UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

DIPARTIMENTO DI AGRARIA

Tesi sperimentale per il conseguimento del titolo di Dottore di ricerca in Scienze e Tecnologie delle Produzioni Agro-Alimentari

Calorie reduction in food: Sensory Performance of a New Sweetener and Fat Replacer Optimization

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To my family

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0. Preface

Consumers in the industrialised world are becoming increasingly aware of the relationship between diet and health. In the context of food health product development worldwide, healthy and functional food are currently some of the most intensive areas (Borgue & Soreson, 2007). Healthy food products can be characterized by several attributes: low to moderate sodium, sugar and trans-fat content and significantly reduced energy density, in comparison to their standard counterparts. In such products mono, oligo- and polysaccharides and triglycerides are replaced by water or air (Palzer, 2009). Food with modified nutrient content, such as low-fat or low-salt products, are not functional foods since they can be regarded as products that support nutritional recommendations, but do not promise specific health effects (Lahteenmaki et. al., 2007).

The food industry plays an important role in healthy choices, it can move in a positive direction, for example by making products with low fat or preparing meals with a high content of fruit and vegetables, or promoting products with low calories. For consumers, the choice of foods that allow them to follow a balanced and healthy diet is very difficult for economic reasons and because they can be deceived by false health message (Anderson, 2007). To try to protect consumers, in 2006 the European Parliament adopted Regulation (EC) No 1924/2006 on nutrition and health claims of foods, although it is still evolving: it will soon be established nutrient profiles, on which the use of nutrition and health claims for specific foods and food categories will be based. In particular, the Annex contains the claims that can be used and the conditions of application, such as the words "low fat" if the product contains no more than 3 grams of fat per 100 grams of solids or 1.5 g per 100 mL of liquid, or " without sodium / salt" if it contains more than 0.005 g sodium or the equivalent value for salt / 100 g product and much more (Reg. 1924/2006). Many government are trying to encourage people to combat obesity mainly through health campaigns and labeling legislation. Their intention to try to reduce the consumption of sugar, fat, saturated fat, trans fatty acids and salts gives the product developer many new challenges and opportunities, but it is important keeping in mind that the reduction of some of these ingredients may affect both sensory and physical properties of modified foods.

Thus, in the 2011 THE EUROPEAN PARLIAMENT issued the REGULATION (EU) No 1169/2011. This Regulation is intended to be the cornerstone of food labeling. The Regulation will apply from 13 December 2014, with the exception of the nutrition declaration which shall apply from 13 December 2016 and some specific requirements which will apply from 1 January 2014.

Generally, to reduce calorie content of a food, fat and sugar are reduced or removed and, subsequently, replaced with other components. Calorie can also ideally reduced in a food, through the incorporation of a certain amount of air.

0.1. References

Borgue J, Soreson D Case study of consumer-oriented food product development: reduced-calorie foods. In *Consumer-led food product development*, MacFie HJH ed., CRC Press, Boca Raton, USA, 2007 pp 524-547

Lahteenmaki L, Lily M, Urala N Consumer attitudes towards functional foods. In *Understanding Consumers of Food Products,* Frewer L & van Trijp H eds, CRC Press Boca Raton, Cambridge, EN, 2007 pp. 412-415.

Palzer S 2009 Food structures for nutrition, health and wellness. *Trends in Food Science & Technology*, 20: 194-200.

REGULATION (EC) No 169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on “the provision of food information to consumers”

REGULATION (EC) No 1924/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 December 2006 on “Nutrition and health claims made on foods”

1. State of the art

Generally, to reduce calorie content of a food, fat and sugar are reduced or removed and, subsequently, replaced with other components. Calorie can also ideally reduced in a food, through the incorporation of a certain amount of air, to obtain a product with the same volume but with a different weight and consequently a reduced calorie content. Considering that lipids and sugar play many roles in food, the reduction or substitution of these ingredients may affect both sensory and physical properties of modified foods.

1.1. **Function of Lipids in Food**

Almost all food raw materials and products contain some quantities of lipids. A few types of foods are devoid of lipids, e.g. saccharose, clarified fruit juices, and wines. Lipids are important for the properties of foods because they contribute to the structure of various tissues and organelles, supply substrates for synthesis of metabolites that are indispensable for the human organism, they serve as the richest dietary source of energy. Indeed, they interact with other components in creating the desirable, rheological properties of food commodities, they determine different products that affect the colour, flavour, texture, nutritive value, and safety of foods and also carry fat-soluble vitamins and other beneficial lipophilic compounds, e.g. carotene, as well as hydrophobic contaminants (Sikorski & Sikorska-Wisniewska, 2006).
Foods are multi-phase materials structured either by nature (plant or animal tissues) or by people. The artificial structured foods use texturing processes such as emulsification processes (e.g. production of margarine, ice cream, mayonnaise), foaming (e.g. production of whipped cream), dough kneading, etc. and the final product has a complex microstructure held together by binding forces between the various phases. Many features are texture-related, including mouth coating, mouthfeel and dryness. Destruction of this texture during mastication is the usual key to final quality and appreciation by the consumer (Van Puyvelde *et al*., 2003).

1.1.1. Food emulsion

Lipids, in different forms, and their interactions with other food constituents, contribute to the rheological properties of food emulsions,. Interactions of lipids with polysaccharides can be utilized in manufacturing low-calorie emulsion-type food commodities. The characteristic texture of some food emulsions is due to the ability of the oil phase to crystallize. Lipid droplets play a structure-forming role in oil-in-water emulsion-type products like milk, cream, ice cream, cheese, dressing, mayonnaise, and various comminute meat foodstuff (Sikorski and Sikorska-Wisniewska, 2006), since their overall viscosity increases with increasing droplet concentration, for example, creams, desserts, dressings, and mayonnaise (Mc Clements, 2005). These droplets are stabilized by interactions with proteins, lecithins, or different synthetic surfactants. Many oil-in water emulsions exhibit creamy characteristics. Creaminess is often related to the food fat content. Kilcast *et al.* (2002) showed that the creaminess may be affected by many factors and it depends of the food system considered. The particle size affected the perceived creaminess of model systems containing solid particles, in fact the addition of small particles increased creaminess; in mousse system the air bubble size was recognized as the primary factor influencing creaminess, followed by air content. In artificial cream, as expected, the perceived creaminess increased with increasing the fat content, but unexpectedly, it also increased with increased fat droplet size. For Akhtar *et al.* (2006) the viscosity was the predominant factor influencing the perception of both thickness and creaminess of model oil in water dairy emulsions. For the high viscosity samples, the fat content was also found to have a significant influence on creaminess perception, but much less than the viscosity. These trends are the same as they previously reported (Akhtar *et al.*, 2005) from a sensory study of emulsions of similar droplet-size distribution containing xanthan or pectin, where it was found that the type of hydrocolloid had no significant effect on perceived thickness or creaminess for emulsions of the same apparent viscosity.
Lipids affect also other properties of food like emulsions. The fat level has also an effect on taste intensity of oil in water emulsions (Arancibia, Jublot, Costell, Bayarri, 2011; Metcalf *et al*., 2001). Metcalf *et al* (2001), showed that, in the emulsion, the dilution with oil, compared to the dilution with water, decreased bitterness, but increased the intensity of salty, sweet, sour and umami taste, because of the higher concentrations of these taste compounds in the aqueous phase when the dilution medium was oil instead of water. Arancibia *et al.* (2011) studied the influence of fat content and thickener type on rheology, structure, stability, in-vivo aroma release and sensory perception in lemon flavored o/w model emulsions. Their results showed that differences in perceived flavour and texture attributes were affected for both fat content and matrix type. Many aroma compounds are hydrophobic or at least have significant hydrophobic fragments. Thus in a food product they accumulate predominantly in the lipid phase. Among the volatile flavour compounds of cheeses generated directly from lipids are FAs, methyl ketones and ketones, alcohols, and lactones. The flavour of butter is due to the volatile components of the fat phase, which are complemented by the compounds of the watery phase. Furthermore, the flavour is enriched by the contribution of different Maillard reaction products. Lipids can also affect food colour because they may contain various lipochromes, lipid-soluble pigments, that are responsible for the colour of the commodities. (Sikorski and Sikorska-Wisniewska, 2006).

1.1.1.1. Milk

The lipid fraction in fresh milk is primarily TAG, about a third of the total dry matter in milk. Fat is present in the form of globules, 500 nm to 10μm in diameter, encased in lipoprotein membranes, about 10 nm thick, which separate them from the aqueous milk serum. This membrane is assumed to consist of phospholipids and a double layer of proteins. The total area of the fat globule membrane is some $80m^2$ per liter, thus its important role in surface phenomena stabilizing the fat emulsion (Lhuch *et al*., 2003). In chilled, natural milk the lipoprotein membranes of fat globules interact on standing with a serum protein, thereby forming clusters of globules and leading to creaming.

1.1.1.2. Yoghurt

In yoghurt, fat contributes in two ways to the perception. Fat in discrete particles (droplets) act as fillers and hence contribute to the thickness of the yoghurt and thereby to the overall smooth perception (Jonhøj, Petersen, Frøst, & Ipsen, 2006). Second, fat is essential for the creamy sensation of full-fat yoghurt, which is a combination of flavour and mouth-feel attributes (Bult, de Wijk, & Hummel, 2007).

1.1.1.3. Cheese

The flavour of unripe cheese is caused by the olfactory action of a large number of volatile compounds present in the fresh milk, in different, small concentration. In the course of ripening, the typical flavour of ripe cheese develops as a result of mainly enzymatic changes in the major components of raw material, lactose, nitrogenous compounds and lipids. Among the volatile flavour compounds of cheeses generated directly from lipids are FAs, methyl ketones, ketones, alcohols, lactones. They differ in flavour perception thresholds and appear in cheese in various concentrations and at different stages of ripening (Sikorski and Sikorska-Wisniewska, 2006).

1.1.1.4. Margarine & Butter

The "spreadability" of water-in-oil (W/O) emulsions, such as margarines and butters, is determined by the formation of a three-dimensional network of aggregated fat crystals in the continuous phase which provides the product with mechanical rigidity. The churning process used in making butter leads to concentration of the fat globules and spreading of the oil at the air-water interface, suppression of the foam, and formation of clumps of the water-in-oil emulsion (Sikorski and Sikorska-Wisniewska, 2006).

1.1.1.5. Mayonnaise and other sauces

Dressings cover a broad spectrum of oil–water composition, from mayonnaise, a 65–84% oil-in-water emulsion, to fat free dressings, which may contain no oil. Of these dressings, only mayonnaise, salad dressing, and French have their own standard of identity. Mayonnaise must have at least 65% fat and salad dressing and French dressing must have 30% and 35% fat, respectively **(**Ford et al., 2004). In mayonnaise, containing the same percentage of fat of margarine and butter, the distribution of the dispersed phase, the droplet size, the amount of fat crystals and the interactions between the droplets influence the product properties, in particular, egg yolk phospholipids stabilize the emulsion **(**Lluch *et al*., 2003**)**. The light mayonnaise contains about 30-40% oil, and seasoning in sauces, low-calorie level drops to 3-10%.

1.1.1.6. Ice cream and whipped cream

The development of a stable structure and a suitable product texture in food emulsions and foam-based food products depends on the interactions between the fat globules and between fat globules and air bubbles. An important factor influencing the stability of food emulsions and foams is the developed fat particle structure. The fat particle structure variation and destabilization during the shearing process are very complex. The presence of solid fat (to promote fat globule rupture) and of liquid fat (to promote clumping) might be necessary for the formation of a stable whipped foam (Wasan *et al.*, 2004).

The creation of products such as ice cream and whipped cream depends on the controlled destabilization of partially crystalline oil droplets in oil-in-water (O/W) emulsions (Mc Clements, 2005); clustering of the fat globules around the air bubbles contributes to the desirable characteristics of ice cream, in particular, fats not only stabilize ice cream structure, but also they improve resistance to melt, creaminess and help to determine the flavour (Gunstone, 2007).

1.1.2. Meat and fish

Many studies have shown an effect on the texture, the flavour, and color of meat and fish. The secondary lipid oxidation products are responsible for the rancid off-flavor, fat, pungent of meat stored for a long time and in poor conditions. In fish products, the flavour of fresh fish is due to a small amount of lipid degradation products, whereas if the degradation is more thrust, it seems to be rancid. The lipid oxidation of hemoproteins can catalyze the browning of the meat, and some carotenoids, fat-soluble, can reduce the oxidation of myoglobin, making a longer color stability. The color intensity increases with the degree of unsaturation of the fish lipids. The marbling of the meat has a positive effect on perceived quality during chewing because the layers of fat act as a lubricant and the product can be perceived more tender in the mouth. The intramuscular fat, if evenly distributed and in small amounts, results in an increase in flavour and juiciness of the meat. Generally, the texture of the fish is more tender than meat, in particular, in fatty fish, the texture is influenced by the presence of intramuscular fat and it is softer when the fat content increases; in fact, salmon, sturgeon, herring, have a very soft texture and are described as viscoelastic bodies (Sikorski and Sikorski -Wisniewska, 2006).

1.1.3. Baked goods

Lipids contribute to the texture of baked products by interacting with proteins and polysaccharides. Wheat flour contains about 1.5±2.5% lipids. One part of these lipids is free, extractable by petroleum ether. The other one, called bond lipids, can be extracted only with a polar solvent, e.g. water-saturated n-butanol. There is also a fraction of lipids, about

0.25% of the total content, firmly associated with starch. All fractions contain neutral lipids, glycolipids, and phospholipids, although in different proportions, and contribute to the behaviour of the dough. The neutral lipids are present in the flour in the form of spherosomes covered with membranes which contain phospholipids. The polar lipids are assembled into hexagonal micelles. On mixing of the flour with water they turn into lamellar liquid crystalline phases by stacking of stretched lipid bilayers separated by water lamellae (Marion *et al.*, 1998). These lamellar phases stabilize the micro-emulsion of the neutral lipids in the dough and are together embedded in the network of the gluten proteins. The shortening effect of the lipids is due to disruption of the structure of the gluten network and contributes to increase in volume and the formation of soft, even-textured crumb grain. Lipid monomers may probably form bridges between the gliadins and glutenins, thus affecting the dough elasticity and extensibility. Furthermore, the polar lipids stabilize the gas bubbles thanks to their surface-active properties and fill the gaps in proteinaceous films, thus preventing the escape of gas. This role is also performed by shortenings or butter added to the dough. In pastry dough and flaky crusts the added butter melts during baking to form impervious layers that prevent steam and carbon dioxide to escape. After cooling, the products have the characteristic flaky texture. Wheat flour contains several low molecular weight proteins that in the non- denatured state have the ability to spontaneously bind lipids or lipid aggregates and are very efficient foam stabilizers. Breads made with defatted flour have poor gas retention and low loaf volume (Sikorski and Sikorska-Wisniewska, 2006).
Lipid interactions with polysaccharides in aqueous systems are due to hydrophobic effects. An amylose-lipid complex is formed and during the baking process of a dough there is an increase in the degree of binding of lipids by amylose. These complexes are able to form gels, affecting the texture of baked goods. The rheological properties of these gels depend on the concentration and type of the lipids and on the crystalline form of the complexes. Addition of lipids to starch gels may retard retrogradation. In baked goods, the fats generally used are plastic, made by mixing liquid and solid components to be solid at a given temperature and deformed by applying some pressure. Lipids most used are butter and margarine, containing about 80% of lipids, but also shortening with 100% lipids. In a cake, the fat initially stabilizes the air bubbles dispersed, then it melts and the water in oil emulsion is inverted with the air trapped in the aqueous phase; afterwards, when it hydrates and starch jelly, proteins bubbles coalesce and become bigger because of steam and carbon dioxide. In the pastry aeration is of secondary importance. The fat used should be solid and distributed in the dough as a thin film; in this case lard, tallow, hardened vegetable oils, were more used than butter and margarine. In puff pastry, fats form a barrier separating the leaves of the dough from each other. The release of gas or steam during baking produces a layered structure. In this case it is necessary to use a fat with a high melting point, high in solid fats with an appropriate degree of hydrogenation (Gunston, 2007). Small amounts of fat are also added to the bread dough and the structure of the crumb became more fine and uniform, more compact and softer to chew, using fats with an high solid fat content. Indeed, adding oils, it requires the addition of emulsifiers to the dough (Piazza and Rainoldi, 1994).

1.2. Strategies for fat reduction

A diet rich in fat has been associated with increased risk of heart disease (Mensink & Plat, 2007), some types of tumors (Glauert, 2007) and obesity (Hausman & Grossman, 2007). Obesity is a major lifestyle factors associated with hypertension and contributes to insulin resistance.
Although public concern about fat in foods is diminished lately, it still remains high. The market need for zero or low-fat food products has become increasingly important and so new ingredients and knowledge are needed in order to manipulate margarine, spreads, salad dressings, dairy, bakery products and so on. For many industrial food designers, the primary difference in low-fat product is the texture, followed by the flavour (Van Puyvelde *et al*., 2003).
An ideal fat substitute must look and function like fat and be able to substantially reduce caloric contributions to food. It is often impossible to replace fat with a single compound, there are actually several types of substitutes with different chemical composition, functional, sensory and nutritional properties (Bednarski and Adamczak, 2003); so a three-ingredient system could be necessary for a good fat mimetic: a thickening agent for lubricity and flow control, a soluble bulking agent for control of adsorption/absorption of the food onto taste perceptors of the tongue, and a microparticulate, generally insoluble, agent that acts like a ball-bearing to create smoothness. It can be postulated, however, that in order to mimic the different functions of fat in a substantially reduced-fat product, we must consider viscosity matching, solids adjustment, and particle size impact and mouthfeel, and we must also carefully balance the perceived flavour characteristics of the system (Jones, 1996).
There are three broad categories of fat replacers on the market, carbohydrate-based, protein-based, and lipid-based fat replacers or combinations thereof.
Since fat replacers may contain calories, food manufacturers using these products should ensure that the final product is not only reduced in fat, but also reduced in calories. In the end the food with fat replacers will be of little value for weight reduction if it fails to cause a significant reduction in calories.
Carbohydrate-based fat replacers use carbohydrate polymers and dietary fibres, such as cellulose, dextrins, maltodextrins, polydextrose, gums, fiber, and modified starch, to replace fat. Carbohydrate-based fat replacers can provide up to 4 kcal/g if the carbohydrate is fully digestible. Often the calories are lower than this since the fat replacers either are dietary fibres, which are not digested or only fermented to some degree or are digestible carbohydrates mixed with water so they provide 0-2 kcal/g. In some cases fibres, such as cellulose, are ground into microparticles that can form gels for use as fat substitutes, e.g. Oatrim and Z-trim. Carbohydrate-based fat replacers are used in a variety of foods including dairy-type products, frozen desserts, sauces, salad dressings, processed meats, baked goods, spreads, chewing gums,

and sweets, but cannot replace fats in frying.
Protein-based fat replacers are made from many different types of protein, but soy, egg, milk, or whey proteins are common. Microparticulation of protein into tiny, spherical particles that provide a creamy mouthfeel similar to fats helps protein to function as a fat replacer. Blending protein with carbohydrates is another way to create fat replacers.
Fat replacers from protein and protein blends do provide 4 kcal/g, but they may provide only 1-4 kcal/g either because they hold water or are used in lesser amounts than fat. For example, 1 g of Simplesse can replace 3 g of fat in cream.
Protein-based fat replacers have been used in fat-free ice cream, low-fat cheese, low-fat baked goods, and reduced-fat versions of butter, sour cream, cheese, yogurt, salad dressing, margarine, mayonnaise, baked goods, coffee creamers, soups, and sauces. Often, a combination of these fat replacers can have tremendous potential in the development of fat-modified foods with greater acceptability while lowering the total energy and fat intake.
Fat-based fat replacers include common fats that have had chemical alterations of fatty acids so that they deliver less than 9 calories per gram. Some fat-based fat replacers pass through the body partially or totally unabsorbed. Thus they provide less than 9 kcal/g or no calories at all. For example, Olestra (Olean) is a sucrose polyester consisting of a mixture of hexa, hepta and octa esters of sucrose, esterified with long chain fatty acids. It has the organoleptic and thermal properties of fat, but cannot be hydrolyzed by gastric or pancreatic lipase. The unhydrolyzed molecule is too large to be absorbed in the gastrointestinal tract and, therefore, cannot be metabolized for energy with a net effect of yielding no calories. Other fat-based fat replacers, Salatrim (short and long chain triglyceride molecule) and Caprenin, a substitute for cocoa butter in candy bars, are only partially digested and absorbed, and provide 5 kcal/g.
Some fat replacers, such as EnovaTM oil, are structured diglycerides and are metabolized differently from triglycerides so that some of the energy is lost as heat rather than stored as adipose (Daniel, 2010; Jones, 1996).
Emulsifiers can be another type of fat-based substances that can be used as a fat replacer. They may be used with water to replace all or part of the shortening content in cake mixes, cookies, icings, vegetable, and dairy products. They provide the same number of calories as fat, but since less is used in the formulation, the resultant product has less total fat and energy. Mono- and diglycerides are currently used in foods as fat replacers and other substances such as dialkyl dihexadecylmalonate, esterified propoxylated glycerol, and trialkoxytriartallate are in various stages of development (Jones and Jonnalagadda, 2006).
Fat replacers are also divided into two main groups: fat mimetics and fat substitutes. The fat mimetics are either proteins or carbohydrates that have been physically or chemically processed to mimic the properties and functions of fats in food systems ; they are not fats. They tend to adsorb a large amount of water, are not stable at frying temperature, and may produce food that is not microbiologically shelf stable; they contribute some calories (1– 4 kcal/g) to the diet. The fat mimetics do not possess all the organoleptic, physical, chemical, and functional properties of fats and cannot replace calories from fat on a 1:1 weight basis . They cannot carry lipid-soluble flavour compounds because they cannot lower the vapour pressure of lipophilic flavour molecules, and most foods prepared with fat mimetics are often perceived by consumers as lacking in taste. Although fat mimetics need a delivery system such as an emulsifier to carry lipid-soluble flavours, they can carry water-soluble flavours. Some fat mimetics have mouthfeel and physical properties approximating those of triacylglycerols but are not suitable for frying operations because they can be denatured (protein- based substances) or caramelized (those based on carbohydrates). They can, however, be used for baking and retort cooking operations (Akon, 2007). Simplesse is a protein-based fat mimetic used in creams, dressings, dairy products, approved by FDA.
Egg, milk, soy and gluten proteins can also be used as fat mimetics. These substitutes are fully digestible, low in calories and are used for the production of sauces, mayonnaise, butter diet, ice cream, yogurt, pastries. Another group is represented by the carbohydrate-based fat substitutes such as starch, cellulose, sucrose, inulin, dextrin, malt obtained from potatoes, wheat, tapioca, corn, and many of these products are low-or zero-calorie (Bednarski and Adamczak, 2003).
The general functions of polysaccharide blends in low calorie foodstuffs are to act as bulking agents, stabilizing agents, and fat-mimetics. Generally, MCC, resistant starch, cereal β glucans, inulins, and pulverized curdlan gel are important. The polysaccharide blends developed are mainly colloidal MCC aggregates or particulates blended with one or two of GG, konjac flour, CMC, CAR, XG, starch components, and microparticulated proteins. These formulations show excellent bulking and stabilizing effects and produce smooth fat-like or oil-like and spreadable textures as well as creamy appearance to the end products. Modified starches coupling with gellan gum, galactomannans–XG blends, TSX, or cellulose are also reported to give low-calorie products with margarine-like spreadable texture and desired mouth feel. Commonly, the fat-mimetic properties of these polysaccharide blends arise from the presence of gel particles with a controlled size of 0.1 to 100 μm (Lai & Lii, 2004).Fat substitutes are compounds that physically and chemically resemble triacylglycerols. They are stable to high- temperature cooking and frying operations, and in theory, can replace fats and oils on a 1:1 weight basis in foods. In the literature, the term ‘‘fat substitutes’’ has been used interchange ably with ‘‘fat replacers’’ but not with ‘‘fat mimetics’’ and this can be confusing. Several lipid-based synthetic low-calorie or zero-calorie fat substitutes belong to the fat substitute group. A good example is sucrose fatty acid polyester (SPE), which was originally developed as Olestra, approved by Food and Drug Administration (FDA) for savory snacks. Other lipid-based fat substitutes are sorbitol polyester, raffinose polyester, stachyose polyester, and alkyl glycoside fatty acid polyesters. Others include Caprenin, Salatrim (short and long acyl triglyceride molecules, marketed as Benefat), structured lipids, medium-chain triacylglycerols (MCTs), mono- and diacylglycerols, esterified propoxylated glycerol (EPG), dialkyl dihexadecylmalonate (DDM), and trialkoxytricarballylate (TATCA). Of all the lipid-based fat substitutes, only sorbitol, trehalose, raffinose, and stachyose polyesters have a chance to compete with Olestra as nondigestible zero-calorie fat substitutes. Others are either partially hydrolyzed or fully hydrolyzed and absorbed, thus contribut-

ing some calories to the diet (Akon, 2007).

1.2. **Fat reduction in food**

1.2.1. Whipped cream

Bazmi *et al.* (2007) studied how the partial replacement of anhydrous milk fat by a high or a low melting temperature milk fat fraction can affect fat droplet size under a whipping test, without freezing, and aeration properties in emulsions containing a low fat content. Three dairy emulsion were prepared, using milk fat samples, skimmed milk powders, sucrose and mixture of polysaccharides and emulsifiers. In two of the three emulsions, one third of the anhydrous milk-fat by weight was replaced by a low or a high melting fraction. The emulsions were whipped and overrun was evaluated. On aged emulsions fat droplet-size distributions and melting behaviour were analyzed. Whipped emulsions were observed both at light and confocal microscope. Results showed that AMF/olein emulsion presented less crystalline fat content and a higher ability for air incorporation than the other two emulsions. Air bubbles formed in the AMF/olein rich emulsion presented a more uniform size distribution, a smaller proportion of bubbles higher than 50 μm, and they appeared to be coated with a thicker layer of fat droplets. So the foam-structure forming properties in reduced milk fat emulsions can be enhanced by lowering the proportion of saturated triglycerides.

1.2.2. Ice cream

Many carbohydrate-based *fat replacers* were used to formulate low-fat ice creams (Li *et al*., 1997; Specter and Setser, 1994). Byars (2002) compared soft-serve ice creams prepared with Fantesk, a fat starch-lipid fat replacer, made with waxy maize starch and butter, to a commercial product. The viscosities of the ice cream mixes were measured on a Rheometrics ARES controlled-strain fluids rheometer using a 50mm diameter, 0.04 radian cone at 6°C. Measurement on the prepared ice cream were conducted on the same rheometer using parallel plates with 25mm diameter at different temperature. Despite the lower level of fat, the overrun and the rheological properties (complex viscosity, storage modulus, loss modulus, stress relaxation modulus) of the Fantesk-based ice creams were similar to the commercial product when differences in the freezing behaviour of the different formulations were accounted for. Aime *et al.* (2001) used modified starch as fat replacer in light (4%), low-fat (2.5%) and fat-free (0.4%) vanilla ice cream. Sensory attributes (intensity of coldness, firmness, viscosity, mouthcoating, degree of smoothness), evaluated by a trained sensory panel, of light sample were not significantly different from regular sample (10%), wherever viscosity, mouthcoating, degree of smoothness of low-fat and no fat were lower than the same of regular samples. Instrumental parameters, such as viscosity, measured using a rotational rheometer with a shear rate sweep between 18.61 and 232.2 s^{-1}, and firmness, evaluated through a *Texture Analyzer*, were related to the same parameters determined through sensory analysis, without sensory differences between regular, light and fat-free samples, while the low-fat sample was considered firmer than others. Aykan *et al* (2008) evaluated the effect of two *fat replacers*, inulin e Simplesse, on sensory, performed by ten judges, and rheological properties of light (control), low-fat and fat free vanilla ice creams. The experimental mixes were significantly more viscous than the control mix due to the water binding capacity of carbohydrate- and protein-based substances. The texture scores, evaluated by the panel, of the ice creams paralleled the viscosity values. An increase in total solids in the mix resulted in better texture, a lower melting rate and better shape retention. In another study only one fat replacer was used, Simplesse, and the purpose of the study was to evaluate the texture of regular (12%), low fat (6%) and fat-free (0.5%) vanilla ice creams by sensory and instrumental analyses. Sensory analysis disclosed that ice creams containing 6% of fat replacer in place of or with milk fat had no demonstrable effect on vanillin flavour. While the sensory attributes of the low fat samples were comparable to the regular vanilla ice cream, the trained sensory panel rated the fat free ice creams to have lower viscosity, smoothness and mouth coating properties. Instrumentally determined apparent viscosity data supported the sensory data. Compared with the fat replacer, milk fat significantly increased the fresh milk and cream flavours of the ice creams. So those results emphasized the importance of fat as flavour modifier and the improvement of texture by addiction of Simplesse (Yilsay *et al.*, 2006). The ‘‘slow-churned’’ice cream process was developed to compensate for the loss of creaminess and foam stability observed with most fat-reduced ice creams. In this process, after freezing, the ice cream mass is extruded at 18 °C in single- or twin-screw extruders. This extrusion step reduces both the air bubbles and the crystal size, increasing the stability. Thus, the product is perceived to be creamier than standard fat-reduced ice creams and the fat content can be reduced by 50-60% while still maintaining consumer preference (Palzer, 2009). However, the storage stability of low-fat ice cream is limited (Crilly *et al*., 2008). During storage, air bubbles coalesce and ice crystals grow. These structural changes are accelerated when the product is exposed to elevated temperatures and temperature variations.

Frozen yoghurt dessert is a complex fermented frozen dairy dessert that combines the physical characteristics of ice cream with the sensory and nutritional properties of fermented milk products, with a lower fat content. Milani and Koocheki (2010) showed that the partial substitution of fat with guar gum determined a softer, sticker and more meltdown stable product than the control one.

1.2.3. Mayonnaise

Generally, industries seek to reduce, as far as possible, the oil content in mayonnaise, also to reduce production costs. Unfortunately, reducing the amount of oil, it also reduces the density of the emulsion droplets, becoming less stable and the interactions between the droplets much more labile. The stability of an emulsion with low or medium amounts of fat, may be increased by reducing the size of the droplets or to increase the viscosity of the continuous phase, in which case it is necessary to use water-soluble gelling agent. Peressini *et al.* (1998), in order to increase the stability of light products and to mimic the effect of fat, added to the aqueous phase gums, modified starches and starch hydrolysates. Their study showed that all the products exhibited a viscoelastic behaviour, but for the traditional mayonnaise samples the elastic modulus G' values were always higher than the viscous module G '' values, meanwhile for light mayonnaise, the observed phenomenon was inverse. The products analyzed were different in terms of rheological properties, texture and appearance, so the light mayonnaise was very different from the classical one. Despite these results, also Palzer (2009) suggests the use of hydrocolloids or starch to improve the quality of low fat or reduced far mayonnaise. Instead, Worrasinchai *et al*. (2008) chosen, as fat replacers, beta-glucans in mayonnaises with different percentages of fats. Both reduced fat mayonnaises and control one showed a thixotropic behavior, without significantly differences, making possible the substitution of fat with beta-glucans until the 50% .

1.2.4. Dairy-based food

The yoghurt is already considered a healthy food for its high content of protein and calcium. The traditional yoghurts contain a 3-4% fat, which can reach up to 10% in the concentrated yoghurt. Consumers in recent years are increasingly interested in the consumption of fat-free or with low fat yoghurts with sensory characteristics similar to traditional ones. Lobato-Calleros *et al*. (2004) compared rheological properties of reduced-fat yoghurts containing whey protein concentrate (WPC), microparticulated whey protein, or a blend of fat replacers to those exhibited by a full-fat yoghurt (FFY). Their results showed that the yoghurt made with WPC had flow and viscoelastic properties that resembled more closely those of the FFY. Also in other studies (Sandoval-Castilla *et al*., 2004; Aziznia *et al.*, 2008), WPC were used to produce low-fat yoghurt, with similar results Although, Alting *et al.* (2009) used an amylomaltase-treated starch (ATS) to enhancer the creaminess in low-fat yoghurt. Small amounts of ATS raised the creaminess perception of yoghurt (1.5%) to that of full-fat yoghurt (5%). In this way, a reduction in fat-related energy value could be achieved from 45 to 21.5 kcal/100 g product. A possible use of β-glucan hydrocolloidal composite as a fat replacer in the manufacture of non-fat yogurts was investigated (Sahan *et al.*, 2008); in that study, yoghurts containing 0.25% or 0.50% β-glucan hydrocolloidal composite were acceptable by expert panels and had scores similar to the control yogurt. The use of inulin as a fat replacer in yoghurt manufacture resulted in low-fat products with acceptable sensory characteristics (Modzelewska-Kapituła and Kłębukowska, 2009).

In low-fat semisolid dairy dessert, the addition of short-chain or native inulin provides low-fat products with creaminess, smoothness and mouth coating properties similar to the control sample prepared with whole-milk, albeit with less thickness. The addition of long chain inulin gives rise to a low-fat product with the same thickness intensity as the full-fat sample, though less creamy, rougher and with more mouth coating (Bayarri *et al.*, 2011).

