linear coefficients; β_{12} is the interaction coefficient; β_{11} and β_{22} are the quadratic coefficients; ε is the random error. In order to determine the significance of each coefficient, the one way analysis of variance (ANOVA) was carried out.

An overall desirability function was constructed as multi-objective optimization (Montgomery, 2001), as already reported by Alamprese et al. (2007).

CCD generation, data analysis and optimization were performed using Design Expert 8 (Stat Ease Inc., Minneapolis, USA).

Table 3.1. Run order of the Central Composite Design, corresponding coded and uncoded factor levels, batter formulations and moisture.

Run	FACTORS		INGREDIENTS (% in batter)					Batter Moisture (%) ^b
	Fat (coded)	Olein (coded) ^a	Fat	Flour	Sugar	Egg product	Baking Powder	
1	+√2	0	30.7	31.2	18.6	18.6	0.9	19.0
2	-√2	0	5.3	42.7	25.4	25.4	1.2	25.9
3	0	0	18.0	37.0	22.0	22.0	1.0	22.5
4	0	-√2	18.0	37.0	22.0	22.0	1.0	22.5
5	0	+√2	18.0	37.0	22.0	22.0	1.0	22.5
6	-1	+1	9.0	41.1	24.4	24.4	1.1	24.9
7	0	0	18.0	37.0	22.0	22.0	1.0	22.5
8	0	0	18.0	37.0	22.0	22.0	1.0	22.5
9	0	0	18.0	37.0	22.0	22.0	1.0	22.5
10	+1	-1	27.0	33.0	19.5	19.5	1.0	19.9
11	0	0	18.0	37.0	22.0	22.0	1.0	22.5
12	+1	+1	27.0	33.0	19.5	19.5	1.0	19.9
13	-1	-1	9.0	41.1	24.4	24.4	1.1	24.9

^a referred to the fat blend

^b calculated on the basis of water content of each ingredient

3.1.5. *Cake preparation*

The reference cake BC was prepared with 37% soft wheat flour, 22% pasteurized liquid whole egg, 22% sugar, 18% butter, and 1% baking powder. The ABC cake was produced with the same formulation of BC, substituting butter with the same weight of anhydrous butter. Both BC and ABC samples were produced in duplicate. In the experimental formulations, butter was replaced by different quantities of PO and SO blends according to the experimental design described in § 2.4, keeping constant the ratios among the other ingredients. Complete cake formulations and batter moisture are shown in Table 3.1.

Cakes were prepared following the creaming method, with a total mixing time of 18 minutes, as suggested by Pylar (1988).

Batters (1500 g each) were prepared by whipping fat and sugar in a N-50G mixer (Hobart, USA) for 8 min (creaming phase, 4 min low speed + 4 min medium speed); then whole egg product was added and mixed for 5 min (3 min low speed + 2 min medium speed). After replacing the mixer whip with a flat beater, premixed flour and baking powder were added and mixed for 5 min (3 min low speed + 2 min medium speed). Equal portions of batter (250 g) were poured in five plum-cake moulds (5.8 x 15.3 cm bottom, 7.8 x 17.3 cm top, 6 cm height) and baked in a forced air convection oven (Moretti, Mondolfo, Italy) for 30 min at 180°C. After baking, samples were cooled down for two hours at room temperature before performing analytical determinations.

3.1.6. *Analytical methods*

Official method Cc 3-25 (AOCS, 2009a) was used to measure the slip melting point (SMP, °C) of fats. SMP was measured on blends containing 0, 20, 40, 42.7, 50, 52.5, 55, 67.5, 70, 75, 85, 90, 92.2, and 100% SO. A SMP curve was built as a function of the olein content in the fat blends, fitting the experimental data using a curve-fitting software (TableCurve 2D, v. 4.00, AISN Software Inc., USA). Solid fat contents (SFC, %) of butter and fat blends were measured following the Cd 16b-93 official method (AOCS, 2009b), by using a Minispec NMS 120 (Bruker BioSpin MRI GmbH, Ettlingen, Germany). Before SFC analysis, butter was melted and filtered on a filter paper containing anhydrous sodium sulphate to remove water. Both SMP and SFC measurements were done at least in duplicate.

Apparent specific gravity of selected batters was measured (n=10) by weighing a standard cup filled with batter or with water and dividing the weights (Dogan et al., 2007).

Cakes were analyzed for specific volume (n=5) by sesame seed displacement.

Moisture content was measured (in triple) as reported by Mariotti et al. (2006).

Colour was evaluated on three plum cakes for each run, using a reflectance colorimeter (Chroma Meter CR210, Minolta Camera Co., Osaka, Japan), with standard illuminant C. On each cake three measurements on the crumb were taken.

Texture analyses were performed using an Instron Universal Testing Machine 3365 (Instron Division of ITW Test and Measurement Italia S.r.l., Trezzano sul Naviglio, Italy) equipped with a 100 N load cell and supported by BlueHill software (BlueHill2, v2.9, 2005, Instron Corp., High Wycombe, UK). Two cakes were evenly sliced obtaining from the central part of each cake four 25±1 mm thick slices. Each slice was compressed in the center using a 27 mm diameter plate. Analyses were carried out at room temperature, with the following conditions: pre-test cross-head speed, 0.2 mm/s; trigger force, 0.05 N; test cross-head speed, 2 mm/s; final strain, 50% of the original sample height. Results are expressed in terms of Young modulus (Pa), representing the slope of the stress-strain curve of compression tests, and load at 25% strain (N).

3.2. Results and Discussion

3.2.1. Slip melting point of fat blends

The slip melting point of the fat blends as a function of SO percentage was studied. In Fig. 3.2 the relation between SMP and SO content is shown. A third degree model ($y=a+bx^3$) was used to fit the experimental data.

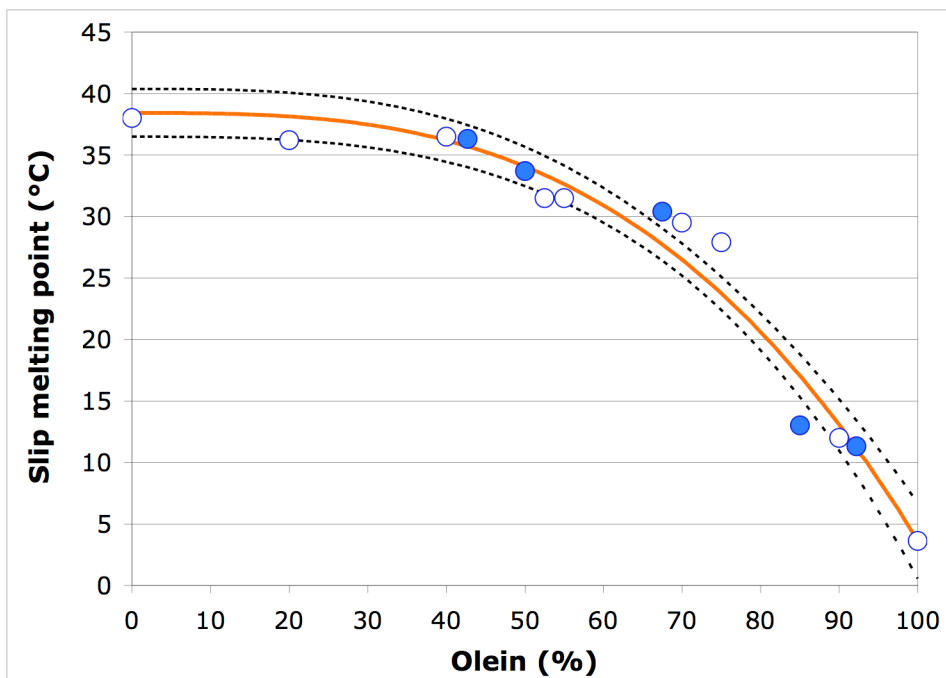


Figure 3.2. Slip melting point curve as a function of olein content in the fat blend; ● : DoE fat blends; — : regression curve; - - - : confidence interval (95%).

