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**PHYSICO-CHEMICAL AND MICROSTRUCTURAL PROPERTIES
OF FOOD DISPERSIONS**

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals. The papers are appended at the end of the thesis

- Paper I.** **Glicerina V.,** Tornberg E. and Bergenstål B. (2013).
The influence of small amount of water on the microstructural and rheological properties of sugar particle dispersions in cocoa butter
Manuscript
- Paper II.** **Glicerina V.,** Balestra F., Dalla Rosa M. and Romani S. (2013).
The influence of process steps on microstructural, rheological and thermal properties of dark chocolate.
Journal on Processing and Energy in Agriculture, 17 (2), 59-63
- Paper III.** **Glicerina V.,** Balestra F., Dalla Rosa M. and Romani S. (2013).
The influence of different process steps on particle size, microstructure and appearance of dark chocolate.
Submitted for publication, 2013
- Paper IV.** **Glicerina V.,** Balestra F., Dalla Rosa M. and Romani S. (2013).
Effect of manufacturing process on the microstructural and rheological properties of milk chocolate.
Submitted for publication, 2013
- Paper V.** **Glicerina V.,** Balestra F., Dalla Rosa M. and Romani S. (2014).
Microstructural and rheological properties of white chocolate during process.
Submitted for publication, 2014
- Paper VI.** **Glicerina V.,** Balestra F., Pinnavaia G.G., Dalla Rosa M. and Romani S. (2013). Rheological characteristics of nut creams realized with different types and amounts of fats.
Journal of Food Quality, 36, 342-350.
- Paper VII.** **Glicerina V.,** Balestra F., Dalla Rosa M. and Romani S. (2013).
Rheological, textural and calorimetric modifications of dark chocolate during process.
Journal of Food Engineering, 119, 173-179.

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1 Introduction

A wide variety of foods are dispersions (suspensions or emulsions), containing solid or liquid particles in a continuous liquid matrix. A typical example of food dispersion is the molten chocolate. Chocolate (a cocoa dispersion), can be defined as concentrated suspensions made up of solid particles, such as sugar, cocoa powder, milk powder, etc., dispersed in a Newtonian liquid, generally cocoa butter (Afoakwa et al., 2008).

Chocolate can be consumed as a candy or used to make beverages. It is also used as a flavouring ingredient or coating for various confectionery and bakery products (Beckett, 2008). The most common types of chocolate are dark, milk and white, characterized by different particles in suspension (depending on the recipe) and the same continuous matrix (cocoa butter). Other commercial products such as cocoa or nut creams, chocolate surrogates obtained by using fats instead cocoa butter, are widespread used.

All chocolate dispersions are very complex system, being non-Newtonian, shear thinning fluids that exhibit yield stress and thixotropic behaviour (Afoakwa et al., 2007). Rheological characteristics (fundamental and empirical) are the most important attribute, affecting several properties such as viscosity, consistency and mouth feel, these properties influenced in strong way the quality and stability of final products (Servais, 2004). In chocolate dispersions all the physico-chemical and microstructural properties are strictly related to their formulation and to the manufacture process (Vavreck, 2004; Schantz & Rohm, 2005). Actually the main steps of chocolate manufacture, mixing, pre-refining, refining, conching and tempering, involves significant modifications in the rheological properties of the mass and consequently in its final quality. An effective control of the process parameters is required in order to achieve a constant and desirable quality of the final product (Muller-Fischer et al., 2005, Baixauli et al., 2007). During chocolate manufacturing, mixtures of sugar, cocoa and fat are heated, cooled, pressurized and refined (Beckett 2000). These steps not only affect particle size reduction, but also break agglomerates and distribute lipid and lecithin-coated particles through the continuous phase, this considerably modify the microstructure of final chocolate (Afoakwa et al., 2009). The interactions between the suspended particles and the continuous phase provide information about the existing network and consequently can be associated to the properties

and characteristics of the dispersions (Sato et al., 2009). Moreover since the macroscopic properties of food materials, as well as the appearance attributes and the thermal ones are strongly determined by their microstructure, the evaluation and study of the microstructural characteristics, can be very important for a through understanding of the food matrices characteristics (Aguilera & Stanley 1999).

1.1 Aim of the thesis

Physico-chemical characteristics and in particular rheological ones are the most important properties in continuous oil food dispersions. The rheological properties depend on particle-particle interactions and on existings network that are directly influenced by formulation and manufacture process.

Understanding the influence of both formulation and manufacture process on the microstructural and consequently on the physico-chemical attributes of the final product, can be a key parameter in order to obtain more detailed informations regarding the complexity of cocoa dispersions matrices. Moreover, to my knowledge no papers are available in literature about the influence of each single process step on microstructural and physico-chemical properties chocolate type dispersions. The most part of the literature (Servais et al., 2004; Afoakwa et al., 2008, 2009; Baldino et al., 2010; et al., 2011) has focused on the study of these characteristics in dark and milk chocolate only after the final tempering process step.

For these reasons the objectives of this investigation were:

- to study the influence of formulation and each process step on the microstructural properties of: chocolate type dispersions model systems (**Paper I**); dark (**Papers II & III**); milk (**Paper IV**) and white chocolate (**Paper V**) types, and cocoa creams (**Paper VI**);
- to investigate the relationships between microstructural changes and the resulting physico-chemical properties of: chocolate type dispersions model systems (**Paper I**); dark (**Papers II & IV**); milk (**Paper IV**) and white chocolate (**Paper V**) products.

2 Chocolate formulation

Chocolate can be defined as a concentrated suspension of solid particles in a continuous phase of semi-crystalline fat (Johansson & Bergensthal, 1992; Afoakwa et al., 2008). The three main types of chocolate are dark, milk and white. Dark chocolate is a suspension of sugar and non-fat cocoa solids in cocoa butter, while milk chocolate contains milk solids and milk fat in the mix. White chocolate, on the other hand, includes milk solids and milk fat but does not include non-fat cocoa solids. Chocolate can also include emulsifiers such as lecithin and PGPR (polyglycerol polyricinoleate) as well as salt, flavorings or spices. The directives 2000/36/EC of the European parliament and of the council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption define:

- **Cocoa powder:** the product obtained by converting into powder cocoa beans previously cleaned, shelled and roasted, and which contains not less than 20 % cocoa butter, calculated according to the weight of the dry matter, and not more than 9 % water.
- **Cocoa butter:** the fat obtained from cocoa beans or parts of them with the following characteristics:
 - free fatty acid content (expressed as oleic acid): not more than 1.75 %
 - unsaponifiable matter (determined using petroleum ether): not more than 0.5 % except in the case of press cocoa butter, where it has to be not more than 0.35 %

For what concern the final product the above reported directive define:

- **Chocolate:** as the product obtained from cocoa products and sugars, containing not less than 35 % total dry cocoa solids, including not less than 18 % cocoa butter and not less than 14 % of dry non-fat cocoa solids.

In particular in order to differentiate in a more detailed way the different formulations, define:

- **Milk chocolate:** as the product obtained from cocoa products, sugar and milk or milk products, which contains: not less than 25 % total dry cocoa solids, not less than 14 % dry milk solids obtained by partly or totally dehydrated full cream milk, semi- or full-

skimmed milk, cream, or from partly or completely dehydrated cream, butter or milk fat not less than 2.5 % dry non-fat cocoa solids, not less than 3.5 % milk fat and not less than 25 % total fat (cocoa butter and milk fat).

- **White Chocolate:** the product obtained from cocoa butter, milk or milk products and sugar which contains not less than 20 % cocoa butter and not less than 14 % dry milk solids, obtained by partly or totally dehydrated full cream milk; semi- or full-skimmed milk; cream, or from partly or completely dehydrated cream; butter or milk fat, of which not less than 3.5 % of milk fat.

2.1 Cocoa Liquor

Cocoa liquor is the product obtained from grinding deshelled and roasted cocoa beans. It is composed by cocoa butter and cocoa solid particles. Chemically it is a mixture of mono- and di-, triglycerides, proteins, cellulose, starch, water, minerals, polyphenols, phospholipids and other organic compounds present in minor quantities. From its composition it is a very complex matrix and contains several compounds that can adsorb large amounts of water (Afoakwa et al., 2009).

In order to separate cocoa butter and cocoa particles from liquor, a pressing system is usually used. Beckett (2008) stated that hydraulic press and expeller extrusion are the two most common ways to extract cocoa butter. In order to obtain the best quality of cocoa butter as well as cocoa powder, a hydraulic press is generally preferred. However, only 55 % of cocoa butter can be extracted with this method and the by-products (e.g. pressed cocoa powder cakes) may contain variable amounts of fat from 8 to 12 % up to 24 % (Hui, 2007). Pressed cocoa cakes need to be further milled in order to obtain a suitable cocoa powder. Cakes are broken into large agglomerates of approximately 3 cm diameter passing through rotating rollers. These agglomerates are finely grounded by hammer mills, and then sieved. Finally, cocoa powder is transported through systems of pipes and packaged immediately. This milling process is carried out at cool temperature, because if temperature is more than 34°C the cocoa butter will melt, leading to a sticky powder. Moreover, during transport should be avoid all contact with moisture because the presence of water tends to aggregate sugar particles creating small aggregates of cocoa powder. This modification of the structural and rheological properties of the product, may be undesirable for the customer (Afoakwa, 2010). Two types of cocoa powder are commonly produced: the high fat cocoa powder (from 20 to 25 % fat content) which is mainly used for

chocolate drinks and the low fat cocoa powder (from 10 to 12 % fat content) which is commonly used for flavourings and coverings of cakes, ice creams or biscuits (Shittu & Lawal, 2007).

2.2 Cocoa particles

The major components of cocoa particles are: proteins (11.5% of the cocoa liquor mass), cellulose (9%), starch (6%), polyhydroxyphenols (6%) and water (5%) (Vernier, 1998; Belitz & Grosch, 1999). Usually cocoa particles amount in chocolate is around 10 %. To release most of the cocoa butter (present in the inner structure of cocoa particles) and hence reduce viscosity, the cocoa particles should be refined to sizes below the size less than that of the cocoa cells (20 to 30 μ m) (Minfie, 1989). This upper size limit is also important because the mouthfeel of chocolate containing particles bigger than 30 μ m is perceived as grainy or gritty (Afoakwa et al., 2007). The cocoa particles are normally coated with cocoa butter, (that reduces the inter-particles force), and are therefore referred to as being hydrophobic (Beckett 2008; Dhonsi & Stapley 2006). Nevertheless, from the composition cocoa particles contain amphiphilic (proteins) or hydrophilic components (starch, cellulose). The considerable water content of the cocoa particles in chocolate is mainly reduced during the conching step.

2.3 Cocoa Butter

Cocoa butter represents the continuous and Newtonian fat phase of cocoa and chocolate dispersions (Baldino et al., 2010). This fat is a triglyceride, made up from three fatty acids attached to a glycerol backbone. It is composed mainly of triacylglycerols of three fatty acids: oleic (about 35%), stearic (about 34%), and palmitic (about 26%), the oleic acid is normally located in the central position (Afoakwa et al., 2007; Beckett, 2008).

Cocoa butter can exist in six different crystalline or polymorphic forms (often denoted by roman numbers I–VI), depending on the temperature reached during productive process and how it is processed, each of which exhibit different thermodynamic stability and melting temperatures (Figure 1) (Rousseau, 2007; Svanberg et al., 2011).

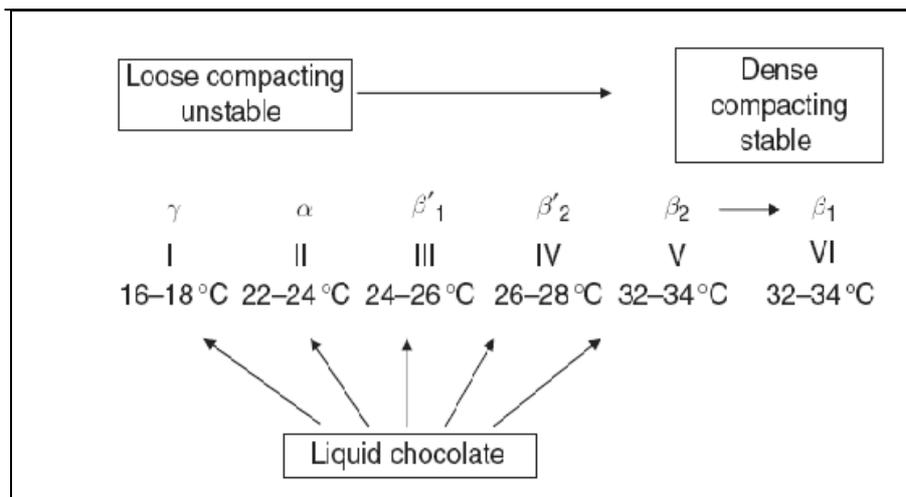


Figure 1. Respective melting points of the polymorphic forms of cocoa butters (Afoakwa et al., 2007).

However, for commercial chocolate production only forms IV to VI are important. Form V is the preferred polymorph, is produced in the factory by machines called temperers, which brings the chocolate through a cooling/ heating cycle while vigorously mixing it. Form IV is found in untempered chocolate, and form VI is found in bloomed samples (Timms, 2002). For the final quality of the chocolate product, it is of utmost importance to obtain polymorphic form V, as it has the capacity to trap liquid oil within its crystal network, thereby obstructing the migration of liquid TAGs (triacylglycerols) to the surface (Dibildox-Alvarado et al., 2004; Smith et al., 2007; Svanberg et. al., 2011). The different forms are due to the individual molecules of fat packing themselves together in different ways. Depending on the countries' regulations and intended use, other types of vegetable fat can be used in chocolate or compound coatings. These fats can either be cocoa butter equivalents (CBE) which have similar chemical and physical characteristics to those of cocoa butter or cocoa butter replacers (CBR), which are similar to cocoa butter only in their physical properties (Talbot, 1994; Samsudin & Rahim, 1996). Typical CBE blends are produced from mixtures of fractionated fats from palm, illipè and shea and must be tempered like cocoa butter (Talbot, 1994). On the other hand, lauric and non-lauric CBRs produced from palm kernel or coconut oil and palm or soyabean oil respectively do not require tempering as they are non-polymorphic (Talbot, 1994).