The cheese is becoming one of the most widely used food ingredients. Recently there was a significant increase in the consumption of low fat or reduced fat cheese, by the growing interest in health. Several studies in the literature reporting the effect of substitution and / or reduction of fats on the physical and sensory characteristics of cheeses. Konuklar *et al.* (2004) studied the textural properties of 3-month-old low-fat Cheddar cheeses manufactured with a b-glucan hydrocolloidal composite, Nutrim, a nutraceutical fat replacer, via instrumental methods and sensory panels. Although the fat levels of Nutrim cheeses were reduced significantly, the hardness, fracturability, and melt-flow index-time values were significantly lower than the low-fat control cheeses. The elasticity and the cohesiveness values were similar for all the cheeses. Most textural attributes from the sensory panel were similar. For flavor attributes, the low-fat control cheeses were significantly less bitter, more buttery, and less starchy than the Nutrim cheeses. Instrumental measurements significantly correlated to the sensory panel and to the proximate analysis. Significant replacement of fat with the Nutrim composites resulted in softer Cheddar cheeses with decreased melt times and decreased sensory scores. Sipahioglu *et al.* (1999) used modified tapioca starch and lecithin, as fat mimetics, to obtain reduced fat and low-fat feta cheeses. Chemical, sensory, SEM, and textural evaluation were performed. Levels of fat and fat mimetics significantly affected moisture, protein, yield, and hardness. Reduced-fat cheeses with modified tapioca starch had the highest moisture (67.6%) and lowest protein (13.5%) content and their hardness was higher. SEM micrographs revealed that the size of protein aggregates increased as the fat content of the cheese decreased. The combination of modified tapioca starch and lecithin improved flavor, texture and overall acceptability of reduced-fat and low-fat Feta cheeses. Different protein-based and carbohydrate-based fat replacers were used to obtain low-fat cheeses (Koca & Metin, 2004; Kavas *et al.*, 2004; Zalazar *et al.*, 2002;), in many cases there was an effect of fat replacer used (Simplesse, Raftline, Santiagel, Perfectamyl) on sensory properties of cheese, but not always the fat replacer chosen (Dairy-Lo) improve the quality of cheese with reduced fat content. In the case of mozzarella cheese, Zizu *et al.*, (2005) showed how by combining EPS cultures with the appropriate fat replacer and pre-acidification, it was possible to increase the yield of low-fat Mozzarella cheeses, achieve textural and behavioural attributes superior to those of untreated low-fat Mozzarella cheeses, and reduce maturation time, thereby reducing storage periods. Recently, also a bacterial cellulose was proposed for cheesemaking in order to reduce calorie content of Turkish Beyaz cheese (Karahan *et al*., 2011).

1.2.5. Meat and fish- based food

Reduce the amount of fat in fish and meat-based products, in order to reduce calories and / or production costs, often means having harder, less juicy and less tasty products than the traditional ones. Low-fat formulations for processed meat products, especially frankfurter sausages, bologna, and surimi gels have been intensively developed. Fat reduction generally results in reduced water holding capacity; visual density and sensory preference; and increased cohesiveness, gumminess, chewiness, and cooking loss or purge loss for processed meat products. Starch-based fat replacers are often

used at preferably ~5 wt% to compensate for most of these shortages. Fat replacement by using a single polysaccharide is more limited, meanwhile polysaccharide blends are more attractive (Lai & Lii, 2004).
In the frankfurters, maltodextrin can be used as a suitable fat replacer since it offset some of the changes brought about by fat reduction, decreasing cook loss and maintaining a number of textural and sensory characteristics (Crehan *et al.*, 2000); also tapioca starch and whey proteins can partially offset some of the changes which occur in low-fat frankfurters when fat is replaced with added water and protein level is constant (Hughes, Mullen, Troy, 1998). Recently, many other fat-replacers were used to formulate low-fat frankfurters, hydrocolloids, such as carrageenan, pectins (Candogan & Kolsarici, 2003; Cierach *et al.*, 2009), but also different vegetable oils, such as olive oil, palm oil, walnut oil (Choi *et al.*, 2010; Ayo *et al.*, 2009; Lopez-Lopez *et al.*, 2009; Tan *et al.*, 2006) or a combination of vegetable oil and marine oil (Delgado-Pando *et al.*, 2011).
In order to reduce the fat content in meatballs, 5% or 10% of rye addition seem to led to acceptable products and can be recommended in the traditional meatball production as a dietary fiber source (Yilmas, 2004).
Short chain fructo-oligosaccharide can be considered a good fat replacer in meat products, in fact it was been possible to manufacture cooked sausages that had 35% less energy value, without sacrificing acceptable sensorial quality (Caceres *et al*., 2004).
The results obtained by Pinero *et al.* (2008) showed that oat fibre in gel form, in certain level, could be used as a functional ingredient in low-fat beef patties by enhancing moisture and fat entrapment, helping on maintaining the uniform tenderness of the products.

1.2.6. Baked goods

Low moisture baked goods, such as biscuits, cookies, are difficult to prepare if you reduce fat because many sensory characteristics of these products are determined by fat and it is difficult to find fat replacers that produce the same effects. In the literature there are several studies on this issue. Zoulias *et al.* (2002) compared five different fat mimetics (carbohydrate or protein-base) for the production of reduced-fat cookies. The replacement of fat with different fat mimetics had various effects on textural properties of the samples depending on the mimetic; an increase in polydestrose or beta-glucan content resulted in harder cookies, meanwhile an increase in inulin or maltodextrin or microparticulated whey proteins had the opposite effect, underlying how these last ones could be used as fat replacers to prepare more tender low-fat cookies. Wekwete & Navder (2008) investigated the effectiveness of partial replacement of butter fat by two different fat replacers, avocado puree and Oatrim, a mixture of soluble beta-glucan and amylodestrins from oat flour, on the physical, textural and sensory properties of oatmeal cookies. Since the overall acceptability was unchanged and the substitutions improved the nutritional content, it is possible using these two ingredient in reduced-fat oatmeal cookies making. Another beta-glucan based mixture, C-trim, was used by the same authors, previously (2006). Also a prune puree was used to partial replace fat in both oatmeal and chocolate chip cookies, determining in both cases, cookies with sensory attributes similar to ideal cookie described by consumer, as shown by the sensory evaluation (Swanson & Perry, 2006). Indeed Rankin & Bingham (2000) determined the palatability and overall acceptability of oatmeal chocolate chip cookies prepared using pureed white beans as a replacement for 25%, 50% and 75% of the fat ingredient, and compared their sensory attributes with those of cookies made with a traditional type and amount of fat. As the fat substitution increased, the acceptability reduced, but all the samples were generally appreciated. Another type of pureed legume was used as fat replacer, green peas, by Romanchick-Cerpovicz *et al.* (2007). Their study showed that moderate fat replacement in chocolate drop cookies was feasible using green peas, thereby creating an acceptable low-fat alternative for individuals on fat-restricted diets. The same authors Romanchick-Cerpovicz *et al.* (2002), previously, used both okra gum and applesauce, as fat ingredient substitute, in making of no-fat chocolate bar cookies. Overall acceptability of fat-free cookies prepared with okra gum did not differ from full-fat cookies or the other ones prepared with applesauce. Pureed white beans were also used to substitute shortening in brownies (Szafranski *et al.*, 2005), resulting in acceptable and more nutritious products, in particular the 50% bean brownies were not different from the control in overall acceptability, tenderness, texture and flavour.
The replacement at 10% of shortening with N-Dulge (a mixture of tapioca dextrin and tapioca starch) gave reduced fat short-dough biscuits with good acceptability, even though it was necessary the substitution of part of the flour with resistant starch in order to balance out the detrimental effects of fat replacement on texture (Laguna *et al.*, 2012).
For what concern reduced-fat cakes, Power *et al.* (2008) conducted a study to explore the possibilities of using silken tofu at levels of 25, 50 and 75 per cent fat substitution (w/w) in shortened cakes. Tenderness of cakes decreased while stiffness and toughness increased with increasing levels of fat substitution. In-house sensory analyses revealed that cakes made with 50% fat substitution scored highest for flavour, indicating that tofu did not have a detrimental impact on the flavour profile of shortened cake. Sensory scores for texture, colour and overall acceptability also improved with fat substitution with tofu up to 50% level. The 50% fat substitution with tofu resulted in a 15% reduction in calories and 43% reduction in total fat per serving. This study revealed that silken tofu can be used to replace up to 50% fat in shortened cakes without compromising the sensory acceptability.
Benefat is a triglyceride composed of one poorly absorbed long chain fatty acid and two short chain fatty acids. It provides 55% of the calories of a typical triglyceride molecule, and is a promising lower calorie alternative to conventional fats. This fat mimetic was used by Cleveland *et al.* (2007) to partially replace conventional fats in cakes . This study involved testing of three versions of a chocolate cake recipe to determine the effects of the fat substitute Benefat on physical structure and sensory qualities. Objective and sensory tests indicated that the control and 50/50 were not significantly different in moistness, preference, compressibility, cohesiveness, shear force, height, or percent moisture con-

tent. In contrast, the statistics for the 100% version indicate less tenderness, moistness, and preference, and greater compressibility, cohesiveness, and density. The results of this study indicate that replacement of 50% of the fat in cakes with Benefat produces highly acceptable results with 22% fewer calories from fat. Inulin, at different levels, was also used as fat replacer in sponge cake formulation (Rodrìguez-Garcìa *et al.*, 2012a). Oil substitution for inulin decreased significantly batter viscosity, giving heterogeneous bubbles size distributions; indeed, cakes with fat replacement up to 70% had a high crumb air cell values; they were softer and rated as acceptable by an untrained sensory panel (n = 51). Zahn *et al.* (2010) showed that the replacement of 50% baking fat in the muffin formulation resulted in products that were comparable or slightly higher than the control ones in crumb firmness. The complete elimination of baking fat with inulin and water, however, led to products, which were downgraded because of high toughness, low volume and the lack of a product-typical taste. Inulin was also used as fat replacer in short dough biscuits; results showed that weight loss during baking and water activity decreased significantly ($P<0.05$) as fat replacement increased. Biscuit dimensions and aeration decreased when fat replacement increased and judges found biscuits with 20% of fat replacement slightly harder than control biscuits (Rodrìguez-Garcìa *et al.*, 2012b).

1.3. Function of Carbohydrates in Food

Carbohydrates are the main source of energy, making up 40-80% of the individual's total energy intake. According to the degree of polymerization, carbohydrates are divided into three principal groups, namely sugars, oligosaccharides and polysaccharides

Carbohydrates determine the texture and structure of many foods, but the functions carried out largely depend on their molecular weight. Polysaccharides, instead, exert multiple functions in food, gelling agent, thickener, clarifier, stabilizer, emulsifier, flocculant, assembling, binding, lubricating and much more. Concentrated aqueous solutions of carbohydrates form viscous liquids. That property is most commonly utilized in practice for texturizing foodstuffs. Blending of various saccharides and polysaccharides can result in the formation of numerous edible glues and adhesives. Such interactions are commonly utilized in texturization of puddings, jellies, and foams. Some oligosaccharides and the majority of polysaccharides form hydrocolloids, which build up their own macrostructure.

They give an impression of jelly formation, thickening, smoothness, stabilization against temperature and mechanical shock, aging, and resistance on sterilization and pasteurization. Plant gums, pectins, and alginates are particularly willingly utilized for this purpose. Such properties can be controlled by the addition of salts because various metal ions form Wernertype complexes with saccharides and polysaccharide ligands. Formation of the calcium ion–sucrose complex, commonly utilized in sucrose manufacture, illustrates that phenomenon well. The effect of the metal ions in texturization is particularly visible in the case of anionic polysaccharides (potato amylopectin, pectin, alginates, carrageenans, furcellaran, xanthan gum, and carboxylic starches from starch oxidation).

The texturizing effect of a given saccharide or polysaccharide and its various blends is developed as a function of the time necessary for the formation of a gel network (a physical cross-linking). If retrogradation does not take place, the texturizing effect is also reversible in time.

Saccharides, oligosaccharides, and polysaccharides also form complexes with proteins and lipids. Such complexes contribute to the texture of foodstuffs. Apart from combinations of natural saccharides, oligosaccharides, and polysaccharides, chemically modified polysaccharides are also utilized for texturization (Lai and Lii, 2004; Anderson *et al.*, 2007).

1.3.1. Low-molecular-weight saccharides

The low molecular weight carbohydrates interact strongly with water and other components, such as polysaccharides and proteins, effectively retain moisture and flavor and reduce the excessive aggregation of the polymers and influence the sweetness . In other words, they act as plasticizers, anti-staling and protect food biopolymers. The low molecular weight carbohydrates can be divided into 3 groups:

1. sugars (including mono-and disaccharides and syrups);
2. oligosaccarides
3. derivatives of sugars.

The sugars and sugar derivatives are used as humectants, plasticizers, anti-staling, as protective agents of food components during freezing, storage or dehydration. In particular, fructose and some sugar alcohols, such as sorbitol, are superior food humectants because of their high hygroscopicities and are widely applied in low-moisture products, such as fruit preserves, peanut butter, chewing gum. In doughs and other bakery or starch-containing products, sugars and their alcohols (sorbitol, mannitol, xylitol, lactitol) also produce plasticizing or antistaling effect, enhancing texture, structure and stability of the product over time. In frozen dough and meat products, the low molecular weight saccharides play a protective action by reducing the freezing point and the amount of free water, the best results are obtained from sugar alcohols such as sorbitol (Lai & Lii, 2004).

Saccharides are usually associated with sweet taste, although some among them are bitter and no-sweet saccharides. Except for sucrose, the sweetness decreases with the number of monosaccharide units going toward oligo- and polysaccharides, because only one monosaccharide unit interacts with the mucoprotein of the tongue receptor. The quantum-mechanical treatment of sweetness, in terms of interaction of sweeteners with receptors, was recently given by Pietrzycki (2004).

Many low-molecular weight saccharides are sweeteners. A key attributes that distinguishes sweeteners from other ingredients is their characteristic and pleasurable sweet taste and intensity. The gold standard for comparing sweetness is sucrose. The relative sweetness of a sweetener can be compared to sucrose in two different ways. The first is to scale a product's relative sweetness against an equal amount of sucrose; the second is to provide a score indicating the amount of sweetener solids needed to match the sweetness of a known amount of sucrose (equal sweetness), even if other conditions should be defined when studying or evaluating sweetness, such as temperature, flavour, solids concentration, pH, salt level. Indeed, the perceived sweetness of two or more blended sweeteners is greater than the relative sweetness of the sweeteners separately.

Sucrose, dextrose, and lactose are the three sugars most likely to demonstrate solubility issues in a food product. Crystalline fructose was mainly used for dry beverage mixes and beverages where clean flavours, and acidity are important. It can also be used to help to reduce the caloric content of a product because it has more sweetness per calorie than sucrose. Dextrose monohydrate is less sweet than sucrose, indeed has a negative heat of solution imparting a strong cooling effect in the mouth as it dissolves. Sweetener solubility plays an important role in many food products, where solubility can be described as how much of a sweetener will dissolve in a liquid before the solution becomes supersaturated with sweetener. Sweeteners can appear, in a given product, completely dissolved, or as a solid, either crystalline or amorphous, or as a mixture of dissolved and crystallized solids, and the solubility is also affected by water availability (free or bound), the total solids of the system, the nature of other ingredients, processing conditions, the viscosity of the final product, and temperatures encountered during processing, distribution, and consumer use. If a "crystallized" sweetener is desired to be present, then the product is often referred to as "grained.", meanwhile if the sweetener is to remain in solution, then the product in considered "ungrained." Graining occurs when a sugar is in a supersaturated solution, and it is able to crystallize, so it is very important controlling conditions that could influence this process, such as temperature, sources of seed crystals, moisture content, the sugars being used, their formula concentration and solubility, and the products finished viscosity. i.e., because undesired graining in a food product is considered a product defect. For what concern the size, the finer the particle size, the greater the surface area and the more rapid a sweetener will dissolve, up to its solubility level. Agitating or mixing speeds dissolution. As the concentration of a sweetener increases, dissolution slows (Helstad, 2006; Talbot, 2009).

The high molecular weight oligosaccharides most commonly used are the maltodextrin, polydextrose and cyclodextrins, the first two are used as sugar replacers in bakery products, energy-reduced, while the latter has the ability to encapsulate hydrophobic compounds and aromas and can be esterified and used as fat replacers (Lai & Lii, 2004).

1.3.1.1. Baked goods

All sweeteners influence the gelatinization point of a starch by raising the temperature where the gelatinization occurs. Applications where gelatinization is important are focused mostly in baked goods, e.g., cakes, cookies, biscuits, pie fillings. In these products fairly high levels of sweeteners are used relative to the starch. The sweeteners are in competition with starch for water, having a stronger affinity than starch, so there is less water available for hydrating and gelatinizing the starch. This phenomenon affect height, in both cakes and cookies, spread, in biscuits and cookies, surface appearance, in cookies, and crumb texture, in cake. Indeed, in presence of a reducing sugar with a protein or other nitrogen source, the Maillard reaction or the Strecker degradation may occur. These reactions help bread crust brown (Helstad, 2006).

Sweet goods contain high levels of sugar, which affect the activity of yeast. Sugar has a strong inhibitory effect on the gassing power of yeast, caused by high osmotic pressure on the yeast cell. Sugar decreases the strength of gluten development due to its competition for water. It inhibits the gliadin–glutenin–water complex, and gluten is thus weakened.

Sucrose is a principal ingredient in cakes. It provides energy and sweetness. It also facilitates air incorporation. It acts as a tenderizer by retarding and restricting gluten formation, increasing the temperatures of egg protein denaturation and starch gelatinization, and contributing to bulk and volume.

Cookies are made from soft and weak flours. They are characterized by a formula that is high in sugar and fat but low in water. Sucrose acts as a hardening agent by crystallizing as the cookie cools and making the product crisp. However, at moderate amounts, it acts as a softener due to the ability of sucrose to retain water. Sugar makes the cooked product fragile, because it controls hydration and tends to disperse the protein and starch molecules, thereby preventing the formation of a continuous mass.

Sugar is also important in the taste and structure of most biscuits. The amount of sugar that goes into solution depends on the particle size of the sugar and influences the spread of biscuits and machining properties of dough to a great extent. The addition of sugar to the formula decreases dough viscosity and relaxation time. Sugar promotes biscuit length and reduces thickness and weight (Indrani & Rao, 2008).

1.3.1.2. Chocolate, coatings, filling

Chocolates contain cocoa liquor, sugar, cocoa butter, milk fat, and milk powder (depending on product category). Fine crystalline sucrose, the main sweetener in chocolate products, compound coatings, and cocoa drinks, is utilized at up to 50% of the formula on a weight basis in chocolate confectionery (Varzakas and Özer, 2011). Sweeteners do not chemically react with fats and oils. Instead, their interaction is physical, impacting a product's rheological characteristics, i.e., viscosity. In chocolate and compound coatings, the rheological characteristics of the paste are critical to its use and function. Sweeteners impact rheology via particle size distribution and trace moisture from amorphous sugar. Both characteristics are affected by milling that could be performed on either roller refiners or hammer mills, reducing the particle size of the sweetener from coarse to fine. As the sweetener's particle size decreases, surface area increases, increasing the need for more fat to maintain the same rheological characteristics for a given application. Milling also creates small amounts of amorphous sugar that could releases its water into the paste determining rheological problems. Of the sugars available for use in chocolate and compound coatings, only sucrose, anhydrous dextrose, and lactose are appropriate. Dextrose monohydrate is a poor choice due to its one molecule of water that releases during processing, leading to dramatic rheological consequences. Crystalline fructose also has poor performance in these systems due to its low glass transition temperature and tendency to absorb moisture from the air during processing (Helstad, 2006).

1.3.1.3. Ice cream and foam

Sweetening agents are added to ice cream mix at a rate of usually 12–17% by weight. Sweeteners improve the texture and palatability of ice cream, enhance flavours, and are usually the most economical source of total solids. Their ability to lower the freezing point of a solution imparts a measure of control over the temperature-hardness relationship. In determining the proper blend of sweeteners for an ice cream mix, the total solids required from the sweeteners, the sweetness factor of each sugar, and the combined freezing point depression of all sugars in solution must be calculated to achieve the proper solids content, the appropriate sweetness level, and a satisfactory degree of hardness. The most common sweetening agent used is sucrose, alone or in combination with other sugars. Sucrose, like lactose, is most commonly present in ice cream in the supersaturated or glassy state, so that no sucrose crystals are present (Goff and Hartel, 2006).

1.3.1.4. Candy

Sugars are utilized for generation of caramel, a brown colorant for food (Tomasik *et al.* 2007). For this purpose, sugar is burned (caramelized). Burning of sugar in non-catalyzed processes results in the formation of particularly high amounts of furan-2-aldehyde and its derivatives. They constitute the flavour and aroma typical of caramel.

In candy making, sugar is first dissolved in water at room temperature to the point at which no more sugar will dissolve (sugar to water ratio 1:1/2) to obtain a saturated solution that is placed over heat and stirred continuously. Then it is heated to boiling (152°C–168°C), creating a supersaturated solution. The degree of sugar concentration of the supersaturated solution can determine the candy's final consistency. Crystallization can be a major determinant of quality in sugar-based products. In products, such as hard candies, the formation of sugar crystals is inhibited during formation of the glassy state; whereas, in products, such as fondants, the presence of crystals is necessary for the desired texture.

Crystallization of an amorphous sugar matrix is affected by numerous factors, including water content, ingredients or additives, and environmental conditions of relative humidity and temperature. In hard candies, corn syrup prevents unwanted crystallization or graining.

There are two groups of crystalline candies: candies with perceptible crystals, such as rock candy, and cream candies in which crystals are too small to be detected by the tongue, such as fondant and fudge.

Non-crystalline or amorphous candies are much simpler to make. The sugar solution must simply contain sufficient interfering agents or cook to a high enough temperature to prevent recrystallization.

Lower-DE syrups are added to chewy candies to increase the viscosity of the formula and produce a less sweet product. Fats from butter and cream help soften caramels and act as release agents that keep the candy from sticking to the teeth (Varzakas and Özer, 2011).

1.3.1.5. Beverages

Carbonated beverage formulations are 90% water and 10% sweetener. Color, carbon dioxide, flavor, acidulants, or preservatives are often added. Many carbonated drinks contain the 42% HFCS although the 55% product is also used. Noncarbonated beverages include those made from fruits, vegetables, and dairy-based ingredients. Sucrose or HFCS are the sweeteners mostly used. Corn syrups or maltodextrins are also used to build body and aid stability and emulsification (Varzakas and Özer, 2011).

Carbohydrate sweeteners determined both sweetness and mouthfeel of beverages. They are soluble solids and influence the volatility of some polar and non-polar compounds, affecting flavour. Beverages prepared using sucrose as a sweetener have the advantage that the breakdown products of sucrose hydrolysis (glucose and fructose) are also sweet. The resulting invert sugar produced delivers sweetness of virtually identical intensity (and quality) as the sucrose from which the breakdown products are derived (Kemp & Lindley, 2009).

1.4. Strategies for sugar reduction

There is no clear evidence that a high-carbohydrate diet leads directly to coronary heart disease; nevertheless, the progression from overeating, through obesity and diabetes mellitus, to coronary heart disease, is well known (Tomasik, 2004). Indeed, sucrose in food with texture permitting adhesion to the teeth produces high risk of dental caries.

The replacement of saccharide sweeteners (first of all, sucrose) in food with various natural and synthetic sweeteners of very high RS (currently, mainly saccharin, aspartame, and cyclamates) is a task. It is also demanded by consumers looking for low-calorie foods. Diabetics are also looking for food free of insulin-requiring saccharides and polysaccharides. Following such demands, problems are encountered in providing the anticipated texture of sweet products manufactured without saccharides (Mazurkiewicz *et al*., 2001).

There are many compounds have been found to be sweet and have no calories. Only a few are approved for use commercially. None yet meet the criteria for the perfect low-calorie sweetener, such as a similar taste profile, non-cariogenic, safe, natural, cheaper, stable, without after taste etc. Low-calorie sweeteners are known under different names, such as non-nutritive, intense, high intensity, high potency, alternative and artificial sweeteners, sugar replacers, sugar substitutes and macronutrient substitutes.

The low-calorie sweeteners approved for use around the world are: Acesulfame-K, alitame, aspartame, aspartame-acesulfame salt, cyclamate, neohesperidin dihydrochalcone (NHDC), neotame, saccharin, stevioside, sucralose and thaumatin (Kemp, 2006).

High-intensity sweeteners (HIS) provide sweetness with negligible calories, although the sensation of their sweetness is often different from that of sugar. As reported by Anderson *et al*., 2007**,** HIS are sweeter than sucrose, in particular acesulfame-K, aspartame, sucralose, saccharin and neotame are 200, 180, 600, 300 and 8000 times sweetener than sugar, respectively. Two other artificial sweeteners, alitame and cyclamate (2000 and 30 times sweeter than sugar, respectively), have been used in foods in Europe but not in the United States. Another group of sweeteners provides sweetness with reduced calories. These are the sugar alcohols, identified as sugar replacers or polyols. Sugar alcohols – including mannitol, sorbitol, xylitol, erythritol and lactitol, which, respectively, provide 1.6, 2.6, 2.4, 0.2 and 2.1 kcal/g – are hydrogenated forms of carbohydrate where the ketone group has been reduced at the primary and secondary hydroxyl group. Although they have the same bulk and texture as sucrose, they are less sweet and provide fewer calories than sugars. Additionally, they can be used to mask the detectable aftertaste of some artificial sweeteners; therefore, they are often used with high-intensity sweeteners**.** Two other sweeteners, trehalose, synthesized by various microorganisms, and tagatose, the fructose isomer, both digestible in small part, although they are more similar to that of sugar alcohols for sugar, behave as polyols (Patra *et al*., 2009). The HFCS, a liquid form sweetener with different percentages of glucose and fructose, has been widely used by food manufacturers for its higher sweetening power than sucrose so it is possible to reduce the amount used, but the problem associated with it is due to the fact that it most contains fructose, which has a different metabolism from glucose and is not able to give the signal of satiety to the brain, so it may promote lipogenesis and thus obesity (Elliot *et al*., 2002).

None of the current low-calorie sweeteners match sucrose in sweet taste quality or temporal characteristics. They are commonly used in blends together and/or with nutritive sweeteners to give an improved sweet taste profile, such as masking off tastes. The sweetener combination gives a higher perceived sweet intensity than would be expected from the sweet tastes of the individual components. The amount of each sweetener that needs to be used in a blend is less than if used individually, lowering costs. The first blend combination was saccharin and cyclamate. It has a less bitter aftertaste than the individual sweeteners and is used in a variety of applications, particularly beverages and table-top sweeteners. Blends commonly used include acesulfame-K/aspartame and aspartame/saccharin. Three way blends (sucralose/acesulfame-K/cyclamate; saccharin/ acesulfame-K/aspartame) and four way blends (aspartame/acesulfame-K/ cyclamate/saccharin) are also used. Aspartame-acesulfame salt is a chemical blend that provides blended sensory properties in a single compound and offers advantages over physically blending the two components. Blending low-calorie sweeteners with nutritive sweeteners can give synergy whilst reducing calories and in some instances cost. For commercial reasons, intense sweeteners are used in combination with sucrose in a range of beverages (Kemp, 2006).

Sweet proteins are an attractive alternative to some of the most traditional sweeteners, because they respond to the consumers need of low calorie products (Uher, 2004). As reported in the review of Picone and Temussi (2012), the protein-sweeteners best known are monellin, thaumatin, and brazzein. In addition, there are two sweet little protein, mabinlin and lysozyme as well as the other two miraculin and curculin, that give a sweet taste when combined with acids and for this reason are considered "taste-modifying" proteins. The fruits of some tropical plants, particularly plants from regions that have fostered the evolution of primates, contain sweet proteins. They are intensely sweet, several orders of magnitude sweeter than sugars, but taste sweet only to primates. Besides their presence in tropical plants, they have very little in common, according to usual ways of comparing proteins with the same function.

They have very different amino acid sequences and different three-dimensional structure. Sweet proteins stand out among sweet molecules because their volume is not compatible with an interaction with orthosteric active sites of the sweet taste receptor. The best explanation of their mechanism of action is the interaction with the external surface of the sweet taste receptor, according to a model that has been named wedge model (Temussi, 2011). It is hypothesized that this mode of action may be related to the ability of other members of their protein families to inhibit different enzymes.

There has been recent research and development activity on three main natural high-potency sweeteners; the steviol glycoside rebaudioside A, an extract of the Chinese lo han guo fruit containing a sweetener known as mogroside, and monatin from a shrub indigenous to South Africa. The progress of these development activities is reviewed. Rebaudioside A is a diterpene glycoside occurring in the leaves of Stevia rebaudiana, a shrub indigenous to Paraguay in South America. The plant produces a number of steviol glycosides (there are believed to be at least eight different glycosides), all of which are sweet, with stevioside and rebaudioside A having been commercial in some parts of the world for many years. Stevioside elicits a clear bitter/liquorice aftertaste that makes rebaudioside A have been compared and the data show clearly products with rebaudioside A as opposed to stevioside. The sensory properties of stevioside and the sensory benefits that will result from formulating beverages and other research has identified rebaudioside A as eliciting a cleaner sweetness, probably due to the fact that it is a more polar molecule than stevioside, and this has resulted in plant-breeding attempts to select for rebaudioside A. Now, steviol glycoside preparations that are predominantly rebaudioside A are available with at least one commercial product claimed to consist of > 99% rebaudioside A. This ability to produce almost pure rebaudioside A has stimulated much development activity into the optimisation of its sensory delivery, culminating in publication of a large body of patent applications that identify formulation routes to improving the taste of rebaudioside A (Prakash and DuBois, 2007). Rebaudioside A is characterised as delivering potent sweetness, being approximately 300 times as sweet as sucrose at practical use levels, with associated bitter and liquorice/anise aftertastes. Sweetener displays an unusual sensory characteristic in that the first taste is very much sweeter than subsequent tastes, leading to potential complications in formulating it into successful beverages.

Siraitia grosvenorii is a Chinese plant of the cucumber or melon family, the fruit of which is used indigenously as a food, beverage and traditional medicine. The sweet principles of the plant are known as mogrosides (derived from the original name of the plant as Momordica grosvenorii), and are triterpene glycosides. Lo han guo is one of the common names of the plant. The plant produces a number of mogrosides, of which the most common have been designated mogroside IV and mogroside V. Isolation of these two mogrosides has been described and their sensory properties established. Mogroside V is the most abundant component, occurring at about 1% in the dried fruit, and its sweetness potency is usually described as being approximately 200–250 times that of sucrose (Kemp and Lindley, 2009).

1.5. Sugar reduction in food

1.5.1. Beverages

An inevitable consequence of removing soluble solids in the form of sucrose, fructose syrups and/or glucose syrups from beverages is a reduction in the perception of 'mouthfeel'. Consumers frequently describe diet or sugar-free beverages as 'thin' or 'watery' and for many these sensations are perceived as a negative (Kemp & Lindley, 2009).
Replacing lost body/mouthfeel is not as straightforward as might be imagined, however, as the seemingly simple solution of adding back solids in the form of ingredients such as maltodextrins clearly is counterproductive in that adding maltodextrin also adds back calories. Use of a low-calorie bulk sugar substitute such as polydextrose, although promoted for this functionality by the commercial supplier due to its Newtonian viscosity characteristics (Auerbach *et al.*, 2006), adds substantial cost. Polysaccharides such as pectin and xanthan gum have also been proposed for use in low-calorie beverages in the expectation that the perception of mouthfeel is directly related to viscosity. However, this formulation approach has not been adopted widely, probably because mouthfeel may be as much a consequence of sweet taste quality as it is dependent on solution viscosity and because viscosity derived from polysaccharides can have quite profound flavour modification effects (Cook, 2006).

1.5.2. Ice cream

Ice-cream, generally, with its high sucrose content, is not suitable for diabetics or people trying to lose weight, although the sucrose has not only the function of sweetener in ice cream but it adds solids to the mix, lowering the point freezing, and contributes to the texture and body of the product. Bordi research team, in several studies, compared the sensory attributes and shelf-life of a vanilla ice cream without added sugar, containing maltitol as sugar replacer, with another one containing sucrose (Bordi *et al*., 2004; Stokols *et al*., 2005; Lee *et al*., 2005). In a preliminary study, using a small number of consumers, participants were asked to judge the liking for the taste, texture, appearance, and for the product, globally. Results showed that there were no significant differences between the standard product and that no added sugar, some attributes of taste, appearance and texture were evaluated and among those only the sweetness distinguished the two products, in particular the traditional ice-cream was sweeter than others, precisely it was considered too sweet. These characteristics were then evaluated by a wider number of participants, divided by age and gender. The results showed that both women and young men preferred the sweetness of sugar-free ice cream as opposed to adult women and men. For what concern the shelf-life, for both ice-cream types, overtime, there was a reduction of sweetness and some texture attributes, such as the increase of ice crystals, and liking for overall taste, appearance and texture. The main differences between the two products were mostly related to the uniformity and intensity of color.
Whelan *et al.* (2008) investigate the suitability of alternative sweeteners for the manufacture of low-GI ice cream, to optimize formulations to match their freezing curves and other physicochemical properties and to enhance the sensory attributes of the most suitable formulations. Three relatively new novel commercial sweeteners, tagatose, erythritol and trehalose, were studied, along with maltitol and polydextrose.
Their work demonstrated that matching the freezing curve was thus the first step to formulate low-GI ice creams with alternative low-GI, low calorie sugars. Most physicochemical parameters of interest to ice cream structure ⁄ texture will balance the control once the freezing curve has been matched. A strong correlation was found between perceived dairy flavour, sweetness liking and vanilla flavour, with vanilla flavour perception lacking in the alternative formulations. From the formulations studied, a combination of tagatose (6%), polydextrose (6%) and maltitol (3%) or maltitol (15%) and trehaolse (2.5%) in a fomulation with milk, cream and milk protein concentrate showed to be a potential formulation that could satisfy both physicochemical and sensory requirements.