As it can be noticed, SMP was barely affected up to 40% SO, while at higher olein contents, even small SO additions definitely lowered SMP values. This means that relatively low olein contents could not destabilize the structure of the high melting triglycerides contained in palm oil. As already mentioned in § 3.1.3, this result was taken into consideration for choosing the factor levels of fat blend composition: only SO contents higher than 40% were included in the experimental design, because they are really effective in SMP variation, enabling the study of a wide range of fat melting characteristics..

3.2.2. Cake characteristics

Table 3.2 shows the results of the response variables considered in the thirteen runs of the Central Composite Design. Before carrying out the CCD analysis, the normal distribution plots of the response variables were checked. All the response variables followed a normal distribution, except for the load at 25% strain, which required a power transformation. The results of ANOVA and lack-of-fit test with R^2 , Adjusted R^2 and Predicted R^2 are presented in Table 3.3: R^2 is a measure of the amount of variation around the mean explained by the

model, Adj-R^2 is the same index adjusted for the number of terms in the model, while Pred-R^2 is a measure of the amount of variation explained by the model in new predicted data. Significant models were calculated for all the response variables, except for colour parameters a^* and b^* . In particular, significant ($p < 0.01$) linear models were calculated for specific volume and Young modulus, while complete quadratic models were found for slice and crumb moisture, load at 25% strain and L^* of crumb. No variables showed significant interaction models (linear and interaction terms), as indicated by 2FI p-values in Table 3.3. Among the variables with significant models, crumb moisture, specific volume, load at 25% strain and crumb L^* had a non-significant lack-of-fit ($p > 0.05$), thus indicating an adequate description of the true shape of the response surfaces. On the other side, slice moisture and Young modulus showed a significant lack-of-fit ($p < 0.05$), which means that the models did not correctly fit the experimental data.

Table 3.2. Mean values of the response variables considered in the Central Composite Design.

RUN	RESPONSES							
	Slice Moisture (%)	Crumb Moisture (%)	Specific Volume (cm ³ /g)	Young Modulus (kPa)	Load at 25% Strain (N)	L* Crumb	a* Crumb	b* Crumb
1	15.13	19.05	2.16	64.31	6.82	68.62	-7.52	68.62
2	20.66	24.16	2.39	133.95	13.37	75.60	-7.60	75.60
3	17.60	20.65	2.24	94.58	8.27	75.08	-7.96	75.08
4	18.45	20.86	2.11	118.32	9.98	75.70	-7.88	75.70
5	17.81	20.78	2.30	94.90	7.92	75.68	-8.03	75.68
6	20.15	23.17	2.42	130.05	11.22	76.13	-7.83	76.13
7	17.43	20.57	2.17	93.42	7.92	75.07	-7.46	75.07
8	17.65	20.92	2.17	86.84	7.55	75.77	-7.71	75.77
9	17.54	20.84	2.18	91.27	7.44	75.12	-7.68	75.12
10	16.12	19.41	2.06	70.20	6.47	70.30	-7.58	70.30
11	17.74	20.78	2.13	94.35	8.15	75.74	-7.86	75.74
12	16.60	19.61	1.98	49.14	5.10	70.58	-7.33	70.58
13	20.32	23.16	2.30	135.82	12.19	75.56	-7.74	75.56

Table 3.3. ANOVA and model fitting for the response variables of CCD; selected models are reported in bold. 2FI: two factors interaction.

Test	Degrees of Freedom	Slice Moisture (%)	Crumb Moisture (%)	Specific Volume (cm ³ /g)	Young Modulus (kPa)	Load at 25% Strain (N)	L* Crumb	a* Crumb	b* Crumb
Sequential Model Sum of Squares (p-values)									
Linear	2	<0.0001	<0.0001	0.0019	0.0001	0.0003	0.0033	0.4412	0.1754
2FI	1	0.4380	0.8272	0.1757	0.3917	0.7537	0.9303	0.4491	0.6803
Quadratic	2	0.0117	0.0001	0.4615	0.2189	<0.0001	0.0001	0.1648	0.5615
Lack-of-fit Tests (p-values) of significant models									
Linear	6	0.0068	0.0139	0.0823	0.0189	0.0020	0.0026	-	-
2FI	5	0.0058	0.0104	0.9232	0.0166	0.0015	0.0019	-	-
Quadratic	3	0.0312	0.5729	0.0684	0.0175	0.1567	0.1343	-	-
Predictive parameters of significant models									
R ²		0.9875	0.9956	0.7159	0.9182	0.9877	0.9759	-	-
Adjusted R ²		0.9785	0.9924	0.6590	0.9019	0.9790	0.9587	-	-
Predicted R ²		0.9202	0.9841	0.4244	0.8371	0.9337	0.8664	-	-

Table 3.5. Values of selected responses for BC, ABC and CCD central point formulations.

SAMPLES	SMP (°C)	RESPONSES							
		Slice Moisture (%)	Crumb Moisture (%)	Specific Volume (cm ³ /g)	Young Modulus (kPa)	Load at 25% Strain (N)	L* Crumb	a* Crumb	b* Crumb
BC ¹	32.8	20.33±0.07 ^a	23.97±0.04 ^a	2.23±0.03 ^a	54.08±0.70 ^a	5.63±0.03 ^a	77.39±0.09 ^a	-7.18±0.02 ^a	29.63±0.94 ^a
ABC ¹	33.0	17.54±0.03 ^b	20.79±0.14 ^b	2.01±0.04 ^b	80.51±4.37 ^b	7.86±0.91 ^b	76.26±1.03 ^b	-7.22±0.18 ^a	31.08±0.79 ^a
CCD central point ²	30.4	17.59±0.11 ^b	20.75±0.14 ^b	2.18±0.04 ^a	92.09±3.21 ^c	7.87±0.36 ^b	75.36±0.36 ^b	-7.73±0.19 ^b	35.73±0.22 ^b

¹ average and SD calculated on two technological replicates; ² average and SD calculated on five technological replicates

^{a,b} Numbers with different letters within the same column are statistically different (p<0.05)

Table 3.4 reports the regression coefficients of the coded factors for the significant and adequate models found, which allow comparing directly the relative magnitude of different factors effects. Thus the fat content (x_1), having the highest absolute values among coefficients (b_i), showed the greatest influence on all responses considered.

Table 3.4. Regression coefficients of polynomial models, according to Eq. (1), for selected experimental response variables.

COEFFICIENTS (coded)	RESPONSE VARIABLE			
	Crumb Moisture (%)	Specific Volume (cm ³ /g)	Load at 25% Strain (N) ^a	L* Crumb
β_0	20.75	2.2	280.09	75.36
β_1	-1.82***	-0.12**	-352.42***	-2.58***
β_2	0.01	0.038	-77.15**	0.1
β_{12}	0.047	0	27.45	-0.075
β_{11}	0.46***	0	185.63***	-1.81***
β_{22}	0.066	0	46.65*	-0.023

^a Coefficients referring to the transformed variable

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The influence of fat content on crumb moisture is a direct consequence of the different water content in batter formulations (Table 3.1); in fact, a highly significant correlation between batter moisture and crumb moisture ($r=0.96$, $p < 0.05$) was found. On the contrary, olein percentage (x_2) did not affect this variable, as confirmed by the non significant value of b_2 and as also shown in Fig. 3.3a.