2.4 Sugar

Sugar is one of the most important component of the cocoa dispersions (Beckett, 2008), its amount in a chocolate recipes is normally around 45 -50 % of the whole formulation. As known by literature (Beckett, 2008) different molecular varieties of sugar exist, all of them are designed for different types of chocolate. Among these varieties, sucrose and lactose are used predominantly. In particular, sucrose is a disaccharide and is made of a molecule of glucose linked to a molecule of fructose. Sucrose can exist in two different polymorphic structures: form I and form with different chemico-physical properties depending on the arrangement of their crystal (Okuno et al., 2003; Lee & Lin, 2007). Moreover the presence of a solvent, such as water, plays an important role influencing sugar crystallization properties and the flow properties of liquid chocolate. The surface properties of the sugar particles shows strong affinity with water molecules. In particular, the damage of sugar particles during the refining step (**paper VII**) can create surfaces with extremely high temperature, that are able to absorb the humidity present in the surrounding, modifying their crystalline structures, that become amorphous, and their rheological properties (Bouzas & Brown, 1995; Vernier, 1998). Appropriate processing conditions, that is low humidity of the air in the factory and intermediate storage under dry conditions, protect the sugar in chocolate from moisture uptake. Upon water uptake, the amorphous sugar re-crystallizes and releases the water. This process can happen immediately after the roller-refining (conching step) or during storage (Beckett, 1999).

2.5 Milk powder and derivatives

Milk powder is one of the main ingredient of milk and white chocolate (being used at about 20% w/w in the formulation). Milk products are complex materials composed of: proteins, lactose, fat and emulsifiers (fat membrane components) (Attaie et al., 2003). Milk powder determines the sensory profile of the chocolate (taste, texture) and influences processing behaviour of the molten chocolate mass, e.g. flow properties (Franke & Heinzelmann, 2008). The latter one is important for chocolate manufacturers with respect to moulding and coating. From its structure, milk powder can be described as a sponge-like material, exhibiting a large specific surface area. The proteins are capable of binding high amounts of water and lactose that can be present in the amorphous state. These physical and chemical attributes give to milk powder its high water

sorption capacity (Dewettinck et al.,1996; Fäldt & Bergenstahl, 1996). Milk and cream powders are milk products, which can be obtained by the partial removal of water from milk or cream; in particular powders for the chocolate industry are usually produced by spray drying or by roller drying. The fat and/or protein content of the milk or cream may have been adjusted, only to comply with the compositional requirements, as shown in detailed way as follow, by the addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted (Codex Alimentarius, 2003).

The principal milk powders used during the chocolate production are:

- ***Full cream Milk Powder***

Full milk powders obtained by using spray- and roller-dried techniques. Specially processed spray-dried powders with a higher level of free fat are produced for the confectionery sector. The composition should be as follows (Codex Alimentarius, 2003):

- Minimum milk fat	42 %	m/m
- Maximum water	5.0 %	m/m
- Minimum milk protein in milksolids-not-fat	34 %	m/m

- ***Skimmed Milk Powder***

This powder is used in combination with milk fat to make chocolate. This means that all the fat is free, so the viscosity is lower, the texture softer and the taste is different compared to a product made with an equivalent amount of full cream milk powder. Sometimes, lipase-free milk powders are requested. This is because the lipase can accelerate decomposition of some milk fat into free fatty acids, which produce a soapy or cheesy flavour (Codex Alimentarius, 2003).

- Maximum milk fat	1.5 %	m/m
- Maximum water	5.0 %	m/m
- Minimum milk protein in milk solid non fat	34 %	m/m

- ***Whey Powder***

Whey powders are milk products obtained by drying whey or acid whey. Whey is the fluid milk product obtained during the manufacture of cheese, casein or similar products by separation from the curd after coagulation of milk and/or of products obtained from milk. Coagulation is obtained through the action of, principally, rennet type enzymes. This powder is normally used at a level up to about 5% of the chocolate mass to increase the “milkiness” of the product. It is used at

higher levels in coatings and chocolate-like products. In general, demineralized whey is used not to impart unwanted flavours into the chocolate (Codex Alimentarius, 2003; Beckett, 2008).

- Maximum milk fat	2.0 % (m/m)
- Maximum water	5.0 % (m/m)
- Minimum Milk protein	10 % (m/m)

- ***Lactose***

A natural constituent of milk normally obtained from whey with an anhydrous lactose content of not less than 99 % m/m on a dry basis. Lactose can be used as a partial alternative to sucrose in order to make chocolate less sweet. As with whey, it must not contain any off-flavours (Codex Alimentarius, 2003).

- ***High-Fat Powders***

Full-cream milk powders are available with more than 55 % milk fat. This anhydrous fat is largely present in a free form and so helps chocolate to flow. The high-fat powders enable the manufacturer to add all the milk fat as a powder and thereby to avoid the cost and inconvenience of additional liquid fat metering systems (Codex Alimentarius, 2003).

- ***Buttermilk Powder***

As with whey powder, this can be used in smaller proportions to adjust the flavor and the flow properties of a chocolate. It is also used in chocolate-flavored coatings and, in the United States a special type of product, known as “buttermilk chocolate” is manufactured. Milk fat is commonly used in chocolate because of its desirable flavour and its lower price compared with cocoa butter. Adding milk fat also influences the physical properties of chocolate masses, such as the crystallization behaviour of cocoa butter and the texture of the final chocolate. A negative correlation between the amount of milk fat used and the hardness of chocolate has been reported (Afoakwa, 2010). Although this softening effect of milk fat in chocolate limits its use; up to 30% of cocoa butter can be replaced by milk fat before the product becomes unacceptable (Hartel, 1996; Codex Alimentarius, 2003).

2.6 Minor ingredients in the chocolate recipe

Minor ingredients are also present in a chocolate recipe, that contribute to the final attribute of the product. Inside this group there are: stabilizers, used in order to improve the dispersion stability throughout the entire shelf life; natural extracts, that contribute to the final aroma of the product; and overall emulsifiers and water. The last two ingredients, even if present in lower amount (around 1 % or less), influence in strong way the microstructural and rheological properties of a cocoa dispersion.

Emulsifiers

Most oil-continuous food dispersions such as cocoa ones, contain emulsifiers, that can regulate the rheological properties, the crystallization of fats and improve the dispersion of the disperse phase particles (cocoa, sugar, milk particles, etc.) in the cocoa butter (Schuster, 1985). The emulsifiers function is mainly related to their ability to adsorb to different surfaces like oil/water, water/air or particle surfaces (Johansson, & Bergensthal, 1992a). The hydrophilic portion of the emulsifier molecules is attached at the hydrophilic surface of chocolate particles. The hydrophobic portion protrudes into the bulk fat phase. This reduces particle-particle interactions due to steric hindrance, modification of Van-der Waals attraction forces and (in the case of charged emulsifiers) modification of particles surface charge (Babin, 2005). The emulsifiers that are mostly used in chocolate type dispersions are: lecithin and PGPR. Their most important feature is the reduction of the final product yield value (PGPR, lecithin) and viscosity (lecithin) (Weyland, 1994). This is important since the cocoa butter content can be reduced in chocolate (which is the most caloric and expensive chocolate component), even if the amount of emulsifiers cannot exceed the limit range, in order to avoid agglomeration effects between micelles. A viscosity increase in chocolate, when lecithin is dosed at concentrations beyond the amount needed for monolayer coverage, was attributed to the thickening effect of the micelles, which may also immobilize fat phase (Finke, 1991).

Water

Cocoa dispersions contain typically from 0.5 to 1.5% of water; with an a_w -value of 0.5 - 0.6 (Jermini, 1980). When water is added to molten chocolate in small amounts, the chocolate thickens irreversibly and can no longer be processed. Water in small amounts (0.5 %) dissolves the sugar particle surfaces, which leads to a concentrated syrup at the sugar surface and strong agglomeration effects (**Paper I**). This results in a strong disperse phase network, that involves a

modification in the rheological properties of sample, causing an increase in its yield stress and viscosity. When water is added to the chocolate at higher percentages, interactions with the disperse phase particles and with the emulsifiers have to be taken into account. Belton, et al., (1995) investigated the effect of water addition to milk chocolate. He demonstrated by NMR and microscopy analysis that the water causes the dissolution of carbohydrates (sugar) and partial dissolution of proteins. This promotes the interactions between carbohydrates and proteins and causes protein swelling and a build-up of a network. The separation of fat and proteins from the matrix could be the cause of the fat weak contraction and the difficult demoulding.

3 Manufacture process of chocolate type dispersions

As previously reported in the manufacture process of chocolate common ingredients requested are: sugar, cocoa nibs, cocoa liquor, cocoa butter, butter fat, some emulsifiers, and milk powder for the production of milk chocolate. The addition of these ingredients must be rigorously performed and should follow several process stages including dosing of the ingredients, mixing, pre-refining refining, conching and tempering (Beckett, 2008). The follow diagram (Figure 2) illustrates the main steps of the chocolate manufacture process.

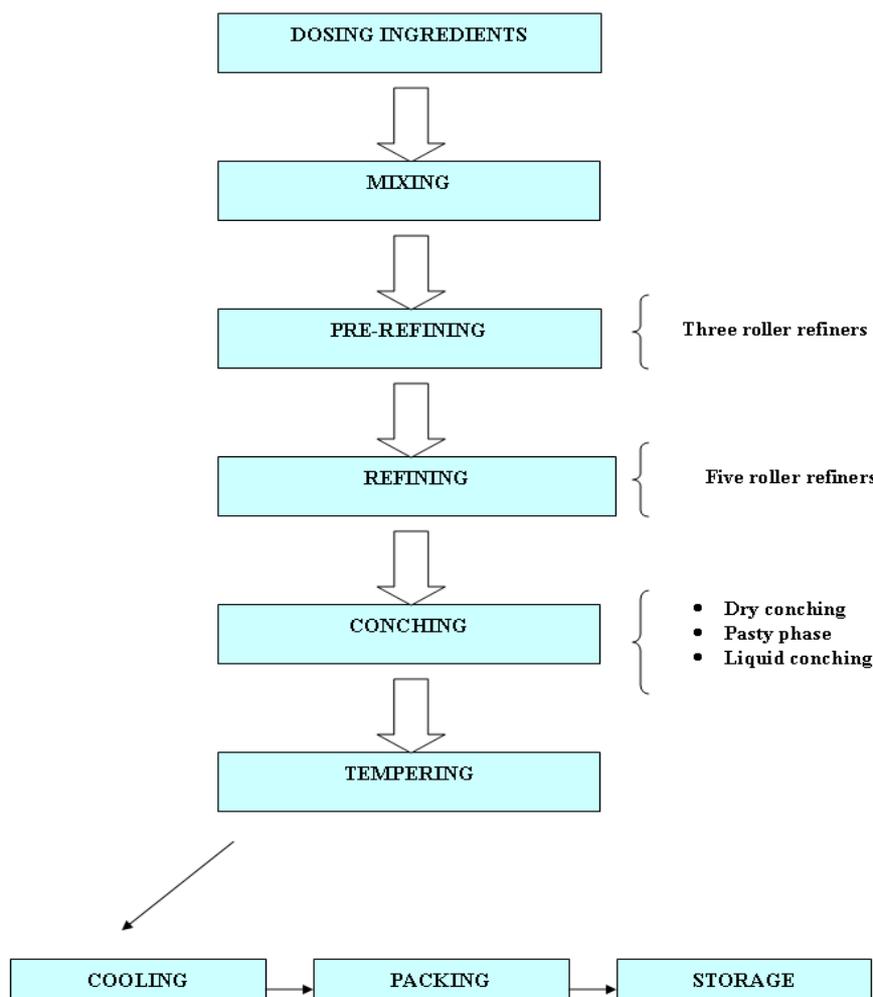


Figure 2. Main phases of a chocolate manufacture process. (Adapted from Afoakwa et al., 2007)

3.1 Continuous plant and roller ball mill

Cocoa dispersions have two major distinguishing characteristics: the flavour and its rheological properties. The processing of chocolate is aimed to obtaining these two main quality characteristics in the final product. Although two different methods of chocolate making exist: the industrial and the small scale one. In the first one, the final product is obtained by pushing all the dosed ingredients through a mixing machine linked to a three roller refiners (in order to obtain a first reduction in particle size around 100 μm) that transport the mass until a five roller refiners (in order to obtain a more refined product, around 30 μm particles). The refined product is then piped to a conche and at the end toward a tempering machine. The process is fully automatic and all the single machine are collected each other by a series of pipes and heat exchangers (Afoakwa et al., 2007). The small scale plant is usually a non-continuous plant, called “roller ball mill”. This manufacture plant consists of a vertical ball mill grinding chamber equipped with a thermostatic control even at night. The inner part of the roller ball is characterized by the presence of small stain ball/beads, that realize at the same time the different steps of: mixing, pre-refining, refining and conching. Alloy steel beads are particularly suitable, because of their high relative density and hardness, for crushing and mixing heavy and hard materials and also favourable in reducing particle size and fine dispersion of highly viscous fluids, such as cocoa mass dispersions (Alamprese et al., 2007). The ingredients passing through and between the steel beads are mixed, refined and at the same time conched because of the crushing and mixing effect of these beads on the particles present in the fluid matrix (Figure 3) (Martin, 1988). The tempering step is then realized in a separated machine called: “tempering machine”. In this step the final product is obtained as it will show in the 3.7 paragraph.

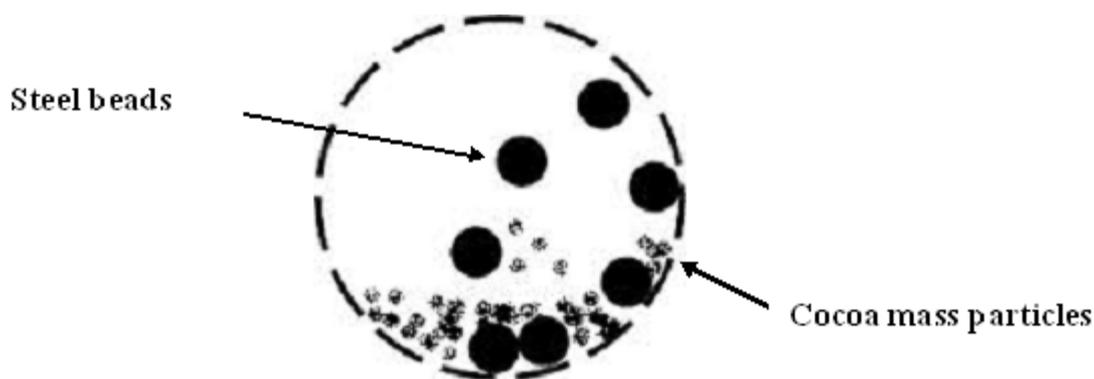


Figure 3. Mechanism of action of a roller ball mill machine (Nanoparticles, 2013).