1.5.3. Dessert

Demirag *et al.* (1999) manufactured a milk pudding type of dessert, kazandibi, according to a standard recipe, replacing sucrose and milk fat by food additives (aspartame, acesulfame K, xylitol, fructose, guar and xanthan gums) and the effect of these additives on sensory quality, chemical components and energy value was investigated. Their results showed that a guar gum-xanthan gum (2.8-1.2) mixture besides rice milk and rice flour increased the textural quality whereas the artificial sweeteners aspartame-acesulfame K (0.25-0.2) together with small amounts of xylitol and fructose were effective in providing sufficient sweetness. Milk-cream aromas significantly affected flavor acceptability. The samples optimized had higher protein contents and lower energy values (83kcal/l00 g) than those of standard katandibi and could be used both for diabetic and dietetic purposes.
Gautam *et al.* (2012) showed that it is possible to develop Chhana kheer, an Indian dairy dessert, using artificial sweeteners (aspartame, acesulfame-K and sucralose) instead of conventional sugar which will lead to manufacture of dietetic foods based on traditional dairy products simulating their taste and texture at the same time offering a product with reduced calorie.

Lassi is a ready-to-serve, fermented dairy product obtained after the growth of selected culture, usually lactic streptococci, in heat-treated milk followed by sweetening with sugar. It has a creamy consistency, sweetish-rich aroma and mild to acidic flavour, which makes the product refreshingly palatable. It is consumed as a cold, refreshing therapeutic beverage usually in summer. George *et al.* (2010) investigated, through sensory analysis, the possibility to use binary sweetener blend, aspartame/acesulfame-k (50:50, 0.05%), instead of sucrose in the preparation of lassi. They also standardized the technology for artificially sweetened lassi in order to obtain the same consistency as the sucrose sweetened lassi (control).

1.5.4. Baked goods

Sugar and fat are two key ingredients of cookies and other baked desserts. Food writers frequently advise reducing the amount of sugar in cookie recipes as a method for arriving at a lower-calorie product. An alternative strategy, reducing the fat content of cookies, may reduce calories even further, while maintaining high overall product acceptability levels. A study conducted by Drewnowski *et al.* (1998) showed that sweetness was the key sensory attribute that determined preferences for cookies. Acceptability ratings for cookies dropped sharply following a 25% reduction in recipe sugar levels, but were relatively unaffected following a 25% reduction in recipe fat content.

Psimouli and Oreopoulou (2011) studied the effect of alternative sweeteners on batter rheology and cake properties. The best results were obtained by using oligofructose, lactitol or maltitol as sugar replacers, which exhibited similar behaviour to sucrose in terms of batter rheology and increased starch gelatinisation temperature. Fructose and mannitol led to cakes of poor quality characteristics, as was demonstrated by instrumental measurements and sensory evaluation. The sensory evaluation indicated that overall acceptance followed closely the scores of tenderness and taste.

Schirmer *et al*. (2012) investigated the effect of sucrose replacement by polydextrose on the physical properties of pound cake and batter. Batter analysis revealed differences between the stability under shear stress as well as in texture when replacing sucrose by polydextrose, which resulted in a less stabile batter due to the interfacial behavior of the polydextrose and the batter components. In the case of the baked pound cake, polydextrose gave a similar effect on all the textural and crumb grain features. The decrease of polydextrose resulted in a significant increase in the pores average sizes, which are typical for an irregular crumb. For what concern staling the polydextrose containing cakes showed similar behavior to the control products containing sucrose.

1.5.5. Chocolate

Guinard *et al.* (1999) studied the effects of sugar and fat on the sensory properties of milk chocolate, throught descriptive analysis and instrumental measures. Nine formulation of milk chocolate were evaluated, varying chocolate liquor, milk solids and cocoa butter, but using three level of sugar and three level of total fat. Lecitin and vanilla levels were kept constant. They found that low sugar samples were more bitter, gritty and roasted than others. High sugar samples had higher milky/dairy, vanilla/caramel, hardness and sweetness intensities. Samples higher in fat were faster melting. Low fat and low sugar samples were associated with viscous, mouthcoating, fatty/oily, cocoa and darker notes. Samples with high levels of both sugar and fat were more cooling and faster vanishing.

The type and ratio of sugar substitutes, inulin (IN), polydextrose (PD), maltodextrin (MD), induced various effects on physicochemical, textural and sensory properties of low-calorie milk chocolate. Sugar replacement with high ratios of sugar substitutes resulted in high moisture content and low hardness values. The lowest moisture content and the highest hardness values were found for the moderate ratio of sugar substitutes. Moreover, higher IN and PD and lower proportions of MD improved sensory attributes of milk chocolates (Farzanmehr and Abbasi, 2009).

1.6. Aeration: a process to reduce calorie content of food

One of the fastest growing food processing operations is aeration. Aerated products vary from hard (confectionary) and elastic (bread) solids to thin liquid foams (beer). Inclusion of air in food products may improve textures, decrease product costs or even create new structures (Minor *et al*., 2009). Incorporation of air into food emulsions imparts much appreciated texture and mouth-feel. A foam-like structure in dairy products, such as natural and artificial whipped creams or ice creams, is characterized by adsorption of networks of partially adsorbed fat droplets onto air bubble surfaces (Bazmi *et al*., 2007). Aeration of these systems is generated by whipping oil-in-water emulsions containing hydrogenated animal or vegetable fat and milk proteins in combination with mixture of emulsifiers and polysaccharides (Goff, 2002; Leser & Michel, 1999). Generally, air gets into the emulsion to form unstable large air bubbles, which are then broken down into smaller ones. Then, depending on shearing conditions (force intensity, time, temperature) fat droplets can be more or less destabilized in the bulk phase, and then adsorb at the surface of air bubbles, contributing not only to effectiveness of the introduction of air bubbles, but also to structure stabilization (Goff, 1997; Vanapalli *et al*., 2001; Van Aken, 2001). The main parameters determining the physical behaviour of food foams are the level of aeration and the bubble size distribution, which are determined by the aeration process and the interfacial properties of surfactants and proteins, and the firmness and fracture properties of the matrix in which the bubbles are dispersed. These parameters will probably also play a key role in oral processing and perception, involving the bulking and structure breaking effect of the foam bubbles, which affects foam rheology, foam breakdown and flavour release. In fat-free foams, at low air contents, sensory perception was determined by matrix properties, meanwhile, at high air contents, it was determined by bubble characteristics and the matrix plays a negligible role in perception. Indeed, bubbles were better noticeable in a semi-solid than in a liquid foams, in particular, in sucrose-ester carrageenan foams, large bubbles were perceived as more airy and less creamy, compared with the same composition with smaller bubbles (Minor *et al*., 2009). Numerous studies performed on aerated food emulsions have focused on possible relationships between microstructure and emulsifier interfacial properties, destabilization effect upon mechanical shearing or air incorporation (Boode, Walstra, & de Groot-Mostert, 1993; Pelan, Watts, Campbel, & Lips, 1997; Segall & Goff, 1999; Smith, Kakuda, & Goff, 2000; Dickinson, 2006; Relkin *et al*., 2006). In most of these studies, fat and protein sources used for manufacture of aerated products were milk fat and skim milk powder (SMP), caseinate or whey protein concentrates, and low-molecular-weight emulsifiers, such as mono- and di-glycerides or polyoxyethylene sorbitan esters, which are often used in combination with milk proteins for their competitive adsorption to fat/serum or air/serum interfaces. It has been shown that emulsion stabilization against fat globule flocculation/coalescence is highest for sodium caseinate, and lowest for SMP (Segall & Goff, 1999). By incorporating air bubbles into gradually flocculating caseinate-stabilized emulsions via simultaneous whipping and slow acidification, aerated emulsion gels of good foam stability can be formulated (Dickinson, 2006). Indeed, replacement of native whey proteins by a mixture of denatured proteins and casein promoted a smaller and more uniform air bubble size distribution, a higher attachment of fat globules to the air–bubble interface, and more aggregation of fat globules. The formation of protein aggregates led to a more heterogeneous distribution of protein at the fat interface, allowing for more aggregation despite a higher total adsorbed protein content (Relkin *et al*., 2006).

The formation of stable aerated products based on milk protein emulsions, such as whipped creams, depends on the physical properties of the emulsion before the whipping step and how the air is incorporated and the created air bubbles are stabilized. The fat in the emulsion should be present in liquid as well as at the crystalline state. The emulsion has to be destabilized in a controlled way after homogenization and pasteurization, because the more stable an emulsion is the more difficult it is afterwards to destabilize it again, and the more difficult it is to make a stable whipped cream (Leser *et al*., 1999). There are at least two mechanisms that degrade the aerated product: buoyancy and Ostwald ripening. Due to buoyancy, the larger air bubbles rise to the top of the container and result in a vertical phase separation (creaming) of the dispersed air. This results in a no uniform product quality, which is undesirable. In addition, the polydispersity in the size distribution results in a change in bubble size due to Ostwald ripening. As the larger bubbles grow in size at the expense of the smaller ones, under certain conditions, the ripening process could serve to accelerate the separation due to buoyancy. It is clear that a good product is one in which the effects of both these destabilizing factors are mitigated (Dutta *et al*., 2004).

Thakur *et al.* (2005) showed that is possible to produce stable unfrozen foamed emulsions similar to an ice cream mix aerated with 100% overrun in a narrow annular gap unit under steady state conditions. This device can be used for pre-aeration in ice cream, or more generally, in frozen dairy emulsion processing. Although the eight dairy emulsion recipes studied in this work differ only slightly in terms of rheological properties and surface tension, far different unfrozen foamed emulsions were obtained. Experimental results showed that there was an optimal rotational speed: over-whipping decreases foam stability. Similarly, there is a minimum residence time for stabilizing unfrozen foams, especially when partial coalescence may be high. Emulsifier type appears to play the major role during foaming, both on bubble size and foam stability. Fat type and residence time can drastically change the extent of fat destabilization, while protein type affects mainly the minimum residence time.

1.7. References

Aime DB, Arntfield SD, Malcolmson LJ, Ryland D 2001 Textural analysis of a reduced vanilla ice cream products. *Food Research International* 34: 237-246.

Akhtar M, Murray BS, Dickinson E 2006 Perception of creaminess of model oil-in-water dairy emulsions: Influence of the shear-thinning nature of a viscosity-controlling hydrocolloid. *Food Hydrocolloids*, 20: 839-847.

Akhtar M, Stenzel J, Murray BS, Dickinson E 2005 Factors affecting the perception of creaminess of oil-in-water emulsions. *Food Hydrocolloids*, 19: 521-526.

Akoh CC 2007 Lipid-based synthetic fat substitutes. In *Food Lipids: Chemistry, Nutrition and Biotechnology,* Akoh CC & Min DB, eds., CRC Press, Boca Raton, USA, pp. 390-420.

Alting AC, van de Velde F, Kanning MW, Burgering M, Mulleners L, Sein A, Buwalda P 2009 Improved creaminess of low-fat yoghurt: the impact of amylomaltase-treated starch domains. *Food Hydrocolloids*, 23: 980-987.

Anderson GH, Akhavan T, Mendelson R Food ingredients implicated in obesity: sugars and sweeteners. In *Novel food ingredients for weight control*, Henry CJK ed., Woodhead Publishing Limited, Cambridge, UK, 2007 pp. 104-127.

Anderson AS. Changing unhealthy food choices. In *Understanding Consumers of Food Products,* Frewer R & van Trijp H. eds, CRC Press, Boca Raton, USA, 2007 pp. 496-500

Auerbach M, Craig S and Mitchell H Bulking agents: multi-functional ingredients. In *Sweeteners and Sugar Alternatives in Food Technology*, Mitchell H ed., Blackwell Publishing Ltd, Oxford, UK, 2006 pp 367–397.

Aykan V, Sezgin E, Guzel-Seydim ZB 2008 Use of fat replacers in the production of reduced-calorie vanilla ice cream. *Eur. J. Lipid Sci. Technol,* 110: 516-520.

Ayo J, Carballo J, Solas MT, Jiménez-Colmenero F 2008 Physicochemical and sensory properties of healthier frankfurters as affected by walnut and fat content. *Food Chemistry*, 107: 1547-1552.

Aziznia S, Khosrowshahi A, Madadlou A, Rahimi J 2008 Whey Protein Concentrate and Gum Tragacanth as Fat Replacers in Nonfat Yogurt: Chemical, Physical, and Microstructural Properties. *Journal of Dairy Science*, 91: 2545-2552.

Bayarri S, González-Tomás L, Hernando I, Lluch MA, Costell E 2011 Texture perceived on inulin-enriched low-fat semisolid dairy desserts. Rheological and structural basis. *Journal of Texture Studies*, 42: 174–184.

Bazmi A, Duquenoy A, Relkin P 2007 Aeration of low fat dairy emulsions: Effects of saturated-unsaturated triglycerides. *International Dairy Journal*, 17: 1021-1027.

Bednarski W, Adamczak M 2003 Modified lipids and fat mimetics. In *Chemical and Functional Properties of Food Lipids,* Sikorski ZE, Kolakowska A., eds, CRC Press, Boca Raton, USA, pp. 309–324.

Boode K, & Walstra P 1993 Partial coalescence in oil-in-water emulsions.1. Nature of the aggregation. *Colloids and Surfaces A: Physiochemical and Engineering Aspects,* 81: 121–137.

Bordi P, Cranage D, Stokols J, Palchak T, Powell L 2004 Effect of polyols versus sugar on the acceptability of ice cream among a student and adult population. *Foodservice Research International,* 15: 41-50.

Bult JHF, de Wijk RA, & Hummel T 2007 Investigations on multimodal sensory integration: texture, taste, and ortho- and retronasal olfactory stimuli in concert. *Neuroscience Letters*, 411: 6–10.

Byars J 2002 Effect of a Starch-Lipid Fat Replacer on the Rheology of Soft-Serve Ice Cream. *Journal of Food Science*, 67: 2177-2182.

Arancibia C, Jublot L, Costell E, Bayarri S 2011 Flavor release and sensory characteristics of o/w emulsions. Influence of composition, microstructure and rheological behaviour. *Food Research International,* 44: 1632–1641.

Caceres E, Garcia ML, Toro J, Selgas MD 2004 The effect of fructo-oligosaccharides on the sensory characteristics of cooked sausages. *Meat Science,* 68: 87-96.

Candogan K, Nuray K 2003 The effects of carrageenan and pectin on some quality characteristics of low-fat beef frankfurters. *Meat Science*, 64: 199-206.

Choi YS, Choi JH, Han DH, Kim HY, Lee MI, Jeong JY, Chung HJ, Kim CJ 2010 Effects of replacing pork back fat with vegetable oils and rice bran fiber on the quality of reduced-fat frankfurters. *Meat Science*, 84: 557-563.

Cierach M, Modzelewska-Kapituła M, Szaciło K 2009 The influence of carrageenan on the properties of low-fat frankfurters. *Meat Science*, 82: 295-299.

Cleveland MA, Erickson RR, Brown SC, Gee DL 2007 Benefat is a successful partial substitute in chocolate cake. *Journal of the American Dietetic Association,* 107 (8) suppl.3: A-74.

Cook D Hydrocolloid-sweetener interactions in food products. In *Optimizing Sweet Taste in Foods*, Spillane W.J. ed, Woodhead Publishing Ltd, Cambridge, UK, 2006 pp 388–403.

Crehan CM, Hughes E, Troy DJ, Buckley DJ 2000 Effect of fat level and maltodextrin on the functional properties of frankfurters formulated with 5, 12 and 30 % fat. *Meat Science*, 55: 463-469.

Crilly J, Russel A, Cox A, Cebula D 2008 Designing multiscale structures for desired properties of ice cream. *Industrial and Engineering Chemistry Research,* 47: 6362-6367.

Daniel JR Advances in Development of Fat Replacers and Low-Fat Products. In *Innovation in Food Engineering,* Passos M L, Ribeiro CP, eds, CRC Press, Boca Raton, USA, 2010 pp. 657-684.

Delgado-Pando G, Cofrades S, Ruiz-Capillas C, Solas MT, Triki M, Jiménez-Colmenero F 2011 Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient: Microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation. *Meat Science*, 89*:* 65-71.

Dickinson E 2006 Structure formation in casein-based gels, foams, and emulsions. *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 288: 3–11.

Dutta A, Chengara A, Nikolov AD, Wasan DT, Chen K, Campbell B 2004 Texture and stability of aerated food emulsions—effects of buoyancy and Ostwald ripening. *Journal of Food Engineering*, 62: 169–175.

Hughes E, Mullen AM and Troy DJ 1998 Effect of fat level, tapioca starch and whey protein on frankfurter formulated with 5% and 12% fat. *Meat Science*, 48: 169–180.

Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ 2002 Fructose, weight gain, and the insulin resistance syndrome. *Am. J Clin. Nutr.*, 76 (5): 911-922.

Farzanmehr H & Abbasi S 2009 Effects of inulin and bulking agents on some physicochemical, textural and sensory properties of milk chocolate. *Journal of Texture Studies*, 40: 536–553.

Ford LD, Borwankar RP, Pechak D, Schwimmer B Dressings and Sauces In *Food Emulsion*, fourth edition, Friberg S, Larsson K, Sjoblom J, eds, Marcell Dekker Inc., New York, USA, 2004 pp. 525-540.

Gautam A, Jha A & Singh R 2012 Sensory and textural properties of chhana kheer made with three artificial sweeteners. *International Journal of Dairy Technology*, 65: 1-10.

George V, Arora S, Wadhwa BK, Singh AK and Sharma GS 2010 Optimization of sweetener blends for the preparation of lassi. *International Journal of Dairy Technology*, 63: 256-261.

Glauert HP Influence of dietary fat on the development of cancer. In *Food Lipid : Chemistry, Nutrition and Biotechnology,* Akoh CC & Min DB, eds., CRC Press, Boca Raton, USA, 2007 pp. 180-200

Goff HD 1997 Instability and partial coalescence in whippable dairy emulsions. *Journal of Dairy Science*, 80: 2620-2630.

Goff HD 2002 Formation and stabilization of structure in ice cream and related products.. *Current Opinion in Colloidal and Interface Science*, 7: 432-437.

Goff HD, Hartel RW Ice Cream and Frozen Desserts. In *Handbook of Food Science, Technology, and Engineering, Volume 4,* Hui YH ed.,CRC Press, Boca Raton, USA, 2006 154: pp. 2-45.

Guinard JX, Mazzucchelli R 1999 Effects of sugar and fat on the sensory properties of milk chocolate: descriptive analysis and instrumental measurements. *Journal of the Science of Food and Agriculture*, 79: 1331-1339.

Gunstone FD Food applications of lipids. In *Food Lipid : Chemistry, Nutrition and Biotechnology,* Akoh CC & Min DB, eds., CRC Press, Boca Raton, USA, 2007 pp. 280-302.

Hausman DB, Grossman BM Dietary fats and obesity. In *Food Lipid : Chemistry, Nutrition and Biotechnology,* Akoh CC & Min DB, eds., CRC Press, Boca Raton, USA, 2007 pp. 160-178.

Helstad S, Ingredient Interaction: Sweeteners. In *Ingredient Interactions: Effects on Food Quality, 2nd* ed" Gaonkar AG & McPherson A, eds., CRC Press, Boca Raton, USA, 2006 pp 167-194.

Indrani D, Rao GV Functions of Ingredients in the Baking of Sweet Goods. In *Food Engineering Aspects of Baking Sweet Goods*, Şumnu SG & Sahin S, eds., CRC Press, Boca Raton, USA, 2008: 31-48.

Jones JM & Jonnalagadda SS 2006 The use of fat replacers for weight loss and control. In *Improving the fat content of foods*, Williams C & Buttriss J, eds,Woodhead Publishing, Cambridge, UK, 2006 pp. 380-390.

Jones SA Physical, Chemical, and Sensory Aspects of Fat Replacement. In *Handbook of fat replacer*, Roller S & Jones SA, eds, CRC Press, Boca Raton, USA, 1996 pp. 59-86.

Jonhøj T, Petersen CB, Frøst MB & Ipsen R 2006 Sensory and rheological characterization of low-fat stirred yoghurt. *Journal of Texture Studies*, 37: 276–299.

Karahan AG, Kart A, Akoğlu A, Çakmakçı ML 2011 Physicochemical properties of low-fat soft cheese Turkish Beyaz made with bacterial cellulose as fat mimetic. *International Journal of Dairy Technology*, 64: 502-508.

Kavas G, Oysun G, Kinik O, Uysal H 2004 Effect of same fat replacers on chemical, physical and sensory attributes of low-fat white pickled cheese. *Food Chemistry*, 88: 381-388.

Kemp SE Low-calorie sweeteners. In *Optimizing sweet taste in foods*, Spillane WJ ed., Woodhead Publishing, Cambridge, UK, 2006 pp. 175-251.

Kemp SE, Lindley MG Developments in sweeteners for functional and speciality beverages. In *Functional and speciality beverage technology*, Paquin P, ed., Woodhead Publishing, Cambridge, EN, 2009 pp. 39-54

Kilcast D & Clegg S 2002 Sensory perception of creaminess and its relationship with food structure. *Food Quality and Preference*, 13: 609-623.

Koca N, Metin M 2004 Textural, melting and sensory properties of low-fat fresh kashar cheeses produced by using fat replacers. *International Dairy Journal,* 14: 365-373.

Konuklar G, Inglett GE, Warner K, Carriere CJ 2004 Use of β-glucan suspension in the manifacture of low-fat Cheddar cheeses: textural properties by instrumental methods and sensory panels. *Food Hydrocolloids*, 18: 535-545.

Laguna L, Varela P, Salvador A, Sanz T and Fiszman SM 2012 Balancing texture and other sensory features in reduced fat short-dough biscuits. *Journal of Texture Studies*, 43**:** 235–245.

Lahteenmaki L, Lily M, Urala N Consumer attitudes towards functional foods. In *Understanding Consumers of Food Products,* Frewer L & van Trijp H eds, CRC Press Boca Raton, Cambridge, EN, 2007 pp. 412-415.

Lai VMF, Lii CY Role of Saccharides in Texturization and Functional Properties of Foodstuffs. In *Chemical and Functional Properties of Food Saccharides,* Tomasik P ed., CRC Press, Boca Raton, USA, 2004 pp. 159-178.

Lee H, Stokols J, Palchak T, Bordi P 2005 Effect of polyols versus sugar on the shelf life of vanilla ice cream. *Foodservice Research International*, 16: 44-52.

Leser M, Michel M 1999 Aerated milk protein emulsions-New microstructural aspects. *Current Opinion in Colloidal and Interface Science,* 4: 239-244.

Li Z, Marshall R, Heymann H, Fernando L 1997 Effect of milk fat content on flavour perception of vanilla cream. *Journal of Dairy Science*, 80: 3133-3141.

Lluch MA, Hernando I, Munuera IP Lipids in food structures. In *Chemical and Functional Properties of Food Lipids,* Sikorski ZE & Kolakowska A eds., CRC Press, Boca Raton, USA, 2010 pp. 9-30.

Lobato-Calleros C, Martinez-Torrijos O, Sandoval-Castilla O, Perez-Orozco JP, Vernon-Carter EJ 2004 Flow and creep compliance properties of reduced-fat yoghurts containing protein-based fat replacers. *International Dairy Journal*, 14: 777-782.

López-López I, Cofrades S, Jiménez-Colmenero F 2009 Low-fat frankfurters enriched with *n*−3 PUFA and edible seaweed: Effects of olive oil and chilled storage on physicochemical, sensory and microbial characteristics. *Meat Science*: 83: 148-154.

Mazurkiewicz J, Rębilas K, and Tomasik P 2001 Aspartame as texturizing agent for foodstuffs. *Z. Lebensm. Unters. Forsch.,* A212: 369.

Mc Clements DJ Characterization of emulsion properties In *Food Emulsions: Principles, Practice and Techniques, 2nd Edition*, Mc Clements DJ ed., CRC Press, Boca Raton, USA, 2005 pp.

Mensink RP & Plat J Dietary Fats and Coronary Heart Disease. In *Food Lipids: Chemistry, Nutrition and Biotechnology,* Akon CC & Min DB eds., CRC Press, Boca Raton, USA, 2008 pp. 551-560.

Metcalf KL, Vickers ZM 2001 Taste intensities of oil-in-water emulsions with varying fat content. *Journal of Sensory Studies,* 17: 379-390.

Meyer D, de Wolf J Inulin in bread and other cereal-based products. In *Technology of functional cereal products*, BR Hamaker ed., Woodhead Publishing, Cambridge, EN, 2008 pp. 409-427.

Milani E & Koocheki A 2011 The effects of date syrup and guar gum on physical, rheological and sensory properties of low fat frozen yoghurt dessert. *International Journal of Dairy Technology,* 64: 121-129.

Minor M, Vingerhoeds MH, Zoet FD, de Wijk R & van Aken GA 2009 Preparation and sensory perception of fat-free foams - effect of matrix properties and level of aeration. *International Journal of Food Science and Technology,* 44: 735–747.

Modzelewska-Kapitula M, Klebukowska L 2009 Investigation of the potential for using inulin HPX as a fat replacer in yoghurt production. *International Journal of Dairy Technology,* 62: 209–214.

Palzer S 2009 Food structures for nutrition, health and wellness. *Trends in Food Science & Technology,* 20: 194-200.

Patra F, Tomar SK, Arora S 2009 Technological and Functional Application of Low-Calorie Sweeteners from Lactic Acid Bacteria. *Journal of Food Science*, 74: 16-23.

Pelan, BMC, Watts KM, Campbel IJ & Lips A 1997 The stability of aerated milk protein emulsions in the presence of small molecule surfactants. *Journal of Dairy Science*, 80: 2631–2638.

Peressini D, Sensidoni A, De Cindio B 1998 Rheological characterization of traditional and light mayonnaises. *Journal of Food Engineering,* 35: 409-417.

Picone D & Temussi PA 2012 Dissimilar sweet proteins from plants: Oddities or normal components? *Plant Science,* 195: 135-142.

Pinero MP, Parra K, Huerta-Leidenz N, Arenas de Moreno L, Ferrer M, Araujo S, Barboza Y 2008 Effect of oat's soluble fibre (β-glucan) as a fat replacer on physical, chemical, microbiological and sensory properties of low-fat beef patties. *Meat Science*, 80: 675-680.

Power L, Williams C, Fremont J, Gupta N, Samuel L, Navder KP 2007 *Journal of the American Dietetic Association,* 107 (8) suppl.3: A-74.

Prakash I and Dubois GE 2007 High-potency sweetener for hydration and sweetened hydration composition. US Patent Application 2007/0116823 A1.

Psimouli V & Oreopoulou V 2011 The effect of alternative sweeteners on batter rheology and cake properties. *J Sci Food Agric,* 92: 99–105.

Rankin LL & Bingham M 2000 Acceptability of oatmeal chocolate chip cookies prepared using pureed white beans as a fat ingredient substitute. *Journal of The American dietetic association*, 100: 831-833.

Relkin P, Sourdet S, Smith AK, Goff HD, Cuvelier G 2006 Effects of whey protein aggregation on fat globule microstructure in whipped-frozen emulsions. *Food Hydrocolloids*, 20: 1050–1056.

Rodrìguez-Garcìa J, Puig A, Salvador A, and Hernando I 2012 Optimization of a Sponge Cake Formulation with Inulin as Fat Replacer: Structure, Physicochemical, and Sensory Properties. *Journal of Food Science,* 77: 189-197.

Rodrìguez-Garcìa J, Laguna L, Puig A, Salvador A and Hernando I 2012 Effect of Fat Replacement by Inulin on Textural and Structural Properties of Short Dough Biscuits. *Food Bioprocess Technol,* 1: 1-12.

Romanchick-Cerpovicz J, Perez ML 2007 Feasibility of using green peas as a fat ingredient substitute in low-fat and fat-free chocolate dropped cookies. *Journal of the American Dietetic Association,* 107 (8) suppl.3: A-74.

Romanchick-Cerpovicz J, Tilmon RW & Baldree KA 2002 Moisture retention and consumer acceptability of chocolate bar cookies prepared with okra gum as a fat ingredient substitute. *Journal of The American Dietetic Association,* 102 (9), 1301-1303.

Sahan N, Yasar K, Hayaloglu AA 2008 Physical, chemical and flavour quality of not-fat yogurt as affected by a β-glucan hydrocolloidal composite during storage. *Food Hydrocolloids,* 22: 1291-1297.

Sandoval-Castilla O, Lobato-Calleros C, Aguirre-Mandujano E, Vernon-Carter EJ 2004 Microstructure and texture of yogurt as influenced by fat replacers. *International Dairy Journal*, 14: 151-159.

Schirmer M, Jekle M, Arendt E, Becker T 2012 Physicochemical interactions of polydextrose for sucrose replacement in pound cake. *Food Research International*, 48: 291-298.

Segall KI & Goff HD 1999 Influence of adsorbed milk protein type and surface concentration on the quiescent and shear stability of butteroil emulsions. *International Dairy Journal*, 9: 683–691.

Sikorski ZE, Sikorska-Wisniewska G The role of lipids in food quality. In *Improving the fat content in foods*, Williams C & Buttriss J eds., Woodhead Publishing, Cambridge, EN, 2006 pp. 250-273.

Sipahioglu O, Alvarez VB, Solano-Lopez C 1999 Structure, physico-chemical and sensory properties of feta cheese made with tapioca starch and lecitina as fat mimetics. *International Dairy Journal*, 9: 783-789.

Smith AK, Goff HD & Kakuda Y 2000 Changes in protein and fat structure in whipped cream caused by heat treatment and addition of stabilizer to the cream. *Food Research International*, 33: 697–706.

Specter SE, Setser CS 1994 Sensory and physical properties of a reduced-calorie frozen dessert system made with milk fat and sucrose substitutes. *Journal of Dairy Science*, 77: 708-717.

Stokols J, Bordi P, Palchak T, Lee H 2005 Profiling of sensory evaluation of a no-sugar-added vanilla ice cream among specific age and gender populations. *Foodservice Research International*, 16: 86-92.

Swanson RB & Perry M 2007 Modified oatmeal and chocolate chip cookies: evaluation of the partial replacement of sugar and/or fat to reduce calories. *International Journal of Consumer Studies*, 31: 265-271.

Szafranski M, Whittington JA, Bessinger C 2005 Pureed cannellini beans can be substituted for shortening in brownies. *Journal of the American Dietetic Association*, 105: 1295-1298.

Tan SS, Aminah A, Zhang XG, Abdul SB 2006 Optimizing palm oil and palm stearin utilization for sensory and textural properties of chicken. *Meat Science*, 72*:* 387-397.

Thakur RK, Vial C, Djelveh G 2005 Combined effects of process parameters and composition on foaming of dairy emulsions at low temperature in an agitated column. *Journal of Food Engineering*, 68 : 335–347.

Tomasik P Saccharides. In *Chemical and Functional Properties of Food Components*, Third Edition, Sikorski ZE ed., CRC Press, Boca Raton, USA 2007 pp. 93-128.

Van Aken GA 2001 Aeration of emulsions by whipping. *Colloids and Surface. A: Physicochemical and Engineering Aspects*, 190: 333-354.

Van Puyvelde P, Moldenaers P Biopolymer systems for low-fat foods. In *Texture in food Volume 1: Semi-solid foods*, McKenna BM ed., Woodhead Publishing Ltd, 2003 pp. 180-197.

Vanapalli S & Coupland JN 2001 Emulsion under shear- The formation and properties of partially coalesced lipid structures. *Food Hydrocolloids*, 15: 507-512.

Varzakas T & Özer B Application of Sweeteners in Food and Drinks (Bakery, Confectionery, Dairy Products, Puddings, Fruit Products, Vegetables, Beverages, Sports Drinks, Hard Candies, Loukoumia, Marmalades, Jams, Jellies, Baked Goods, Sorbet). In *Sweeteners Nutritional Aspects, Applications, and Production Technology*, Varzakas T, Labropoulos A, Anestis S, eds., CRC Press, Boca Raton, USA, 2012 pp. 209-254.

Wasan DT, Xu W, Dutta A, Nikolov A Structure and Stability of Aerated Food Emulsions. In *Food Emulsion*, fourth edition, Friberg SE, Larsson K, Sjoblom J, eds, Marcell Dekker Inc., New York, USA, 2004 pp. 221-241.

Wekwete B & Navder KP 2008 Effects of avocado fruit puree and oatrim as fat replacers on the physical, textural and sensory properties of oatmeal cookies. *Journal of Food Quality*, 31: 131-141.

Whelan AP, Vega C, Kerry JP, Goff HD 2008 Physicochemical and sensory optimization of a low glycemic index ice cream formulation. *International Journal of Food Science and Technology*, 43: 1520-1527.

Woods E & Navder KP 2006 Effect of C-trim as a fat replacer on the physical, textural and sensory properties of chocolate chip cookies. *Journal of the American Association*, 106 (8): A55.

Worrasinchai S, Suphantharika M, Pinjai S, Jamnong P 2006 β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. *Food Hydrocolloids*, 20: 68-78.

Yılmaz L 2004 Effects of rye bran addition on fatty acid composition and quality characteristics of low-fat meatballs. *Meat Science*, 67: 245–249.

Yilsay TO, Yilmaz L, Bayizit AA 2006 The effect of using a whey protein fat replacer on textural and sensory characteristics of low-fat vanilla ice cream. *Eur. Food Res. Technol.*, 222: 171-175.

Zahn S, Pepke F & Rohm H 2010 Effect of inulin as a fat replacer on texture and sensory properties of muffins. *International Journal of Food Science and Technology*, 45: 2531–2537.

Zalazar CA, Zalazar CS, Bernal S, Bertola N, Bevilacqua A, Zaritzky N 2002 Effect of moisture level and fat replacer on physicochemical, rheological and sensory properties of low-fat soft cheeses. *International Dairy Journal*, 12: 45-50.