The flat response surface of cake specific volume illustrates the significant linear negative influence of fat content on this parameter (Fig. 3.3b). Probably, as fat content in the formulation increased, a higher amount of gasses escaped from the batter during chemical leavening, due to a progressive weakening of the gluten network and a lower viscosity of the batter. On the contrary, the olein content in the fat blend had no significant influence on cake volume, to indicate that fat melting point did not influence the amount of gas retained in the baked product, at least in the range of olein content/SMP considered. The percentage of fat in batter was a significant factor for cake texture parameter (load at 25% strain), both for linear and quadratic coefficients. This variable was also the only one significantly affected by olein content; however, observing coefficient values, it can be noticed that fat still had the highest importance: load decreased as fat and olein content increased. The significant quadratic coefficients resulted in a curvature of the response surface (Fig. 3.3c) with an area of minimum in which cake hardness reached the lowest values, yielding softer cakes.

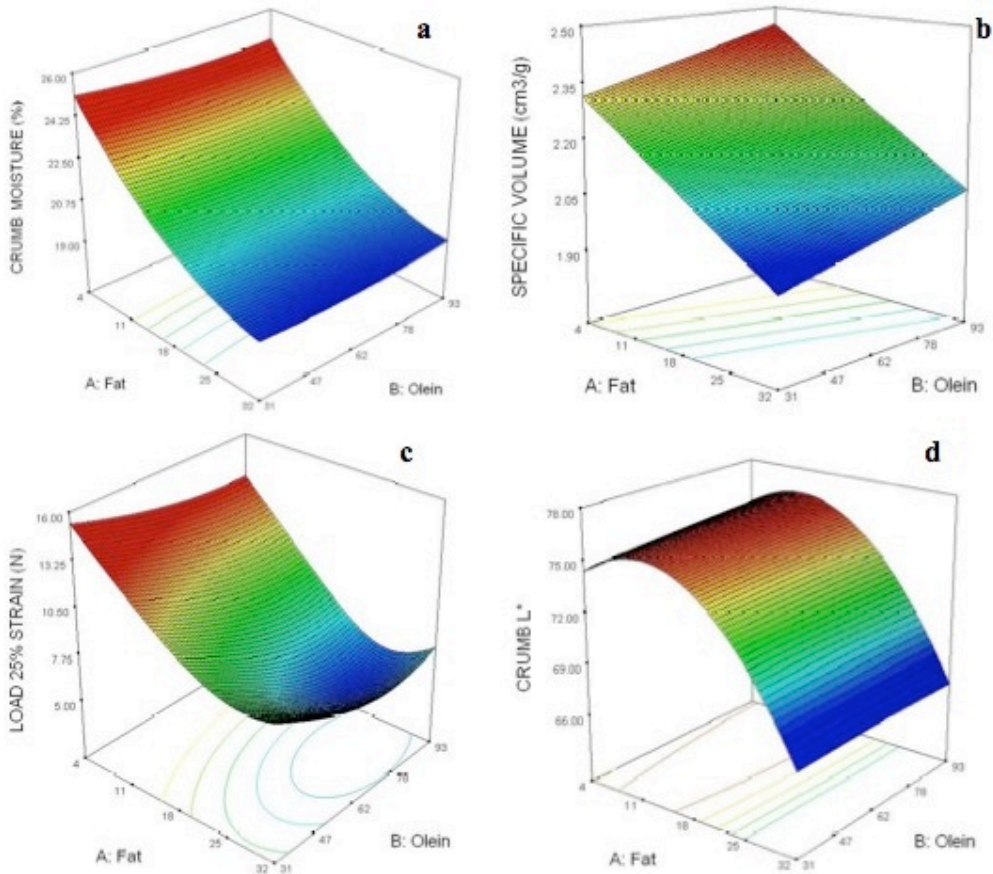


Figure 3.3. Response surfaces for crumb moisture (a), specific volume (b), load at 25% strain (c) and crumb L* (d).

Observing the response surfaces of the three variables discussed so far, it appears that high fat contents generally lead to products with low values of moisture, specific volume and hardness. This could seem in contrast with the tenderizing action of water on cake texture, described by Mizukoshi (1985) who studied the effect of cake formulation on shear modulus of batter and cake. Actually, analysing our results, we can assume that fat content had a larger tenderizing effect on cake texture than water, but in high fat cakes with very low moisture contents, load slightly increased following a quadratic curvature (Fig. 3.3c).

As regards the colour parameters, crumb lightness (L*) decreased as fat content increased (Fig. 3.3d); this is probably due to the lower specific volume, i.e. a lower air content in the product. The two variables, in fact, were positively correlated ($r=0.58$, $p<0.05$): a higher specific volume corresponded to a brighter crumb in the cake. Olein content instead did not significantly affect L* values ($p>0.05$) as well as a* and b*, which showed no significant models due to the similar colours of the two blended oils.

Selected samples among the runs of the experimental design are shown in Fig. 3.4.

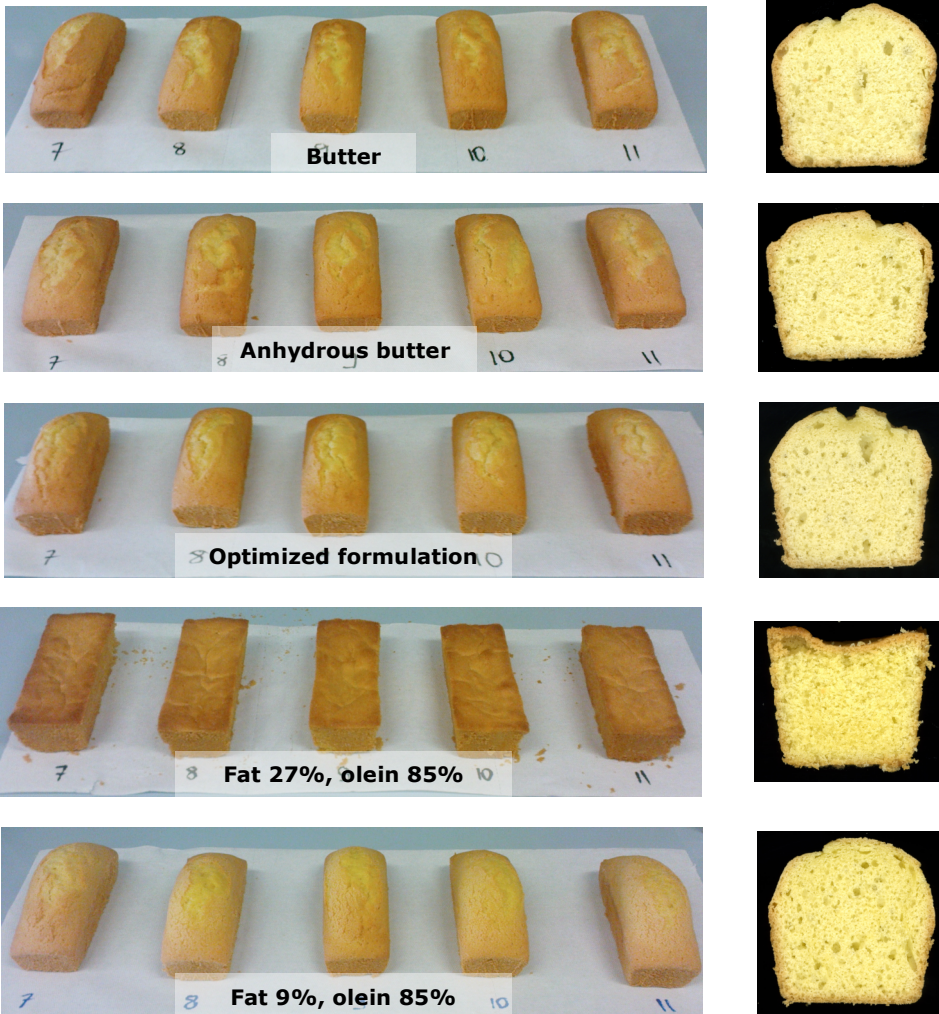


Figure 3.4. Selected samples of CCD runs: five baked cakes (left) and central slice of the cake (right).