3.2 Dosing ingredients

The first step of the manufacture process of chocolate is the dosing of the ingredients. An accurate blending and mixing is a basic requirement for making high-quality products. Usually an automatic tubular screw conveyor is used (Figure 4) in order to collect (from the cylindrical raw materials storage unit) each individual ingredient, to weigh hoppers and then to the mixers. This machine is also usefully suited to conveying of materials in applications which must meet elevated sanitation requirements. Usually a sanitation-focused design and bearings that are separated from the product stream, and a completely enclosed housing allow flexible application even if demanding requirements must be satisfied (Buhler, 2013)



Figure 4. Automatic tubular screw conveyor for the dosing ingredients (Buhler, 2013)

3.3 Mixing

Mixing is the second phase process achieved for the chocolate production as previously seen in Figure 2. The basic ingredients, including cocoa liquor, sugar, cocoa butter, milk products (for milk chocolate) are blended together. The machine is generally equipped with a thermostat control, with either open or closed water circuit and in conjunction with a hot water system energy-efficient, in order to provide accurate temperature control (Figure 5). The outcome is a paste with a rough texture and plastic consistency. Commonly from 2 to 10 minutes of mixing are necessary in large-scale manufacturing (Afoakwa, 2010; Beckett, 2008).



Figure 5. Mixer machine used to blend chocolate ingredients (Buhler, 2013).

3.4 Pre-refining

After the mixing step in many industrial plant there is a phase of pre-refining. This step is done with a series of three roller refiners, in which the chocolate mass is pushed through the rollers with a defined gap. This refiner was set up vertically, with the first two rollers in the feeding area and the third roller collecting the flakes (Figure 6). This stage allows the reduction of the particle

size smaller than 100 μm , but this size is still larger for what concern the optimization of chocolate rheological and sensorial properties, so a further refining step is necessary.

However the pre-refining step is very useful in order to obtain:

- homogeneous particle size distribution and fluidity of the chocolate mass;
- increased productivity of dosing and mixing line thanks to reduced mixing time;
- improved refiner efficiency (Afoakwa et al., 2007).



Figure 6. Pre-refiner machine equipped with three roller refiners (Buhler, 2013).

3.5 Refining

Refining is an important processing stage, carried out in order to obtain a reduction of mass particles size, and the releasing of some fat, that uniformly have to coat the different kinds of particles (Vernier, 1998). Refining of chocolate that is an important step to for the production of smooth texture that is a required quality attribute in modern chocolate confectionery. Final particle size and the microstructure of chocolate (sugar and cocoa networks, fat distribution, etc...), influence the rheological and sensory properties of the cocoa mass (Afoakwa et al., 2009; Fernandes et al., 2013). The refining step is done with a series of roller refiners, generally with five rollers, used to reduce the particles size lower than 30 μm , which is the optimum size of particles in chocolate products. The cocoa liquor is pushed through the rollers with a defined gap. The 5-roller refiner is set up horizontally whereas the 2-roller refiner is set vertically. The first roller is commonly used for feeding, whereas the fifth roller collects the flake by a knife

blade. Each roller is thermostatically controlled via inner cooling/heating systems (Figure 7). In this phase the control of temperature plays an important role; for instance, if the roller is too cold, the fat will crystallise on it. Moreover, the local pressure and the shearing rate are the two main factors which control the extent of size reduction of the particles. The pressure in the gap is hydraulically controlled, and the shearing action depends on the relative roller speed. Shearing causes the particles to be pulled apart and pushed towards the faster moving roller. By adjusting the gap size, the operator can generate different shear entities and so different amounts of product pushed towards the faster roller. The average size of the particles obtained in the final stage depends greatly on the gap between rollers, the speed of the refining rollers and the original size of the particles (Minifie, 1989; Beckett, 1999; Beckett, 2008). The refiners, in summary, not only affect the particle size reduction, but allows also the release of fat necessary in the next step.

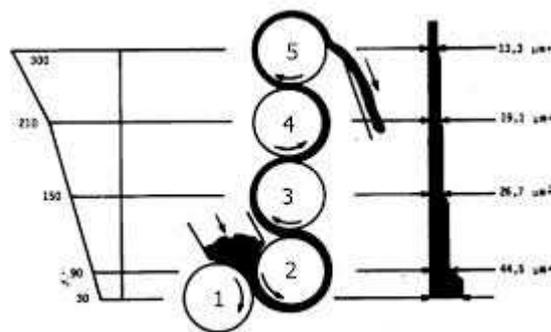


Figure 7. Mechanism of action of the five roller refiners (Cacaochocolade, 2013).

3.6 Conching

Conching is an essential operation that contributes to develop of the viscosity, texture and flavour of the final product. The principle of conching is to enhance the flavour and the texture of the powdery state by a series of periodic mixings. Conching is normally carried out by shaking chocolate at more that 50 °C for few hours (Beckett, 2008). During this step, the chocolate texture evolves in three stages: dry conching, pasty phase and liquid conching (Figure 8). The aim of this step is to mix the powder as long as possible in the dry state in order to allows the removal of undesirable volatile compounds, e.g. ethanoic acid, through its powerful mixing. It also permits the disruption of aggregates and the further release of fat to coat more of the

particles. Moreover, it has been observed that, during the earlier stage (Cabras & Martelli, 2004), the chocolate paste loses part of its water content thereby contributing greatly to the fluidity of the system. This water release, under vaporised form, is enhanced by the temperature of the conche (70-80 °C). As the cocoa butter melts and water is lost, the chocolate is transformed from a powdery state (or flakes) into a thick paste. The slow motion of the rotary blades allows uniform mixing of cocoa butter and lecithin (that is usually added during this step) throughout the paste (Minifie, 1989; Awua, 2002; Beckett, 2008). The viscosity of the sample gets thinner as more cocoa butter is present in the liquefied state and more water is lost during the process. In the last stage of the conching, the mixing time is increased and the remaining ingredients such as cocoa butter and emulsifiers are added to the system. These ingredients bring more fluidity to the dispersed system and play an important role in determining the viscosity of the final product. At this stage of the process, few more processing procedures are required before the product can be packaged (Awua, 2002, Whitfield, 2005; Afoakwa, 2010).

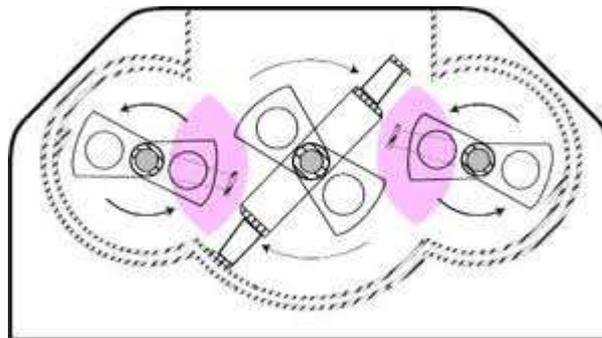


Figure 8. Mechanism of action of the conche (Adapted from Afoakwa, 2010).

3.7 Tempering

Cocoa butter exists in six different crystals states, however only state V, which has the most stable form of crystals, has the full requirements for making a good chocolate, as previous explained in the section 2.3.

In order to obtain the right type of cocoa butter crystals, the chocolate needs to be tempered. This means that the chocolate should become crystallised at a temperature below 32°C. Once seed crystals are formed, chocolate follows a heating and cooling phases steps (Figure 9). This

process promotes the formation of the crystals in the V form rather than in the VI form. While it is still warm, melted chocolate flows easily into moulds, or it can be used to enrobe different types of sweets or biscuits. Prior to packaging, the chocolate is cooled down online to approximately 15°C. Finally the chocolate is stored in chilled area away from direct light exposure (Afoakwa et al., 2007). The nutritional value and quality of chocolate may last up to a year, but it is generally recommended to be eaten within a month for best results (Cabras & Martelli, 2004).

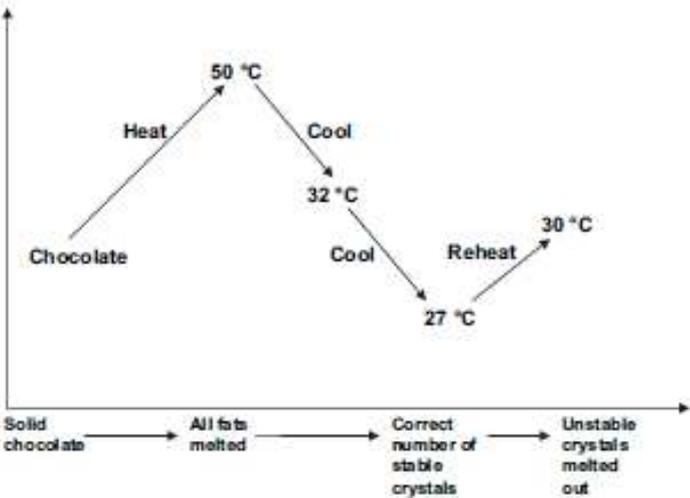


Figure 9. Tempering sequence and cocoa butter crystallization (Talbot, 1994).

4 The microstructure of chocolate type dispersions

Cocoa dispersions can be defined as complex systems. An extended and rigorous characterisation using detailed methodological advancements is important in order to predict and evaluate chocolate dispersions properties and relate them to quality characteristics and performances (cream stability, creaminess and processability) (Peressini et al., 2006).

It is known and well emphasizes in literature (Aguilera, 2005; Larrea et al., 2007; Lillford, 2010; Van de Velde & Klok, 2011; Moreno & Bouchon, 2011; Zdunek & Kurenda, 2013) the importance of understanding food microstructure in both food process and food design.

The macroscopic properties of food, such as rheology and mechanical strength, sensory attributes (e.g. mouth feel, texture and even flavour release) as well as engineering properties are strongly determined by the microstructure (Figure 10) (Bayod et al., 2008), that is considered a key parameter in the understanding of the foods behaviour (Aguilera, 2005).

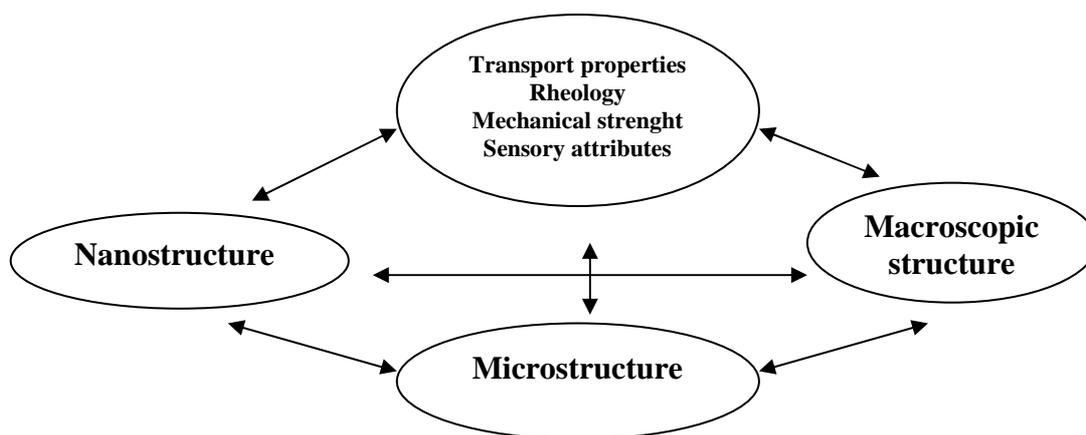


Figure 10. Schematic showing the hierarchy of food structure (Bayod et al., 2008).

The physical properties, rheological behaviour and sensory perception of food products are influenced largely by its processing techniques, particle size distribution and composition of ingredients (Afoakwa et al., 2007). Processing and formulation in fact can drastically change the

physico-chemical and structural properties, as well as the microstructure of foods. Important examples are fruit juices, vegetable purees, cocoa creams and ground meats that have chemical composition similar to their sources, but very different physical properties, rheological behaviour and sensorial attributes (Aguilera, 2005).

In particular food dispersions on the basis of their microstructure can be classified as being dilute, in the transition region or as concentrated (Figure 11) (Steeneken, 1989). In dilute systems the particles are swollen to their equilibrium size, they have maximum volume and are free to move in the suspension under Brownian forces. In the transition region, the particles are in contact with each other, but still have their maximum volume. In highly concentrated suspensions, such as cocoa dispersions, the particles are deformed and fill the space available, the matrix is thus fully packed. Another definition was given by Coussot & Ancy (1999), who described concentrated suspensions and granular pastes from a physical point of view, as “complex systems within which particles interact strongly, giving rise to viscosities much higher than the viscosity of the suspending media”. In concentrated systems the interactions and contact between particles clearly dominate over the Brownian forces.

Understanding the macroscopic properties governing food systems involves the characterization and quantification of their microstructure (Bayod et al., 2008). Microstructural techniques are necessary in order to study structure-property relationships. There is no single technique that is superior in all applications (Wu et al., 2004). A plethora of new instrumentations has become available to probe the foods microstructure (Aguilera et al., 2000), and there are different techniques to measure it, each technique has its own limitations, such as the amount of sample required, the particle density, etc. The most widely used techniques, in order to analyze the structure of foods are: the microscopy and the laser light diffractions (as showed in detailed way in the further paragraphs).

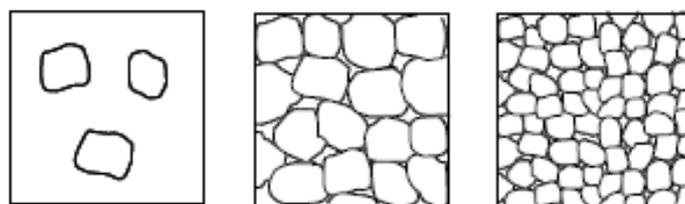


Figure 11. Concentration regime in suspensions. From left to right: dilute, transition and concentrated (Adapted from Steeneken, 1989).