Zisu B, Shal NP 2005 Textural and functional changes in low-fat Mozzarella cheeses in relation to proteolysis and microstructure as influenced by the use of fat replacers, pre-acidification and EPS-starter. *International Dairy Journal*, 15: 957-972.

Zoulias EI, Oreopoulou V, Tzia C 2002 Textural properties of low-fat cookies containing carbohydrate- or protein-based fat replacers. *Journal of Food Engineering*, 55: 337-342.

2. Aim of the thesis

The aim of the thesis was the calorie reduction in food. The research have been focused on: the characterization of a new sweet protein, as alternative to sugar, particularly, for beverage industry application; the optimization of a fat replacer system for fat based fillings.

As first case study, a sensory characterization of the new sweet protein, MNEI, obtained by recombinant expression in cells of E. coli with high yields, was performed through the determination of its taste detection and recognition thresholds, in different experimental conditions. In order to determine the recognition threshold of MNEI protein a modified version of a classical method was adjusted. Then, sensory performance of MNEI was compared with other market sweeteners, in a model beverage, through dynamic sensory methods: time-Intensity and Temporal Dominance of Sensation.

In the second case study, since it is not always possible to replace fat with a single compound, a three component fat replacer system was developed in order to reduce the calorie content of a model fat based filling.. The chosen fat replacer system was constituted by a thickening agent as lubricant and flow regulator, a soluble bulking agent as regulator of adsorption/absorption of the food onto taste receptors of the tongue, and an insoluble micro-particulate as mimetic of the typical fat smoothness. Previously, the effect of different sugar and milk proteins on ability to bind water and incorporate air was investigated in order to obtain a stable product with a reduced weight/volume ratio.

3. I Study Case: Sensory characterization of a new sweet protein

Abstract

Sweet proteins are an attractive alternative to the most traditional sweeteners. This I study case focuses on the sensory characterization of MNEI, obtained by recombinant expression in cells of E. coli with high yields. Since MNEI is an High Intensity Sweeteners (HIS) it could be used in the beverage industry meeting the consumer needs about the low calorie content and the sweetness of beverage. Thus, the specific objectives of this study were, first, the determination of taste detection (DT) and recognition threshold (RT) of MNEI in different conditions typical of beverage processing and of beverage consumption, concerning mineral content, serving temperature of the product, pH and thermal treatment, and then to compare dynamic sensory performance of MNEI, with that of aspartame, saccharin and sucrose.

A selected panel evaluated DT and RT of MNEI by using a modified version of difference from Reference method. Through the modified method, assessors were also asked to give a qualitative information about the perceived taste, thus it was also possible to obtain the RT of MNEI.

Then, equi-sweet concentrations of different sweeteners were found through difference from Reference method, by using a selected panel. Next sweeteners, at those concentrations, were analysed thought time-Intensity (t-I) and Temporal Dominance of Sensation (TDS), to determinate sweetness evolution over time and the interaction between sweetness and other stimuli, respectively, by using a trained panel.

The results demonstrated that MNEI DT and RT were much lower than those of sucrose. Besides, both mineral content and serving temperature affected MNEI DT, whereas pH and thermal treatment did not affect perceived sweetness. The modified version of difference from Reference method was useful for RT determination.

Equi-sweetness values of MNEI and of three market sweeteners were determined. Time-Intensity results showed that MNEI differed from sucrose, meanwhile TDS results showed that, in presence of other stimuli, these differences were reduced The dominant attributes of model beverages were, first, strawberry flavour and, then, sweet, for all sweeteners, with some differences.

Keywords: sweet proteins, sweetness, detection thresholds, recognition thresholds, time intensity, Temporal Dominance of Sensation.

3.1. Introduction

Saccharides are usually associated with sweet taste, although some among them are bitter and no-sweet saccharides. Except for sucrose, the sweetness decreases with the number of monosaccharide units going toward oligo- and polysaccharides, because only one monosaccharide unit interacts with the mucoprotein of the tongue receptor. The quantum-mechanical treatment of sweetness, in terms of interaction of sweeteners with receptors, was given by Pietrzycki (2004). Many low-molecular weight saccharides are sweeteners. A key attributes that distinguishes sweeteners from other ingredients is their characteristic and pleasurable sweet taste and intensity. The gold standard for comparing sweetness is sucrose (Helstad, 2006).

Over the centuries, several hundreads among synthetic and natural molecules have been proven to be sweet, not only sugars but also, for example, amino acids, peptides, proteins, olefinic alcohols, nitroanilines, saccharin, chloroform and many other organic compounds (Temussi, 2006). Indeed some sweet compounds, after a short period of popularity, were apparently lost or forgotten, such as osladin, hernandulcin, phyllodulcin and other ones, as reported by Bassoli *et al.* (2011).

The sweet molecules can be divided by chemical characteristics, molecular weights and size. In addition, some of these sweeteners have the same bulk and texture as sucrose (Patra *et al*. 2009) and they are characterized by high sweetening power, low caloric power and a marked persistence of taste sensation. Many compounds have been found to be sweet and have no calories. The sugar substitutes, also known as replacers or alternative sweeteners, are both natural and artificial. Only a few are approved for use commercially. Some of these sweeteners are reflected in the terms used to describe them, such as non-nutritive, intense, high intensity, high potency, alternative and artificial sweeteners, sugar replacers, sugar substitutes and macronutrient substitutes.

Sweet proteins are an attractive alternative to some of the most traditional sweeteners, because they respond to the consumers need of low calorie products. However, sweet proteins do exist and are sweeter than sucrose and most non-caloric sweeteners. Some of them are indeed intensely sweet, orders of magnitude sweeter than sugar (Picone & Temussi, 2012).The intensely sweet protein best known are monellin, thaumatin, and brazzein. In addition, there is another sweet little protein, mabinlin and the other two proteins miraculin and curculin, that give a sweet taste when combined with acids and for this reason are considered “taste-modifying” proteins. Mechanism of interaction of sweet- proteins with the T1R2-T1R3 GPCR receptor is different from that of low molecular mass sweeteners **(**Temussi, 2006). In contrast with previous studies aimed to identify a “sweet finger” protruding from the protein surface, it has been recently proposed that sweet proteins activate the sweet receptor by a mechanism of interaction, called “wedge model” in which proteins fit a large cavity of the receptor with wedge-shaped surfaces of their structures (Temussi, 2002; Tancredi *et al*., 2004). Recently Temussi (2011) showed that the wedge model is also supported by experimental information.

Monellin was one of the first sweet proteins to be discovered, isolated from Dioscoreophllum cummninsii, by Inglett and May (1969). The sweetness of the monellin was quantified 3000 times higher (based on weight) and 90000 higher (on a molar basis) than sucrose. However Hung *et al.* (1999), reported a sweetness of monellin molecule 100000 times higher (on a molar basis) than sucrose. The monellin consists of two non-identical subunits, formed respectively by 42 and 50 amino acid residues, called A and B chains, linked by a secondary binding forces (Bohak & Li, 1976). This structural feature makes the native protein quite unstable with respect to the temperature increase, so that when heated above 50 °C monellin loses its sweetness. To overcome this drawback, that limits the potential applications of monellin as sweetener, two modified forms of monellin have been designed: the first is called SCM (Single chain monellin) where the C terminus of the B chain is directly united with the residue of the A-chain N-terminus (Kim *et al*.,1989), the second modified form, called MNEI is a construct of 96 amino acid residues engineered by linking, with a Gly-Phe dipeptide, chains B and A of monellin, so its molecular structure is similar to both native monellin and SCM (Spadaccini *et al.*, 2001), even if it is more stable than the native protein with respect to temperature and pH changes (Esposito *et al*., 2006) and can be described as an alpha helix inserted into a beta-sheet made-up by five antiparallel strands. This secondary structure element seems to play a specific role in orienting the protein during the binding process to sweetness receptors (De Simone *et al*., 2006).

High-intensity sweeteners provide sweetness with negligible calories, although the sensation of their sweetness is often different from that of sucrose. The sensory performance of a sweetener changes over time and, when added in beverages or food, interacts with other stimuli (taste, olfactory, texture). Dynamic sensory techniques consider that sensory perception is a process that evolves over time: the sensations perceived by assessors are constantly monitored to describe and quantify the continuous changes that occur at the receptor level for a given stimulus (Lawless and Heymann, 2010). The time intensity method (t-I) is used to obtain the temporal profile of an attribute in a certain product, monitoring, measuring and recording the perceived intensity of a given attribute over time. A growing number of studies have used TI methods for different reasons (Lawless and Heymann, 2010). Many studies were focused on taste and flavour sensation tracking, such as sweetness (Calvino & Garrido, 2000; Zamora *et al*., 1998; Palazzo *et al*., 2011), bitterness, salty taste (Drake and Drake, 2011). Also trigeminal and chemical/tactile sensation could be evaluated through this method, such as the oral heat from capsaicin in various meat products **(**Reinbach *et at*., 2007, 2009). Temporal release of flavour compounds was also studied through this technique (Chung *et al*., 2003; McGowan *et al*., 2006).

Temporal Dominance of Sensations method (TDS), during the phases of product evaluation, determines the dominant sensation or prevailing one over others. Different definitions of “dominant attribute” were made; for Pineau *et al.,* (2009) it was the attribute associated to the sensation catching the attention at a given time, Saint-Eve *et al.* (2011) defined it as the sensation that triggers the most attention during the eating process. For someone an attribute was per-

ceived as dominance if it was the most intense sensation at that moment (Albert *et al*., 2012) even though Pineau *et al.* (2009) said that the dominant attribute was not necessarily the one with the highest intensity. This method has been used to characterize different products, such as candies (Saint-Eve *et al.*, 2011; Deleris *et al*., 2011), fish sticks (Albert *et al*., 2012), wine (Meillon *et al.*, 2009; Sokolowski and Fischer, 2012), hot beverages (Le Reverende *et al.*, 2008), dairy products (Pineau *et al*., 2009), blackcurrant squashes (Ng *et al.*, 2012), breakfast cereals (Lenfant *et al*., 2009). Dinella *et al*. (2012) used TDS to assess the impact of two Italian extra-virgin olive oils with different sensory properties on the perceived sensory profiles of pureed beans and tomatoes.
This research focuses on the sensory characterization of MNEI, obtained by recombinant expression in cells of *E. coli* with high yields.
Thus, the specific objectives of this study were:

1. To find taste detection thresholds (DT) and recognition thresholds (RT) of MNEI, in different experimental conditions, typical of beverage industry, by using a modified version of difference from control test (DiffC). The following effects, on DT and RT were evaluated: mineral content, serving temperature of the product, pH and thermal treatment.
2. To compare dynamic sensory performance of MNEI, with that of aspartame, saccharin and sucrose. First equi-sweet concentrations were found and next sweeteners, at those concentrations, were analysed thought t-I and TDS, to determinate sweetness evolution over time and the interaction between sweetness and other stimuli, respectively.

3.2. Materials and Methods

3.2.1. Detection (DT) and Recognition (RT) Thresholds

3.2.1.1. Subjects

Fourteen assessors (7 males and 7 females) participated in the study; they were selected for having the same DT and RT for the basic tastes in order to avoid errors because of different thresholds (Lawless and Heymann, 2010). The mean age of the subjects was 36 (22-50) years. All were either students and employees at the University of Naples, Federico II. All the participants provided a verbal consent before the test. The analyses were performed in the Sensory Laboratory of The Department of Agriculture of the University of Naples, Federico II.

3.2.1.2. Materials

The single chain monellin (SCM) mutant MNEI has been produced in *E.coli* and purified following the protocol described in detail in Spadaccini *et al*. (2001), with an average yield of 50 mg/L. Having assessed the protein purity and folding by SDS-PAGE under non-reducing conditions and circular dichroism analysis, respectively, the sample was extensively dialyzed against double distilled water. Aliquots of 10 mg were prepared on the basis of UV absorbance at 280 nm, using a molar extinction coefficient of 1.29 (cm/mg/mL), lyophilized and stored at -20°C until use. The coefficient was calculated on the basis of the amino acid sequence, and it corresponds to a molar ratio of 14,770/M/cm, as reported in Morris and Cagan (1972).
Citric acid ("J.T. Baker", 1995, Denveter, Holland) was used as acidifying agent in MNEI solutions and to evaluate the sourness threshold during disguising tests.
Sodium chloride ("J.T. Baker", 0277, Denveter, Holland) was also used to evaluate the saltiness threshold during disguising tests.
Disguising tests were performed in order to avoid that assessors focalized their attention only on sweet taste recognition, making an expectation error.
Two different commercial waters were used: water A, mineral water with a low mineral content, water B, standard mineral water. The fixed residual parameter, index of the degree of mineralization, was equivalent to 22.3 mg/L for A and 420 mg/L for B. Water pH was 6.8 for A and 7 for B.

3.2.1.3. Preparation of MNEI protein solutions

A stock solution of 1 mg/mL of MNEI was prepared by dispersing the protein in water.
In order to determine the detection threshold, a wide range of concentrations was explored (Jellinek, 1985); a dilution factor of 1.3 was used to prepare, from stock solution, samples from to 0.84 mg/L to 9 mg/L of MNEI. The range of concentrations was chosen considering that the protein monellin was 3000 times sweeter than sucrose (Temussi, 2006) and that average recognition threshold of our judges for sucrose was 4 g/L. All the experimental conditions were shown in table 1.
In order to evaluate the effect of salt on thresholds, samples were prepared either with water A (E_1) and water B (E_2).
Two serving temperature were considered: 20°C (E_1) and 10°C (E_3).
Two different condition of pH were investigated: 6.8 (E_1) and 4.3 (E_4). Samples were acidified by adding citric acid at a concentration of 24 mg/L.
To study the effect of thermal treatment MNEI solutions, at pH 6.8 (E_5) and 3.8 (E_6), were heated up to 90°C, kept at 90°C for 15 minutes, then rapidly cooled up to 20°C. Samples were acidified by adding citric acid at a concentration of 50 mg/L. Both the concentrations of citric acid used were below the sour detection threshold of our judges (data are not shown).

Table 1: Experimental condition of MNEI solution evaluation

Experimental condition	water	pH	Thermal history	Serving temperature
E1	A	6.8	held at 20°C	20°C
E2	B	7.0	held at 20°C	20°C
E3	A	6.8	held at 10°C	10°C
E4	A	4.3	held at 20°C	20°C
E5	A	6.8	heated from 20 to 90°C - held at 90°C for 15 min - cooled from 90 to 20°.	20°C
E6	A	3.8	heated from 20 to 90°C - held at 90°C for 15 min - cooled from 90 to 20°C	20°C

3.2.1.4. Evaluation of MNEI thresholds

A modified version of *difference from Reference* method was used to determinate DT and RT of MNEI. Through the original method only the detection threshold (DT) may be evaluated (Lundahl *et al*., 1986; Brown *et al.*, 1978). Using the modified method, judge was also asked to give a qualitative information: "00", if he thought that the sample was water; "?", in the case he had not recognized the taste; the name of perceived taste, if he recognized it. Through this method, it was also possible to obtain the recognition threshold (RT) of MNEI.

During each session, the judges made two tests, one to determinate the thresholds of MNEI protein, and the other one, as disguising test, by using other stimuli, such as citric acid and sodium chloride. The differences from the reference were evaluated by using a 10cm linear scale, anchored at the extremes (0 = equal to R, 10 = extremely different from R).

For each experimental condition the presentation of the samples was carried out according to an incomplete block design: during each session, subjects compared a reference sample of pure water (R), with a hidden reference and two samples containing different concentrations of stimulus. The assessors received 10 mL of each sample, in plastic cups coded with three-digit random numbers. For each sample, assessors had to first taste the reference and then the sample. They were instructed to spit the sample out. Among two evaluations they had to rinse the mouth with water and wait 30 seconds. The different conditions were tested in the order as shown in table 1. Each sample was evaluated in duplicate.

3.2.1.5. Data analysis

For each assessor, the scores of all samples were corrected by subtracting the hidden references score. Corrected data have been submitted to the t-Student statistical test to evaluate if they were significantly different from zero (Lundhal *at al.*, 1986; Gonzàlez, Salvador & Martin-Alvarez, 1997). The general group detection threshold was calculated as the geometric mean of the highest undetected concentration and the lowest detected one.

The recognition threshold (RT) was determined by calculating the percentage of correct responses of sweet taste. The concentration at which 50% of subjects correctly recognized the sweet taste was defined as recognition threshold.

3.2.2. Dynamic Evaluation and Sensory Performance

3.2.2.1. Assessors

Different assessors participated in this study. A selected panel of 7 assessors (3 males and 4 females) participated in the first sensory test. The mean age of the assessors was 36 (22-50) years. The same assessors, excepted one female, took part to the time Intensity test. For the TDS test, other 4 assessors were selected, for a total of 10 assessors (6 males and 4 females). All of them were either students or employees at the University of Naples Federico II. All the participant provided a verbal consent before the test. The analysis were performed in the Sensory Laboratory of the Agricultural Department of the University of Naples, Federico II.

3.2.2.2. Stimuli

Four different sweeteners were used: sucrose (Eridania), saccharin sodium (BP, A.C.E.F. spa.), aspartame (granular aspartame PSL E951, Tillmanns spa.) and MNEI. All the solutions were prepared dissolving sweeteners in a mineral water (Sant'Anna), with a fixed residual parameter of 22.3 mg/L.

Citric acid ("J.T. Baker", 1995) as acidifying agent and strawberry flavour (Ingredients: water, propylene glycol, natural flavors D.L.107 25/01/92) were added to the water solutions to obtain model beverages.

3.2.2.3. Equisweetness determination

The range of concentrations was chosen on the basis of literature data: aspartame was 125-238 times (Swiader *et al*,

2009) and saccharin 300-500 times sweeter than sucrose (Spencer, 1971); Temussi (2006) reported the sweetness of monellin 3000 times higher than sucrose on weight basis. Thus, solutions of sucrose (30 g/L; 40 g/L), aspartame (0.18 g/L; 0.21 g/L), saccharin (0.08 g/L) and MNEI ($1.308x10^{-2}$ g/L; $0.981x10^{-2}$ g/L) were evaluated using a sip- and- spit method. The presentation of the samples was carried out according to a complete block design: during each session assessors compared a reference sample (R=40 mg/L sucrose) with five samples. Among samples a hidden reference could be present. The assessors received 10 mL of each sample, at room temperature, in plastic cups and coded with three-digit random numbers The assessors were asked to rinse well between each sample with water and wait at least 30 seconds between each sample. The intensity of sweetness was measured using a 10 cm linear graphic scale, anchored from 0 (much less sweet than reference) to 10 (much sweeter than reference) and 5 in the middle (equal to the reference).

3.2.2.4. Time intensity

The equisweet concentrations were evaluated by assessors in order to determine dynamic evolution of sweetness by means of method. Prior to participation in the experiment, the assessors took part into a training session to evaluate the sweetness intensity of the stimulus. Subsequently, the same panel took part in the experiment; five replicates were carried out, during which the samples were served in plastic cups and coded with three digit random codes. Assessors sipped an aliquot of sample, swirled it around the mouth and spat it after 5 seconds; the software showed a linear anchored scale from 0 (no perceived) to 10 (highest intensity) and the mouse cursor could slide freely so that the assessors could continuously indicate the intensity perceived over time. The Fizz software collected the position of the pointer along the scale every second during 60 second. A period of 90 seconds between two samples evaluation allowed for the assessors to clean their palate with water.

3.2.2.5. Temporal Dominance of Sensation

Four model beverages containing sweeteners at equisweet concentration of stimulus, 0.09g/L of citric acid and strawberry flavour (50µl/L) were prepared in order to evaluate the dominant attributes over time. The final pH was 3.2 which is very similar to common fruit juices, such as orange juice (pH 3.5-4) or apple juice (pH 3.5).
A training session of 60 minutes was initially dedicated to attribute generation; after tasting the sample, the assessors recorded the perceived attributes. The chosen attributes were those cited by at least 50% of assessors. Suddenly, three training sessions were conducted to familiarize assessors with TDS method. After training sessions, the real evaluation started.
The samples (10 mL) were always served in coded plastic cups, balanced and randomized. Also the order of attributes on the screen was randomized among all the assessors to minimize the effect due to the attribute sequence. The test duration was 100 seconds and a break of 90 seconds was done among two samples evaluation. Once the assessors had swallowed the product, they clicked on a start button on the screen to begin the evaluation. During an evaluation, the assessors had to select the attribute considered as dominant; when the dominant perception changed, the assessors had to score the new dominant sensation (Labbe *et al.*, 2009). Five replicates in two blocks were made.

3.2.2.6. Data analysis

All the data were obtained by FIZZ software (ver. 2.45 G, Biosystemes). The SPSS v.17 (SPSS statistics, Illinois) was used in order to determine the iso-sweet concentrations of the four sweeteners obtained from difference from control test and to analyze the parameters extracted from the curves. The dominance rate of the sensory attributes of each product evaluated by TDS, was calculated through the software FIZZ Calculation (ver. 2.45 G, Biosystemes).
One sample t-test was used in order to evaluate if sweetness intensity of each stimulus differed significantly from 5, score given to the reference sample (R= sucrose 40 g/L).
Several parameters extracted by each time-intensity curves were defined as follows: Istart: intensity of the first acquisition point of the curve, Iend: intensity of the last acquisition point of the curve, Imax: maximum intensity observed of the stimulus, tstart: computed start time, tend: computed end time, tspl: plateau start time, tepl: plateau end time, DurPl: duration of the plateau, DurInc: duration of the increasing phase, DurDec: duration of the decreasing phase, SIMInc: maximum slope measured in the increasing phase, SIMDec: maximum slope measured in the decreasing phase, AREA: total area under the curve, AreaInc: area under the increasing phase, AreaDec: area under the decreasing phase, AreaPl: area under the plateau. Average parameters (subject x replication) of each sweetener were submitted to paired t-test in order to find difference between MNEI and other sweeteners. Also, a simplified trapezoidal curve for each sweetener was constructed by connecting four point having time value, as x coordinate, and intensity value, as y coordinate (Lallemand *et al.*, 1999). The first point had these coordinates I_{start};T_{start}, the second I_{max},, $T_{startPL}$, the third I_{max},, T_{endPL}, and the last one I_{end}, T_{end}.
For each product, the proportion of runs, “n” (subject x replication) for which the given attribute was assessed as dominant was computed. These proportions, transformed through a smoothing B Spline (Fizz Calculator) were plotted against time and called TDS curves (Pineau et al.2009).
In order to get more insight into the TDS graphical display, two lines were drawn. The first one P_0, called “chance level”, was the dominant rate that an attribute can obtain by chance:

$$P_0 = \frac{1}{p} \qquad (3.1)$$

where p was the number of attributes.
The second one Ps, called "significance level", was the minimum value this proportion should equal to be considered as significantly higher than P_0 (a=0.05). It was calculated using the confidence interval of a binomial proportion based on a normal approximation:

$$P_S = P_0 + 1.645\sqrt{\frac{P_0(1-P_0)}{n}} \qquad (3.2)$$

When a confidence interval of a proportion based on a normal approximation is calculated, it is recommended that:

$$np(1-p) > 5 \qquad (3.3)$$

n being the number of trials and p the probability of success.

3.3. Results and Discussion

3.3.1. Detection and Recognition thresholds

Sweeteners are generally added to a food or beverage at a concentration that is higher than its DT and RT, but considering that this study was focused on a new sweet protein, produced in laboratory and in small quantity, it was chosen to evaluate the effect of experimental conditions, typical of beverage industry, on the lowest detectable MNEI concentrations. The detection (DT) and recognition (RT) thresholds of MNEI in different experimental conditions were listed in table 2.

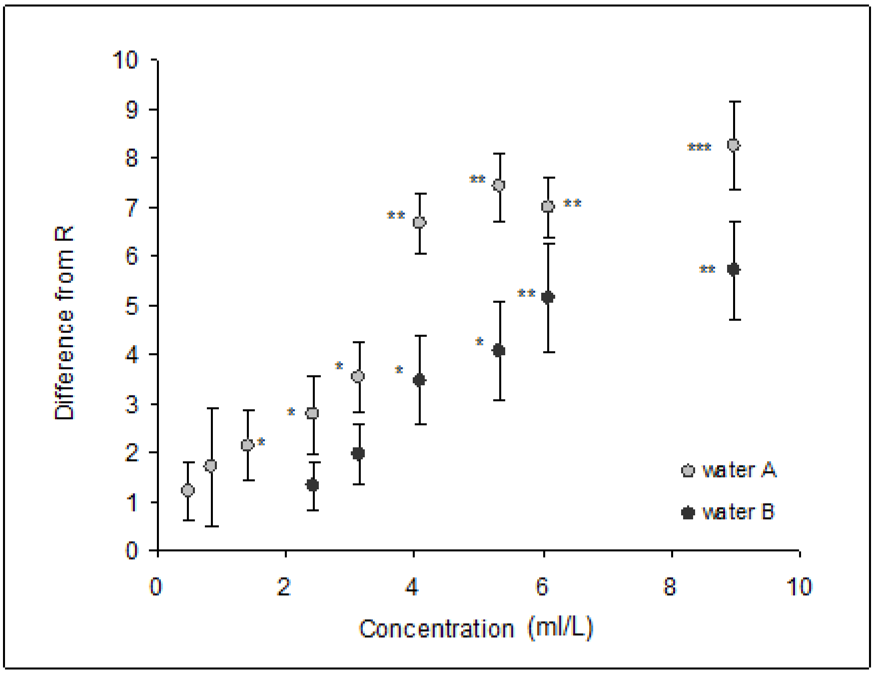
Table 2: Detection (DT) and recognition (RT) thresholds of MNEI in different experimental conditions

Experimental condition	DT	RT	Corrected answers (%)
E_1	1.10mg/L	1.43mg/L	50%
E_2	3.59mg/L	-	-
E_3	2.76mg/L	3.15mg/L	65%
E_4	1.10mg/L	1.43mg/L	75%
E_5	0.64mg/L	0.84mg/L	71%
E_6	0.64mg/L	1.43mg/L	83%

RT= MNEI concentration at which 50% of subjects correctly recognized the sweet taste

3.3.1.1. Mineral content effect

Salts are often placed in many beverages, such as soft drinks, so it is important to consider their effect on the sweetness performance. In order to study this effect on MNEI sensory perception, the E_1 and E_2 conditions were compared.
The average differences from reference for each MNEI concentration, both in the water A and B, are illustrated in the figure 1.
Experimental data showed that with increasing concentration of MNEI, the differences from reference increased too and at higher MNEI concentration, the percentage increase decreased. The samples prepared with water A presented higher differences from the reference than those prepared with water B, for all MNEI concentrations. At high MNEI concentrations, sensory scores became almost constant.
When the protein was diluted in the water A, the first significantly ($P<0.05$) different concentration of MNEI from reference was 1.43 mg/L, whereas in the water B the first significant difference was observed for the concentration of MNEI of 4.09 mg/L ($p<0.05$). Hence, the DT of MNEI in water A was 1.10 mg/l, while that in water B was 3.59 mg/l, i.e. the geometric mean values among the highest undetected and the lowest detected concentrations.
The RT of MNEI in the E_1 condition was 1.43mg/l, a concentration at which the 50% of assessors correctly recognized the sweet taste. The RT of MNEI evaluated in the condition E_2 was not calculated.



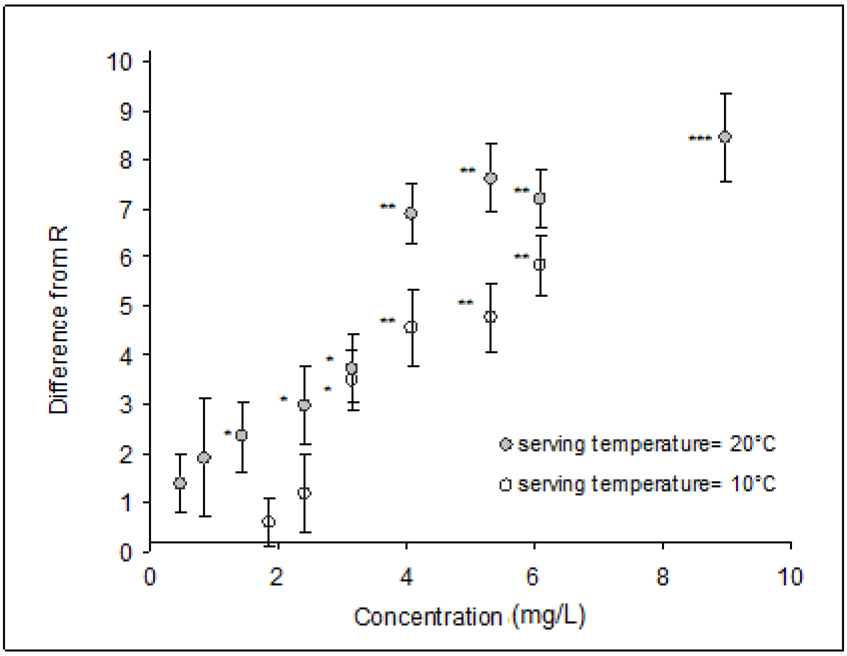
*= MNEI concentration significantly different from R (p≤0.05)
**= MNEI concentration significantly different from R (p≤0.01)
***= MNEI concentration significantly different from R (p≤0.001)

Figure 1. Mineral content effect: Average differences (±SE) from R, for each MNEI concentration.

As expected, mineral content significantly increase the detection threshold of MNEI. This could be due to the contemporary presence of different molecules that interact with the taste buds, determining the mixture suppression effect (McBurney and Bartoshuk, 1973). Recognition threshold of MNEI in water B was not calculated, although it is known that the recognition threshold of a stimulus is often a bit greater than the detection threshold of the same (Lawless & Heymann, 2010). What we found is in contrast with Schiffmann *et al.* results (2000) that showed no or very small effects of salts on perceived sweetness given by several different sweeteners. The disagreement among our results and Schiffmann *et al.* (2000) results, could be due to two main reasons. The first one is that we studied a sweetener which was not investigated by them. The second one is that we studied the salts effect, by using waters which differ in terms of fixed residual parameters meanwhile Schiffmann and co-workers added well-known and selected salt concentrations to the samples. In general, the interaction between sweet and salty compounds have not been widely examined (Lindley, 2006). Although the mixture suppression effect have been reported in mixture of sucrose and sodium chloride (Bartoshuk,1975), other researchers found that some salts at specific concentrations can cause enhancement of sweet taste (Birch, 1999; Kilcast *et al.*, 2000). The perceived effect on sweetness depends on the sweetener type concentration and on the type of salt. No literature was found on the effect of salts on sweet proteins, with except for Schiffmann *et al.* (2000) that studied thaumatin,

3.3.1.2. The serving temperature effect

Since we hypothesized the use of MNEI in a soft drink or, in general, in a beverage, it was necessary to ascertain whether serving temperature could affect both detection and recognition thresholds of MNEI.
In order to study the serving temperature effect on MNEI sensory perception, the E_1 and E_3 conditions were compared.
The average differences from R of each concentration of MNEI evaluated at the serving temperature of both 20˚C and 10˚C are illustrated in the figure 2.
The figure showed that after the first detected concentration, different percentage increases occurred in the two experimental conditions. Indeed, at the serving temperature of 10˚C, with increasing concentration of MNEI, the differences from the reference also increased. Meanwhile at the serving temperature of 20˚C, the percentage increase enhanced to a concentration 4mg/L MNEI and the sensory scores became almost constant.
When the MNEI was evaluated at the serving temperature of 20˚C, the first significantly (P<0.05) different concentration from the reference was 1.43 mg/L, whereas 3.15 mg/L was the first MNEI concentration that significantly differed from the reference (P<0.05) at the serving temperature of 10˚C. Thus, the DTs of MNEI were 1.10 mg/L and 1.76 mg/L for the serving temperature of 20˚C and 10˚C, respectively .
The RT of MNEI calculated at 10˚C, was 3.15 mg/L, a concentration corresponding to 65% of corrected answers.



*= MNEI concentration significantly different from R (p≤0.05)
**= MNEI concentration significantly different from R (p≤0.01)
***= MNEI concentration significantly different from R (p≤0.001)

Figure 2. Serving temperature effect: Average differences (±SE) from R, for each MNEI concentration.

Serving temperature may affect both DT and RT of a stimulus. Foods served at different temperatures display different properties, as found by Zellner *et al*. (1988). For example, the intensities of odour and flavour attributes increased with increasing product temperature for custard dessert as well as for mayonnaise (Engelen *et al*., 2003). Further, the findings of Drake *et al*. (2005) showed the same effect of serving temperature on sour taste intensity of Cheddar cheese. In our case, lowering the tasting temperature, thresholds of MNEI increased. This result was in accordance with Ross and Weller (2008) who found that sweetness and acidity intensities of white wines were significantly influenced by the interaction between the assessors and serving temperature., This result also agreed with those ofSchiffmann *et al*. (2000), by which temperature affected perceived sweetness intensities, for the most part of investigated sweeteners, when they were added at the lowest concentrations.

Bartoshuck et al. (1982) demonstrated that sucrose sweetness depended on temperature as well as the concentration of solution. Relatively low concentrations gain sweetness at the temperature increased, this effect diminished with progressively higher concentration and finally became negligible at about 0.5M. Fry et al. (2011) showed that Rebiana, a zero calorie, natural HPS, derived from *Stevia rebaudiana*, was more potent in the cold than at room temperature, an important practical consideration for the formulation of products using it and intended to be consumed refrigerated.