In Table 3.5 values of some selected responses for butter (BC), anhydrous butter (ABC) and the average of the five central points are reported, all produced with 18% fat content. A different behaviour among the three samples is shown, especially for moisture and texture: butter cake was significantly ($p < 0.05$) softer and moister than the other two samples, which showed similar load and water content both in slice and crumb. This is related to differences in batter moisture (Table 3.1), since butter, containing 18% water, adds around 3% moisture to batter. Cakes produced using anhydrous butter had the lowest specific volume and a high load, in accordance with findings of NorAini et al. (1996), who showed that butterfat had the worse performance in terms of cake softness, when comparing cakes produced with 100% palm oil or butterfat or blends of the two fats.

3.2.3. Optimization of cake formulation

One of the main purposes of response surface methodology is to find the optimum conditions for a process to obtain the desired responses (Kahyaoglu & Kaya, 2006). In order to identify a cake formulation optimized for texture and volume characteristics, a desirability function was constructed. This function reaches a maximum value of 1 when the aims set for the chosen variables are fully achieved. Cake optimization was based on both minimization of load at 25% strain and achievement of a specific volume similar to that obtained with butter reference recipe. To the two variables, both significant for cake performances, was given the same importance because they were both representative of a baked product quality, two of the major quality indexes of a baked product. A linear desirability function (weight=1) was chosen. The highest desirability corresponded to an optimized formulation (OF) containing 19.7% fat in the batter and 92% olein in the fat blend (SMP = 11°C), with a predicted value of specific volume and load at 25% strain of 2.23 cm³/g and 7.09 N, respectively. The desirability function reached a value of 0.87 (Fig. 3.5), which is quite good considering that the constraints for the two variables headed for opposite directions, while the trends of the two surfaces as a function of fat quantity were similar (Fig. 3.3).

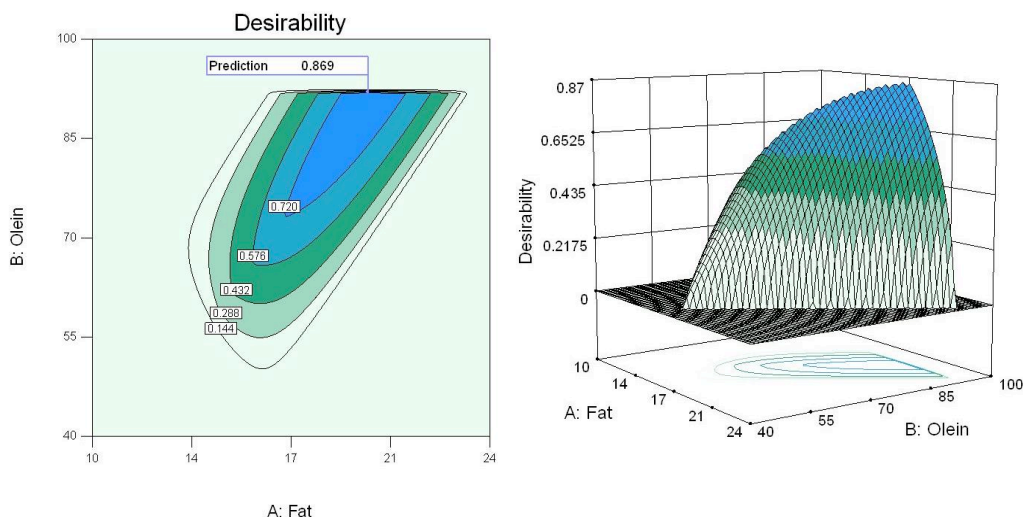


Figure 3.5. Desirability function contour plot (left) and 3D representation (right).

The OF was experimentally tested twice, obtaining specific volume and load at 25% strain values of $2.25 \pm 0.03 \text{ cm}^3/\text{g}$ and $7.68 \pm 0.27 \text{ N}$, respectively. The validation values were fairly consistent with those predicted, showing a full correspondence for specific volume and a minor discrepancy for load at 25% strain. Comparing the values of OF with those of BC reported in Table 3.5, cakes showed the same volume, as desired, but OF cake had a higher consistency ($p < 0.05$), due to the lower batter moisture content (21.7% vs 25.7% of butter) as calculated on the basis of water content of ingredients. Comparing OF with ABC, optimized cakes were more developed in volume ($p < 0.05$) although with a similar consistency, in spite of the slightly higher fat content of OF cake that could have negatively affected cake volume. The different SMP could have played a role in the specific volume difference, being SMP of OF blend 11°C and that of anhydrous butter 33°C . However, this finding is not consistent with previous reports (Podmore, 2002; Stauffer, 1996) indicating that a minimum content of crystalline fat at the working temperature is required to develop a well aerated structure (at least 5%, according to Podmore, 2002).

To further investigate this point, Fig. 3.6 presents SFC values of the different fats used; butter (B) and anhydrous butter (AB) have the same profile because B is dehydrated before the analysis. Considering a working temperature of 20°C , it is possible to observe SFC values of about 25% for PO, 19% for B-AB and 1% for OF, while olein is liquid at temperatures higher than 10°C . Consequently, our findings suggest that in a plum cake formulation a well aerated structure can be achieved also with a SFC lower than 5%.

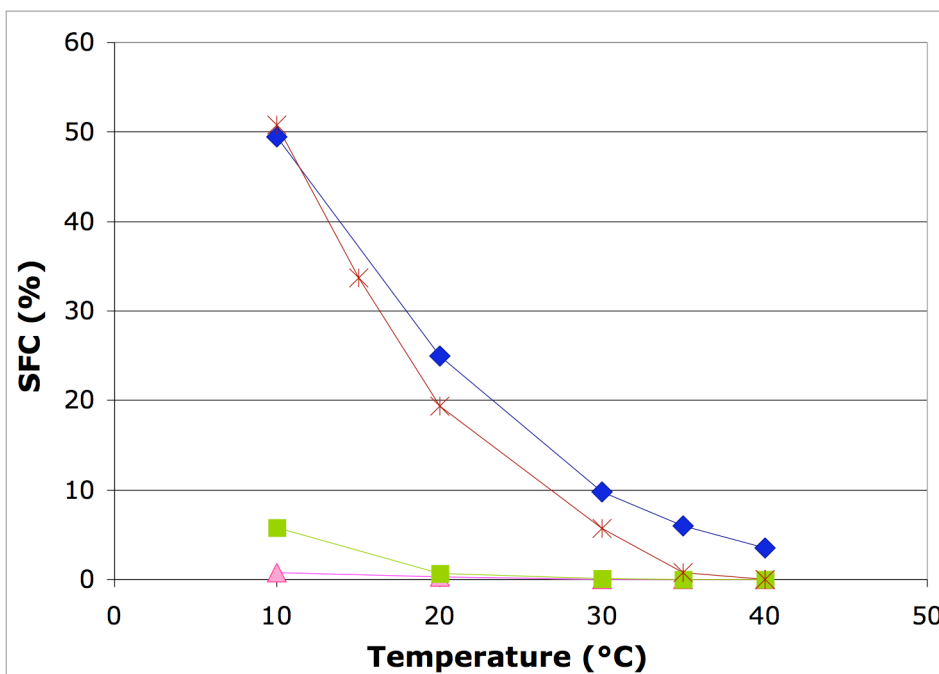


Figure 3.6. Solid fat content (SFC) of palm olein (PO, \blacklozenge), palm superolein (SO, \blacktriangle), optimized formulation blend (OF, \blacksquare) and butter – anhydrous butter (B-AB, \times).