4.1 Techniques to study food microstructure

Microscopy

Microscopy (optical or light, electron and atomic) coupled with imaging analysis, are the most directly and appropriate techniques used to evaluate food structure because they are the only analytical methods that produce results in the form of images rather than numbers. The images may then also be converted into numerical data to allow a statistical evaluation (Kalàb et al., 1995). Microscopy provides valuable information on the shape and arrangement of the particles in food systems (Bayod et al., 2008). Several microscopical techniques are used in both fundamental and applied research: *light microscopy in bright-field* in order to study network structures and particle size of cocoa dispersions (Afoakwa et al., 2009); *polarised light microscopy* to study fat crystal morphology and aggregation behaviour (Marangoni & McGauley, 2002); *fluorescence microscopy* to study lipid migration phenomena (Marty et al., 2005); *scanning electron microscopy* or *environmental electron microscopy* for the examination of crystal morphology, network structure and particle sizes (Kinta & Hatta, 2005); *magnetic resonance imaging* to examine migration (Walter and Cornillon, 2002; Choi et al., 2005; Deka et al., 2006); *atomic force microscopy* to investigate surface structure (Hodge & Rousseau, 2002; Smith & Dahlmann, 2005; Sonwai & Rousseau, 2006; Khan & Rousseau, 2006; Rousseau, 2006). Light microscopy and scanning electron are the most suitable techniques to analyze the microstructure of cocoa dispersions, since provide a lot of detailed information regarding the internal structure of the matrices, such as, the type and distribution of networks, the quantity and size of spaces filled by the particles, the empty spaces, the size, shape and distribution of the individual particles, etc. (Kalab et al., 1996; Afoakwa et al., 2009; Dahlenborg et al., 2010). In the studies carried out from this PhD thesis an environmental electron microscopy (ESEM) (**Papers II, III, IV**) and a light microscope (**Papers I and V**) were used to investigate the microstructural properties of chocolate type dispersions in terms of network structure, particle size and particle-particle interactions. For all obtained images was necessary a subsequently correction of their illumination. This is a common problem in microscopic images, when external sides are darker than the central ones. The difference in illumination was corrected by using the rolling ball technique, and by using several imaging filters (Image pro-plus manual, v. 6.0). Image analysis is commonly performed on binary images or by using grey scales. In these works (**Papers I, II, III, IV and V**) a grey 8 scale was used in order to elaborate images and better show areas occupied by particles and void spaces.

Particles in food dispersions are usually non spherical and poly - dispersed. For a non-spherical particle, several equivalent diameters, which are usually based on equivalences either in geometric parameters (e.g., volume or surface) are defined. Thus, for a given non spherical particle, more than one equivalent diameter can be defined. An equivalent diameter of a particle is usually defined in relation to a specific sizing method developed on the basis of a certain equivalency criterion. In particular, for what concern the sizing of particles based on the microscopic analysis, three different diameters are normally used: projected area, Martin's diameter and Feret diameters (Merkus, 2009) (Figure 12).

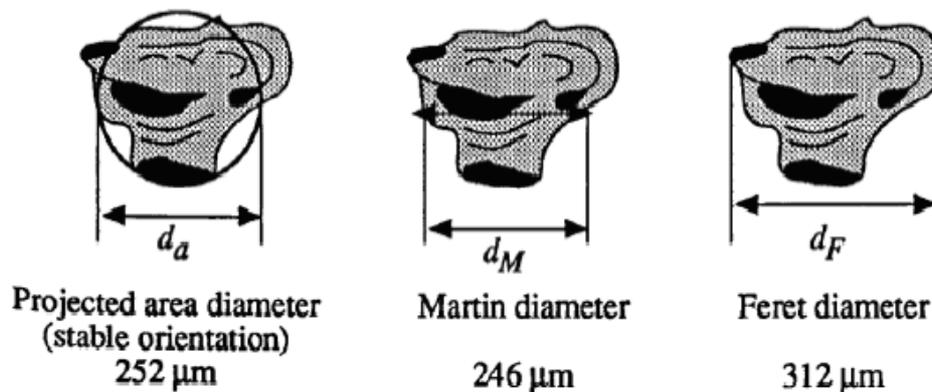


Figure 12. Schematic representation of the Project area, Martin and Feret diameters (Fan & Zhun, 2009).

Specifically, the projected area diameter is the diameter of a sphere having the same projected area as the particle; Martin's diameter is defined as the averaged cord length of a particle which equally divides the projected area and Feret's diameter is the averaged distance between pairs of parallel tangents to the projected outline of the particle. Since Martin's diameter, Feret's diameter, and projected area diameter are based on the two-dimensional image of the particles, they are generally used in optical and electron microscopy (Fan & Zhun, 2009). In **Papers III, IV and V** Feret's diameter was largely applied, discriminating in significantly way particle size of samples obtained from different steps of the manufacture process, as shown in Table 1.

Table.1. Feret diameters (μm) of milk chocolate samples obtained from different process steps. (corresponding to Table 1 in Paper IV).

Samples	Particle size (μm) (Feret diameter)
A (mixed)	103.00 ^a \pm 2.57
B (pre-refined)	67.00 ^b \pm 3.54
C (refined)	29.00 ^c \pm 2.37
D (conched)	22.00 ^c \pm 2.56
E (tempered)	17.91 ^c \pm 3.75

^{a-c} values in the same column followed by different letters differ significantly at $p < 0.05$ level

Laser light diffraction

Particle sizing can be obtained also by using indirect techniques such as light scattering and diffraction. The laser diffraction technique is based on degree of light diffraction. The degree of diffraction depends on the particles size. In general small particles cause large angle diffraction, big particles cause small angle diffraction (Merkus, 2009). Usually this technique is coupled to microscopic ones, because only by using the laser light diffraction is not possible to distinguish between primary particles and agglomerates. Compared with other sizing techniques (microscopic ones e.g.), laser light has the advantage of high speed, good reliability, high reproducibility and it is a non intrusive approach (Zhenhua et al., 2000). Several studies are present in literature highlight the relationship between particle size (PS) and the final properties of the product. Barbosa - Canovas et al., (2005) studied the importance of the particle size distribution on the bulk density, compressibility and flowability of food powders; Huang et al., (2001) studied the relationship existing between emulsion stability and the size distribution of the oil droplets present inside; Soh et al., (2006) investigated the influence of starch particle size on the final properties of the resulting dough; Bayod et al. (2008), analyzed the importance of different particle size of tomato products on the final rheological properties of ketchup. For what concern chocolate type products, some authors: Servais et al., (2002), Sokmen & Gunes, (2006), Afoakwa et al., (2007), Do et al., (2007), observed that the particle size (PS) and determinate particle size distribution (PSD) had a considerable effect on the macroscopic properties of the final product and in particular on the rheological ones, underlining the presence of a correlation between these parameters. However many aspects are still unclear and others studies are necessary.

This technique has been widely used throughout the **Papers I, III and V**, and the diffraction data were analysed using the Fraunhofer diffraction method. The Fraunhofer method can be applied to particle sizes between 1 and 200 μm (Annappagada & Adjei, 1996), and can handle polydisperse systems, such as cocoa ones. It assumes that the particles are spherical, but it adequately describes the particle size of sugar, cocoa and milk powders. The use of the Fraunhofer theory in determining the PSD of cocoa products is rather common (Attaie et al., 2003; Beckett, 2008; Gould et al., 2013).

For a given size distribution, various averaged diameters can be calculated, depending on the forms of weighing factors. The selection of an appropriate averaged diameter of a particle system depends on the specific needs of the application. For cocoa dispersions the most suitable diameters are:

- the Sauter mean diameter, that represent the diameter of a sphere with the equivalent surface to volume ratio of all the particles in the size distribution (1);

$$d_{32} = \sum_i n_i d_i^3 / \sum_i n_i d_i^2 \quad (1)$$

- the weight mean particle diameters that represents the diameter of a sphere having the average weight of all the particles in the size distribution (2) (Black et al., 1996).

$$d_{43} = \sum_i n_i d_i^4 / \sum_i n_i d_i^3 \quad (2)$$

where n_i is the percentage of particles with diameter d_i .

Particle size distributions are often expressed as the percentage of particles found in each size class. Foodstuffs often consist of poly - dispersed particles, with continuous particle size distributions containing several peaks, i.e. particles of all sizes are present, but most of them are of one or two specific size. In chocolate dispersion products, the PSD is usually considered to be bi-modal in the first two stages of the manufacture process, becoming unimodal in the last three steps (**Papers III and V**), as shown in Figure 13. Laser light diffraction technique describes in very accurate way the changes in the suspensions during processing (**Papers III and V**) and due to the formulation (**Papers I**).

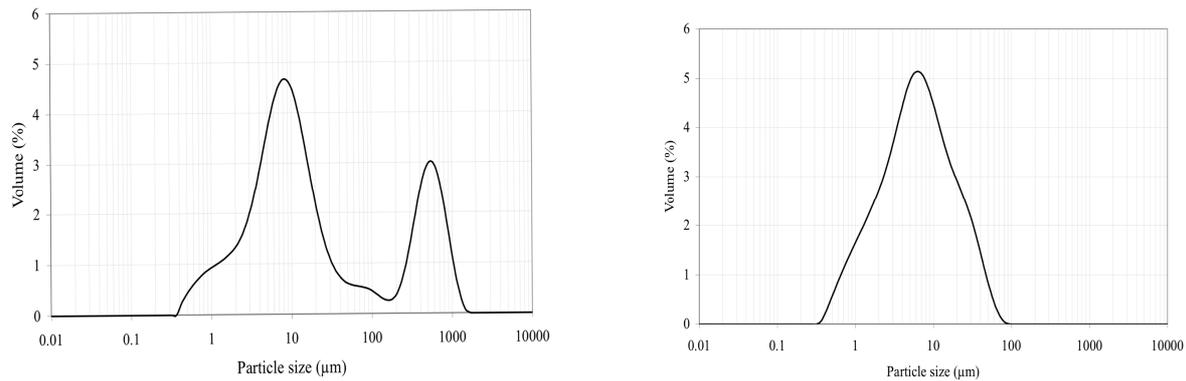


Figure 13. Bimodal and unimodal PSD of dark chocolate particles obtained after the mixing and tempering step respectively (*corresponding to Figures 2 a, e in Paper III*).

4.2 Influence of formulation on microstructure

In literature several studies related to the relationships between food microstructure and formulation are present. Bayod et. al (2008) showed the influence of incorporation of different tomato paste in the microstructural properties of the final ketchup products; Bengtsson (2009) demonstrated the effect of different vegetable fibre suspensions on the microstructural characteristics of low fat sausages; Lee et al. (2001) focused their studies on the relationship between different formulated doughs and its microstructure.

Model systems

For what concern chocolate type dispersions various ingredients and different amounts of them can be used during their manufacture. Chocolate composition, in terms of type and amount of ingredients, plays a fundamental role in influencing its microstructural and physico-chemical properties. The role of fat, that is the most expensive and important component in a chocolate recipe has been for long time investigated. In particular several studies (Marangoni, 2002; Awad & Marangoni, 2003; Shi et al., 2005) were carried out on fat model systems and showed the relationship between size, shape, amount and type of crystalline fat material and microstructural characteristics.

Other studies (Johanson & Bergensthal, 1992) report the influence of minor component, such as emulsifiers, on the structural properties of model chocolate type systems. All these studies confirm how the interactions between the suspended particles (e.g. sugar, cocoa, milk powder) and the

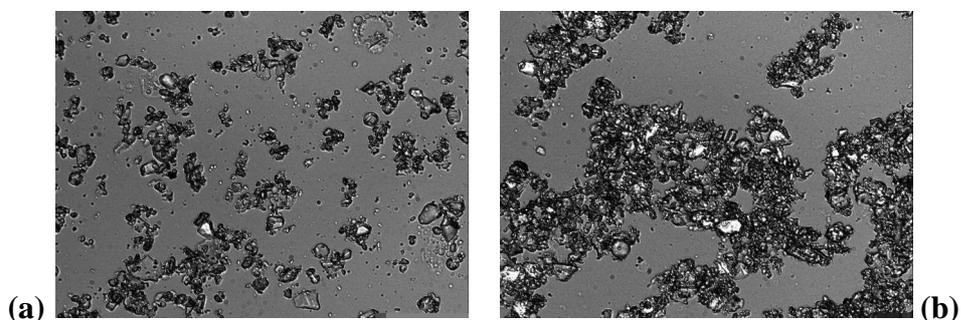
continuous phase influences the existing network and consequently can be associated to the rheological properties and structural characteristics of the final product (Sato et al., 2009).

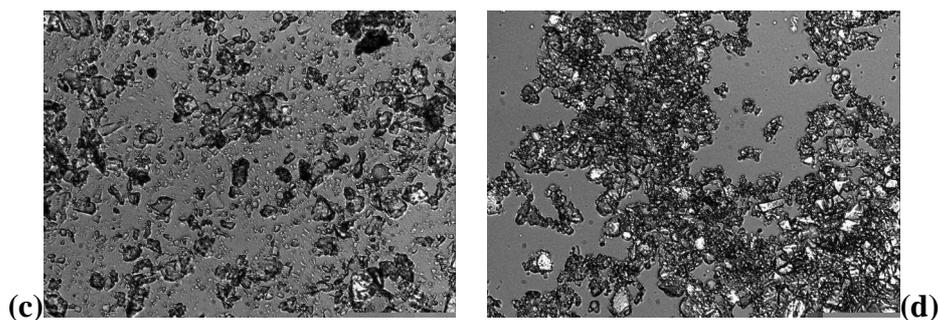
The microstructural behaviour of chocolate type dispersions can be influenced by the presence of minor constituents, such as water (Gaonkar, 1998; Beckett, 2008). It is known that water affects stability, quality and physical properties of foods because of the interaction of its molecules with other food constituents (Lewicky, 2004). The water binding influence on the solid disperse or aggregate phase was no longer considered important for microstructural and rheological behaviour of suspensions, especially at low water content, but this does not reflect the reality (Windhab et al., 2000). In chocolate type suspensions the final water content is around 0.5-1.5%, and despite this scarce amount, its presence can influence the microstructure of the product (**Paper I**).

New findings

Paper I studied the influence of different formulations on microstructural properties of dispersed model systems. In particular the influence of small amount of water on the final properties of model samples made up by sugar, water and cocoa butter was evaluated.

From microstructural analysis, carried out by using a polarized light microscope, increasing the sugar solid content from 45 to 60 ϕ with 0.1 ϕ of water, an increase in the aggregation was observed. This is due to the increase of the volume fraction of particles that involves an increase in the contact point between them (Afoakwa et al., 2009). Increasing the water amount from 0.1 to 0.5 ϕ for all sugar concentrations, these interactions between particles become even stronger as shown in Figure 14. According to the studies of Lewicky (2004), increasing the water content, the macromolecules of sugar acquire structure, due to their interactions with water molecules (Figure 14).