3.3.1.3. The pH effect

The high intensity sweeteners are mono-functional ingredients, determining only sweetness of the foods and beverages in which they are added. In these circumstances, the sweetness potentiation or inhibition of HISs derived by acids may have important commercial implication, since HISs are often used in beverages that have pronounced acid taste.

In order to study the pH effect on MNEI sensory perception, the E_1 and E_4 conditions were compared.

The effect of the pH on the DT of MNEI is illustrated in the figure 3, as the average differences from R of each concentration of MNEI determined at a pH of both 6.8 and 4.3.

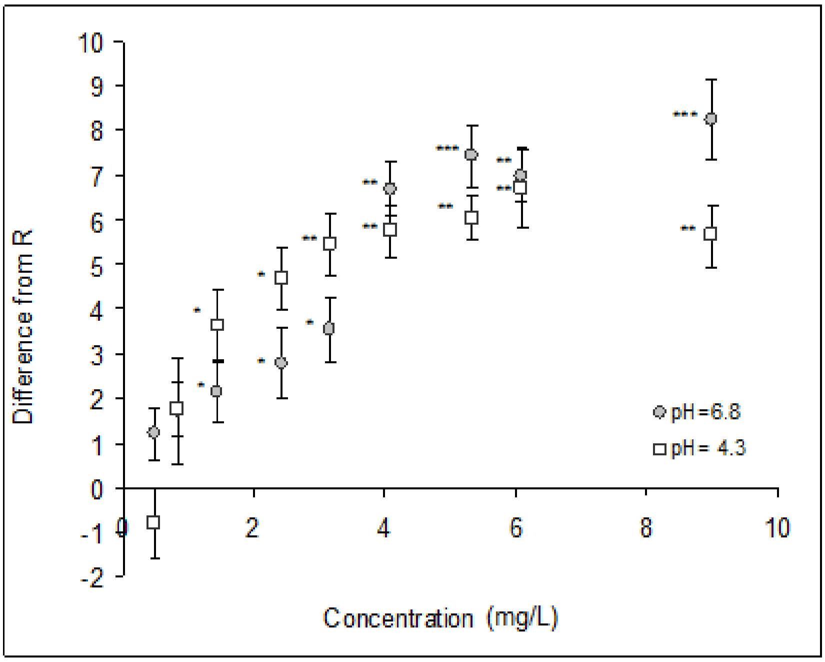
Figure 3 shows that, after DT values, at low MNEI concentrations, the percentage increase of samples at pH 4.3 was higher than that of samples at pH 6.8; whereas at high MNEI concentrations, the contrary occurred.

The first concentration of MNEI found significantly different from reference ($P<0.05$) was 1.43 mg/L for both the pH 6.8 and the pH 4.3. Thus, the DT of MNEI was 1.10 mg/L in both the pH conditions. At the highest MNEI concentrations, samples at pH 4.3 showed greater differences from reference than the samples at pH 6.8

The RT was 1.43 mg/L with the 50% and 75% of corrected answers for MNEI at pH 6.8 and 4.3, respectively.

There is very little published literature on the influence of food acids on the sweet taste delivered by high intensity sweeteners and their blends. Indeed, different food acids exhibit different sensory properties. For example, malic acid is marketed as delivering a longer-lasting acid taste than that of citric acid. This difference is claimed to make malic acid more ideally suited for use with high potency sweeteners, particularly with those sweeteners that deliver longer-lasting sweet aftertastes (Lindley, 2006). Shamil (1998) identified tannic acid compounds as additives that have the capability to reduce the lingering sweet aftertaste of sucralose, for the “mixture suppression” sensory phenomenon. Prakash *et al.* (2001) found that cinnamic acid reduced the duration of sweetness of neotame, even though in this case the effects are

due to general taste modification rather than sweetener-acid interactions.
In the most part of the studies, as well as in the present one, the sweetener performance was examined in citric acid buffers (Lindley, 2006). Our results showed that MNEI sweetness was not affected by pH changes, since detection and recognition thresholds did not change in different pH conditions. This confirms the stability of MNEI at acidic pH, already highlighted by Esposito *et al.* (2006). This was also in accordance with Cardello *et al.* results (1999) that showed no difference in sweetness intensities of some sweeteners in different pH conditions (3.0 and 7.0). Our results could be also explained considering the buffering capacity of $NaHCo_3$ of saliva. In fact, when one tastes a food, he produces saliva and this phenomenon depends also on the tested amount (Bradley and Beider, 2003).

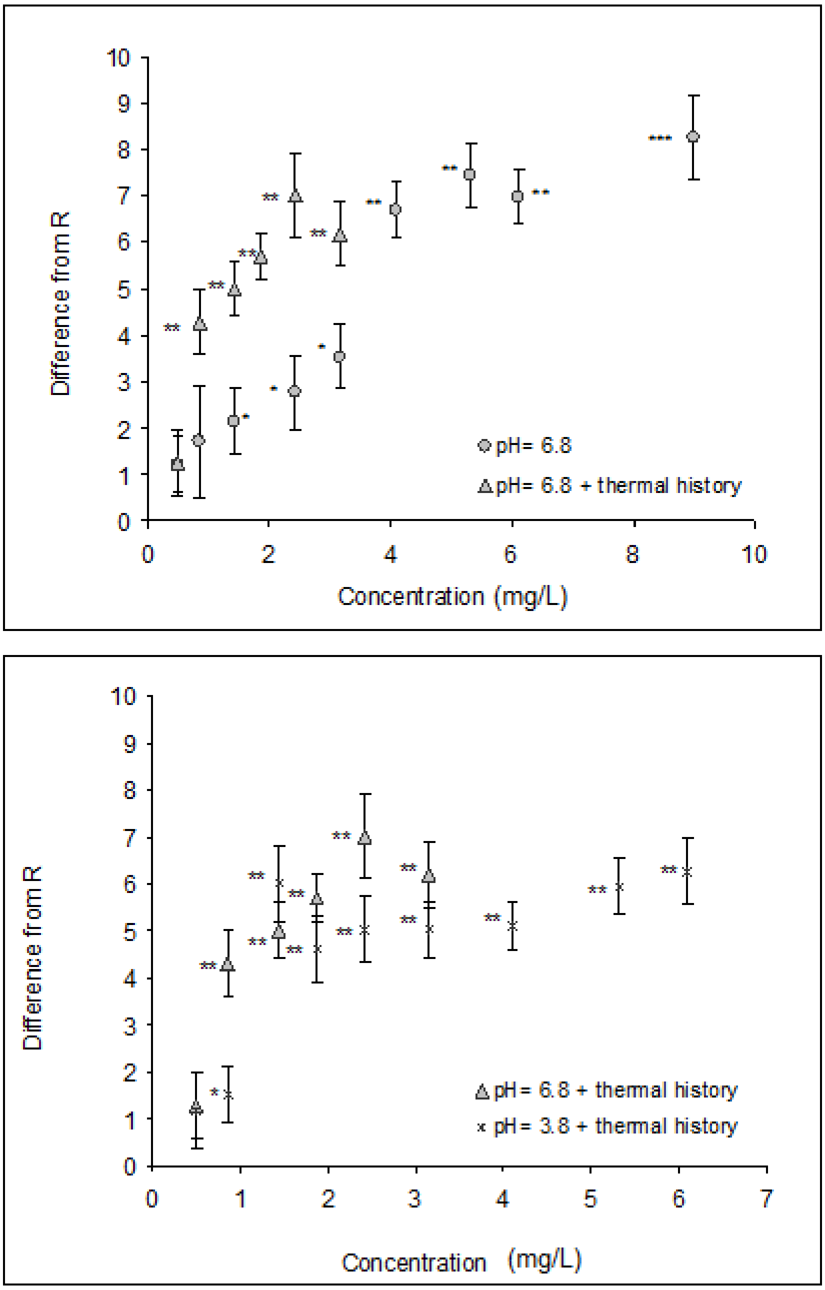


*= MNEI concentration significantly different from R (p≤0.05)
**= MNEI concentration significantly different from R (p≤0.01)
***= MNEI concentration significantly different from R (p≤0.001)

Figure 3. pH effect: Average differences (±SE) from R, for each MNEI concentration.

3.3.1.4. The thermal treatment and combined pH effects

In order to study the thermal treatment effect on MNEI sensory perception, the E_1 and E_5 conditions were compared.
The effect of thermal treatment on DT of MNEI is illustrated in figure 4a.
When the assessors evaluated the sweetener after it was submitted to thermal treatment (E_5), the 0.84 mg/L was the first concentration of MNEI significantly different from reference (p<0.05), corresponding to a DT equal to 0.64 mg/L, concentration lower than that corresponding to the E_1 condition (1.10 mg/L). Samples submitted to thermal treatment, already at low MNEI concentration, presented differences from reference greater than untreated samples. Hence, the percentage increase of the samples submitted to thermal treatment was higher than the other one, in the same range of MNEI concentration. The RT of MNEI in E_5 condition was 0.84 mg/L corresponding to 71% of corrected answers (Table 2). These results could be due to an adaptation error of the assessors involved, considering that the samples submitted to thermal history were evaluated in the last part of the study.
In order to study the combined effect of thermal treatment and pH on MNEI sensory perception, the E_5 and E_6 conditions were compared.
The effect of the thermal treatment at different levels of pH (6.8 and 3.8) on the DT of MNEI, is illustrated in the figure 4b as the average difference from R of each concentration of MNEI. The first concentration of MNEI found to be significantly different from reference (P<0.05) was 0.84mg/l for both experimental conditions. Thus, the DT of MNEI was 0.64 mg/L in both cases. A similar percentage increase occurred, even though at pH 6.8even though at pH 6.8 the first detected concentration presented higher differences from reference than the same at pH 3.8. The RT of MNEI in E_6 condition was 1.43 mg/L corresponding to 83% of corrected answers (Table 2).



*= MNEI concentration significantly different from R ($p \leq 0.05$)
**= MNEI concentration significantly different from R ($p \leq 0.01$)
***= MNEI concentration significantly different from R ($p \leq 0.001$)

Figure 4. Combined effect of thermal history and pH: Average differences (±SE) from R, for each MNEI concentration.

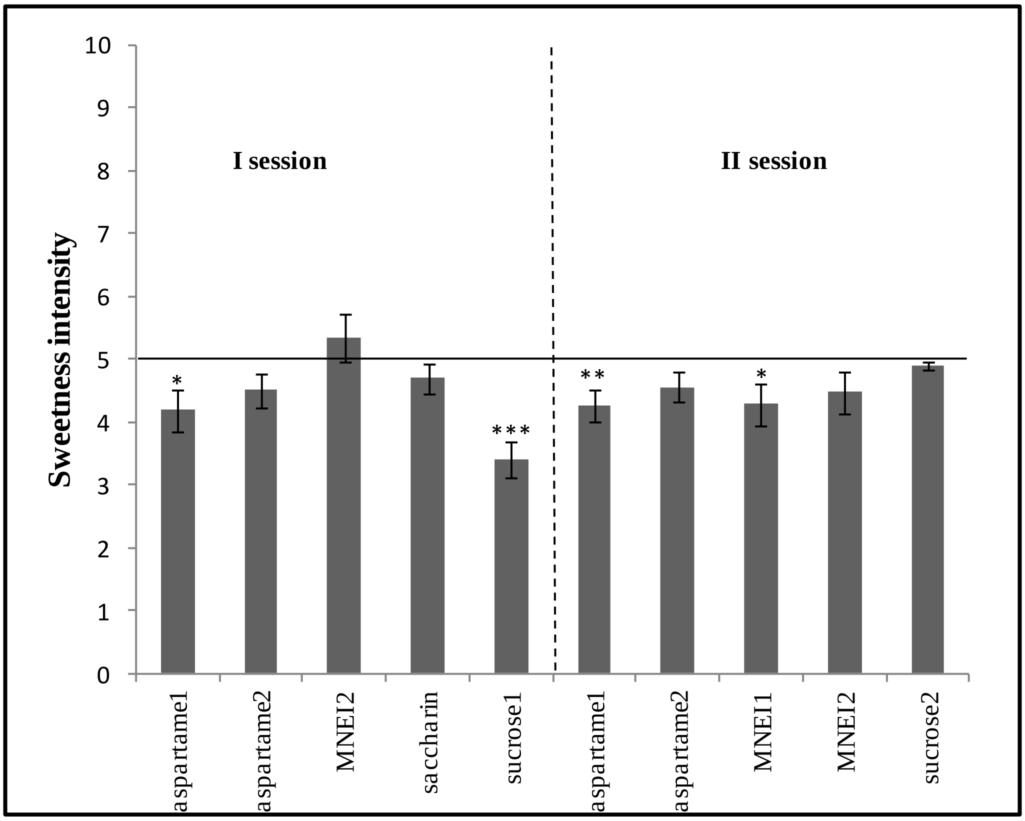
As regards the combined effect of pH and thermal treatment, this significantly affected only the recognition threshold of MNEI, but not the detection one, confirming the stability of protein at acid pH. Indeed, only the first detected concentration of MNEI were significantly judged differently under the two conditions.

3.3.2. Dynamic Evaluation and Sensory Performance

3.3.2.1. Isosweetness

The results of *Difference from control test*, the method used to determinate the isosweet concentrations of, aspartame, mnei,saccharin and sucrose are given in figure 5

On the graph, the horizontal line corresponding to the value of 5 on the 10 cm scale, indicates the intensity of the reference sample (sucrose 40 g/L). The results obtained in the first session showed that saccharin (0.08 g/L), MNEI 2 (1.308×10^{-2}) and aspartame 2 (0.21 g/L) were not significantly different from reference; instead sucrose (30 g/L) and aspartame 1 (0.18 g/L) were significantly less sweet than Reference ($p \leq 0.05$).



*= sweetener concentration significantly different from R (p≤0.05)
**= sweetener concentration significantly different from R (p≤0.01)
***= sweetener concentration significantly different from R (p≤0.001)

Figure 5. Equisweetness determination results

In the second session a lower concentration of MNEI (MNEI 1 = $0.981x10^{-2}$) was used in order to verify if a small reduction in concentration was perceived; also a hidden reference and two concentrations of aspartame were tested, to confirm the previous results,. Sodium saccharin was not revived because the isosweet concentration was lower than other found in literature. It was worth noting that the isosweet concentration of Saccharin was lower than other data in the literature; Swiader *et al*. (2009), in fact, found that the sweetness equivalence corresponding to 40 g/L of sucrose was 0.21 g/L, instead, Tunaley *et al*. (1987) reported a concentration of 0.143 g/L. For a cyclamate/saccharin blend, Moraes & Bolini (2009) in instant coffee beverage at 9.5% of sucrose found the isosweet concentration of 0.14 g/l, Cardello *et al.* (1999) at 3% of sucrose a sweetness equivalency of 0.01034 g/l. Reis *et al.* (2011) showed that the sweetness concentration equivalent to strawberry yoghurt sweetened with 115 g/Kg was 0.43 g/kg. In this study, instead, saccharin was 500 times sweeter than sucrose, in accordance to Spencer (1971).
The results for aspartame in the two session were the same, i.e. the less concentration was that differed from the reference, although the equisweet concentrations were slightly lower than other studies. Tunaley *et al* (1987) and Swiader *et al*. (2009) reported that the isosweet concentration corresponding to 40 g/L of sucrose was 0.37 g/L and 0.3 g/L respectively; Reis *et al*. (2010) at 115 g/L of sucrose found a sweetness equivalency of 0.72 g/Kg. According to this study, Moraes & Bolini (2009) identified at 39.1 g/L of sucrose, an isosweet concentration of 0.21 g/L. In this study aspartame was 190 times sweeter than sucrose, according to Swiader *et al*. (2009).
Comparing the concentrations of MNEI to equivalent 40g/L sucrose concentration equaling to, $1.308x10^{-}2$ g/L was the concentration found to be equal to the reference; thus MNEI had a sweetness potency of 3000 times higher than sucrose. There are not other studies about MNEI in order to make a comparison, but there are several studies on Monellin. Inglett & May (1969) reported that monellin was 3000 times sweeter than sucrose on weight basis and 9000 times on molar basis while Hung *et al*., (1999) 100000 times higher on molar basis.
Differences may be determined by several factors such as the intensity of sweeteners used as reference, temperature, the number of subjects. (Pangborn, 1963). There were also different sensory methods used to examined the sweetness potency of sweeteners, such as paired comparison (Yamaguchi *et al*., 1970; Hyvonen *et al*., 1977), magnitude estimation (Stone & Oliver, 1969; Moskowitz, 1970), time Intensity (Palazzo *et al*., 2011, Calvino & Garrido, 1998). The used methodology to determine the isosweetness might contribute to reduce or increase the differences.

3.3.2.2. Time Intensity

The sweeteners, at the concentrations found equisweet to 40g/L of sucrose, were characterized through dynamic sensory

tests.
Each assessor displayed an unique T-I curve (Figure 6) and the variability may be due to the individuality in moving the tongue or salivary flow; these factors influence the perception of the intensity and persistence of a sensation and In figure 6 the curves relative to the four sweeteners of judge were reported. The large degree of differences observed among individual judges was also found for sweeteners in solutions (Calvino *et al*.,2000; Zamora *et al*., 1998) and in other products, as chewing gum (Mc Gowan & Lee, 2006). Indeed, by considering also that replications could be source of variability (Lawless & Heyman, 2010), the parameters extracted from the single t-I curve of each sweetener, of each judge, of each replication, was averaged for sweetener were analyzed in order to test differences between MNEI performance and other sweeteners.

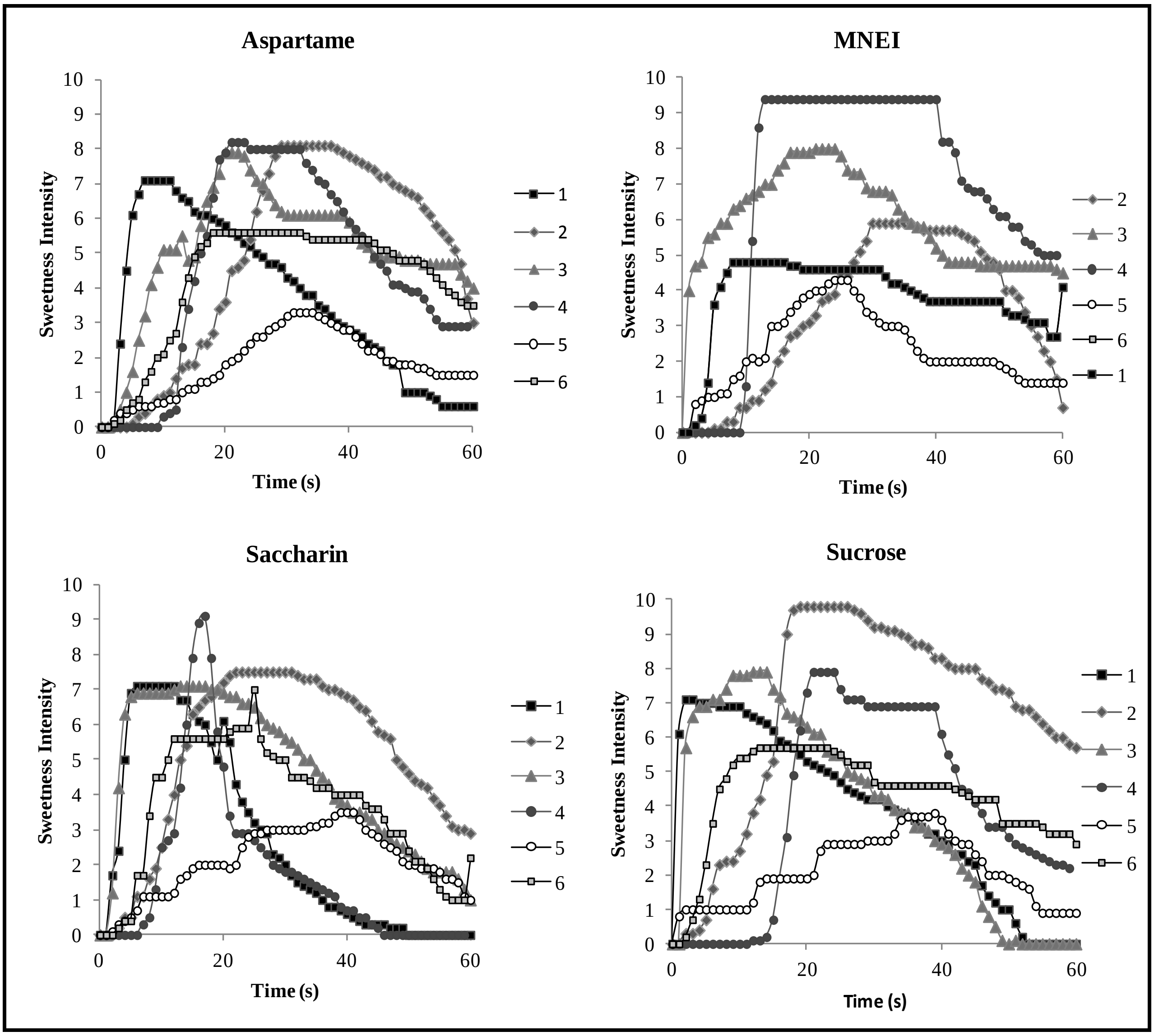


Figure 6. Raw t-I curves of each judge during replication 3

Paired t-test results showed that MNEI differed from other sweeteners for some of extracted parameters, as listed in the tables 3-5. By using the average parameters extracted from the t-I curves, simplified trapezoidal curve (Lallemand *et al.*, 2005) for each sweetener was constructed, as shown in the figure 7-9. Standard error was also reported for each coordinate.
Figure 7 shows that the intensity of sweet taste given by MNEI differed from that given by sucrose, for what concern Tstart and Tend, indicating a delay in sweetness detection for MNEI. Sucrose and MNEI differed also for TSpl, TePl, DurPl, in fact sweetness imparted by MNEI achieved the maximum intensity later than sucrose and this sensation persisted at maximum intensity for a longer time. They were different also for AreaDec parameter.
In figure 8 sweetness evolutions of MNEI and aspartame are compared. This figure shows that MNEI and Aspartame had a temporal profile similar to each other. Results of paired t-test between MNEI and aspartame indicated that they differed for parameters related to the decreasing phase of the sensation.
For what concern the last comparison, MNEI-saccharin (figure 9), they were significantly different for Iend, which also determined a different SIMDec ; in fact the intensity MNEI sweetness decreased more slowly than saccharin and at the final time, sweetness of MNEI remained at a high intensity, about 3 on the 10 cm scale.

Lastly, there were no significant differences among sweeteners regarding area total or intensity (tables 3-5), in accordance with equisweetness results.

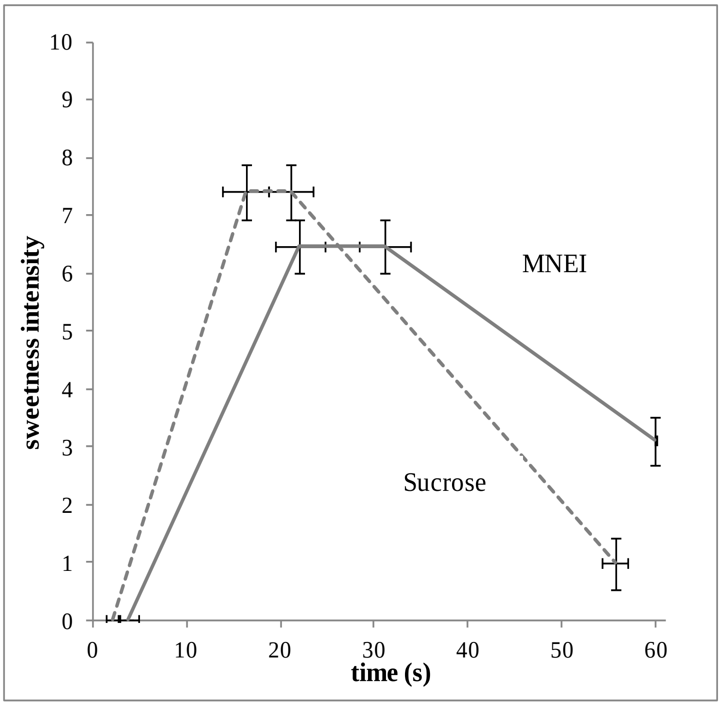


Figure 7. Average Trapezoidal curve (±SE of mean for each coordinate) of sucrose and MNEI

Table 3. Paired t-test between MNEI and sucrose

	MNEI	Sucrose	p
Iend	2.42	1.46	0.121
Tstart	5.11	2.00	0.027
Tend	59.89	56.22	0.015
IMax	5.93	7.08	0.092
TSPl	24.94	16.67	0.025
TEPl	32.94	20.72	0.002
DurPl	8.00	4.06	0.018
DurInc	19.83	14.67	0.145
DurDec	25.22	36.31	0.009
SlMInc	1.42	2.34	0.076
SlMDec	0.51	0.74	0.099
AREA	228.11	239.61	0.690
AreaInc	61.56	48.78	0.300
AreaDec	112.89	170.56	0.032
AreaPl	49.56	29.72	0.140

p values less than 0.05 indicate significantly differences between sweeteners.

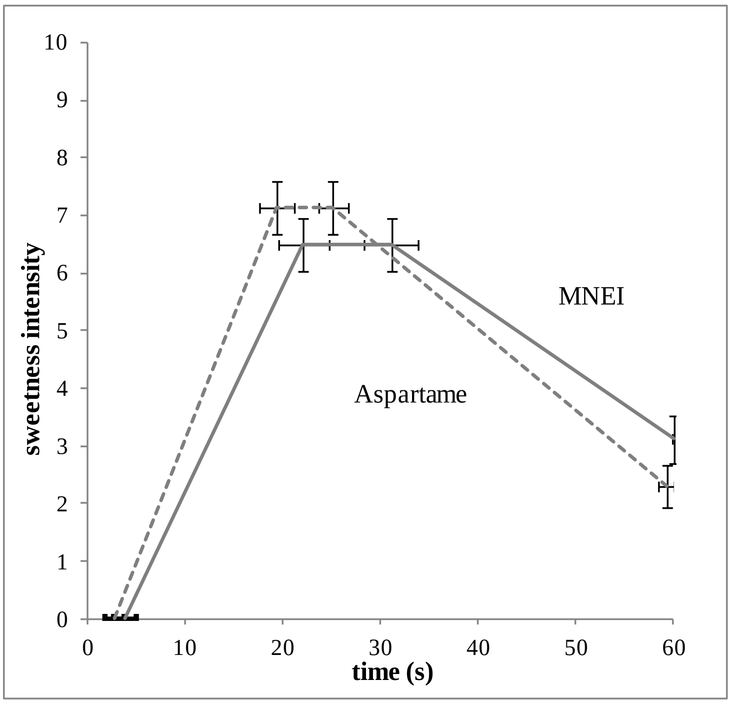


Figure 8. Average Trapezoidal curve (±SE of mean for each coordinate) of aspartame and MNEI

Table 4. Results of paired t-test between MNEI and aspartame

	MNEI	Aspartame	p
Iend	2.42	2.12	0.595
Tstart	5.11	2.78	0.096
Tend	59.89	59.06	0.329
IMax	5.93	7.03	0.099
TSPl	24.94	19.06	0.072
TEPl	32.94	24.83	0.015
DurPl	8.00	5.78	0.241
DurInc	19.83	16.12	0.244
DurDec	25.22	34.28	0.012
SlMInc	1.42	1.76	0.406
SlMDec	0.51	0.56	0.638
AREA	228.11	255.44	0.303
AreaInc	61.56	51.00	0.368
AreaDec	112.89	162.33	0.031
AreaPl	49.56	40.06	0.535

p values less than 0.05 indicate significantly differences between sweeteners.

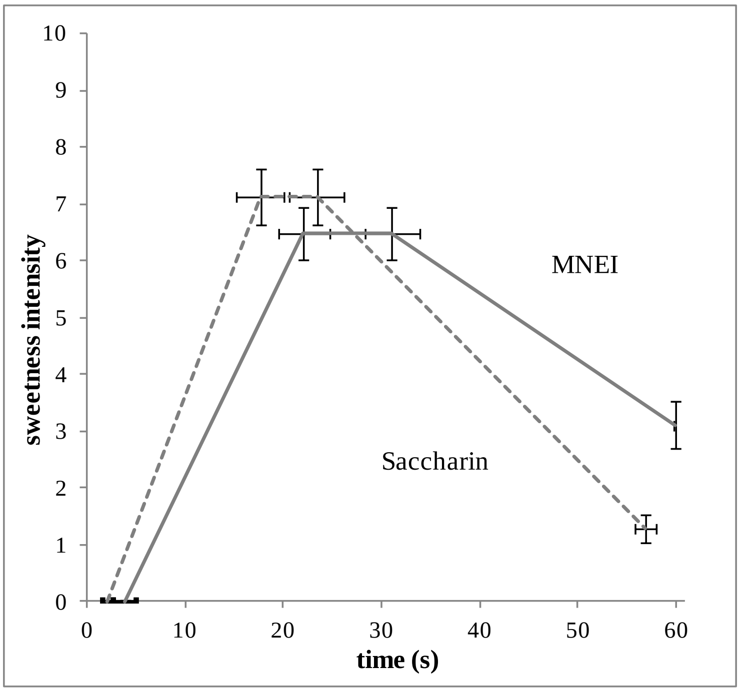


Figure 9. Average Trapezoidal curve (±SE of mean for each coordinate) of saccharin and MNEI

Table 5. Results of paired t-test between MNEI and saccharin

	MNEI	Saccharin	p
Iend	2.42	1.26	0.023
Tstart	5.11	2.28	0.033
Tend	59.89	58.06	0.116
IMax	5.93	6.93	0.146
TSPl	24.94	20.17	0.186
TEPl	32.94	25.22	0.056
DurPl	8.00	5.06	0.099
DurInc	19.83	17.18	0.437
DurDec	25.22	32.65	0.076
SlMInc	1.42	1.95	0.285
SlMDec	0.51	0.79	0.03
AREA	228.11	225.17	0.907
AreaInc	61.56	61.41	0.991
AreaDec	112.89	133.82	0.357
AreaPl	49.56	34.28	0.288

p values less than 0.05 indicate significantly differences between sweeteners.

3.3.2.3. Temporal Dominance of Sensation

The attributes selected were: bitterness, metallic, salty, sourness, strawberry flavor and sweetness.
Thus for the number of attributes chosen (p=6) chance level (P_0) and significance level (P_s) , calculated using the equation 3.1 and 3.2, were respectively 0.16 and 25,.
Figure 10 shows the TDS graphs for the four model beverage; each curve represent the evolution of the dominance rate of each attribute over time. The panel dominance rate represents the percentage of judges who recognize an attribute as dominant at a given time.
The dominant attributes in all the curves were sweet and strawberry flavor. Acid taste was dominant only for saccharin and aspartame in small part. The other attributes were never dominant and ever lower than chance level (P_0). The sweet panel dominance curves did not differ one from each other beverages, except for the aspartame.
This dominance rate of sweetness given by judges to aspartame was strongly higher (67%) than the significance level, then there was a high consensus of the panel according sweet taste from 5 to 68 seconds for this sweetener. The other dominant attribute for this product was strawberry flavour, from 1 to 12, with a maximum dominance rate at 4 s; sour was dominant just at 13 s.
For sucrose, the first dominant attribute was strawberry flavour, from 1 to 18 and from 23 to 33 s with a maximum dominance rate at the beginning, and sweet, from 2 to 55 seconds with a maximum dominance rate at 44 seconds. The other attributes were never dominant.
Dominant attributes for Saccharin were strawberry flavour, which was also the most dominant, from the beginning to 18 seconds, with a maximum panel dominance rate of 47% at 5 seconds, sweet, which was also dominant, except a small period of time, from 14 to 60 seconds, and sour, which was dominant at 18 seconds.
For MNEI, sweet and strawberry flavour were both dominant, but sweet was mostly dominant at the beginning and from 10 to 60 seconds. Strawberry flavour was the most dominant at 5 seconds but has never been more dominant for the rest of the evaluation.
The presence of sucrose and strawberry flavor might mask the perception of acid taste, or ensure that this attribute was never dominant.