OF was compared with BC and ABC recipes also in terms of apparent specific gravity of batters. This parameter is strictly related to the creaming performance of fat: the lower the specific gravity, the higher the air content in the batter (Dogan et al., 2007). BC formulation

showed the lowest specific gravity (0.97 ± 0.02) compared to those of OF (1.19 ± 0.02) and ABC (1.19 ± 0.03). The better creaming performance of BC is probably related with its physical state, i.e. a water in oil emulsion, which helped air incorporation and air bubble stabilisation, thanks to the naturally present emulsifiers, represented by the residue of the milk fat globule membranes. Furthermore, creaming performances seemed not to be affected by SMP, as shown by the similar values found for ABC and OF. However, the different specific volumes of the final cakes observed for these two formulations could be ascribed to differences in SMP as well as in batter rheology and fat content.

3.3. Conclusions

Response surfaces, obtained from the experimental design and representing quadratic and linear models, adequately describe the effects of fat quantity and composition on texture and specific volume of plum cakes. Fat quantity had a more significant effect on cake characteristics than olein content in the fat blend. The increase of fat content enhances plum cake softness while lowering the volume; higher olein contents, corresponding to lower slip melting points, also improve plum cake texture. No interactions between the two factors have been observed.

The optimized plum cake formulation showed good structural properties, comparable to those of the reference butter cake. These results demonstrate the possibility to replace butter or shortening with a vegetable fat blend, thus increasing unsaturated fat content and reducing total cholesterol in the final cake.

3.4. References

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4. Batter properties and cake behaviour during storage of three plum cake formulations

Most bakery products are stored under ambient conditions and marketed as fresh; consequently, considerable research has focused on studying their shelf-life and the influence of ingredients on quality characteristics (Heenan et al., 2010). The stability of cakes during storage can be defined as the maintenance of the physical and sensorial characteristics associated with freshness, such as softness, compressibility and humidity, with minimal alterations associated with staling (Gómez et al., 2010). In fact, two products that result similar few hours from production, may have very different behaviour during shelf-life. This may be due to storing conditions but also to ingredients and/or process used, which at the same time influence dough/batter characteristics. In particular, rheological properties of the batter can be correlated with the characteristics of the baked cake, such as volume and texture (Sakiyan et al., 2004).

Cake batter is a complex fat-in-water emulsion, composed of air bubbles as the discontinuous phase and of an egg-sugar-water-fat mixture as the continuous phase, in which flour particles are dispersed (Kocer et al., 2007). Fats contribute to the soft and tender eating properties required for cakes; this benefit comes, on one side, from the effect on batter aeration and, on the other side, from the lubricating effect that fat has in the mouth (Dogan et al., 2007). In general, bakery products contain plastic fats, which vary in consistency according to their temperature, melting point and proportion of liquid oil within the network of solid fat crystals; these factors greatly influence properties like structure, consistency, mouthfeel and shelf life of the products (Daglioglu et al., 2004). Moreover, fats may eventually decompose, rising unpleasant (rancid) flavours through the process of oxidation. Long storage times may be involved between preparation and consumption of the foodstuff (Daglioglu et al., 2004); consequently, properties and functions of the selected bakery fat are of major importance for the total quality of the finished product (Manley, 1991).

In this part of the research, rheological characteristics of three fats -and their corresponding batters- were studied, searching for a possible relationship with the performances of baked products. Furthermore, cakes underwent a storage study in order to monitor changes in moisture, primary oxidation products (hydroperoxides) and texture characteristics, as these are markers for baked products quality.

4.1. Materials and Methods

4.1.1. *Materials and cake preparation*

Raw materials used for cakes production were the same used for the DoE runs and previously described in § 3.1.1. The three formulations produced were BC, ABC and OF, prepared as reported in § 3.1.1, using respectively butter, anhydrous butter and the optimized fat blend. Anhydrous butter and the optimized fat blend were crystallized as reported in § 3.1.2.

Chemicals used for Peroxide Value analysis (chloroform, glacial acetic acid, potassium iodide, starch) were purchased from Sigma-Aldrich (Sigma-Aldrich Co., Saint Louis, MO, USA).

4.1.2. *Rheological determinations*

Viscosity measurements are commonly used to monitor changes in fat rheology due to crystallisation, but the application of shear can destroy some of the delicate interactions present in fat systems; consequently a controlled stress rheometer was used for the present studies, as suggested by Bell et al. (2007). The choice of a plate-plate geometry was made also referring to Lupi et al. (2011).

Fat and batter rheological behaviour was evaluated using a Physica MCR 300 rheometer (Anton Paar, Graz, Austria), supported by Rheoplus/32 software (v3.00, Physica Messtechnik GmbH, Ostfildern, Germany), and equipped with a plate-plate geometry (d=25 mm, rough surface for batter analyses; d=50 mm, plain surface for fat analyses), gapped to 1 mm. All tests were run at least in duplicate.

For fats, temperature sweep (TS) tests, simulating the temperature profile of crystallization (70°C to 0°C for O, 70°C to 15°C for AB; cooling rate, 2.5°C/min) were performed, applying a strain (γ) of 0.1% and an oscillation frequency (f) of 1 Hz. Frequency sweep (FS) tests were performed at 25°C, with $\gamma=0.1\%$ and $f=0.01-100$ Hz. These tests conditions were within the linear viscoelastic range, previously determined by strain sweep (SS) tests carried out at 25°C, with $\gamma=0.01-100\%$ and $f=1$ Hz.

For batters, TS tests (25°C to 70°C; heating rate, 2.5°C/min) were performed with $\gamma=0.01\%$ and $f=1$ Hz. FS tests were performed at 25°C and 60°C, with $\gamma=0.01\%$ and $f=0.01-100$ Hz. The test conditions were within the linear viscoelastic range, previously determined by SS tests performed at both 25°C and 60°C, with $\gamma=0.001-100\%$ and $f=1$ Hz. The exposed edges of the sample were covered with a thin layer of paraffin oil to prevent dehydration during measurements.

In both fats and batters characterization, the following parameters were registered: storage modulus (G' , Pa), representing the elastic component of the sample; loss modulus (G'' , Pa), representing the viscous component of the sample; complex modulus (G^* , Pa), defined as the ratio of the shear stress to the applied deformation, measuring the overall resistance to deformation of the material; complex viscosity (η^* , Pa·s), the frequency-dependent viscosity obtained from the ratio between G^* and angular frequency.

4.1.3. *Cake storage*

The three cake formulations were monitored during storage. After baking, samples were cooled down for two hours at room temperature; cakes were then packed and sealed in oriented polypropylene (OPP) bags, previously sprayed inside with ethanol, in order to prevent microbial growth. Packaging material was chosen for its good moisture barrier properties and for screening out light, due to the white-pearly appearance. Packed cakes were stored at 25°C for 35 days; PV and texture analysis were performed on 0 - 2 - 5 - 7 - 10 - 14 - 21 - 28 - 35 days from production.

PV was measured following the EU method for olive oil (Reg. CE 2568/91, 1991). Fat was extracted by milling two slices of cake using a rotating blade mixer; 12 g of the milled cake (in duplicate) were weighed, 100 mL chloroform was added and the suspension was stirred for 45 min. The chloroformic extract was then filtered with Whatman filter paper n.4 (Whatman Int. Ltd, UK). Fat content was measured by evaporating 20 mL of extract; another 20 mL of extract were sampled and subjected to PV analysis.

Texture measurements were performed as reported in § 3.1.6.

4.1.4. *Statistical analyses*

In order to determine the significant difference among samples, the one way analysis of variance (ANOVA) was carried out, followed by Fisher's Least Significant Different (LSD) test in order to discriminate samples. Data was elaborated using the Statgraphics Plus 5.1 (Statistical Graphics Corp., 1994-2001, Princeton, NJ, USA).