Figures 14 a, b, c, d. Micrographs of model dispersions obtained with: 55 ϕ of sugar and 0.1 ϕ of water, 55 ϕ of sugar and 0.5 ϕ of water, 58 ϕ of sugar and 0.1 ϕ of water and 58 ϕ of sugar and 0.5 ϕ of water respectively (*corresponding to Figure 5 a, b and Figure 6 a, b in Paper I*).

From **Paper I** it seems that small amount of water has an impact on the aggregation of particles higher than sugar. This study showed that water presence even at very small amount is an important factor able to affect both network strength and the rheological properties of food dispersions, as we can see in the further chapter 5.

Real systems

There are three different main type of chocolate: dark, milk and white, according to their formulation in terms of cocoa solids, milk fat and cocoa butter, the final products have different compositions of carbohydrate, fat, proteins, etc.. (Fernandes et al., 2013). Chocolate composition influence during processing the different interactions that take place among ingredients, hence involving changes at microstructural level. Studies on the fat amount in dark chocolate recipes (Beckett 1999; Dhonsi & Stapley 2006; Afoakwa et al., 2009) highlighted how the greatest amount of cocoa butter in formulation involves wide variations in sugar crystalline network structure and inter-particle interaction, reducing the aggregation state of matrix.

Some other studies (Attaie et al., 2003; Franke & Heinzelmann,2008) showed the influence of cocoa particles and milk powders on the microstructural properties of cocoa type dispersions. In particular the high porosity of these two kind of solid particles, can influence in different way the aggregation with sugar and the microstructure system.

New findings

The influence of (each single real) formulation on microstructural properties of dark (**Papers II, III**), milk (**Paper IV**) and white (**Paper V**) chocolate was investigated.

Even if, all this kind of chocolate were subjected to the same manufacture process, results in microstructure compositions were different, due to their different formulations.

A comparison between the micrographs obtained from dark and milk chocolate after the refining step (Figure 15 a, b) show strong differences in the microstructure of the matrix.

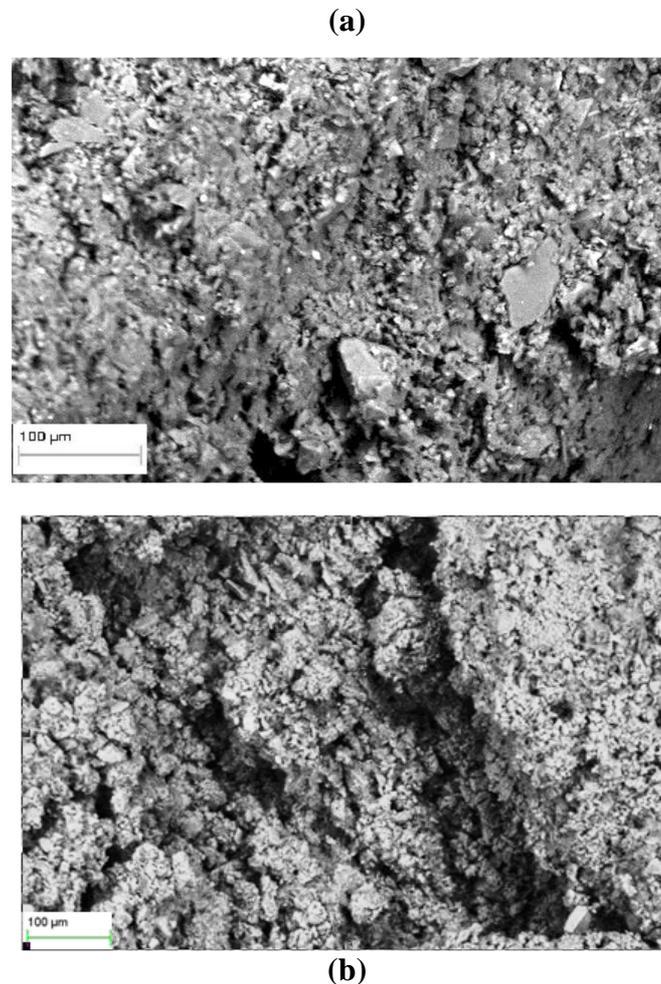


Figure 15 a, b. Micrographs of dark and milk chocolate samples respectively obtained from the refining step of the manufacture process (*corresponding to Figures 3c in Paper III and Figure 2c in Paper IV respectively*).

As reported in chapter 5, these different structures due to the different amount of cocoa butter involves different rheological final characteristics. In milk chocolate, the presence of milk powder with an inner high porosity, involves an immobilization of fat on particle surface (surface fluid immobilization, SIF), mainly after the refining step that led to a break of their structure, and inside to the formed aggregates (volume fluid Immobilization VIF). The latter is related to particle cavities and pores as well as to inner voids in particle aggregates. Volume immobilization will increase with the inner porosity of particles or particle aggregates. The portion of immobilized fat can be reduced during the manufacture process.

4.3 Influence of manufacture process on microstructure

Several studies were conducted on the influence of the processing (Parada & Aguilera, 2001; Muller-Fischer & Windhab, 2005; Agbisit et al., 2007; Zepon, 2013) on the microstructural properties of several food matrices, but to our knowledge there are no researches on chocolate types matrix. Final quality attributes and physico-chemical properties of food dispersions, are affected by a number of factors, such as: particle size (PS), particle size distribution (PSD) and interactions and are strictly related to both raw materials characteristics and to the manufacture process (Servais et al., 2002; Granger et al., 2005; Sato et al., 2009; Baldino et al., 2010). The manufacture process with its different steps (mixing, pre-refining, refining, conching and tempering) can affect chocolate properties and final characteristics. During chocolate manufacturing, mixtures of sugar, cocoa and fat are heated, cooled, pressurized and refined (Beckett, 2008). These process steps determine PS reduction and the breakage of agglomerates and distribution of lipid and lecithin-coated particles through the continuous phase, thus modifying the microstructure of final product (Afoakwa et al., 2009). The interactions between the suspended particles and the continuous phase provide information about the existing network and consequently can be associated to the properties and characteristics of the dispersions (Sato et al., 2009).

New findings

In **Papers II, III, IV** and **V**, the influence of the different process steps on microstructural properties of dark, milk and white chocolate was evaluated. Samples were obtained at each phase of the manufacture process: mixing, pre-refining, refining, conching and tempering. Laser light diffraction technique was used to study the particle size distribution (PSD), ESEM was used to analyze modifications in the network structure of dark and milk chocolate. Micrographs of white chocolate were obtained by using a light microscope. White chocolate in fact thanks to its optical properties in terms of lightness, due to the absence of cocoa particles, can be easily observed by using a light microscope. Each processing step influences in strong way the microstructural characteristics of the product and mainly the PSD. A reduction in particles size was observed for all samples, from the mixing to the tempering step. However, microstructural analysis showed an increase in contact points and in the aggregation state of particles in all formulations from the mixing to the refining step

(Figures 16, a, b ,c). The result is a fully packed suspension in which the smallest particles fill spaces between the largest ones (Bayod, 2008).

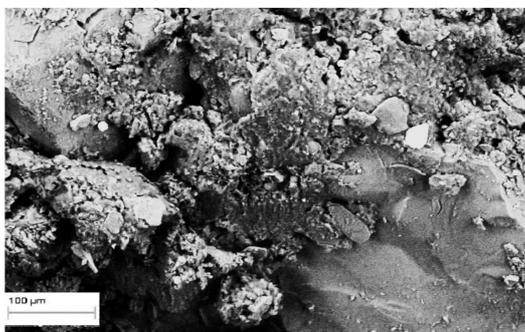


Figure 16 a. ESEM micrograph of mixed dark chocolate samples (*corresponding to Figure 1a in Paper II and 3 a in Paper III*).

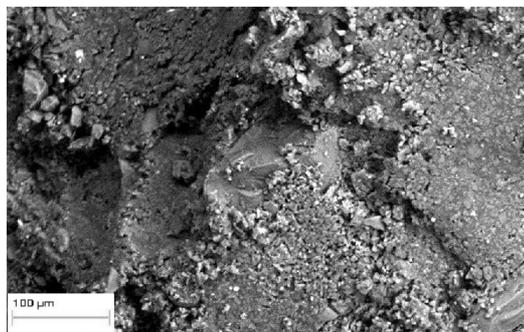


Figure 16 b. ESEM micrograph of pre-refined dark chocolate samples (*corresponding to Figure 1 a in Paper II and 3b in Paper III*).

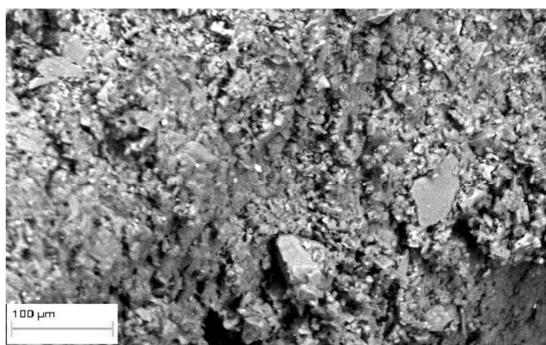


Figure 16 c. ESEM micrograph of refined dark chocolate samples (*corresponding to Figure 1 c in Paper II and 3 c in Paper III*).

The conched and tempered samples (Figures 16 d, e), even if made of particles with the smallest size diameter, were constituted by a less dense sugar crystalline network. This could be related to the further addition of cocoa butter and lecithin, during the conching step, in dark and white chocolate samples, that, wet the suspension, filling the gaps within the crystal network, and open the structure (Afoakwa et al., 2009). In milk chocolate samples the less dense structure in the last process steps is due to a redistribution of cocoa butter and to a breakage of the previous agglomerates.

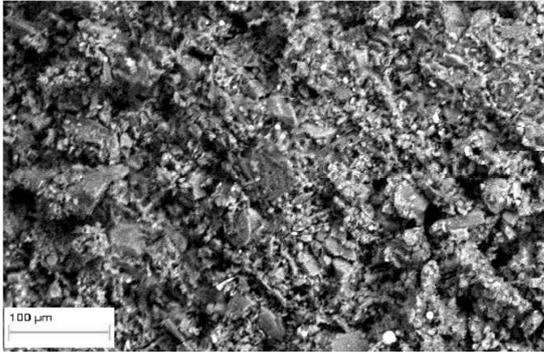


Figure 16 d. ESEM micrograph of conched dark chocolate samples (*corresponding to Figure 1d in Paper II and 3d in Paper III*).

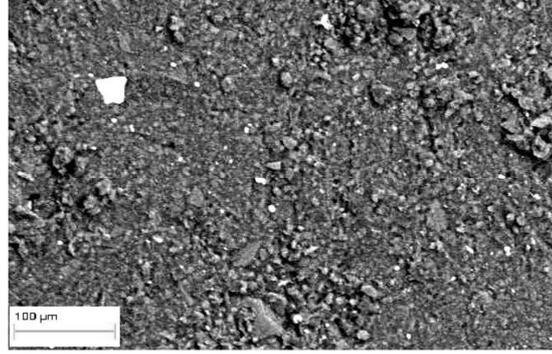


Figure 16 e. ESEM micrograph of tempered dark chocolate samples (*corresponding to Figure 1 e in Paper II and 3 e in Paper III*).

5 Rheological behaviour of chocolate type dispersions

Rheology is the study of the deformation and flow of matter (Barnes & Hutton, 1989) and it investigates how materials deform under application of external forces. Deformation expresses the relative movements of the "particles" (atoms, molecules, solid particles, etc...) present in the matrix. Consequently the way in which a material deforms depends upon the structure of the material.

The rheological behaviour of dilute suspensions, that assume no interactions between particles, is notoriously described by the Einstein equation (3) that stated that the viscosity of a dilute suspension of hard spheres ($\phi \leq 0.03$), can be defined as:

$$\eta = \eta_s (1 + 2.5 \phi) \quad (3)$$

in which η_s is the viscosity of the dispersion medium and ϕ represent the volume of a cubic centimetre occupied by the particles in suspension (Mark & Simha, 1940).

The Krieger-Dougherty equation (4) describes the viscosity of more concentrated suspensions of hard spheres ($\phi \leq 0.63$) at low shear rates $\dot{\gamma} \rightarrow 0$, when hydrodynamic effects dominate and particle interactions are negligible:

$$\eta = \eta_s \cdot \left[\frac{\phi}{\phi_m} \right]^{-2.5 \phi_m} \quad (4)$$

In highly concentrated suspensions, as in the case of cocoa dispersions, the interaction between particles dominates over the hydrodynamic forces, especially at low shear rates, and the material exhibit a complex flow behaviour. The viscosity of suspensions can be divided into three areas as shown in Figure 17.

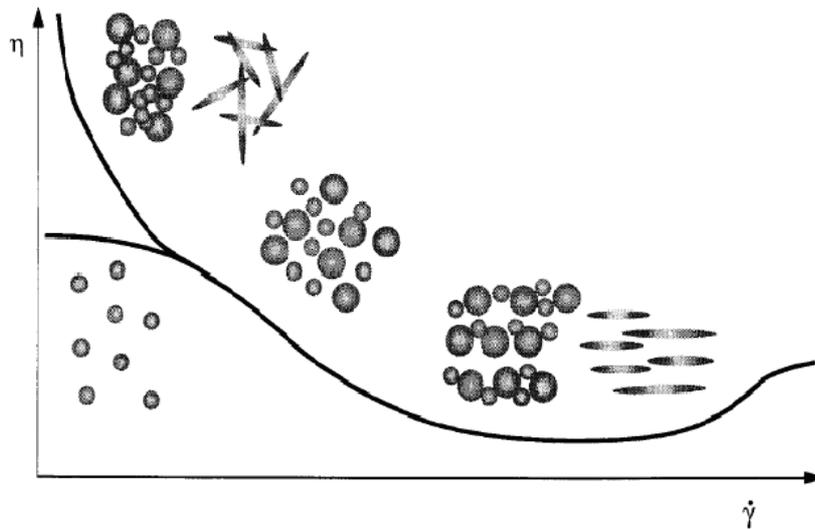


Figure 17. Area of high, medium and low viscosity of dispersion (Adapted from Banerjee et al., 2010).

At very low shear rates and depending on the type of suspension it can either exhibit a Newtonian plateau or a yield value. Increasing shear rate will lead to a shear thinning region which levels off to an upper Newtonian plateau. At some point in the upper Newtonian region there can be an increase in viscosity which is based on instability effects (Banerjee et al., 2010). The structures of matrix can be related to each segment of the flow curve. In cocoa dispersions distance between the individual particles decreases and continuous networks are formed. In this case the system exhibits a "yield" value. The liquid phase is often, immobilized within the agglomerates and thus the freely available liquid phase is less than the total amount of liquid phase. Increasing the shear rate, the forces acting on the particles increase, and the structures are altered. The agglomerates will break up, release the entrapped liquid phase and dilute the system. This decreases the resistance to flow and hence viscosity.