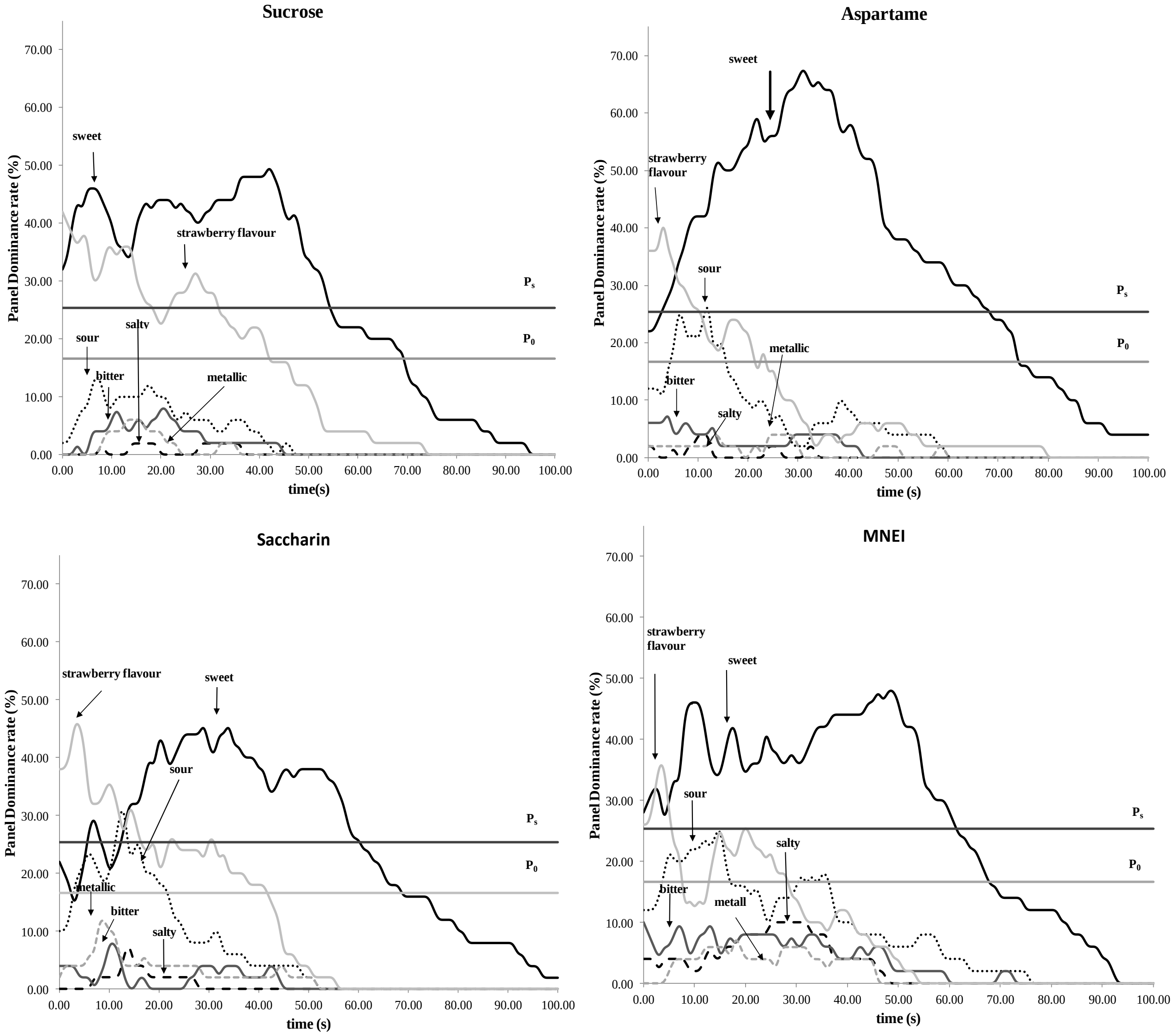


Figure 10. TDS graphical representation for model beverages containing different sweeteners

In order to compare results of t-I and TDS for what concern the sweetness evolution, in the next figures (11-12-13) only the sweetness dominance rates of MNEI versus the other sweeteners are reported.

Figure 11 shows that MNEI and sucrose presented a similar evolution of dominance rate for sweet attribute, in fact initial time and ending time were almost similar one to each other, so, also duration of dominance was similar, but they differed only for dominance rate.

Also aspartame and MNEI presented the same times and durations of dominance, even if aspartame showed a higher panel dominance (fig 12).

Meanwhile the main differences were observed between saccharin and MNEI, in fact the attribute sweetness for saccharin became later dominant than for MNEI and presented a smaller dominance duration (fig 13).

These results suggested that in a beverage model, containing different stimuli, the sweetness imparted by MNEI was almost similar to the one given by sucrose and aspartame. Meanwhile from t-I results, MNEI resulted more similar to aspartame for the evolution of the sweetness over time, and very different from sucrose. The obtained results were not in contrast each other, but complementary, because underlined that in distillated water the sweetness imparted by MNEI was perceived later comparison with that provided by sucrose and evolved in a different way, maybe due to their different mechanism of interaction with taste receptors (Temussi, 2011), but in presence of other stimuli, it was very difficult to find these differences.

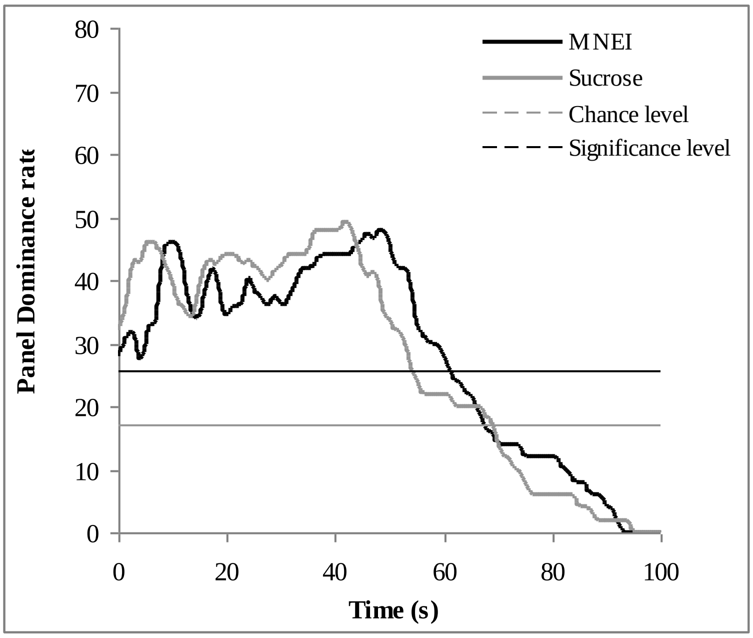


Figure 11. Sweetness dominance rate for two beverages respectively containing MNEI and sucrose

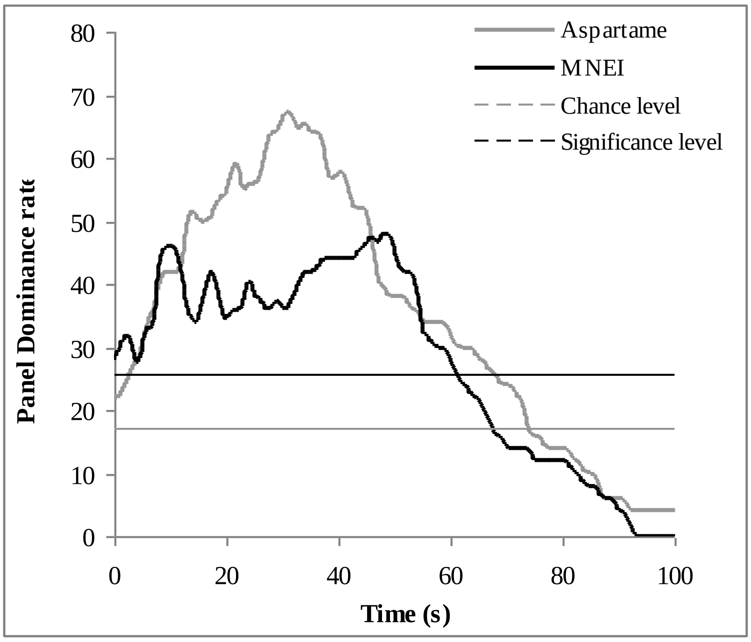


Figure 12. Sweetness dominance rate for two beverages respectively containing MNEI and aspartame

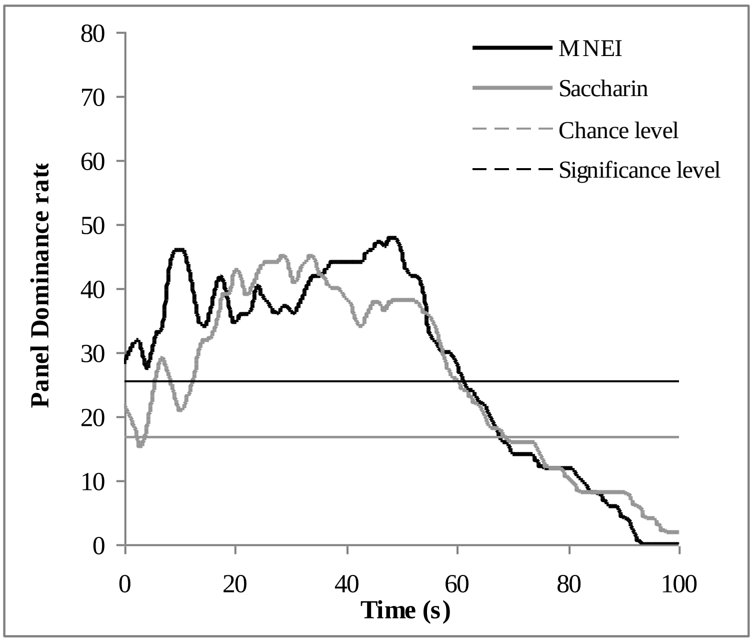


Figure 13. Sweetness dominance rate for two beverages respectively containing MNEI and saccharin

3.4. Conclusions

Detection and recognition thresholds of sweet protein MNEI were found. Different factors may affect both thresholds. We studied only the effect of conditions that may concern beverages production and consumption. Salts are often placed in many beverages, for example soft drinks, so it is important to consider an effect of salts on the sweetness performance. In our case, the concentration of salts negatively affects both detection and recognition thresholds of MNEI protein.Thus, further studies should be focused on the effect of selected salts on detection and recognition thresholds of MNEI, in order to verify and to clarify our results.

Both detection and recognition thresholds increase as the serving temperature decreases

Our results showed that MNEI sweetness is not affected by pH changes, since detection and recognition thresholds do not change in different pH conditions. This confirmed the stability of MNEI at acidic pH, already highlighted.

Detection and recognition thresholds are affected by thermal treatment, even though MNEI is more stable than the native protein with respect to temperature and pH changes (Esposito *et al*., 2006); in particular, when the MNEI protein was submitted to thermal treatment, detection and recognition thresholds of judges decreased.

Dynamic evaluation of sweeteners is a useful tool to obtain information about the sensory performance of sweeteners studied, in particular of MNEI that was unknown in literature. Considering that the average t-I curves of sweetness imparted by MNEI and aspartame, a HIS very used in a lot of foodstuff, were quite similar to each other and the dominant attributes of model beverages, containing also flavour and sour stimuli, were almost the same for all the sweeteners studied, with small differences, this modified new protein MNEI could be used as sugar substitute in different foodstuff, even if other studies should be employed to verify its efficacy also in more complex foodstuffs, where e.g. sugar plays a role on the structure of the final product too.

Indeed TDS is a very interesting sensory technique that gives information concerning what really happens if you taste a product. TDS method is also a valuable method, since it requires a small number of judges and a small training.

T-I and TDS are both dynamic sensory techniques but give different complementary information that could help to better explain the sensory performance over time of a stimulus, considering that it is important not only to know how the perception of a single stimulus change during its evaluation but also how it could interact with other stimuli that are included in a food.

3.5. References

Albert A, Salvador A, Schlich P, Fiszman S 2012 Comparison between temporal dominance of sensations (TDS) and key-attribute sensory profiling for evaluating solid food with contrasting textural layers: Fish sticks. *Food Quality and Preference* 24:111-118.

Bartoshuk LM 1975 Taste mixtures: is mixture suppression related to compression? *Physiology & Behavior*. 14: 643-649.

Bartoshuk LM, Rennert K, Rodin J, Stevens JC. 1982. Effect of temperature on the perceive sweetness of sucrose. *Physiology & Behavior*. 28: 905-910.

Birch GG 1999 Modulation of sweet taste. *BioFactors* 9: 73-80.

Bohak Z, Li SL 1976 The structure of monellin and its relation to the sweetness of the protein. *Biochimica et Biophysica Acta* 427: 153-170.

Bradley Z & Li SL Saliva: Its role in taste function. In *Handbook of Olfactory and Gustation,* Second Edition Revised and Expanded, Doty RL, ed., Marcel Dekker, Inc, New York (USA) 2003, pp. 1054-1071

Brown DGW, Clopperton JF, Meilengard MC, Mall M 1978 Flavour Thresholds of added substances. *Journal of the American Society of Brewing Chemists* 36: 73-80.

Calvino A & Garrido D 2000 Potency of sweetness of Aspartame, D-Tryptophan and Thaumatin evaluated by single value and time-intensity measurements. *Journal of Sensory Studies* 15:47-64.

Cardello HM, Da Silva MA, Damasio MH 1999 Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. *Plant Foods for Human Nutrition* 54(2):119-130.

Chung SJ, Heymann H, and Grün IU 2003 Temporal Release of Flavor Compounds from Low-fat and High-fat Ice Cream During Eating. *Journal of Food Science* 68 (6): 2150-2156.

De Simone A, Spadaccini R, Temussi PA, Fraternali F 2006 Toward the understanding of MNEI next term sweetness from hydration map surfaces. *Biophysical Journal* 90: 3052-3061.

Déléris I, Saint-Eve A, Dakowski F, Sémon E, Le Quéré JL, Guillemin H, Souchon I 2011 The dynamics of aroma release during consumption of candies of different structures, and relationship with temporal perception. *Food Chemistry* 127: 1615-1624.

Dinnella C, Masi C, Zoboli G, Monteleone E 2012 Sensory functionality of extra virgin olive oil in vegetable foods assessed by Temporal Dominance of Sensations and Descriptive Analysis. *Food Quality and Preference* 26: 141-150.

Drake MA, Yates MD, Gerard PD 2005 Impact of serving temperature on trained panel perception of cheddar cheese flavor attributes. *Journal of Sensory Studies* 20: 147–155.

Drake SL & Drake MA 2011 Comparison of salty taste and time intensity of sea and land salts from around the world. *Journal of Sensory Studies* 26: 25–34.

Duizer LM, Bloom K & Findlay CJ 1997 Dual-attribute time-intensity sensory evaluation: a new method for temporal measurement of sensory perceptions. *Food Quality and Preference* 4: 261-269.

Engelen EL, De Wijka RA, Prinz JF, Anke M, Janssen AM, Weenen H, Bosman F 2003 The effect of oral and product temperature on the perception of flavor and texture attributes of semi-solids. *Appetite* 41: 273–281.

Esposito V, Gallucci R, Picone D, Saviano G, Tancredi T, Temussi A 2006 The importance of electrostatic potential in the interaction of sweet proteins with the sweet taste receptor. *Journal of Molecular Biology* 360: 448-456.

Ganchrow JR, Steiner JE, Daher M 1983 Neonatal facial expressions in response to different qualities and intensities of gustatory stimuli. *Infant Behavior and Development* 6:189–200.

Gonazlez V, Salvador MD, Martin-Alvarez PJ 1997 Comparison of two simple methods for the measurement of detection thresholds for basic, umami and metallic tastes. *Journal of Sensory Studies* 13: 299-314.

Hivonen L, Kurkela R, Koivistoinen P & Merima P 1977 Effects of temperature and concentration on relative sweetness of fructose, glucose and xylitol. *Lebensmittel Wissenchaft und Technologie*, 10: 316-320.

Hung LW, Kohmura M, Ariyoshi Y & Kim SH 1999 Structure of an enantiomeric protein, D-monellin at 1.8 A resolution. *Department Biologie*, 54: 494-500.

Inglett GE & May JF 1969 Serendipity berries-source of a new intense sweetener. *Journal of Food Science* 34: 408-411.

Jellinek G, *Sensory Evaluation of Food*. Ellis Horwood Ltd, Chichester, England,1985.

Kilcast D, Portmann M, Byrne BE 2000 Sweetness of bulk sweeteners in aqueous solution in the presence of salts. *Food Chemistry* 70: 1-8.

Kim SH, Kang CH, Kim R, Cho JM, Lee YB, Lee TK 1989 Redesigning a sweet protein, increased stability and renaturability. *Protein Engineering* 2: 571-572.

Kobue-Lekalake RI, Taylor JRN &. de Kock HL 2012 Application of the dual attribute time-intensity (DATI) sensory method to the temporal measurement of bitterness and astringency in sorghum. *International Journal of Food Science and Technology* 47: 459–466.

Lallemand M, Giboreau A, Rytz A, Colas B 1999 Extracting parameters from time-intensity curves using a trapezoidal model: The example of some sensory attributes of ice cream. *Journal of Sensory Studies* 14: 387-399.

Lawless HT & Heymann H *Sensory Evaluation of Food: Principles and Practice*. Springer, New York, USA, 2nd Edition, 2010

Le Reverende FM, Hidrio C, Fernandez A & Aubry V 2008 Comparison between temporal dominance of sensation and time intensity results. *Food Quality and Preference* 19: 174- 178.

Lenfant F, Loret C, Pineau N, Hartmann C, Martin N 2009 Perception of oral food breakdown. The concept of sensory trajectory. *Appetite* 52: 659-667.

Lindley M Taste-ingredient interactions modulating sweetness. In:. *Optimising sweet taste in foods*, Spillane WJ, ed., Woodhead Publishing Limited, Cambridge, UK, 2006, pp. 66–84.

Lundhal DS, Lukes BK, McDaniel M, Henderson LA 1986 A semi- ascending paired difference method for determining the threshold of added substance to background media. *Journal of Sensory Studies* 10: 205-306.

McBurney DH, Bartoshuk LM 1973 Interactions between stimuli with different taste qualities. Physiology *and Behaviour* 10:1101–1106.

McGowan BA & Lee SY 2006 Comparison of methods to analyze time-Intensity curves in a corn zein chewing gum study. *Food Quality and Preference* 17: 296-306.

Meillon S, Urbano C & Schlich P 2009 Contribution of the Temporal Dominance of Sensation (TDS) method to the sensory description of subtle differences in partially dealcholized red wines. *Food Quality and Preference* 20:490-499.

Moraes PC & Bolini HMA 2010 Different sweeteners in beverages prepared with instant and roasted ground coffee: ideal and equivalent sweetness. *Journal of Sensory Studies* 25: 215-225.

Moskowitz HR 1970 Ratio scales of sweetness. *Perception and Psyhchophysics* 7: 315-320.

Ng M, Lawlor JB, Chandra S, Chaya C, Hewson L, Hort J 2012 Using quantitative descriptive analysis and temporal dominance of sensations analysis as complementary methods for profiling commercial blackcurrant squashes. *Food Quality and Preference* 25: 121-134.

Palazzo AB, Carvalho MAR, Efraim P & Bolini HMA 2011 The determination of isosweetness concentrations of sucralose, rebaudioside and neotame as sucrose substitutes in new diet chocolate formulations using the time-intensity analysis. *Journal of Sensory Studies* 26:291–297.

Pangborn RM 1963 Relative taste intensities of selected sugars and organic acids. *Journal of Food Science* 28: 726-733.

Patra F, Tomar SK, Arora S 2009 Technological and Functional Application of Low-Calorie Sweeteners from Lactic Acid Bacteria. *Journal of Food Science* 74: 16-23.

Peyvieux C and Dijksterhuis G 2001 Training a sensory panel for TI: a case study. *Food Quality and Preference* 12: 19-28.

Picone D, Temussi PA 2012 Dissimilar sweet proteins from plants: Oddities or normal components? *Plant Science* 195, 135-142.

Pineau N, Schlich P, Cordelle S, Mathonniere C, Issanchou S, Imbert A, Rogeaux M, Etievant P & Koster E 2009 Temporal dominance of sensation: Construction of the TDS curves and comparison with time- intensity. *Food Quality and Preference* 20:450-455.

Prakash I, Bishay IE, Desai N, Walters DE 2001 Modifying the temporal profile of high potency sweetener neotame. *Journal of Agricultural and Food Chemistry* 49: 786-789.

Reinbach HC, Meinert L, Ballabio D, Aaslyng MD, Bredie WLP, Olsen K, Møller P 2007 Interactions between oral burn, meat flavor and texture in chili spiced pork patties evaluated by time-intensity. *Food Quality and Preference* 18: 909-919.

Reinbach HC, Toft M, Møller P 2009 Relationship between oral burn and temperature in chili spiced pork patties evaluated by time–intensity. *Food Quality and Preference* 20: 42-49.

Reis RC, Minim VR, Bolini HMA, Dias BRP, Minim LA & Ceresino EB 2011 Sweetness equivalence of different sweeteners in strawberry flavored yogurt. *Journal of Food Quality* 34: 163–170.

Ross CF, Weller K 2008 Effect of serving temperature on the sensory attributes of red and white wines. *Journal of Sensory Studies* 23: 398–416.

Saint-Eve A, Deleris I, Panouill M, Dakowski F, Cordelle S, Schilch P, Souchon I 2011 How Texture Influences Aroma and Taste Perception Over Time in Candies. *Chemosensory Perception* 4:32-41.

Shamil SH 1998 Reduction of lingering sweet aftertaste of sucralose, World Patent Application WO 98/20753.

Sokolowsky M, Fischer U 2012 Evaluation of bitterness in white wine applying descriptive analysis, time-intensity analysis, and temporal dominance of sensations analysis. *Analytica Chimica Acta* 732: 46-52.

Spadaccini R, Crescenzi O, Tancredi T, De Casamassimi N, Saviano G, Scognamiglio R, Di Donato A, Temussi PA 2001 Solution structure of a sweet protein, nmr study of mnei, a single-chain monellin. *Journal of Molecular Biology* 305: 505-514.

Spadaccini R, Trabucco F, Saviano G, .Picone D, Crescenzi O, Tancredi T, Temussi PA 2003 The Mechanism of Interaction of Sweet Proteins with the T1R2-T1R3 Receptor: Evidence from the Solution Structure of G16A-MNEI. *Journal of Molecular Biology* 328: 683-692.

Spencer HW Taste panels and the measurement of sweetness. In: *Sweetness and Sweeteners*, Birch GG, Green LF, Coulson CB, eds. Applied Science Publishers, London, England, 1971 pp. 112-129.

Stone H & Oliver SM 1969 Measurement of the relative sweetness of selected sweeteners and sweetener mixtures. *Journal of Food Science* 34: 215-222.

Swiader K, Waszkiewicz-Robak B, Swiderski F & Kostyra E 2009 Sensory properties of some syntetic high-intensity sweeteners in water solutions. *Journal of Science and Food Agricultural* 89:2030-2038.

Tancredi T, Iijima H, Saviano G, Amodeo P, Temussi PA 1992. Structural determination of the active site of a sweet protein: a 1H NMR investigation of MNEI. *FEBS Letters* 310: 27-30.

Tancredi T, Pastore A, Salvadori S, Esposito V, & Temussi PA 2004 Interaction of sweet proteins with their receptor. A conformational study of peptides corresponding to loops of brazzein, monellin and thaumatin. *European Journal of Biochemistry* 271, 2231–2240.

Temussi PA 2002 Why are sweet proteins sweet? Interaction of brazzein, monellin and thaumatin with the T1R2–T1R3 receptor. *FEBS Lett*. 526: 1–3.

Temussi PA 2006 Natural sweet macromolecules: how sweet proteins work. *Cellular and Molecular Life Sciences* 63:1876-1888.

Temussi PA 2011 Determinants of sweetness in protein: A topological approach. *J. Mol. Recognit*. 24: 1033–1042.

Tunaley A, Thomson DMH & McEwan JA 1987 Determination of equi-sweet concentrations of nine sweeteners using relative rating technique. *International Journal of Food Science and Technology* 22:627-635.

Yamaguchi S, Yoshikawa T, Ikeda S & Ninomiya T 1970 Studies on the taste of some sweet substances part I. Inter- relationships among them. *Agricultural and Biological Chemistry* 34:181-186.

Zamora MC, Buratti FM and Otero-Losada ME 1998 Temporal study of sucrose and fructose relative sweetness. *Journal of Sensory Studies* 13: 213-228.

Zellner DA, Rozin WF, Brown JM 1988 Effect of temperature and expectations on liking for beverages. *Physiology & Behavior* 44: 61–68.

4. II Study Case: Fat replacer Optimization

Abstract

*The effect of different sugar and milk proteins on some properties of a fat-based filling cream were investigated by using a three-component (icing sugar, dextrose monohydrate, skimmed milk powder) D-optimal mixture design (Design Expert 8). The best formulation was used as reference and powders percentage were considered the constant ingredients. In order to reduce calorie content, a D-optimal mixture design with five component (water, margarine, simplesse, microcrystalline cellulose and maltodextrin) was used, 25 formulations resulted. As response variables, a_w, density, colour parameters and mechanical parameters were evaluated. The best formulation was chosen through the desirability function (**Nath & Chattopadhyay, 2007**) to determine areas of overlap where all responses were simultaneously optimized. To validate the model, formulations in the optimized region were prepared and submitted to a paired t –test ($p \leq 0.05$). Quadratic models were used to explain almost all parameters considered, except for a_w, highlighting that the components interacted with each other and determined effect on some physical parameters. The optimized formulation had 3.5 kcal/g ,so, considering that the full fat formulation had 5.1kcal/g, it was possible to develop filling cream reducing the caloric content at least of 30%.*

Keywords: D-optimal design, formula optimization, filling cream, fat replacer, squeezing flow

4.1. Introduction

Bakery filling creams are used in various baked goods. Creams are important components in different confectionary foods, in which they provide taste, texture and adhesion of the baked items. From a processing viewpoint, these creams belong to the categories: heavy creams, light creams and creams with water. Pralines, truffles, nougat, are the names frequently given to fat-based fillings and centres. These fillings are used with chocolate, biscuits, wafer, cakes to provide an enormous variety of products. These products can be made suitable for all markets, from the premium market to the low cost, high volume market (Birkett, 2009).

A filling is usually called 'cream'. In some countries 'creams' are called 'cremes', in others 'fillings'. The amount of cream in a sandwich ranges from about 17 to 36% with an average amount of 26%. Sweet creams are basically sugar and fat mixtures, even if also other ingredients are included in the recipe and they influence many characteristics of the final product. There are also non-sweet cream used as fillings. The basic cream cannot have icing sugar as its main ingredient, so non-sweet powders like milk powders, cheese powder, maltodextrin, starch and cracker dust must be used as bulking agents with the fat. These powders do not dissolve in the mouth as readily as sugar so it is necessary to have a higher fat content in the cream to make them more palatable. The flavouring agents, apart from bottled liquids, include meat extract powders, dried autolyzed yeast and monosodium glutamate. (Manley, 2001).

The quality of the fat and the type of the sugar largely determine the eating quality.

Sugar is generally the main ingredient of fat-based fillings and confectionery products. Highly refined white sugar is 99.9% sucrose, a non-reducing disaccharide (Birkett, 2009). The greater the quantity of sugar in the cream recipe the harder and 'drier' will be the cream, the larger the sugar crystal size the more gritty will be the cream in the mouth. It is not necessary to have the sugar particle size as small in creams as in chocolate because the cream is mixed with biscuit while eating. Thus a maximum sugar particle size of about 40μm will be acceptable for sugar in creams. The use of dextrose monohydrate as a partial replacement for sugar is interesting. The dextrose is less sweet than sucrose and dissolves in the mouth with a significant and pleasant cooling effect, but too much dextrose can lead to splitting problems because of a water activity problem (Manley, 2001). Indeed, dextrose has the same disadvantages of sucrose, being cariogenic, unsuitable for diabetics and having a calorific value of 4 kcal g^{-1}. Fructose is slightly sweeter than sucrose and is suitable for diabetics but is hygroscopic.

The fat content has an enormous effect upon the sensory and rheological properties of fat-based fillings. Generally the fat content is about 30% of the filling but it can be up to about 60%. Since fat is the continuous phase in a fat-based filling, the viscosity of the filling decreases as the fat content increases because the powder content decreases. The type of fat that is used affects the sensory properties of the filling, its compatibility with the other components of the product and the shelf life of the product. There are different ways to categorise filling fats. Generally, three types of fat can be used: polymorphic filling fats, non-lauric (cocoa butter), suited for covering with chocolate or supercoating composed of cocoa butter equivalents; non-polymorphic, non lauric filling fats, such as aerating filling fats with a density of 0.6 g cm^{-3} and; non polymorphic, lauric filling fats, based upon coconut and palm kernel oil. Fat based upon hydrogenated rapeseed, soyabean oil and palm fractions have been used successfully as filling fats for many years because of their excellent oxidative stability, speed of solidification, direct crystallising in the β' form, but they had a high trans content. In the last years the use of filling fats composed of interesterified blends of palm fractions and palm kernel oil fractions has increased significantly (Birkett, 2009). Basically the consistency of a cream is determined by the solids content of the fat. Clearly the higher the temperature the lower will be the fat solids and the softer will be the cream. The cream has to be soft at the time of stencilling or depositing, but firm at the time of eating. Furthermore, the cream will taste better if the fat in the cream melts quickly in the mouth and has a very small fraction of solids that melt above body temperature. If there are significant amounts of high-melting solids in the fat a waxy film will be left in the mouth. The best fats for creams are, therefore, those with a very steep melting curve which release sugar and flavours. The lauric fats, coconut and palm kernel oils, and their blends are commonly used. These fats melt rapidly and draw latent heat from the mouth to give an attractive cool eating sensation. The hardness of a cream, at ambient temperature, is also affected by the crystal size of the fat. Fats which have been mechanically agitated, while cooling, have small free crystals and are said to be plasticised. Fats which cool passively from liquid are much firmer at ambient temperature because the crystals have grown together in an interlocked form. So if there is a big difference between the temperature of the fat and the ambient temperature, at the sandwiching time the cream will be firmer than if the temperature difference is small. In addition, if the cream is significantly aerated this will also make the cream softer when the cream cools (Manley, 2001).

Most fat-based fillings contain some milk component, often a milk powder, in order to have good flavor and texture. A simple fat and sugar mix is not generally considered to be acceptable. In order to meet specific industry requirements, many milk powders may be used, such as whole or skimmed milk powder, cream powder, demineralized whey powder

Lecithin is the most frequently used emulsifier in fat-based fillings because it gives the filling an acceptable viscosity with less fat and to bind water if it is present. Lecithin can be sourced from soya, rape and sunflower (Birkett, 2009). This emulsifier speeds the mixing of the cream but tends to give softer creams after cooling.

Also flavours can be added to the formulation. There is a great range of cream flavours. Brown 'chocolate' creams are the most popular and vanilla and 'creamy' vanilla the next. Others include fruit flavours such as lemon, orange, strawberry and raspberry. The chocolate creams are flavoured with cocoa or cocoa mass (milled roasted cocoa beans, the precursor of chocolate). The creamy vanilla creams have skimmed or full cream milk powder to mellow the vanilla flavour and the fruit flavoured creams include bottled fruit extracts or oils together with an appropriate amount of fruit acid (citric, tartaric or malic) to give tartness. In all cases the optimum effect is achieved if colour is added to 'suggest' the flavour. The flavour may be enhanced with small quantities of salt which has a very fine particle size (Manley 2001). Vanilla extract is a spice that has been traditionally used in fat-based fillings, at concentrations up to 0.05% (Birkett, 2009).

For what concern the technology process, creams are generally made mixing plasticized fat with warm or cold icing sugar. Sugar do not chemically react with fats, but they impart product's rheological characteristics (Helstad, 2006). During mixing, air becomes entrained, if mixing continues, even more air is sometimes included, and the cream becomes 'fluffy'. As the temperature rises, the amount of air in the cream decreases. It is difficult to apply process control to give cream of a specific density when batch mixing. When creams are made in a continuous process warm (liquid) fat is mixed with sugar and other ingredients and the mixture is passed through a scraped surface heat exchanger which plasticises the fat as it cools. At the same time air (or nitrogen) is metered into the mixture and a cream of desired density is obtained. Thereafter, the cream is pumped continuously to the cream sandwiching machine, usually in a ring main. The problem is that the air included in the cream coalesces to form larger bubbles and is readily released as soon as the pressure drops when it comes out of the ring main. This means that the cream deposited in the sandwich has a higher density than is expected. Cream densities vary from 0.75 to 1.15 g cm^{-3}. Those of lower density give deposits which are thicker and therefore appear more generous for the same weight. If the fat content of the cream is too low there may be a problem with 'splitting' of the cream from the biscuit shells because there are not enough fat crystals on the biscuit–cream interface. So, fat reduction should be replaced by the right fat replacer which determinate the appropriate texture characteristics, in fact if the cream is too soft it will squeeze out eating the product, meanwhile if it is too hard, the cream will seem dry (Manley, 2001). Non-nutritive sweeteners are often used with polyols, to correct the lower sweetening effects of the last ones. The combination of lactitol, polydextrose and aspartame has been shown to give a good sensory effect (Birkett, 2009)

Replacing sugar or fat enables a variety of nutritional claims to be made, as shown in table 1, permitted by European Regulation (EC) No 1924/2006 of the European Parliament and of Council of 20 December 2006 on nutrition and health claims made on food.

Table 1. Permitted nutritional claims conserving energy, sugar and fat

Nutrition claim	Definition
Energy reduced	Reduction in energy of at least 30% compared to a similar product
Low energy	Energy less than or equal to 40 kcal (170 kJ)/100 g for solids or 20 kcal (80 kJ)/100 ml for liquids. For table-top sweeteners the limit of 4 kcal (17 kJ)/portion, with equivalent sweetening properties to 6 g of sucrose.
Energy free	Energy less than or equal to 4 kcal (17 kJ)/100 ml. For table-top sweeteners the limit of 0.4 kcal (1.7 kJ)/portion, with equivalent sweetening properties to 6 g of sucrose
Low sugars	Less than or equal to 5 g sugar per 100g or 100 ml of product
Reduced sugars	Reduction in sugar content of at least 30% compared to a similar product
Low fat	Less than or equal to 3g of fat per 100 g for solids or 1.5 g of fat per 100 ml for liquids (1,8 g of fat per 100 ml for semi-skimmed milk).
Fat-free	Less than or equal to 0.5 g of fat per 100 g or 100 ml.
Reduced fat	Reduction in fat content of at least 30% compared to a similar product
Low saturated fat	Less than or equal to 1.5 g per 100 g for solids or 0.75 g/100 ml for liquids and in either case the sum of saturated fatty acids and trans-fatty acids must not provide more than 10 % of energy.
Saturated fat-free	Less than or equal to 0.1 g of saturated fat per 100 g or 100 ml.

[a]Directive 90/496/EEC defines sugars as all monosaccharides and disaccharides present in food but excludes polyols.