4.2. Results and Discussion

4.2.1. Rheological determinations

Fats

In the FS tests, carried out at 25°C, the three fat samples showed storage modulus values higher than those of loss modulus; only for O a clear dependence of G' and G'' from frequency was observed, with both moduli increasing with the increasing of frequency (Fig. 4.1). G' and G'' values for O blend were substantially lower than the values of B and AB, due to its lowest SMP.

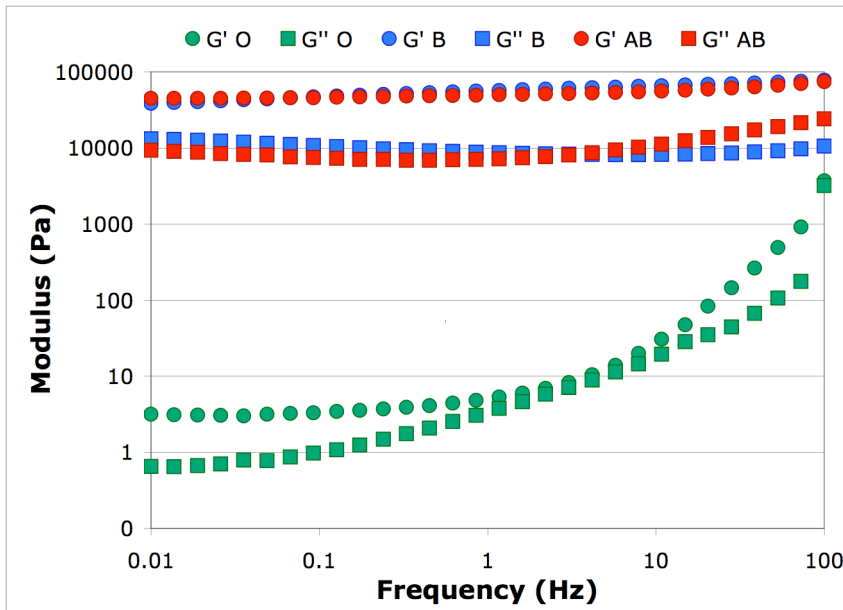


Figure 4.1. G' and G'' values in Frequency Sweep test for B, AB and O fats at 25°C.

Observing the TS curves of AB and O fats (Fig. 4.2), an expected and similar slow increase of moduli with the decrease of temperature could be noticed. At temperatures around 45°C, a first crossover could be observed for both samples, with G'' exceeding G' . A clear crossover of G' and G'' , indicating the initial solidification of the fat, was also observed around 21°C for AB only.

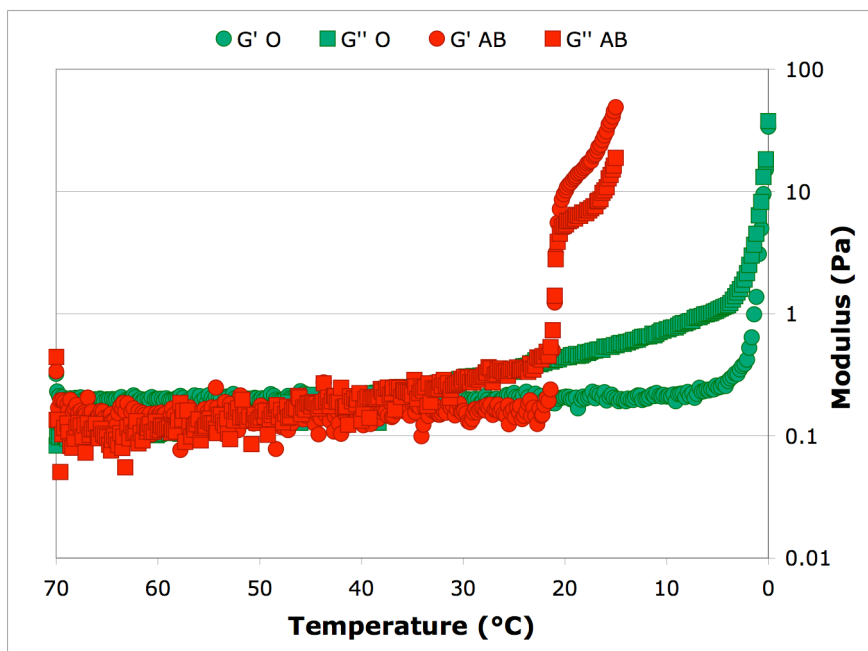


Figure 4.2. G' and G'' values in Temperature Sweep test for AB and O fats.

A steep increase of both storage and loss modulus corresponds to the solidification of the fat. As De Graef et al. (2006) observed, the primary crystallization process can also be followed by means of viscosity changes as a function of time (or temperature), as shown in Fig. 4.3. With the formation and growth of crystals, in fact, the viscosity increases almost linearly with the amount of crystals in the suspension until it reaches a thermodynamic equilibrium (De Graef et al., 2006). Furthermore, the crystallization phenomenon was observed at higher temperatures than the SMP previously measured (§ 3.2.1). This is in accordance with the data reported by Pyler (1988), indicating that the solidification point of a fat does not coincide with its melting point, but is normally considerably lower; in our case, the gap between the two temperatures was about 10°C for AB sample.

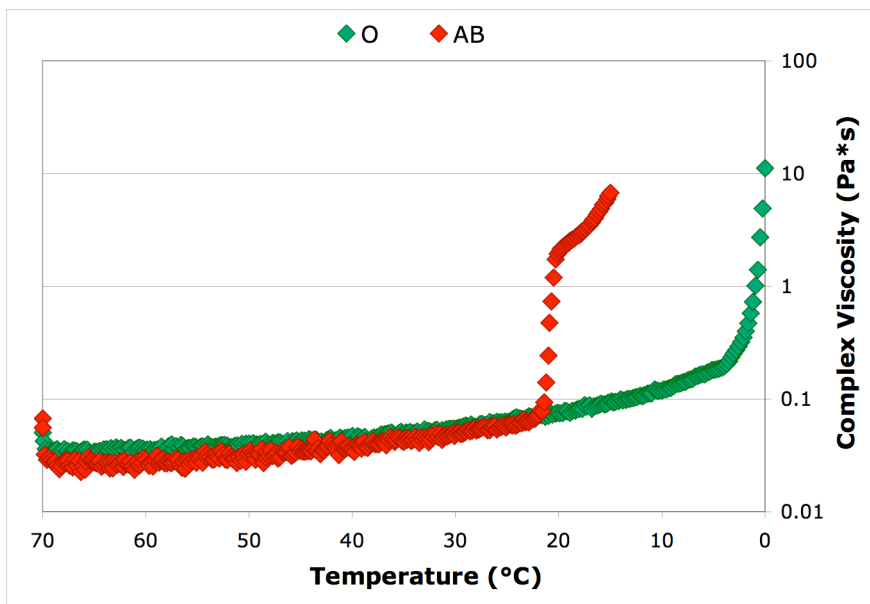


Figure 4.3. Complex viscosity values in Temperature Sweep test for AB and O fats.

Batters

Chesterton et al. (2011) highlighted that inclusion of air - a key aspect of batter preparation - not only determines the structure of the baked product but also affects batter rheology, which governs the expansion of the material before the continuous phase sets during baking. Sanz et al. (2008) reported that a muffin with a higher number of air bubbles in the batter corresponded to better baking performances. A clear influence of the fat type on batter structural characteristics has been in fact demonstrated in the studied formulations, even if rheological behaviour of batters did not always reflect differences evidenced among baked cakes (Sanz et al., 2008).