The fundamental rheological properties investigated in this study were the flow behaviour and the solid-like character of cocoa dispersions.

5.1 Fundamental and empirical properties

Flow measurements

The way in which a material deforms following an applied stress defines its flow behaviour. The flow behaviour of materials can be described by the relationship between the shear stress (τ) and the

shear rate ($\dot{\gamma}$). The shear stress can be defined as the force divided by the area parallel to the force direction, while the shear rate is defined as the velocity gradient or deformation rate.

The viscosity (η) can be expressed as a constant linking stress and shear rate as follow (5):

$$\eta = \frac{\tau}{\dot{\gamma}} \quad (5)$$

For a Newtonian fluid such as cocoa butter, shear stress and shear rate have a linear dependence, but in concentrated suspension, these two parameters are not linear related and fluids exhibit a Non-Newtonian flow (Dickinson, 1992). The cocoa dispersions, a typical example of Non Newtonian fluid, as all the major part of the concentrated suspensions are characterized in particular by the presence of a yield stress (σ_y), defined as the minimum stress required to achieve flow. When the stress applied to a material is below a certain value ($\sigma < \sigma_y$), the material exhibits little or no deformation. When the stress exceeds a certain value ($\sigma > \sigma_y$), the material begins to flow. The yield stress is related to the strength of the network structure, which in turn results from attractive particle-particle interactions (Larsson, 1999; Coussot & Ancy, 1999). The magnitude of the yield stress is affected by a number of factors, such as the density of the network, particle concentration and particle size (Dzuy & Boger, 1983).

Flow behaviour of cocoa dispersions is usually evaluated according to the International Confectionary Association (ICA, 2000), increasing the shear rate from 2 to 50 s⁻¹. Several models were developed in order to describe the chocolate behaviour and evaluate the yield stress and the infinity viscosity of these complex matrixes as after specified, on the basis of the experimental curves obtained.

As know by literature (Barnes, 1999) the flow behaviour of cocoa matrix is complex, the use of structurants together with the increasing presence of flocculated and aggregate structures, due to the influence of formulation and manufacture process, leds to the appearance of thixotropy phenomena. The thixotropy designates the energy required to break down the structure that is not recovered during the experimentation period (Roopa & Bhattacharya, 2009) and represents the rate of the internal breakdown of matrix (Dolz et al., 2000).

Oscillatory measurements

Flow properties are not enough to analyse the chocolate structure and to understand as formulation and operating conditions can affect it. Therefore, it should be necessary to adopt techniques able to

measure parameters related to material structure and independent on deformation history (Baldino et al., 2000). This is the case of the so-called “asymptotic kinematics” carried out in conditions of linear material behaviour where measured material functions are independent from the magnitude of the stress/strain and are only the function of microstructure (Van Der Vaart et al., 2013).

In order to identify the linear viscoelastic range (LVR), in which the viscoelastic properties become independent on the level of stress/strain applied, stress/strain sweep test is applied. This test consists of increasing the magnitude of the stress or strain, while keeping the frequency of oscillation constant, usually 1 Hz. In food materials, strains are often kept below 1% to avoid non-linear effects (Steffe, 1996). In cocoa dispersions (**Papers II, IV, VI, VII**) results showed a narrow viscoelastic region, low intensity stresses can destroy the structure of system. Subsequently frequency sweep test were carried out in the LVR in order to evaluate the storage modulus (G'), and the loss modulus (G''). Storage modulus is an index of the elastic behaviour of a sample, and represents the deformation energy stored in the sample during the shear process and the loss modulus (G'') that corresponds to the viscous or dissipative component of a sample is a measure of the sample energy lost during the shear process (Angioloni & Collar, 2009).

For solid-like materials $G' \gg G''$, whereas for liquid-like materials $G' \ll G''$. The complex modulus, is defined by:

$$G^* = G' + G'' \quad (6)$$

and the complex viscosity is thus defined by

$$\eta^* = [G^*(\omega)]/\omega \quad (7)$$

where ω is the frequency of oscillation.

Rheological models

Several rheological model functions were developed in order to describe and predict the chocolate behaviour; models are mathematical equations, utilized to describe the various flow behaviour curves on shear stress–shear rate diagrams.

Pseudoplastic and plastic fluids (e.g. cocoa dispersions), are assumed to have an initial viscosity at the origin η_0 which then decreases with increasing shear rate. The decrease is assumed to be the consequence of the loss of structure/network in the material, as previous shown. When the molecular

structure/network in the fluid has reached a steady state (where the intermolecular forces acting to build the structure/network and those acting to break it down by shear, are in equilibrium) no further decrease of viscosity is observed. This viscosity is called equilibrium viscosity η_{∞} , (Figure 18).

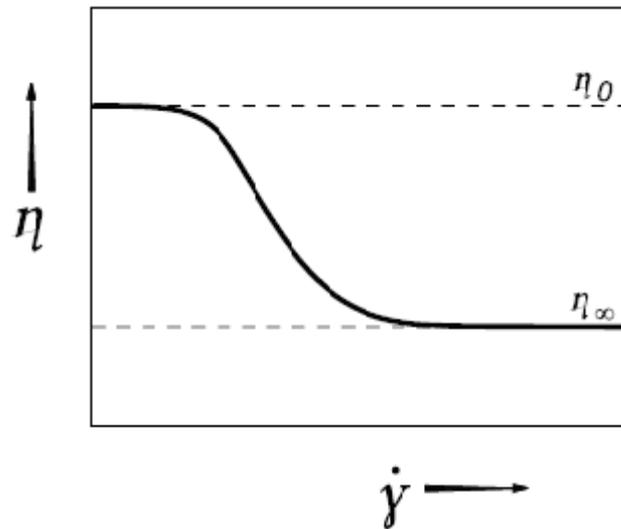


Figure 18. Typical pseudoplastic and plastic behaviour (Adapted from Spetch et al., 2007).

In this type of material, the viscosity changes from η_0 to η_{∞} with increasing shear rate. For a mathematical description of these sigmoid shaped curves, a number of model functions have been developed. In general, most of these functions require two or three parameters, depending on its complexity. These parameters are constants in the model function, whose numerical values make the function specific for a given material.. Normally, model parameters are measured experimentally, or they are obtained from other researchers working on same material, who report their findings in the scientific literature.

Different models were elaborate and applied to concentrated dispersions, but the most suitable in the case of cocoa dispersions were: the rheological model of *Ostwald*, commonly referred to as the Power Law model (Holdsworth, 1993; Hugelshofer, 2000), the model of *Casson* (ICA, 1973), and the model of *Windhab* (IOCCC, 2000).

- The *Power Law* model is represented by the following equation (8)

$$\sigma = K \cdot \dot{\gamma}^n \quad (8)$$

where σ is the shear stress (Pa), K is the consistency index (Pa sⁿ), γ is the shear rate (1/s) and n is the dimensionless flow behaviour index (**Paper VII**). When the flow behaviour index is less than unity ($n < 1$) the function describes a pseudoplastic flow behaviour curve (convex profile). When the flow behaviour index is greater than unity ($n > 1$) the function describes a dilatant flow behaviour curve (concave profile). For Newtonian fluids, the flow behaviour index becomes 1.

- The rheological model of *Casson* is normally expressed by (9):

$$\tau^{0.6} = \tau_0^{0.6} + n_{PL} y^{0.6} \quad (9)$$

where τ_0 is the yield stress and η_{PL} is the so-called “plastic viscosity”. The model of *Casson* was originally introduced for suspension of pigments and describes the rheological behaviour of viscoplastic fluids (Bolenz & Tischer, 2013). In the 1973 the ICA recommended the use of the *Casson* model for shear rates between 5 and 60 (s⁻¹) (Ludger et al., 2007). For this reason, the *Casson* model is the most known and used to study the rheological behaviour of cocoa dispersions; however, according to Weipert et al. (1993), sometimes this model does not reflect in accurate way the physical properties of chocolate because its rheological properties do not fit exactly to the *Casson* equation. For this reason modification to *Casson* model were generated (**Paper IV**) and further model developed such as the *Windhab* one.

- The *Windhab* model has been recommended for shear rates in the range between 2 and 50 s⁻¹ at 40°C (10):

$$\tau = \tau_0 + \eta_{\infty} * \gamma + (\tau_1 - \tau_0) (1 - e^{-\gamma / \gamma_*}) \quad (10)$$

This model assumes that when liquid chocolate is put under shear, there is a change in structure of the molten chocolate. This can be observed by noting a change (decrease) in viscosity from an initial value (structure of no shear) to a steady state value. Here, the ordering forces (“building structure”) and disordering forces (“breaking down structure”) are in equilibrium and the chocolate shows an equilibrium viscosity. When the shear stress is increased further, an equilibrium viscosity is reached which no longer decreases any further, and a final viscosity η_{∞} is reached. In the region of this final viscosity η_{∞} , there is a straight line with a constant slope in the flow curve. This straight line can be extrapolated back to the point of zero shear rate in order to find the intercept. This intercept would give the parameter τ_1 which is an hypothetical yield stress (Ludger & Teixeira, 2007) (Figure 19).

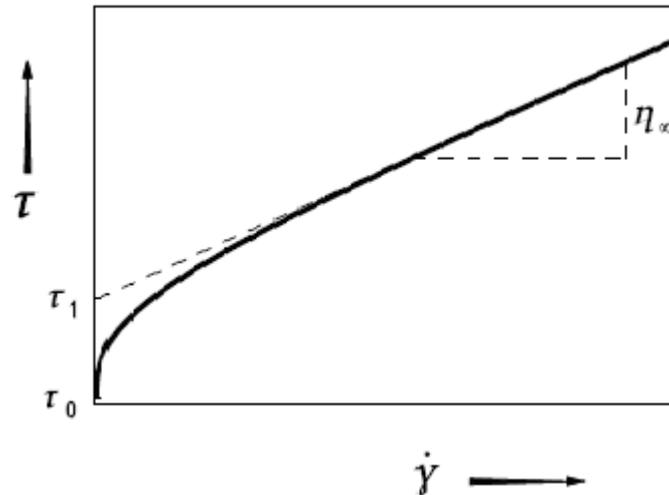


Figure 19. Schematic diagramme of the *Windhab* model (Ludger & Teixeira, 2007).

In order to find the point on the curve where the final viscosity η_∞ is reached, the *Windhab* model uses a second parameter $\tau^* = \tau(\gamma^*)$ indicating that the shear-induced loss of structure is at a maximum when $\gamma = \gamma^*$ is reached. For shear rates higher than γ^* the material behaves like a plastic fluid and the Bingham equation would apply.

Empirical properties

Empirical properties of a food derive from a group of mechanical and rheological properties (not from a single property) depending on the structural parameters of the product and the way in which these are perceived (Afoakwa, 2010). These properties often are called "textural properties" of a food, designed as a set of physical characteristics resulting from the structural elements of the product, perceptible by the senses, related to the deformation or flow of the food under stress (Steffe, 1996).

Empirical tests are often used in food engineering and are well-known as quick, cheap test to identify the flow behaviour of complex fluids. These tests are useful to simulate the real conditions of the process and to obtain objective results comparable with the sensory evaluations (Perrot, 2012). The empirical imitative tests, in fact, are carried out by specific tools, called dynamometers, that mechanically reproduce the conditions in which the product is located in reality. Textural parameters are extremely important for the evaluation of the mechanical properties of chocolate (strictly related to the sensory characteristics during consumption) and also to predict its rheological behaviour during processing (Beckett, 1999; Bourne, 2002). For these kind of matrix dispersions the most suitable empirical test are: the back extrusion and the hardness evaluation. The back extrusion

technique can be a very useful tool in assessing the rheological behaviour of non-Newtonian fluids (e.g. cocoa matrix) (Alviar & Reid, 2006). Using this test four structural parameters can be obtained: firmness, consistency, cohesiveness and index of viscosity. These measurements are performed to evaluate the degree of spreadability, consistency and resistance to flow (viscosity) of a material (Ziegler & Hogg, 1999).

Back extrusion test consist in a compression and subsequently de-compression of sample, hold in a back extrusion container by using a disk attached to an extension bar.

From previous studies (Beckett, 2008; Bueschelberger, 2004), a strictly relationship was observed between particle size (PS) and back extrusion index in cocoa products. In particular, particles with smaller diameters are firmer, more consistent, cohesive and viscous than those with larger diameters. The presence of particles with smaller diameters involves an increase in their number and of their contact points due to the increase in the specific surface area. The increase in the state of aggregation of particles involves the presence of higher values of firmness, consistency and cohesiveness, restricting spreadability and viscosity for a specific solid concentration.

A reduction in all textural parameters was also observed increasing the fat amount in the formulation, probably due to its lubricant effect.

Another habitual empirical analyzed parameter is the hardness of chocolate. Studies presents in literature (Beckett, 2008; Afoakwa et al., 2009; Afoakwa, 2010) showed inverse relationships between hardness and particle size, fat and lecithin content. The greater hardness levels were noted in presence of small particle size and low amount of cocoa butter, as previous showed for back extrusion index. Significant reductions were noted (Afoakwa et al., 2009) with the increase of lecithin content from 0.3 to 0.5 % and with the increase in particle size.

These results showed that the combined effects of PSD, fat and lecithin could be manipulated to control softness and/or hardness of tempered dark chocolate, with implications in quality control and production cost.

5.2 Influence of formulation and manufacture process on rheological properties

Rheological properties are a nodal point in the oil- continuous foods. They govern properties such as: flow behaviour, consistency, mouth feel, spreadability and handling of the product during different production and transportation steps (Servais et al., 2002). The rheological properties depend on

particle-particle interactions and particle microstructure and are directly influenced by the adsorption of different molecular species on particles (Johanson & Bergensthal, 1992c). It is important to control the viscosity of chocolate and of related cocoa products in order to obtain good quality products. In particular flow properties of cocoa dispersions, a concentrated lipophilic suspension of solid particles dispersed in a continuous fluid, are strongly influenced by its formulations, in terms of solid fraction and in terms of fat composition (Attaie et al., 2003; Franke & Heinzelmann, 2008). Confectionery manufacturers optimizing the process and formulation can control and manipulate the chocolate rheological characteristics. The relationships between all ingredients present in cocoa dispersions and the continuous phase influencing the microstructural properties of the final matrix, affect strongly its rheological properties. The type, quality and quantity of each ingredient used in a recipe, particularly fats, affect the chocolate rheological and textural properties and also the flow parameters (Fang & Zhang 1997). Also single component present in formulation in small amount, such as, water should be take into account. Chocolate rheology exhibits a complex rheological behaviour, strictly dependent also on manufacture process (Bourne, 2002; Servais et al., 2004; Afoakwa et al., 2008). For this reason understanding how the single process step can affect the final rheological properties of these matrixes, could be very useful in order to manipulate and optimize the process efficaciously, and mainly to predict and improve quality of final product (Ahmed & Ramaswamy, 2006). During chocolate processing the ingredients are mixed, pre-refined, refined, conched and tempered in order to obtain desired rheological properties for a final defined product texture (Chevalley, 1999; Servais et al., 2004).