In literature there are few information about filling for cakes, rolles and similes. These kind of filling creams can be heated or made at ambient temperature (20°C±2). They are similar to biscuit filling, because sugar and fat are the main ingredients, but they also contain a certain percentage of water, that could influence its performance and shelf life. Considering that in the last years partially-hydrogenated fats have been replaced by no-trans fats (Talbot, 2009), and sucrose and dextrose are the most used sugars in this type of foodstuffs (Birkett, 2009; Helstad, 2006), the objective of this work was, first, to study the effect of sugar, dextrose and skimmed milk powder on properties of a model filling cream for cakes, made with margarine without trans acid fats. Indeed, considering that the aeration of filling can help to providing lower calorie alternatives, it was also investigated the effect of powder on whipping ability of the filling. Then, new filling creams, with different level of fat substitution and containing different fat replacers, were studied in order to optimize the fat replace system to use in the reduced –fat filling cream.

4.2. Materials and Methods

4.2.1. Materials

Samples were prepared using margarine without trans fatty acids (Flo'c, Unigrà s.r.l.); dextrose monohydrate (J.T. Beker), powdered sucrose (Eridania), skimmed milk powder (Irish rived by Morgan Chemicals S.p.a.), deionised water (W) and soy lecitine (Lecinova).
Microionized whey proteins (Simplesse 100, Kelco), microcrystalline cellulose (Tabulose, Blanver Farmoquimica LTDA), maltodextrin 6 DE (Glucidex, Roquette Preres SA) were used to partially or completely replace the fat phase.

4.2.2. Sample preparation

The mixing was made using an emulsifier (Stephan electronic 2011 UMC 5). The lecithin was added to water under constant stirring until complete dissolution was obtained. Then, the powders were added and mixed for 1 minute, to hydrate the powders. Meanwhile, the margarine, at 20°C was processed. Next, the powders hydrated were added to the margarine and mixed at 1500 rpm for 15 minutes, at 20°C. The aeration was made by whipping through a mixer (Phillips HR 1453) at the highest speed for 5 minutes. Samples were stored at 4°C for one night, to stabilize them, then analysed at 20°C.
When fat substitution was made, the fat replace system was added to the fat phase. A similar procedure was followed, but a difference device was used (Kenwood chef), because of the reduction of viscosity of the fat phase. For these formulations, whipping phase was made inside the same device, only changing the tool and increasing the revolution per minute (rpm).

4.2.3. Full-fat formula optimization

To optimize the filling cream formula a three-component D-optimal mixture design was used (Design Expert 8). Mixture Design is defined as a special type of Response Surface Method (RSM) where the factors are the components of the mixture, and the response is a function of the proportions. In many mixture designs, the restrictions on the component proportions x_j take the form of lower (L_j) and upper (U_j) constraints.
The general form of the constrained mixture problem is:

$$\sum_j x_j = 1 \text{ and } L_j \le x_j \le U_j \qquad (4.1)$$

D-optimal design that focuses on estimating model coefficients with good especially for constrained mixture regions is used if the determinant of $(X'X)^{-1}$ is minimized (Gacula, 1993).
Constant ingredients were water, lecithin and lipids, representing 45% of the mixture. The remaining part (55%) consisted of icing sugar (x_1); dextrose monohydrate (x_2) and skimmed milk powder (x_3). The proportions for each ingredient were expressed as a percentage of the mixture, and for each treatment combination, the sum of the component proportions was equal to one hundred, where:

$$x_1 + x_2 + x_3 = 100 \qquad (4.2)$$

Through preliminary studies of pastry-mixing workability (Miele, 2010), ranges for each ingredient were determined as follows: icing sugar (x_1) 30–64%; dextrose monohydrate (x_2) 30–64% and skimmed milk powder (x_3) 6–20%.
As regression model, a cubic model was used and 18 experimental points were determined, four points were replications.

4.2.4. Fat replacer system optimization

The optimal formulation obtained from the previous experimental design was used as reference and powders percentage were the constant ingredients now. A D-optimal mixture design with five component (water, margarine, simplesse, tabulose and glucidex) was used and their sum represents the 45% of the total. The proportions for each ingredient were expressed as a percentage of the mixture, and for each treatment combination, the sum of the component proportions was equal to one hundred, where:

$$x_1 + x_2 + x_3 + x_4 + x_5 = 100 \qquad (4.3)$$

The percentage of each ingredient used was chosen according to the literature and preliminary experiments. In particular, ranges for each ingredient were determined as follows: water (x_1) 11-65%, maltodextrin (x_2) 0-25%, cellulose microcrystalline (x_3) 0–10%, simplesse (x_4) 0-35%, margarine (x_5) 0-89%.
Indeed, the following mixture constraints were imposed, in order to reduce fat content at different percentages:

$$0 \le x_2 + x_3 + x_4 \le 89 \qquad (4.4)$$

$$11 \leq x_1 + x_2 + x_3 + x_4 \leq 100 \qquad (4.5)$$

As regression model, a quadratic model was used and 25 experimental points were determined, five points were replications. Only 20 formulations were evaluated as reported in table 2.

Table 2. Composition of the fat phase of formulations evaluated

Formulation	A:W	B:MD	C:MC	D:S	E:M
1	65.00	11.52	4.55	18.92	0.00
2	65.00	11.52	4.55	18.92	0.00
3	65.00	0.00	0.00	35.00	0.00
4	41.78	23.22	0.00	35.00	0.00
5	65.00	18.07	10.00	0.00	6.93
6	42.52	4.38	9.74	35.00	8.36
7	65.00	25.00	0.00	0.00	10.00
8	39.30	25.00	6.50	13.98	15.22
9	39.30	25.00	6.50	13.98	15.22
11	55.73	0.00	0.00	22.12	22.13
13	30.09	6.95	0.00	35.00	27.96
14	32.06	24.21	10.00	0.00	33.73
16	38.45	8.48	0.00	14.91	38.16
17	38.45	8.48	0.00	14.91	38.16
19	53.75	0.00	4.34	0.00	41.92
20	53.75	0.00	4.34	0.00	41.92
22	16.86	14.19	6.78	0.00	62.18
23	16.86	14.19	6.78	0.00	62.18
24	11.11	0.00	10.00	13.50	65.39
25	11.11	0.00	0.00	0.00	88.89

4.2.5. Water activity

Water activity was measured by AcquaLab (Decagon Devices, Inc., 4TE series) at 20 ±3°C. It uses the chilled-mirror dewpoint technique to measure the water activity of a sample. The mirror temperature is controlled by a thermoelectric cooler. Three replicates for each sample were made.

4.2.6. Colour measurement

Fat-based model cream colour, was measured by colorimeter Minolta Chroma Meter II Reflectance Cr-300, set on the L*, a*, and b* system. The chromameter was previously calibrated with a white tile using Illuminant D-65. Three replicates of each sample were made.

4.2.7. Density and overrun

A small Plexiglas cylinder of known volume and weight, was filled with the sample and weighted. Sample density values were obtained as ratio between the sample weight (grams), and the cylinder volume (cm^3).
Overrun was evaluated immediately after whipping, by sampling from the middle of the backer. The overrun was calculated according to Bazmi (2007):

$$Overrun = \frac{p_1 - p_2}{p_2} \cdot 100 \qquad (4.6)$$

Where p_1 and p_2 were the weight before and after whipping of the sample at fixed volume, respectively. The overrun value was determined in triplicate for each sample.

4.2.8. Mechanical properties

Lubricated squeezing flow at constant diameter of the sample was performed with an Instron Universal Machine (Instron Ltd., mod. 4467) equipped with a 100N load cell. The test was based on the uniaxial compression of a cylindrical sample between two parallel plates with different diameter, where the upper compression plate and the sample had the same diameter (40mm), but the lower place was much larger. Sample was gently poured on the lower plate and the uneven surface of the sample was flattened with a spoon, which did not affect results. The exact initial height of the sample, approximately 8 mm, was determined as the sample height at the moment the compression force exceeded 0.1 N.
Measurement were performed at room temperature (20°C ±2). Before test started, the samples were allowed to relax and thermally equilibrate for 1 min. Test was performed at different constant crosshead rate (1, 5, 10 mm/min). Meas-

urements were made in triplicate.
As reported by Steffe (1996), during compression of a cylindrical sample in the z-direction, the Hencky strain along this direction (ε_z) was:

$$\varepsilon_z = Ln(h(t)/h_0) \quad (4.7)$$

where *h* was the height of the sample at time *t* and h_0 was the initial height of the sample

The biaxial (radial) strain, ε_b , was:

$$\varepsilon_x = \varepsilon_y = \varepsilon_b - 0.5[Ln(h(t)/h_0)] \quad (4.8)$$

The corresponding biaxial strain rate $\dot{\varepsilon}_b$ was then:

$$\dot{\varepsilon}_b = -0.5(dh/hdt) \quad (4.9)$$

At constant diameter the normal stress difference, σ_b ,that drives the flow was:

$$\sigma_b = F(t)/\pi R^2 \quad (4.10)$$

where *F* was the force at time *t* to compress the sample and *R* was the radius of the sample

The biaxial extensional viscosity η_b was:

$$\eta_b = \sigma_b / \dot{\varepsilon}_b \quad (4.11)$$

4.2.9. Data analysis

ANOVA was performed to evaluate formulation effect on data (Design Expert 8). In the first part, as response parameters, aw, density, overrun, colour parameters, at 20 ±3°C were measured. Then, as response variables, aw, density, colour parameters and A, m, n, were used. These last three parameters were obtained, by fitting σ_b , $\dot{\varepsilon}_b$ and ε_b average values of each filling cream, with model reported by Launay and Michon (2008) (Statistica 98, StatSof. Italia).

$$Ln\sigma_b = A + nLn\dot{\varepsilon}_b + m\varepsilon_b \quad (4.12)$$

Principal component analysis (PCA) (Unscrambler 9.1., Camo) applied to the matrix of formulation x average response parameters was used in order to examine if formulations fell into differentiated patter of responses.
In both experimental designs, attributes which significantly varied were used as response variables in models generated to determine the optimal levels of ingredients. The best formulation was chosen through the desirability function (Nath & Chattopadhyay, 2007) to determine areas of overlap where all responses were simultaneously optimized. To validate model chosen, formulations in the optimized region were prepared and submitted to a paired t –test ($p \leq 0.05$). Parameters which significantly varied due to the powder effect were used as response variables in cubic regression models generated to determine the optimal level of ingredients (13), whereas parameters which significantly varied due to the fat replacer system mixture were used as response variables in quadratic regression models generated to determine the optimal levels of ingredients (14):

$$Y=B_1X_1+B_2X_2+B_3X_3+B_{12}X_1X_2+B_{13}X_1X_3+B_{23}X_2X_3+B_{123}(B_1B_2B_3)+B_{12(1-2)}X_1*X_2*(X_1-X_2)+B_{13(1-3)}X_1*X_3*(X_1X_3)+B_{23(23)}X_2*X_3*(X_2-X_3)+error \quad (4.13)$$

$$Y=B_1X_1+B_2X_2+B_3X_3+B_4X_4+B_5X_5+B_{12}X_1X_2+B_{13}X_1X_3+B_{14}X_1X_4+B_{15}X_1X_5+B_{23}X_2X_3+B_{24}X_2X_4+B_{25}X_2X_5+B_{34}X_3X_4+B_{35}X_3X_5+B_{45}X_4X_5+error \quad (4.14)$$

where Y was the response variable, X the percentage of each ingredient, B the coefficient generated from the model, and the number of each subscript represented the ingredients.

Contour plots for each parameter were generated and superimposed (Design-Expert v.8.00 software, Stat-Easy Inc. Minneapolis, USA) by means of the desirability function (Nath & Chattopadhyay, 2007) to determine areas of overlap where all responses were simultaneously optimized. Optimal conditions were generated by defining the constraints. Optimal conditions were ascertained by preparing the sample with the highest desirability in the optimized region and by determining significant differences between observed and predicted values (paired *t* -test, $p \leq 0.05$) (SPSS v. 13.0 for windows).

4.3. Results and Discussion

4.3.1. Full fat formula optimization

ANOVA revealed that formulation significantly affect fat-based cream properties. In particular, ANOVA results showed that varying levels of icing sugar, dextrose monohydrate and skimmed milk powders affected a_w, overrun, density after whipping and colour parameters. Density before whipping was almost the same for all the formulations considered (≈1g cm^{-3}). This could be due to the similar size distribution of the powders, in fact more than 50% of each powder involved had an average distribution size bigger than 107 micron, even if they had different ability to bind water, to incorporate air, sensory profile.
The parameters influenced by the formulation were used as response variables in regression models built to determine the optimal levels of ingredients. Estimated parameters of prediction models for each parameter are listed in table 3. The parameters of fat-based filling creams were significantly affected by independent variables (dextrose monohydrate, icing sugar and skimmed milk powders). a_w, density and overrun were linearly affected by ingredient levels, meanwhile L* was also affected by ingredient interaction, as shown in table 3.

Table 3. Estimate coefficient parameters for variables used in prediction model for parameters of full fat filling cream models.

Variables	Overrun	a_w	Density(g cm^{-3})	L*
Icing sugar (S)	0.19	$7.02\cdot10^{-3}$	$8.49\cdot10^{-3}$	0.49
Dextrose monohydrate (D)	0.08	$8.26\cdot10^{-3}$	$9.82\cdot10^{-3}$	1.26
Skimmed milk powder (M)	0.48	$7.29\cdot10^{-3}$	$4.94\cdot10^{-3}$	10.79
S*D*(S-D)				$2.24\cdot10^{-4}$
R^2	0.4157	0.7322	0.5799	0.8525
p-value	0.0178	< 0.0001	0.0015	0.0154

Only the coefficients that significantly affected attributes were reported ($P < 0.05$).

Predicted models were used to generate contour plots for parameters significantly predicted by independent variables. a_w, density, overrun were predicted through a linear model; for L* a special cubic model was used, even if many parameters were not significant. Among them, L* was the best predicted parameter, meancwhile the overrun was the worst.
The contour plots were superimposed to obtain areas of overlap. The contour plots had contour lines which represented constant values of each response variable. A contour line, corresponding to an optimal value of each response, were selected on each map to obtain desirable levels of ingredients (Gacula, 1993).
Area of overlap of each response variable are shown in figures 1-4. Figures 1-2 show that the higher was the dextrose monohydrate content the higher were a_w (0.79) and density (0.95 g.cm^{-3}) parameters, meanwhile the skimmed milk powder, for its ability to incorporate air, reduced the density (0.83) (fig. 3) of the product and increased L* (91) (fig 4), so the formulations were lighter than the others.
In literature there were no studies about filling cream containing water to compare results, a similar product could be an ice-cream, containing, fat, sugar, air, milk proteins. The low overrun values (14-23%) of the fillings could be related to the inefficiency of the air incorporation system and not to the ingredients. Also in the study conducted by Bahramparvar *et al*. (2009) low overrun values of soft ice-cream were due to the same reasons.
BahramParvar and Razavi (2012) studied the effect of sucrose, skimmed milk powder and emulsifier, as key ice cream constituents, on the rheological properties of selected hydrocolloids (basil seed gum, guar gum and carboxymethyl cellulose). They showed that the addition of sucrose or emulsifier led to more viscous and more pseudoplastic solutions, whereas skimmed milk decreased viscosity and pseudo-plasticity in some cases.
Sugar do not chemically react with fats, but they impart product’s rheological characteristics (Helstad, 2006). In our case the effect of powders on mechanical properties was not investigated in this part because the fat phase was constant and only powder percentage varied in a range defined according the results of a previous study (Miele, 2010). In that study, the effect of sugar and dextrose on the structure of a fat-based cream model was tested. Two type of fat, margarine with 70% of fat and fractionated palm oil, with or without water, were used. The addition of to the fat phase, with a very low water content, determined an increase of consistency. Dextrose monohydrate gave a very compact structure to the samples but the water activity was higher. So, to obtain a good product both sugar types must be used..
In order to find the best powders mixture, for each response parameter, the best performance was established among the analyzed formulations (Vatsala, Saxena, & Rao, 2001; Di Monaco *et al*., 2010).
The optimal formulation should had the lowest a_w and density and the highest overrun. Different optimum percentages of ingredients were generated by the Design-Expert software through the desirability function (Castro et al., 2003). Figure 5 showed the graph of the first optimal formulation with the highest desirability value (0.95), the best formulation should contain 50% of icing sugar, 30% of dextrose monohydrate and 20% of skimmed milk powder.
The results of the paired t-test (p 0.05) indicated that there were no significant differences between predicted and observed values for different attributes validating the model chosen, according to Chu and Resurreccion (2004), Holt, Resurrecion, and McWatters (1992), Nath and Chattopadhyay (2007) and Seog *et al*. (2008), Di Monaco *et al*., (2010).

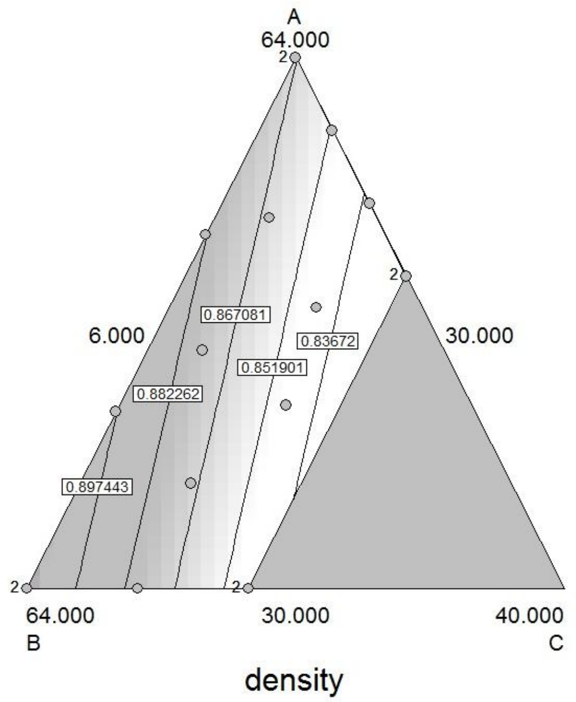


Figure 1. Areas of overlap for density

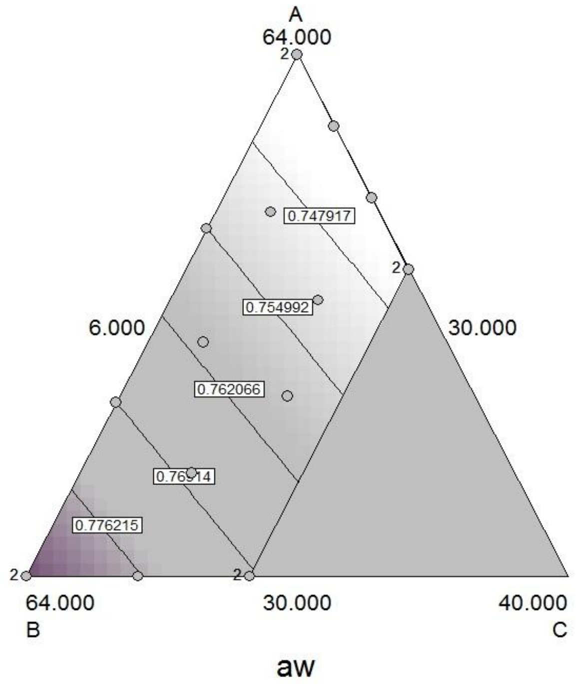


Figure 2. Areas of overlap for a_w

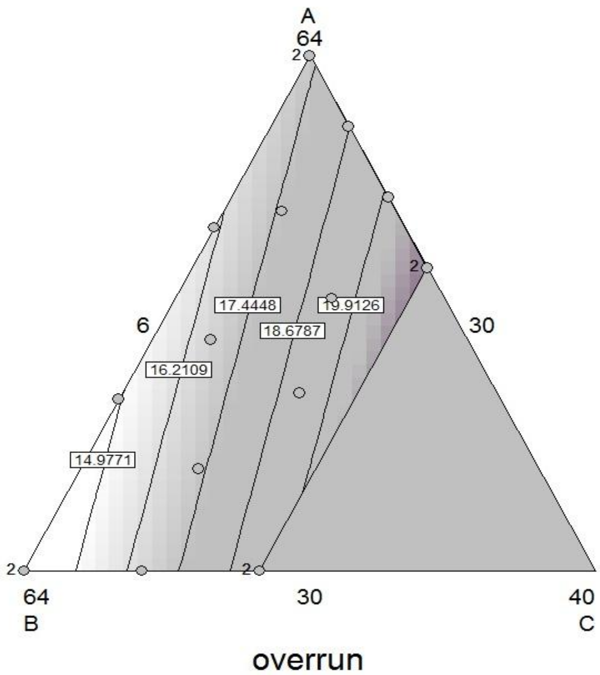


Figure 3. Areas of overlap for overrun

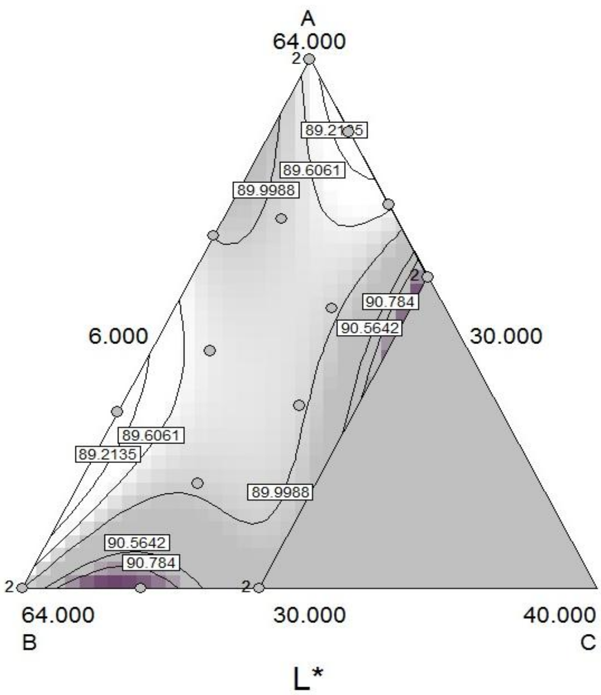


Figure 4. Areas of overlap for L*

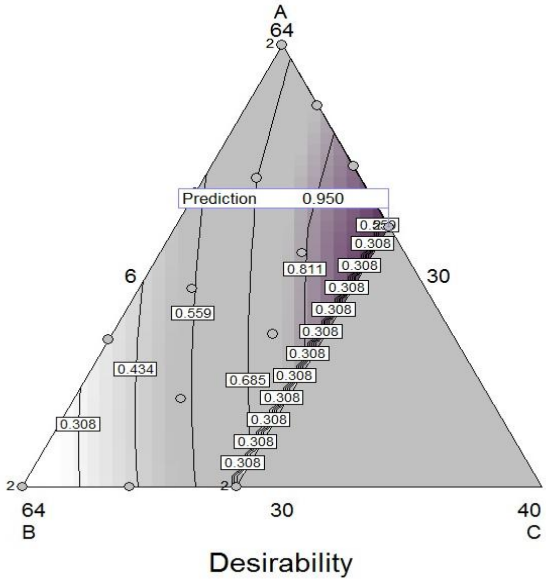


Figure 5. Graphical visualization of the first optimal formulation

4.3.2. Characterization of filling creams with different fat content

The best formulation was used as reference sample for investigate the possibility to reduce the fat content. From the experimental design 25 formulations resulted, of which 5 have not been analyzed because they were instable or not workable. ANOVA, applied to the 20 tested formulations revealed that fat replacer system significantly affected fat-based cream properties, such as a_w, color, density parameters. In table 4 average values for each filling cream with different fat content were reported.

Table 4. Average values (±SE) of parameters for each filling cream

	a_w	Density ($g.cm^{-3}$)	L*	a*	b*
1	0.845±0.001	0.421±0.002	90.107±0.081	-1.657±0.008	5.27±0.02
2	0.846±0.001	0.420±0.002	90.110±0.082	-1.656±0.009	5.267±0.02
3	0.843±0.001	0.338±0.002	90.78±0.067	-1.343±0.013	5.32±0.017
4	0.759±0.001	0.769±0.006	88.797±0.032	-1.80±0.040	6.493±0.061
5	0.870±0.001	1.255±0.006	78.963±0.347	-5.457±0.165	11.35±0.295
6	0.793±0.001	1.063±0.004	84.35±0.14	-3.227±0.028	13.117±0.101
7	0.86±0.001	1.175±0.005	83.713±0.349	-4.523±0.14	11.31±0.315
8	0.788±0.001	1.194±0.001	82.077±0.088	-4.383±0.044	13.71±0.265
9	0.766±0.001	1.094±0.004	82.89±0.043	-3.696±0.040	15.057±0.053
11	0.849±0.001	1.00±0.006	82.200±0.131	-4.36±0.050	13.85±0.060
13	0.766±0.001	1.095±0.003	82.89±0.040	-3.70±0.041	15.06±0.054
14	0.783±0.001	1.135±0.007	85.04±0.04	-4.006±0.044	13.14±0.16
16	0.808±0.001	0.757±0.007	88.227±0.112	-2.543±0.004	9.766±0.054
17	0.809±0.001	0.760±0.008	88.229±0.113	-2.537±0.003	9.770±0.053
18	0.858±0.001	1.137±0.002	81.743±0.39	-4.783±0.062	14.113±0.337
19	0.857±0.001	1.139±0.002	81.741±0.38	-4.783±0.061	14.111±0.338
22	0.761±0.001	0.957±0.001	89.267±0.257	-3.267±0.058	12.617±0.182
23	0.76±0.001	0.958±0.001	89.269±0.258	-3.268±0.057	12.62±0.181
24	0.761±0.002	0.949±0.004	87.307±0.094	-3.277±0.077	13.46±0.240
25	0.782±0.001	0.849±0.006	89.21±0.219	-2.84±0.015	11.957±0.119

As shown in table 3, filling creams presented different a_w values, from 0.76-0.86. a_w was higher in fillings with a high water content and low fat replacer content (1-2-3-5-7-19), meanwhile the presence of maltodextrin, in the right water-powder ratio (4-13-14),determined lower a_w value because at very low DE (Dextrose Equivalent value) acted as hydrocolloid stabilizer, in order to structure the aqueous phase, thickening and gelling the aqueous phase droplets with a reduction of free water (Jones, 1996).
The values of density of the different filling creams were within the range described by Manley (2001); cream densities, in fact, generally vary from 0.75 to 1.15 g cm^{-3}. Those of lower density, such as 1-2-3 formulations, were not good because gave deposits which were thicker and therefore appeared more generous for the same weight. Fillings with high cellulose microcrystalline content were very dense, because MC generally forms, when dispersed in water using sufficient shear, a three dimensional network of crystals, but it is insoluble and do not contribute to incorporate air during the mixing phase, but only simulate network of fat crystal. It was chosen because in reduced fat aqueous based foods it imparts much of the body and opacity usually contributed by fat and is a non-caloric source of insoluble solids (Jones, 1996), but generally it was not used alone, but it was a part of an overall fat-mimetic system, which also includes hydrocolloids, fat flavors, antimicrobial agents.
For what concern color parameters, L* and b* mainly characterized filling creams. The brightest (high L*) and lightest (low b*) filling creams were generally those with the lowest density values, which resembled whipping creams. Other filling creams were very yellow (high b*), such as 9-18-13.

4.3.3. Mechanical properties filling creams

In order to study the mechanical properties of the filling creams with different level of fat reduction, lubricated squeezing flow tests were performed. Lubricated squeezing flow test was previously applied to study both viscoelastic food, such as wheat dough (Launey and Michael, 2008), and non elastic food, such as butter, peanut butter (Campanella & Peleg 2002; Sight *et al*. 2005). In Figure 4 the stress-strain curves obtained at three different crosshead speed, for the full fat formulation, are reported. A fully developed squeezing flow regime cannot be achieved instantaneously. The region of transient flow regime can easily be identified and corresponds to that before the yield stress (Corradini, 2005). Because normal stress depends on both the strain and the strain rate, through eq. 4.8-4.11 stress-strain rate curves and extensional viscosity- strain rate curves were obtained (figures 5 and 6)

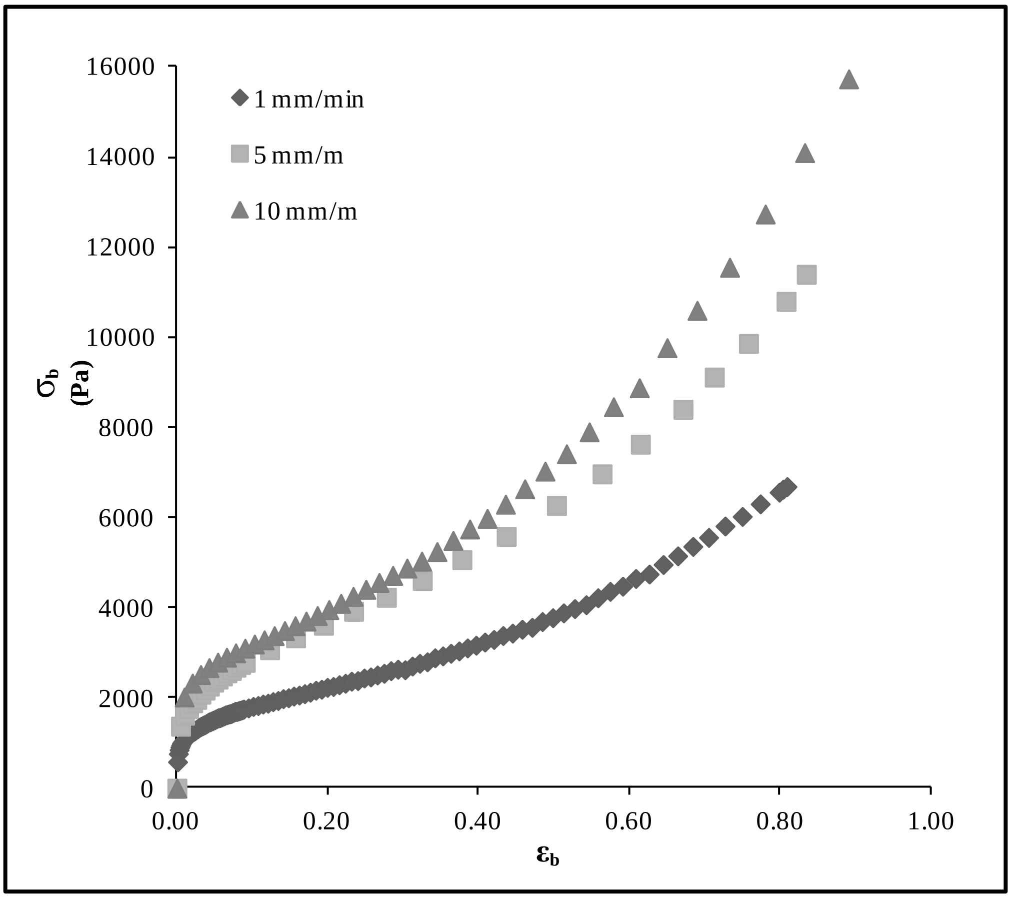


Figure 4. Normal stress versus biaxial strain of full fat formulation

As shown in figure 5 and 6, full fat filling had a shear thinning behaviour. A similar behaviour was observed for all investigated fillings (results not shown).