The mechanical spectra of the three samples, measured at 25°C (Fig. 4.4) and at 60°C (data not shown), presented a similar trend, but with definitely lower values for OF. All the batters showed a solid-like behaviour, with the elastic modulus higher than the viscous modulus. In order to simulate structural changes in cakes during the first stages of baking, TS tests from 25 to 70°C were done. Unfortunately, it was not possible to study temperature values higher than 70°C, thus the real structure modifications due to cooking were not observed. A steep decrease of the complex viscosity (Fig. 4.5) with the increase in temperature was observed for BC and ABC batters, mainly due to the fat melting. The viscosity decrease was, in fact, definitely less evident for OF batter, in which the fat is already liquid at room temperature. Moreover, observing FS curves, the highest G' and complex viscosity values were found for BC, which also resulted in a more aerated batter (BC had the lowest specific gravity, as reported in § 3.2.3) and cakes with the lowest hardness and a high specific volume (§ 3.2.2). Thus, it seemed that the more structured batter helped in the retention of air in cakes, confirming previous findings reported by Kalinga and Mishra (2009). Moreover, Sanz et al. (2008) showed that the increase in batter viscosity and elasticity contributed to higher bubble stability before and during baking, since lower batter viscosity during heating has been associated with a decrease in the end product volume. On the contrary, a highly viscous batter would then give a greater capacity to retain the expanding air nuclei during heating and to prevent coalescence before the batter sets

(Sanz et al., 2008). Nevertheless, for the other two samples (ABC and OF) no clear relation between viscoelastic properties of batter and baked product characteristics could be found. Regarding viscosity changes in FS tests, Sakiyan et al. (2004) found a shear thinning behaviour in cake batters, confirming the behaviour of BC, ABC and OF samples, shown in Fig. 4.6: complex viscosity, in fact, decreases with increasing frequency.

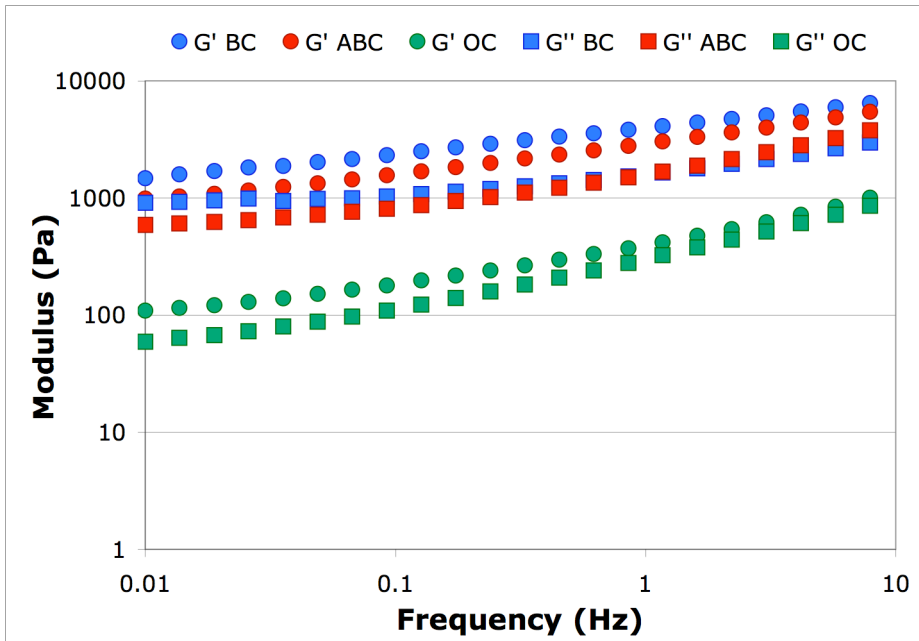


Figure 4.4. G' and G'' values in Frequency Sweep test for BC, ABC and OF batters at 25°C.

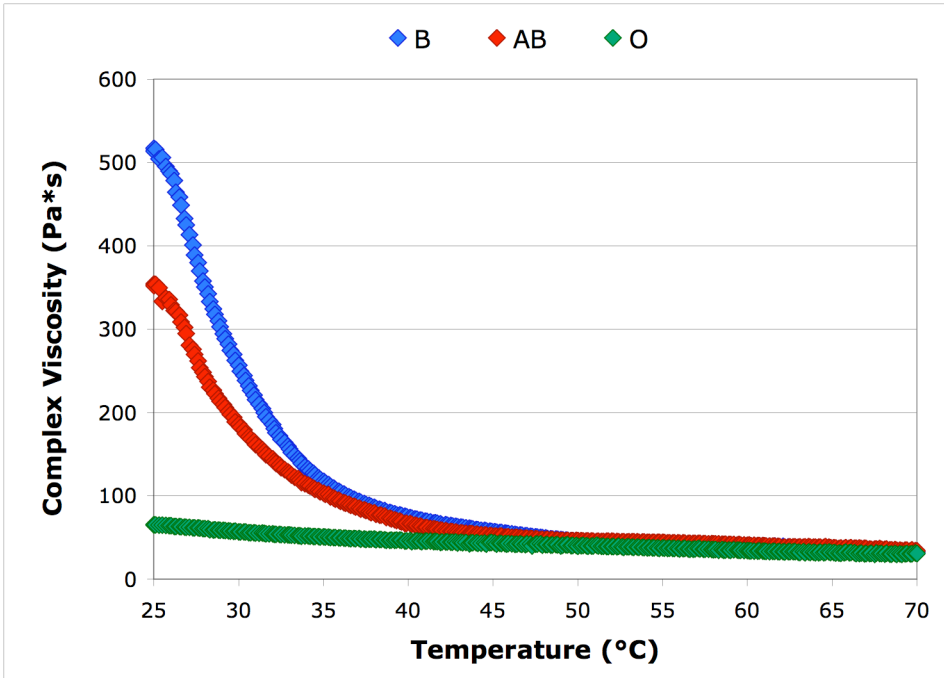


Figure 4.5. Complex viscosity values in Temperature Sweep test for BC, ABC and OF batters.

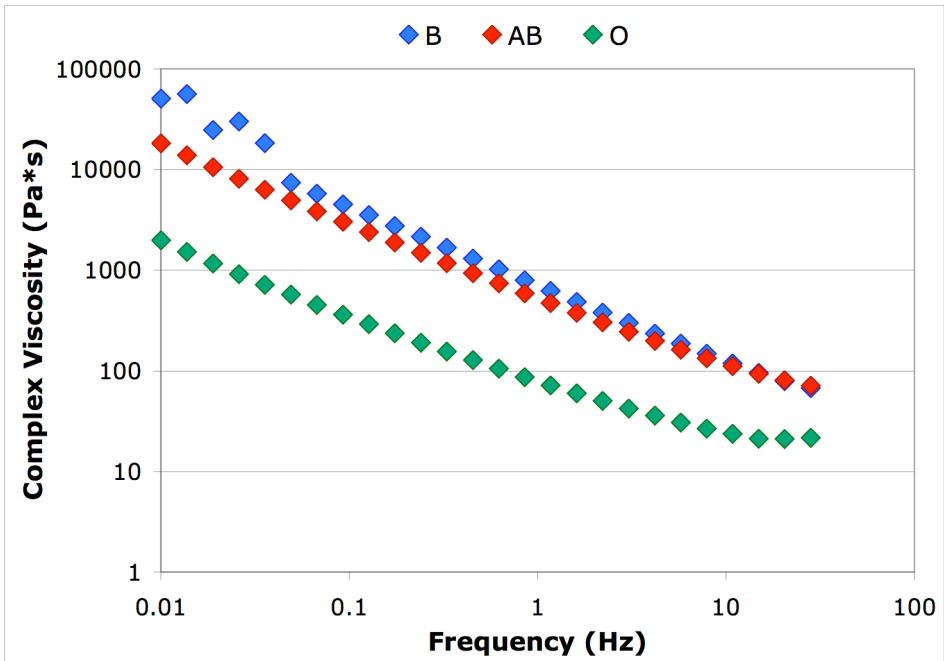


Figure 4.6. Complex viscosity values in Frequency Sweep test for BC, ABC and OF batters at 25°C.