New findings

Formulation

In **Paper I** the rheological properties of model dispersions were investigated. Model dispersions were obtained by using different volume fraction (from 45 to 60 ϕ) of icing sugar, dispersed in different cocoa butter concentration (45 to 55 ϕ), in presence of small amount of water (0.1 ϕ and 0.5 ϕ).

Rheological properties were evaluated by using a stress controlled rheometer equipped with a vane geometry (Kinexus, Malvern, UK). This kind of geometry lets to measure the properties of non-Newtonian fluids reducing the slip effects at walls. It also offers a way to introduce the testing element, a thin-bladed vane, into a structured liquid with the minimum amount of disturbance to the sample, making it very suitable for materials such as chocolate and other gelled liquids (Barnes &

Nguyen, 2001). This geometry forms a cross shape at the end of a central shaft and can be inserted into the sample with minimal disturbance of the microstructure. The fluid circumscribed by the vane moves as a solid cylindrical body therefore slip is effectively eliminated. Inserted into the fluid is an easy and convenient method of performing accurate rheology measurements on such samples (Stokes & Telford, 2004; Baker et al., 2006)

From the flow curves analysis it was possible to highlight the presence of an apparent yield stress in all formulations, and how the increase of the sucrose volume fraction (45 to 60 ϕ) involves an increase of the yield stress values for all dispersion samples, according to the studies of Den Ouden & Van Vliet (1997) and Dzuy & Boger (1983), that evidenced a relationship between yield value and particle concentrations. These results are in agreement with the microstructural ones that stated an increase in the network of the structure increasing the sugar amount. Plotting the sugar concentration as a function of the yield values, an exponential correlation was observed with high coefficient of determination ($R^2= 0.99$).

The presence of an apparent yield stress in these concentrated suspensions was corroborated by results of creep –recovery test, as shown in Figure 20.

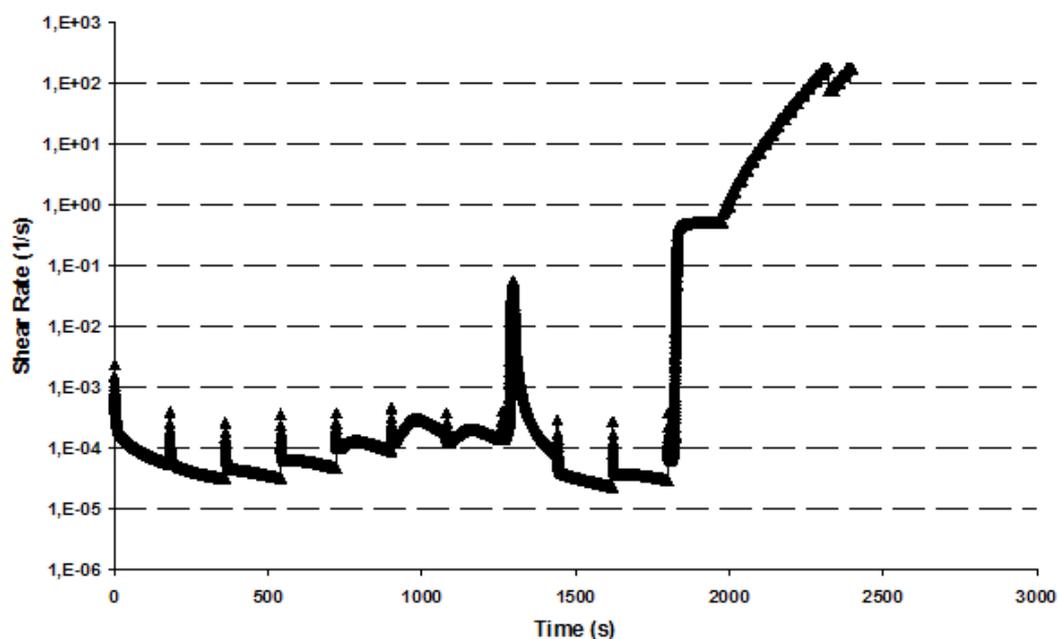


Figure 20. Creep recovery test on a model sample (*corresponding to Figure 3 in Paper I*).

From graph it is possible to notice that when the applied shear stress is below a certain stress value, samples recover the initial structure immediately after stress removing, indicating that a solid-like

behaviour is predominant. If the stress is above a particular limit, the sample structure is broken and it starts to flow exhibiting a liquid-like behaviour (Baldino et al., 2010), showing the presence of a yield stress as previously observed from flow tests. In particular, shear rate and time needful to obtain the breakage of the structure seem to increase from sample with the lowest amount of sugar (45 ϕ) to sample with the highest volume of sugar (60 ϕ), probably related with the arise of the solid volume fraction of samples, as noted previously by using flow measurements.

These kinds of concentrated dispersions are characterized by the presence of a yield stress. The second aim of this study (**Paper I**) was to evaluate the influence of small amount of water on rheological properties of samples expressed in terms of yield value. Figure 21 represents the yield stresses of dispersions at different considered sugar and water concentrations.

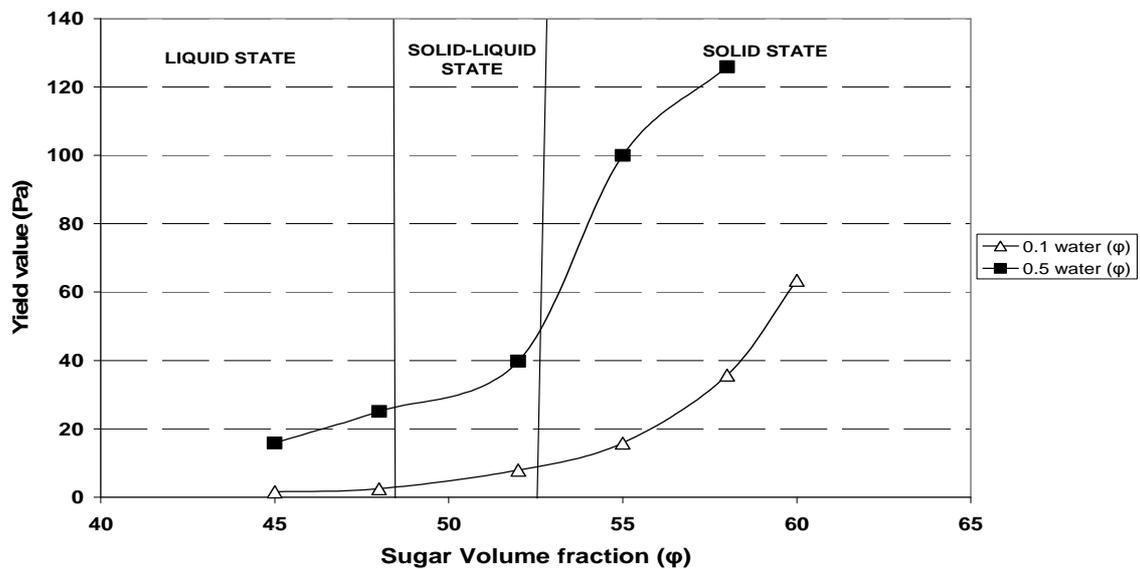


Figure 21. Yield stresses of dispersions at the different considered sugar and water concentrations (corresponding to Figure 4 in Paper I).

As the concentration of sugar and water increases, three viscosity and yield stress regions may be identified. According with the model suggest by Goodwin (2004), it is possible to divide graph samples, showing the sugar volume fraction versus yield value, into three main regions, depending on their state of aggregation, obtaining a volume state diagram (liquid, solid-liquid and solid). The first region, which represents the low concentration suspensions (from 45 % to 48% ϕ of sugar), behaves like Newtonian fluids showing low viscosities and low yield values. The second region that corresponds to the intermediate level of concentration (from 48 % to 52% ϕ of sugar) exhibits slight

viscosities with a shear thinning behaviour and intermediate yield values. Above this concentration range, there is the region of highly concentrated samples corresponding to values of sugar >52 % ϕ . The behaviour of the curves in this region may suggest the formation of "structured" aggregates with a strong affinity between the particles. As a consequence, the fluidity of the samples is reduced and this involves an increase the yield values and a consequent drastically increase of samples viscosity. When the water content increases, even if in very low amount, the interaction between crystal changes, influencing in strong manner the yield value of dispersions. These results according to Johansson & Bergenståhl, (1992c), could be interpreted as the effect of the water adsorption onto sugar. Aggregations between sugar particles become more stronger, probably due to the formation of water bridges, involving an increase of the yield value of the experimental dispersions. From the curves it is possible to highlight how small amount of water on the yield values of dispersions has an impact much higher compared to the sugar one.

In **Paper VI** the influence of different kind and amount of fats on rheological and textural properties of cocoa creams was studied. The type of fats used in the cream formulations were fractionated palm oil and hydrogenated fats (HFs), follow the mixture design (Ruguo, 1999) shown in table 4.

Table 4. Mixture Design used for the Fat formulation in cream samples (*corresponding to Table 2 in Paper VI*).

Cream samples	Palm oil (%)	HF (%)
A _{nut}	100	0
B _{nut}	75	25
C _{nut}	50	50
D _{nut}	25	75
E _{nut}	0	100

Two commercial nut creams produced by Italian confectionery factories were used as controls. These samples were indicated as: X1, the medium-standard quality and X2 the market leader. From the product label, the ingredient composition for X1 cream is as follow: sugar, vegetal and hydrogenated fats (5%), nut paste (13%), cocoa powder, skimmed milk powder (4%), milk whey, lactose, soy lecithin, natural extracts; for X2 cream: sugar, vegetal fats, nuts (13%), cocoa powder, skimmed milk powder (5%), milk whey, soy lecithin, natural extracts.

Rheological tests were carried out only on A, B, C, X1 and X2 samples, being D and E creams extremely consistent and too viscous to conduct textural or oscillatory measurements; this was probably due to the high amount of HF in their formulations.

Flow curves of the analyzed nut cream samples are shown in Figure 22.

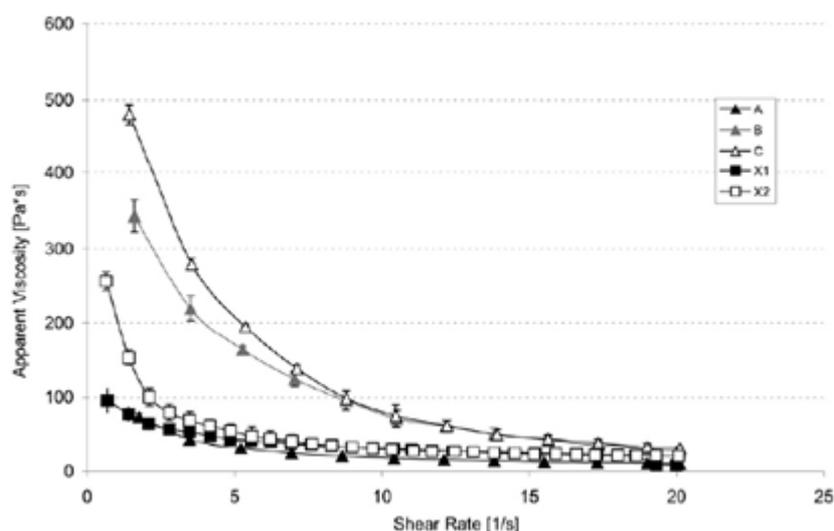


Figure 22. Flow behaviour of nut creams realized with different type and amount of fats (corresponding to Figure 4 in Paper VI).

The viscosity of the A_{nut} and X1 samples was very similar; X2 had an intermediate initial viscosity value (206 Pa s). Sample A_{nut} , made up of palm oil and a 1.5% of solid fat content (SFC), had a lower consistency, a less aggregated and elastic structure than B_{nut} and C_{nut} , made with 25 and 50% of HF. X1 commercial sample showed a plastic structure similar to A_{nut} , while X2 cream presented a structure more consistent and elastic close to that of B_{nut} sample, probably due to the presence of hard vegetable fats or HF. These results were supported also, by the consistence index obtained from the Power Law model, by the dynamic and textural results. This work confirms that the use of high amount of HFs and/or hard vegetable fats leads to a strong fat network, which implies more aggregated structure, influencing the final rheological properties of the cocoa matrix. For this reason the choice of the right type and amount of fats during the formulation is fundamental in order to improve and optimize the rheological properties of the final product.

The relationship between formulation and in particular between different fat amounts (e.g. cocoa butter), and the rheological properties of cocoa matrix was further studied in **Papers V, VII**.

Values of yield stresses were evaluated in dark (**Paper VII**), milk (**Paper IV**) and white chocolate (**Paper V**) formulations according with ICA (2000) and Servais et al., (2002) by evaluating the values of stress at shear rates of 5 s^{-1} and by using different rheological model, in order to better explain the rheological values obtained by the flow curves. Milk and white chocolate samples that showed higher amount of cocoa butter compared to the dark one, showed lower rheological parameters respect this latter formulation. In order to easy underline the differences between yield stress values of chocolate samples, these are reported in Table 5.

Table 5. Yield values of the three cocoa chocolate formulations after tempering process step

Chocolate samples	Yield Stress (Pa)
DARK	33.07 ^c
MILK	14.56 ^b
WHITE	11.15 ^a

^{a-c} values followed by different letters differ significantly at $p < 0.05$ level

As know by literature (Chevalley, 1996) cocoa butter, due to its free-moving lubricating plastic flow, coats particles and decreases forces between them. This effect, reducing the resistance to flow and improving the mobility of the matrix (Beckett, 2008), gives arise to lower values of viscosity and yield stress. For this reason milk and white chocolate, having higher amount of cocoa butter than dark, need less energy to starts flowing.