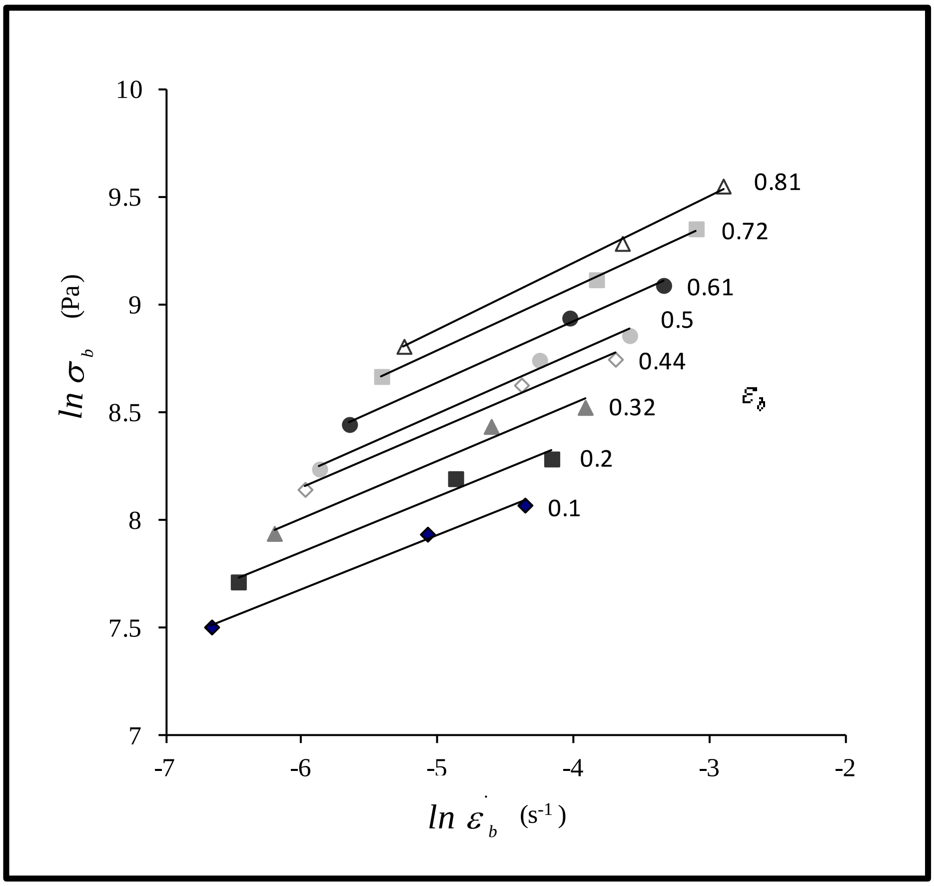


Figure 5. Stress versus biaxial strain rate, at different values of biaxial strain ε_b ,of full-fat filling

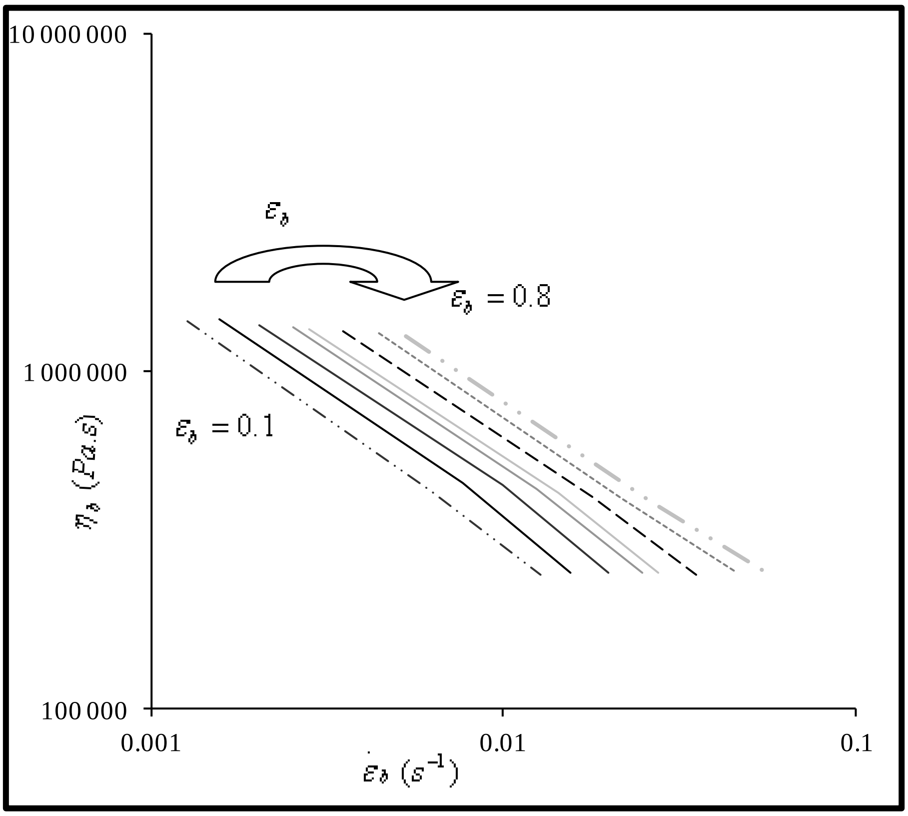


Figure 6. Extensional viscosity versus biaxial strain rate, at different biaxial strain, of full fat filling.

Experimental data were fitted by using a non linear regression model, as reported by Launey and Michon (2008). The model used fitted very well experimental data for each sample, as shown in figure 7 for full fat filling, as example.

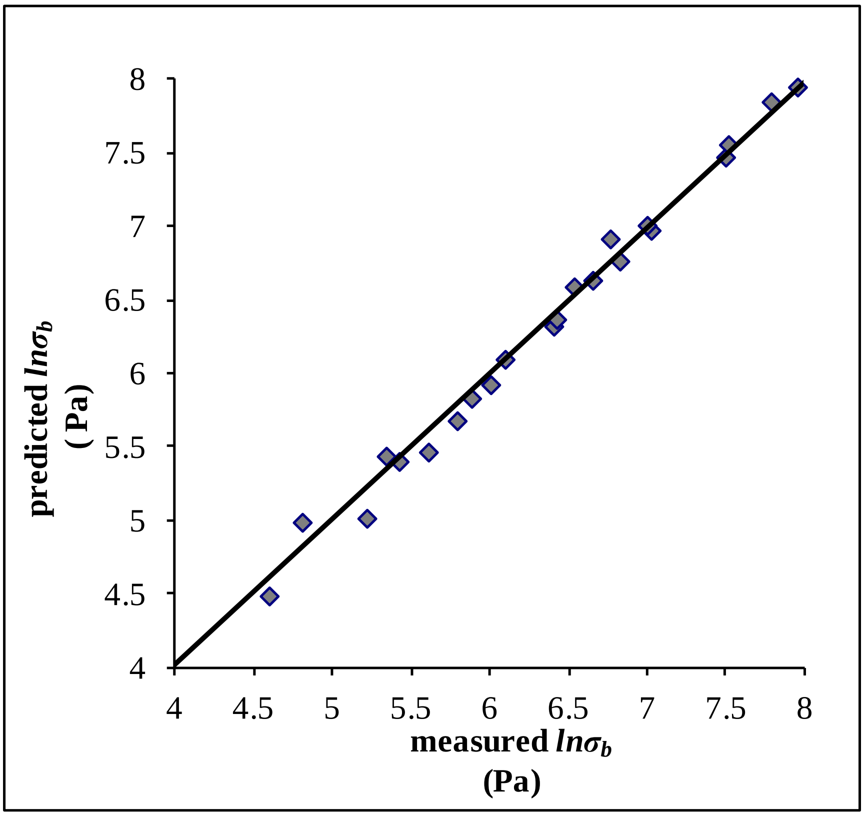


Figure 7. Predicted versus measured stress values for full fat filling cream.

In table 5 the estimated parameters of each tested fillings were reported. As reported in this table, the reduction in fat content generally reduced A values, determining fillings with a lower consistency. The value of m was purely descriptive and had no unequivocal rheological meaning, even if it could depend on the viscoelastic property of a material (Launey and Michon, 2008). For tested filling creams, m depended both on fat content and fat replacer composition. It was higher for samples in which maltodextrin formed a gel. Also n parameter increased as fat content decreased, but it was mainly influenced by fat replacer composition. Filling with high m and n values were hardly spreadable. Sight *et al*. (2005) showed that a fat reduction in peanut butters accentuated the degree of pseudoplasticity, determining products more spreadable than the full fat formulation.

The full fat filling (25) was quite similar to margarine, underlining that sugar and milk powder did not interacted with fat. Filling creams with 10-15% of fat reduction, showed similar properties to full fat cream.

Among filling creams with 55-70% of fat reduction, only formulation 16 determined a filling spreadable as full fat filling, but with a lower consistency. while the 13 and 14 samples presented a marked viscoelastic character, not present in the full fat filling.

Formulations 6 and 8 with low fat content, were characterized by high n and m values, but low A values, so different from full fat formulation.

Among free fat formulations, sample 4 was the most similar to the full fat filling; while samples 2-3 had a foam structure, such as whipping cream. Results could be due to the ratio water-fat replacer powder, more appropriated for sample 4.

Table 5. Average estimated mechanical parameters (±SE) of different formulations

Formulations	A (Pa)	n (Pa.s)	m (Pa)	R^2
Margarine	9.93±0.06	0.23±0.01	1.21±0.05	0.99
Margarine and water	9.52±0.06	0.23±0.01	1.38±0.04	0.99
25	9.21±0.04	0.28±0.01	1.34±0.03	0.99
22	8.55±0.05	0.16±0.01	1.94±0.04	0.99
24	9.28±0.04	0.21±0.01	1.93±0.04	0.99
16	7.76±0.07	0.30±0.01	1.81±0.05	0.99
14	7.54±0.10	0.37±0.02	2.79±0.08	0.99
13	8.28±0.16	0.51±0.03	2.98±0.12	0.99
8	7.68±0.14	0.58±0.02	2.83±0.11	0.99
6	7.57±0.16	0.49±0.03	2.69±0.12	0.99
4	8.11±0.16	0.41±0.03	2.88±0.13	0.99
3	6.73±0.10	0.39±0.02	2.07±0.08	0.99
2	6.05±0.12	0.37±0.02	2.19±0.10	0.99

Formulation were ordered according their fat content: from the highest (margarine with 80% fat) to the lowest one (2 no fat)

4.3.1. PCA of filling creams with different fat content

To better explain the effect of the variables and to underline similarities among the formulations, a PCA was done and the results are shown in figure 8. The first two principal components (PCs) explained 48% and 28% of the variance, respectively. The first PC was connected to colour parameters and density, meanwhile the second PC was connected to a_w and A (fig. 8). In the first quadrant there were formulations (14-13-8-6) with high m and density values, yellow colour and low a_w. The high m value could be due to a viscoelastic behavior of the filling, determined by the gel formed by maltodextrin. In the second quadrant there were formulations (22-24-25-4) characterized by low a_w, low moisture, with similar low n value, but different A values. Formulation 16 was quite similar to the 4 one, even if with higher a_w and different m value. Formulations in the third and fourth quadrants all had high value of a_w. While formulations 2-3 were very airy, light in colour and with the lowest m values, formulations 11-19-5-7 had a low consistency and a high n value. Their viscosity was low and not very dependent on the strain rate.

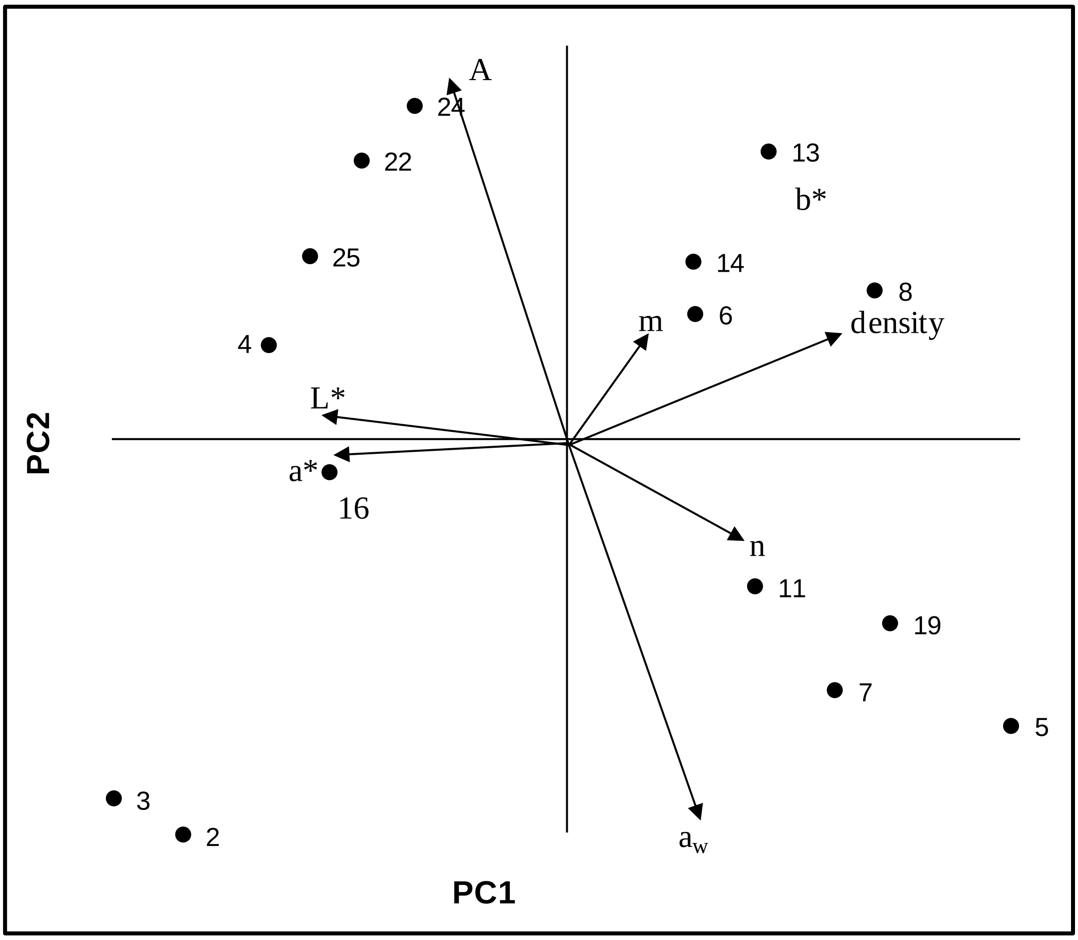


Figure 8. Bi-plot of weighted average scores and x-loadings

4.3.2. Fat replacer system optimization

Response parameters were explained through linear and quadratic models. In particular, as shown in table 6, quadratic models were used to explain almost all parameters considered, except for a_w and n, highlighting that the components interacted with each other and determined effect on structure and other parameters.

Table 6. Final Equations in Terms of Real Components

	A (Pa)	n (Pa.s)	m (Pa)	a_w	density (g/cm^3)	L*	a*	b*
W	**-34.10**	**-0.93**	**2.08**	**0.96**	**14.50**	**-91.18**	**-70.30**	**171.59**
MD	**18.00**	**10.36**	**-82.70**	**0.61**	**48.40**	**-599.00**	**-224.76**	**509.35**
MC	**-610.26**	**-50.29**	**-80.50**	**0.71**	**244.45**	**-2731.98**	**-1123.43**	**2193.53**
S	**-13.04**	**1.44**	**20.70**	**0.63**	**7.54**	**17.37**	**-27.75**	**101.26**
M	**7.94**	**0.28**	**0.50**	**0.75**	**2.05**	**73.71**	**-8.71**	**25.85**
W * MD	**121.00**	-11.40	**113.24**		**-1.13**	**15.62**	**5.38**	**-12.60**
W * MC	**879.00**	88.48	**-21.18**		**-2.93**	**33.31**	**13.55**	**-28.28**
W * S	**147.20**	2.05	**-28.70**		**-0.52**	**6.33**	**2.38**	**-6.23**
W * M	**60.20**	1.96	**6.37**		**-0.26**	**3.42**	**1.29**	**-3.05**
MD * MC	**348.16**	34.80	**498.60**		**-3.16**	**38.91**	**14.44**	**-25.53**
MD * S	**-131.20**	-23.94	**88.57**		-0.09	**1.95**	0.32	-0.69
MD * M	**-54.80**	-15.89	**66.71**		**-0.22**	**3.74**	**1.00**	**-2.22**
MC * S	**484.81**	61.37	**145.40**		**-2.13**	**24.88**	**9.84**	**-16.73**
MC * M	**720.00**	70.02	**110.00**		**-2.96**	**34.95**	**13.64**	**-27.24**
S * M	7.80	0.45	**-26.75**		**0.08**	**-1.52**	**-0.47**	**0.91**
R^2	0.99	0.74	1.00	0.98	1.00	1.00	0.99	1.00
p	< 0.0001	0.04	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0004	< 0.0001

Significant terms of the model were reported in bold case

n parameter was the worst estimated parameter among all with the lowest R^2 value and containing a lot of insignificant

terms in the model. Meanwhile m was estimated very well by the model chosen and it was influenced by maltodextrin and cellulose microcrystalline.
The best formulation of the fat replacer system was found, through the desirability function, imposing the constraints indicated in table 7:

Table 7. Constrains of optimal formulations

	Goal	Lower Limit	Upper Limit
A	maximize	5.40	9.28
N	in range	0.25	0.40
m	in range	1.50	2.50
a_w	minimize	0.76	0.87
kcal/g	in range	2.47	3.50
density	in range	0.70	1.00

The best formulations and the predicted properties were presented in table 8. They had the same caloric content ,the same water and margarine content, the same water-fat replacer ratio, but different content of each fat replacer. These results underlined the strong interaction among components of the fat replacer system .

Table 8. Composition and characteristics of optimal formulation with the highest desirability

Solutions	1	2	3
W	33.49	35.00	29.88
MD	9.74	4.23	6.44
MC	0.06	10.00	9.63
S	29.38	23.03	18.55
M	27.34	27.74	35.50
Moisture	19.86	16.96	17.23
A	8.10	8.48	7.93
n	0.40	0.40	0.27
m	2.50	1.78	2.50
aw t1	0.77	0.79	0.78
kcal	3.49	3.50	3.50
density	1.00	0.96	0.92
L*	84.53	85.26	86.71
a*	-3.38	-3.13	-3.00
b*	12.91	11.70	12.08
Desirability	**0.78**	**0.76**	**0.73**

Formulation one, with the highest desirability, was used to validate the model. The results from paired t-test, between predicted and measured scores, showed no significant differences The filling cream had 3.5 kcal/g , so, considering that the full fat filling had 5.1kcal/g, it can be defined as “reduced fat” filling cream.

4.4. Conclusion

Areas of overlap generated by the desirability function allowed to determinate optimum formulation for manufacturing fat-based model cream. Through whipping was not possible obtain a reduction of calorie content, because the system incorporated at least 25% of air.

D-Optimal design was useful to study the effect of fat reduction on properties of filling creams. The strengths of this method lie in reducing the number of formulations to run. Results obtained confirmed that it is often impossible to replace fat with a single compound, considering that the fat plays different roles in food. Evaluated filling creams presented different structure, such as pseudoplatic, foam, viscoelastic, depending on fat and fat replacer content. Lubricated squeezing flow tests gave important information about mechanical properties of the filling cream at relatively low biaxial rates, concerning spreading properties and consistency. Test results cannot be used to calculate flow properties that are pertinent to pumping or other engineering operations, where shear flow are also involved.

The quadratic model explained the effect of almost all ingredients of the fat replacer system Through the desirability function it was possible to develop a reduce fat filling cream.

4.5. References

BahramParvar M & Razavi SMA 2012 Rheological interactions of selected hydrocolloid–sugar–milk–emulsifier systems. *International Journal of Food Science and Technology*, 47: 854–860

BahramParvar M, Haddad Khodaparast MH, Razavi SMA 2009 The effect of Lallemantia royleana (Balangu) seed, palmate-tuber salep and carboxymethylcellulose gums on the physicochemical and sensory properties of typical soft ice cream. *International Journal of Dairy Technology*, 62: 571-576.

Bazmi A, Duquenoy A, Relkin P 2007 Aeration of low fat dairy emulsions: Effects of saturated-unsaturated triglycerides. *International Dairy Journal*, 17: 1021-1027.

Birkett J Fat-based centres and fillings. *In Science and technology of enrobed and filled chocolate, confectionery and bakery products*, Talbot G ed., WP & CRC Press, Cambridge, England. 2009 pp. 101-122.

Chu CA & Resurreccion AVA 2004 Optimization of a chocolate peanut spread using response surface methodology (RSM). *Journal of Sensory Studies* , 19: 237–260.

Campanella OH, Peleg M 2002 Squeezing flow viscosimetry for non elastic semiliquid food: Theory and Applications. *Critical Behaviour in Food Science and Nutrition*, 42: 241-264.

Castro A, Silva RSF, Tirapegui J, Borsato D, Bona E 2003 Simultaneous optimization of response variables in protein mixture formulation: constrained simplex method approach. *International Journal of Food Science & Technology,* 38: 103–110.

Corradini MG & Peleg M 2005 Consistency of dispersed food systems and its evaluation by squeezing flow viscometry. *Journal of Texture Studies*, 36: 605-629.

Di Monaco R, Miele NA, Cavella S, Masi P 2010 New chestnut-based chips optimization: Effects of ingredients. *LWT - Food Science and Technology*, 43: 126–132

REGULATION (EC) No 1924/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 December 2006 on “Nutrition and health claims made on foods”

Gacula MCJr *Design and analysis of sensory optimization*. Food & Nutrition Press, Trumbull, CT, USA, 1993.

Helstad S, Ingredient Interaction: Sweeteners. In *Ingredient Interactions: Effects on Food Quality, 2nd* ed” Gaonkar AG & McPherson A, eds., CRC Press, Boca Raton, USA, 2006 pp 167-194.

Holt SD, Resurrecion AVA & McWatters KH 1992 Formulation, evaluation and optimization of tortillas containing wheat, cowpea and peanut flours using mixture response surface methodology. *Journal of Food Science*, 57: 121–127.

Jones SA Physical, Chemical, and Sensory Aspects of Fat Replacement. In *Handbook of fat replacer*, Roller S & Jones SA, eds, CRC Press, Boca Raton, USA, 1996 pp. 59-86.

Launay B and Michon C 2008 Biaxial extensional of wheat flour doughs: lubrificated squeezing flow and stress relaxation properties. *Journal of texture Studies*, 39: 496-529.

Manley DJR Recipes for secondary processes. In: *Biscuit, Cracker and Cookie Recipes for the Food Industry*, Manley DJR ed., Woodhead Publishing Ltd., Cambridge, England. 2001 pp. 135-154.

Miele NA 2010 Effect of main ingredient on physical properties of fat-based filling. In *Proceedings of XV Workshop on the Development in the Italian PhD Research on Food Science Technology and Biotechnology*, 15 - 17/09/2010, Università degli Studi di Napoli, Portici.

Nath A & Chattopadhyay PK 2007 Optimization of oven toasting for improving crispness and other quality attributes of ready to eat potato-soy snack using response surface methodology. *Journal of Food Engineering*, 80: 1282–1292.

Seog EJ, Kim HR & Lee JH 2008 Optimization of onion cookie baking process using response surface methodology. *Journal of Food Quality,* 31: 306–322.

Singh SK, Castell Perez ME, Moreira RG 2005 Viscosity and Textural Attributes of Reduced-fat peanut pastes. *Food Engineering and Physical Properties*, 65: 849-853.

Steffe JF *Rheological Methods in Food Process Engineering*, 2nd Ed., Freeman Press, East Lansing, MI 1996.

Talbot G Fats for confectionery coatings and fillings. In *Science and technology of enrobed and filled chocolate, confectionery and bakery products*, Talbot G ed., Woodhead Publishing Ltd, Cambridge, EN, 2009 pp. 50-70.

Vatsala CN, Saxena DC & Rao HP 2001 Optimization of ingredients and process conditions for the preparation of puri using response surface methodology. *International Journal of Food Science and Technology*, 36: 407–414.

5. Acknowledgments

I would like to thank the following people for their help in making this thesis possible:

- prof. Silvana Cavella, my supervisor, for giving me the opportunity to do this work, for believing in me, for her scientific advice and availability;
- dr. Rossella Di Monaco, my co-tutor, for her scientific advice, support, patience and availability;
- prof. Delia Picone and her group, for the protein MNEI;
- dr. Nadia Perone, for her friendship, encouragement, scientific and human support;
- Stefania e Raffaele, for their help in conducing sensory test and friendship;
- sensory judges, for their devoted time and tireless effort;
- all my new and old colleges of Department of Agricultural and CAISIAL, for the long time spent together;
- prof. Giancarlo Barbieri, the coordinator of the PhD course;
- prof. Paolo Masi, the director of the Department of Agricultural.

It was a pleasure to work with all of them. Thanks to all of them, each of them, for better or for worse, contributed to professionally and humanly grow me up.

And finally, special thanks to Piero, Viola and the rest of my family, my friends, for their unconditional love, support, patience, concern, and advice.

6. Appendix

LIST OF PAPERS :

This PhD thesis was in part based on the following paper:

Di Monaco R**, Miele NA**, Picone D, Masi P and Cavella S 2013 Taste Detection and Recognition Thresholds of The Modified Monellin Sweetener: MNEI. *Journal of Sensory Studies 28* (1): 25–33. 1745-459X

Journal of Sensory Studies

Journal of Sensory Studies ISSN 0887-8250

TASTE DETECTION AND RECOGNITION THRESHOLDS OF THE MODIFIED MONELLIN SWEETENER: MNEI

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Accepted for Publication November 8, 2012

doi:10.1111/joss.12020

ABSTRACT

This research focuses on the sensory characterization of modified monellin protein (MNEI), extracted from the plant *Dioscoreophyllum cumminsii*. The specific objective of this study was to determine the detection threshold (DT) and recognition threshold (RT) of MNEI in different conditions typical of beverage processing and beverage consumption. The following effects on DT and RT were evaluated: mineral content, serving temperature of the product, pH and thermal treatment.

A selected panel of 14 assessors evaluated the DT and RT of MNEI by using a modified version of difference from the reference method. Through the modified method, assessors were also asked to give qualitative information about the perceived taste.

MNEI DT and RT were effectively obtained though this modified method. This study confirms that MNEI is a natural high-potency sweetener because the sweet taste RT is about 3,000 times lower than the sweet taste RT of the sucrose measured with the same assessors. Besides, both mineral content and serving temperature affected MNEI DT, whereas pH and thermal treatment did not affect the perceived sweetness. The modified version of difference from the reference method was useful for RT determination.

PRACTICAL APPLICATION

This study represents the first step of modified monellin protein (MNEI) sensory characterization. Because MNEI is a high-potency sweetener, it could be used in the beverage industry, meeting consumer needs for low-calorie content. Although sweeteners are not consumed at their threshold concentrations, the data in this article are an important scientific contribution to the knowledge of MNEI sensory performance.

INTRODUCTION

Sweetness is one of the most important tastes for humans and many animal species. The consumption of sweet food products yields a positive hedonic response in consumers. Sweet taste itself is generally given by soluble carbohydrates present in sufficient concentrations in the oral cavity, conveying the introduction of a large number of calories (Ganchrow *et al.* 1983). However, over the centuries, several hundred synthetic and natural molecules have been found to be sweet, such as sugars, amino acids, peptides, proteins, olefinic alcohols, nitroanilines, saccharin, chloroform and many other organic compounds (Temussi 2006). Indeed, some sweet compounds, after a short period of popularity, were apparently lost or forgotten, such as osladin, hernandulcin, phyllodulcin and other ones, as reported by Bassoli *et al.* (2011). Sweet molecules can be grouped in terms of chemical characteristics, molecular weight and size. In addition, some of these sweeteners have the same bulk and texture as sucrose (Patra *et al.* 2009) and are characterized by high sweetening power, low caloric power and a marked persistence of taste sensation. Sugar substitutes, also known as replacers or alternative sweeteners, may be either natural or artificial. It is very difficult to find natural or artificial

Journal of Sensory Studies **28** (2013) 25–33 25

List of other papers written during PhD:

Miele NA, Di Monaco R, Cavella S, Masi P 2010 Effect of meal accompaniments on the acceptability of a walnut oil-enriched mayonnaise with and without a health claim. *Food Quality and Preference* 21: 470-477. ISSN: 0950-3293

Food Quality and Preference 21 (2010) 470–477

Contents lists available at ScienceDirect

Food Quality and Preference

journal homepage: www.elsevier.com/locate/foodqual

Effect of meal accompaniments on the acceptability of a walnut oil-enriched mayonnaise with and without a health claim

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ARTICLE INFO

Article history:
Received 23 October 2008
Received in revised form 11 January 2010
Accepted 11 January 2010
Available online 18 January 2010

Keywords:
Mayonnaise
Acceptability
Meal accompaniments
Health claim

ABSTRACT

This study aimed to evaluate the effect of meal accompaniments on the acceptability and sensory attribute liking for a mayonnaise enriched with walnut oil, in the presence and absence of a health claim. Through focus group interviews we chose the foods to accompany the mayonnaise and the terminology to provide the health claim to subjects during the subsequent consumer test. During the first phase of the consumer test, the subjects responded to some questions about their food habits and evaluated three samples of mayonnaise with different contents of walnut oil without any information. During the second phase, the subjects were divided into two homogeneous groups and evaluated the samples associated to three different foods. The informed group tasted the samples accompanied by the health claim, the control group without any information. When the meal accompaniments were hamburger or French fries, acceptability of mayonnaise increased, whereas boiled squid rings had a negative effect on acceptability. The health claim did not affect the average consumer response.

1. Introduction

The development of new products is expensive and risky for the food industry. As most new foods are failures in terms of consumer acceptance, it is important to know what factors facilitate and what factors hinder consumer acceptance of new food products (Siegrist, 2007).

Although consumers may not always be able to vocalize their needs, it is important to understand how they perceive products, how their needs are shaped and influenced and how they make product choices based on their needs (Van Kleef, van Trijp, & Luning, 2005a). Food choice is the result of interactions between the food itself, the consumer and the context or the situation within which this interaction takes place. This type of choice incorporates not only decisions based on conscious reflection, but also those that are automatic, habitual and subconscious (Gains, 1994).

Food itself is characterized by intrinsic and extrinsic characteristics. Intrinsic characteristics such as sensory and nutritional properties, resulting from ingredients, processing and storage variables, are the determinants of product choice (Cardello, 2007). However, extrinsic characteristics, such as the information associated with the product, are also important. The information can be related to the brand label (Di Monaco, Cavella, Di Marzo, & Masi, 2004; Guinard, Uotani, & Schlich, 2001), health claim (Di Monaco, Ollila, & Tuorila, 2005; Kähkönen & Tuorila, 1999), price (Bower & Turner, 2001; Di Monaco et al., 2005), origin (Caporale, Policastro, Carlucci, & Monteleone, 2006; Iaccarino, Di Monaco, Mincione, Cavella, & Masi, 2006), technology (Iaccarino et al., 2006; Siret & Issanchou, 2000) and packaging (Marshall, Stuart, & Bell, 2006; Murray & Delahunty, 2000).

Product information may have different effects on consumer choice. In several studies that examined the effect of low-fat labels on sensory and hedonic expectations, e.g. Tuorila and Cardello (1994), Kähkönen, Tuorila, and Rita (1996) and Kähkönen and Tuorila (1998), label manipulation altered sensory expectations in the product, lowered hedonic expectations and modified sensory perception. In other studies using the same label with different foods (Kähkönen, Hakanpää, & Tuorila, 1999) sensory expectations were altered. When the tested product is already considered a low-fat healthy food, the information about its fat content does not affect the sensory and hedonic response of consumers (Kähkönen, Tuorila, & Lawless, 1997).

In the context of food health product development worldwide, functional food is currently one of the most intensive areas (Borgue & Sorenson, 2007). Functional foods are products that beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or a reduction in risk of disease (Diplock et al., 1999).

A functional food is usually accompanied by a health claim that may influence food choice. Roe, Levy, and Derby (1999) identified three types of potential biases in consumer inferences from nutri-

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0950-3293/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodqual.2010.01.001

Di Monaco R, **Miele NA**, Cavella S, Masi P 2010 New chestnut based chips optimization: effects of ingredients. *LWT- Food Science and Technology* 43: 126-132. ISSN: 0023-6438

LWT - Food Science and Technology 43 (2010) 126–132

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

New chestnut-based chips optimization: Effects of ingredients

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ARTICLE INFO

Article history:
Received 29 July 2008
Received in revised form
25 March 2009
Accepted 7 July 2009

Keywords:
Chestnut chips
Formula optimization
Sensory analysis
Consumer test

ABSTRACT

The objective of this study was to develop, produce and optimize new chestnut-based chips. The effect of three ingredients, lipids (x_1), glucose syrup (x_2) and albumin (x_3), was investigated in a mixture experiment. The sum of the component proportions ($x_1 + x_2 + x_3$) was always equal to one. Experimental design points were determined by the extreme vertices design. The effect of three different lipids (palm oil, butter and a mixture of maize oil and margarine) was also investigated. Quantitative descriptive analysis was performed by eight trained judges who evaluated nine sensory attributes. ANOVA results showed that varying the levels of ingredients does not affect adhesiveness, oily mouth coating or astringency. All the other attributes (colour, crispness, hardness, oiliness, sweetness and chestnut flavour) were used as response variables in regression models built to determine the optimal levels of ingredients. Contour plots for sensory attributes and lipid type were generated and superimposed to determine areas of overlap. Optimal conditions were generated by setting the best sensory performance as a goal for each response. Three optimal formulations were found for each lipid, two of which were used for a consumer test to evaluate chip acceptability.

1. Introduction

Snack foods are products that are highly appreciated and consumed throughout the world; they are designed to be less perishable, more durable and more appealing than natural foods. Different types of snack foods are available; the new generation of snacks falls into several categories such as low fat, baked but not fried, high fibre products made from rice/wheat bran and coated snacks (Mazumder, Roopa, & Bhattacharya, 2007).

The effect of replacing wheat flour with bean and corn flour on consumer acceptability was studied by Ward, Resurreccion, and Watters (1998), who found that African consumers appreciated the new snack proposed more than Americans. Formulations with the highest percentage of bean flour and medium wheat flour contents were the most preferred. White bean puree used as a fat substitute to produce biscuits with chocolate drops was inappropriate since consumers did not like biscuits when a large amount of puree was added (Rankin & Bingham, 2000). Good results were achieved in assessing the acceptability of a new product prepared with soybean, corn and cassava flour (Senthil, Ravi, Bhat, & Seethalakshmi, 2002).

In the process of new food development, optimization of ingredients is an essential step to obtain the best product under a given set of criteria, i.e. the best sensory performance and the highest acceptability with the lowest costs of production (Gacula, 1993). In the search for the best formulation, the main objective is to determine the optimum levels of the components or key ingredients. The ingredients are the independent variables or factors and the dependent variable or response is the objective to be optimized (Castro, Silva, Tirapegui, Borsato, & Bona, 2003).

Response surface methodology (RSM) is one of the most widely used optimization techniques due to its efficiency and simplicity, but it does not consider the linear dependence between the components of the formulation. RSM was successfully applied to optimize the onion biscuit baking process and many polynomial models developed by RSM were highly effective at describing the relationship between the studied factors and the response (Seog, Kim, & Lee, 2008). This method was also used to optimize formulations of chocolate peanut spread (Chu & Resurreccion, 2004), malted milk beverage (Singh, Tiwari, Singh, Tyagi, & Arora, 2008), extruded snack food (Thakur & Saxena, 2000), reduced calorie tropical mixed fruits jam (Abdullah & Cheng, 2001), robusta coffee (Mendes, de Menezes, Aparecida, & da Silva, 2001), pasta from rice brokens (Raina, Singh, Bawa, & Saxena, 2005).

Castro et al. (2003) used a complex constrained simplex method to optimize a ternary mixture of protein ingredients used to prepare a milk drink. Three proteins were mixed according to

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0023-6438/$ – see front matter
doi:10.1016/j.lwt.2009.07.005