4.2.2. Cake storage

The increasing trend of cake hardness during the 50 days of storage was similar for the three formulations; in particular, after 8 days of storage, load values almost tripled (Fig. 4.7). The increasing in hardness can be only partially explained by the variations in moisture, which decreased both in crumb and in the whole slice (Fig. 4.8). In fact, hardness increase was much faster than water loss to indicate that starch retrogradation phenomena were certainly involved, especially in the first 10 days of storage.

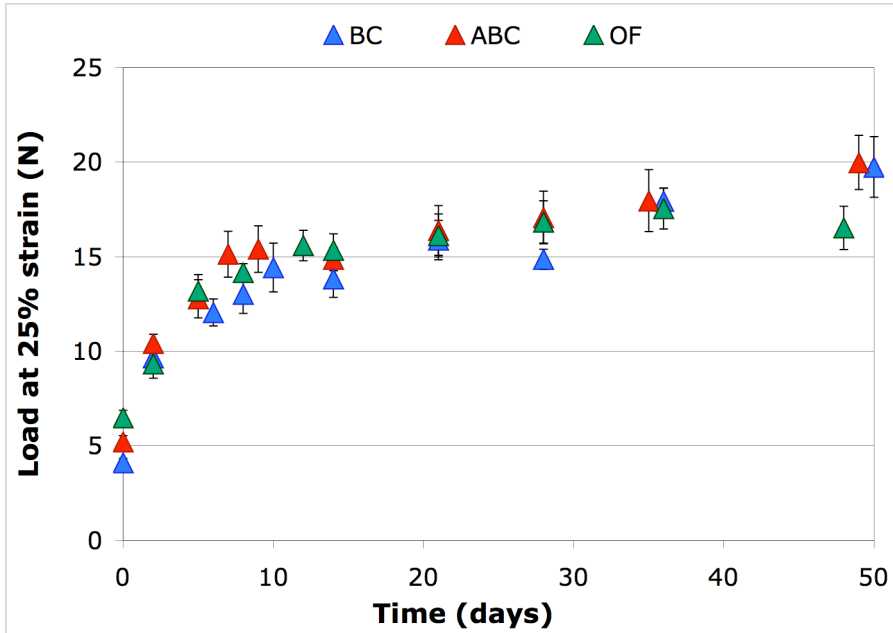


Figure 4.7. Load at 25% strain values in compression test of cakes during storage at 25°C.

Moisture decrease (Fig. 4.8) showed a similar trend for OF, BC and ABC, indicating that water loss is not influenced by the type of fat used. The different moisture values of the three formulations at the beginning of storage were due to a different moisture content in cake formulations. Observing moisture loss for crumb and slice, for every sample there was an initial discrepancy (around 4%) between the two values, being the internal part of the cake moister and indicating the uneven distribution of water across the slice, typical for baked products. As long as storage went further, the slice and crumb moisture tended to merge to the same value, due to water migration from crumb to the external region of the cake; the equilibrium was reached after 20 days of storage. After this time, another 15 days were required to gain a stable moisture content in the samples.

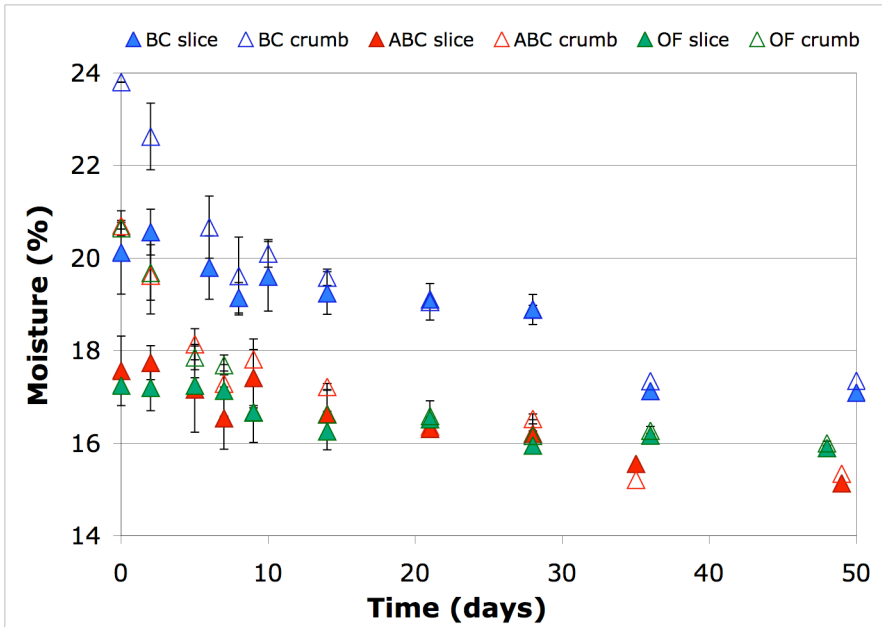


Figure 4.8. Slice and crumb moisture values of cakes during storage at 25°C.

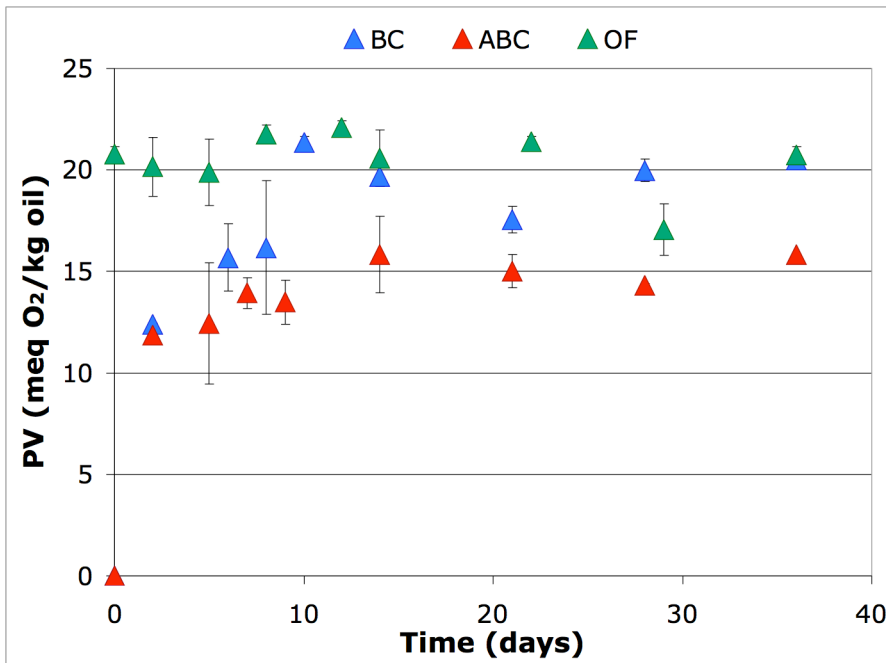


Figure 4.9. Peroxide value of cakes during storage at 25°C.

Observing the oxidation index (PV), OF resulted more stable to oxidation (although starting from a higher PV); BC and ABC showed similar trends, with a steady increase in the first 2 days of storage (Fig. 4.9). ABC sample was characterized by the lowest Peroxide Values.

4.3. Conclusions

Batter viscoelastic behaviour was influenced by the fat type used, with more structured batters corresponding to fats with a higher elastic component. Moreover, creaming performances were related to batter characteristics, even if rheological behaviour of batters did not always reflect differences evidenced among baked cakes.

A higher content of unsaturated fatty acids, such as in the optimized formulation, resulted to be positive for baking performances, if adequately balanced in the formulation. In fact, structural properties of optimized cake were not significantly different from butter reference, revealing in the meantime the best oxidation stability and a healthier nutritional profile. In general, regarding texture and moisture changes, the type of fat did not influence staling and drying phenomena.

4.4. References

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