A similar trend was observed for what concern the dynamic measurements (Table 6), the elastic modulus (G') in fact showed the higher values in dark chocolate sample than in milk one, highlighting the presence of more elastic structure in the first one. As reported in previous studies (Johansson & Bergensthål, 1992; Glicerina et al., 2013) high values of G' are related to a high level of interactive forces between particles; this confirms the high amount of stress needed for dark chocolate samples to start flowing. Milk chocolate samples showed the lowest values of G' probably due to the lubricating effect of the major amount of cocoa butter present in formulation.

Table 6. Storage (G') and loss (G'') modulus of dark and milk temperate chocolate samples evaluated at 1Hz

Chocolate samples	G' (Pa)	G'' (Pa)
DARK	4133 ^b	1396 ^b
MILK	2873 ^a	798 ^a

^{a-b} values followed by different letters differ significantly at $p < 0.05$ level

Empirical results (reported in Table 7) were in agreement with that obtained from the fundamental rheological analysis. Dark chocolate samples in fact showed the highest values of firmness, consistency, cohesiveness and index of viscosity.

Table 7. Textural parameters of dark, milk and white temperate chocolate samples evaluated at 1Hz

Chocolate samples	Firmness (N)	Consistency (N s)	Cohesiveness (N)	Viscosity index (N s)
DARK	20.10 ^b	110.14 ^b	16.07 ^a	105.07 ^a
MILK	0.79 ^a	0.28 ^a	0.40 ^a	1.17 ^a
WHITE	0.92 ^a	0.87 ^a	1.83 ^a	0.18 ^a

^{a-c} values followed by different letters differ significantly at $p < 0.05$ level

Lower rheological values in milk and white cocoa samples can be further attributed to the presence of milk powders that contains milk fat. Milk fat is commonly used in chocolate because of its desirable flavour and its lower price, compared with cocoa butter. The presence of milk fat also influences the physical properties of chocolate masses, such as the crystallization behaviour of cocoa butter and the rheological properties of the final chocolate. Previous studies (Hartel, 1996) highlighted a negative correlation between the amount of milk fat and the hardness of processed chocolate.

Manufacture process

In the researches carried out in this doctoral thesis, the rheological properties of dark (**Papers II, VII**), milk (**Paper IV**) and white (**Paper V**) chocolate were also evaluated during all process steps: mixing (A_{Dark, Milk, White}), pre-refining (B_{Dark, Milk, White}), refining (C_{Dark, Milk, White}), conching (D_{Dark, Milk, White}) and tempering (E_{Dark, Milk, White}).

For each sample the apparent viscosity (η) against shear rate ($\dot{\gamma}$) was graphically used to represent the rheological behaviour in steady state conditions. It was evident that the apparent viscosity decreased increasing the shear rate in all cases (Figure 23 a, b, c), proving the pseudoplastic or shear thinning nature of chocolate.

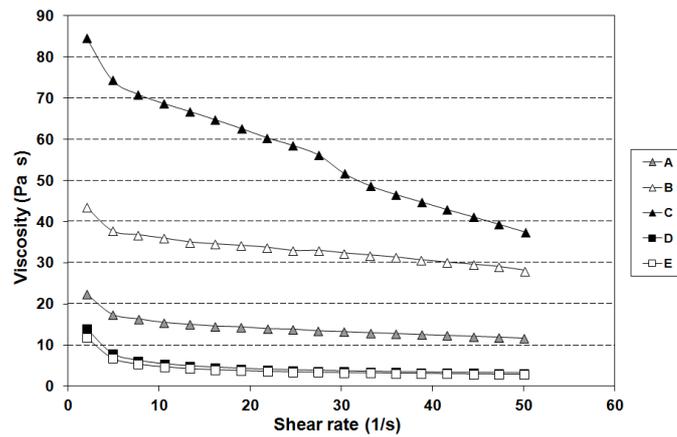


Figure 23 a Flow curves of dark chocolate samples, after mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) steps (corresponding to Figure 2 in Paper VII).

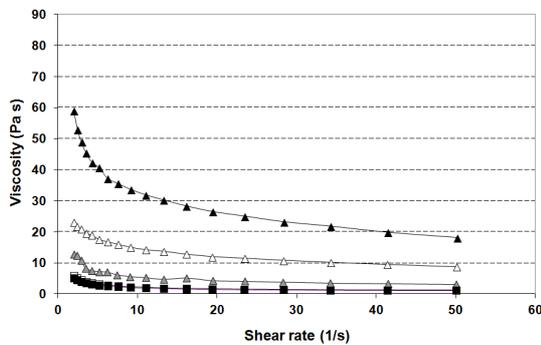


Figure 23 b Flow curves of milk chocolate samples, after mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) steps (corresponding to 3 Figure in Paper IV).

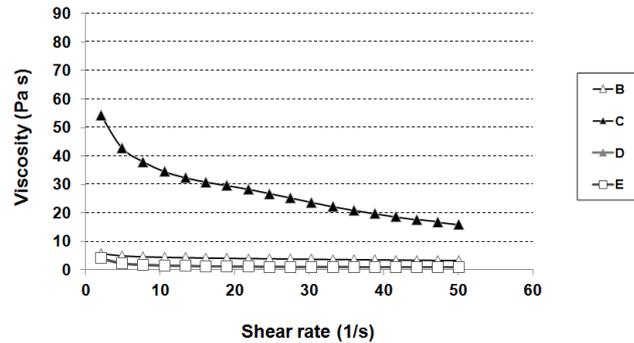


Figure 23 c Flow curves of white chocolate samples, after pre-refining (B), refining (C), conching (D) and tempering (E) steps (corresponding to Figure 5 in Paper V).

In order to better explain the rheological values obtained by the flow curves, several rheological models were applied. Each model was chosen in order to obtain better coefficient of determinations (R^2).

In particular for what concern dark chocolate samples, the obtained flow curves were evaluated and fitted according to the rheological model of Ostwald, commonly referred to as the Power Law model (section 5.1). Power Law model provided high coefficients of determination (R^2), varying from 0.75 to 0.99 (**Paper VII**).

As illustrated in Figure 23a and as confirmed by the yield stress and the apparent viscosity values (evaluated according to Servais et al., 2002) and on the basis of the k obtained (Table 1 in **Paper VII**) the viscosity showed a drastic and significantly increase ($P < 0.05$), from sample A_{dark} to C_{dark} . The highest values of these rheological parameters, as for the sample C, made up from the smallest particles, could be attributed to the increase of the contact point between particles, that forming very aggregated structures (as previous shown by the microstructural analysis, Chapter 4.3), In these samples is necessary a major amount of stress to break them and initiate to flow (Afoakwa et al. 2009). Samples D_{dark} and E_{dark} presented the lowest rheological parameters compared to other samples, probably related with their less aggregate packing structure network. In these steps, in fact the addiction of lecithin and further cocoa butter, because of its lubricating action, reduced the particle–particle interactions, increasing their mobility that involved a reduction of viscosity (Vernier, 1998). These results are supported by the studies of Dzuy and Boger (1983), Coussot and Ancy (1999) and Larsson (1999) that noticed a high dependence of yield stress and apparent viscosity on particles size and their interaction (**Papers II and VII**)

Frequency sweep tests results were also in agreement with the steady state ones (Table 8):

Table 8. Storage (G') and loss (G'') modulus of dark chocolate samples evaluated at 1 Hz (corresponding to Table 2 in Paper VII).

Samples	G' (Pa)	G'' (Pa)
A_{dark}	16346 ^b	2046 ^b
B_{dark}	18593 ^c	3161 ^c
C_{dark}	167086 ^d	21473 ^d
D_{dark}	5966 ^a	1406 ^a
E_{dark}	4133 ^a	1396 ^a

^{a-d} values followed by different letters differ significantly at $p < 0.05$ level

B_{dark} and C_{dark} samples showed the highest significantly G' values compared to the others. Sample A showed viscoelastic properties with intermediate values of G' and G'' inside the clusters $B_{\text{dark}}-C_{\text{dark}}$ and $D_{\text{dark}}-E_{\text{dark}}$. A significantly lower parameters of G' and G'' were found for the samples D_{dark} and

E_{dark} , constituted by a weakly structured system, due to the lubricating and emulsifier effect of fat and lecithin. These results are in agreement with the studies of Johansson and Bergensthal (1992), that highlighted how the effect of emulsifiers on the sugar particles, reducing the changing in the interaction particles and in the network structure ones, involves a decrease of the elastic component G' .

Results of textural properties of dark chocolate samples are shown in Table 9 (**Paper VII**).

Table 9. Textural properties of dark chocolate samples (*corresponding to Table 3 in Paper VII*).

Samples	Firmness (N)	Consistency (N s)	Cohesiveness (N)	Viscosity index (N s)
A_{dark}	54.90 ^b	498.26 ^b	36.47 ^a	414.40 ^a
B_{dark}	146.57 ^c	739.80 ^c	84.83 ^c	718.90 ^c
C_{dark}	378.82 ^d	1624.40 ^d	199.90 ^d	1106.00 ^d
D_{dark}	29.30 ^a	147.76 ^a	22.33 ^a	147.00 ^a
E_{dark}	20.10 ^a	110.14 ^a	16.07 ^a	105.07 ^a

^{a-d} values followed by different letters differ significantly at $p < 0.05$ level

C_{dark} and B_{dark} dark chocolate samples showed, for all the considered parameters, significantly higher values compared to other samples. These results are in agreement with the ones obtained from the fundamental rheological analysis, support the presence of a more consistent structure that arise a strong resistance to its compression. Sample A_{dark} showed intermediate values of consistency and index of viscosity, between those of samples $C_{\text{dark}}-B_{\text{dark}}$ and $D_{\text{dark}}-E_{\text{dark}}$; while results of D_{dark} and E_{dark} samples demonstrate the presence of a weak network structure, more sensitive to the breakage. For what concern milk chocolate samples (**Paper IV**) data were well fitted by the *Casson* model modified by Chevalley (1991), providing high coefficient of determination R^2 ranged between 0.75 to 0.99. Obtained values of the *Casson yield value*, *Casson plastic viscosity*, and of the yield stress and apparent viscosity (Afoakwa et al., 2008 and ICA, 2000) are shown in Table 10 .

Table.10. Casson yield values, Casson Plastic Viscosity, Yield stress and Apparent Viscosity of milk chocolate samples (corresponding to Table 2 in Paper IV).

Samples	Casson Yield value (Pa)	Casson Plastic Viscosity (Pa s)	Yield stress (Pa)	Apparent Viscosity (Pa s)
A _{milk}	6.82 ^b	4.38 ^b	37.10 ^b	3.84 ^b
B _{milk}	11.97 ^c	7.82 ^c	91.10 ^c	10.84 ^c
C _{milk}	35.70 ^d	15.36 ^d	209.33 ^d	23.23 ^d
D _{milk}	2.75 ^a	1.55 ^a	16.93 ^a	1.53 ^a
E _{milk}	1.95 ^a	0.21 ^a	14.56 ^a	1.32 ^a

a-d values in the same column followed by different letters differ significantly at $p < 0.05$ level.

A significantly increase in both Casson obtained parameters and in the yield stress and apparent viscosity was observed from sample A_{milk} to C_{milk}. This could be attributed in part to the increase of the contact point between particles (Do et al., 2007), as confirmed by microstructural analysis results (Ch.4.3) and in part to the presence of large void spaces between aggregates (Ch.4.3) that immobilized cocoa butter. The immobilized fat, cannot contribute to the flow as lubricant, reducing matrix viscosity values (Franke & Heinzelmann, 2008). Samples D_{milk} and E_{milk} were characterized by the lowest and significantly similar values of all rheological analyzed parameters, underlining the presence of a weak structure, as observed from microstructure results, that involves a less resistance to flow. In the conching step in fact, a destruction of the previous agglomerates, coupled to a reduction of the larger voids between aggregates and a re-distribution of cocoa butter between particles, was noticeable (Attaie et al., 2003). Cocoa butter, due to its free-moving lubricating plastic flow, coats particles and decreases forces between them, reducing the aggregation of particles and improving their mobility (Beckett, 2008).

Results of dynamic measurements (Table 11) supported ones obtained in steady state conditions, confirming the high amount of stress needed for B_{milk} and C_{milk} milk chocolate samples to start flowing, compared with the others samples (**Paper IV**).

Table 11. Storage (G') and loss (G'') modulus of milk chocolate samples evaluated at 1 Hz
(corresponding to Table 3 in Paper IV).

Samples	G' (Pa)	G'' (Pa)
A_{milk}	8416 ^b	1281 ^b
B_{milk}	13673 ^c	2357 ^c
C_{milk}	72746 ^d	16873 ^d
D_{milk}	3983 ^a	807 ^a
E_{milk}	2873 ^a	798 ^a

^{a-d} values followed by different letters differ significantly at $p < 0.05$ level

Results obtained by empirical analysis on milk chocolate samples are shown in table 12.

Table 12. Textural parameters of milk chocolate samples

Samples	Firmness (N)	Consistency (N s)	Cohesiveness (N)	Viscosity index (N s)
A_{milk}	11.76 ^b	21.38 ^b	1.41 ^a	2.67 ^a
B_{milk}	24.14 ^c	37.49 ^c	6.20 ^c	16.70 ^c
C_{milk}	61.15 ^d	469.40 ^d	20.81 ^d	90.93 ^d
D_{milk}	1.42 ^a	0.39 ^a	0.63 ^a	2.10 ^a
E_{milk}	0.79 ^a	0.28 ^a	0.40 ^a	1.17 ^a

^{a-d} values followed by different letters differ significantly at $p < 0.05$ level

Samples C_{milk} and B_{milk} also in this case showed significantly higher values for all the considered parameters respect the other samples, demonstrating the presence of an aggregate and very consistent structure. Sample A_{milk} , characterized by a structure made up from larger particles and more empty spaces, presents intermediate values of consistency and hardness significantly different from the other samples. D_{milk} and E_{milk} , chocolate samples, had the lowest values of all analyzed parameters, confirming the presence of a structure affected by weak bonds, more susceptible to breakage. These results are positive for the sensory perception of product by the consumer (Bourne, 2002).

The last part of this research was related with the study of the rheological properties of white chocolate (**Paper V**). To our knowledge no previous studies are found in literature regarding the rheological properties of white chocolate. Because of this lack of studies on white chocolate, in this research, two different models were compared (the *Casson* model and the *Windhab* model), in order to accurately study the rheological behaviour of white chocolate during process. Results provided high coefficient of determination, underlined the usefulness of both methods in studying white chocolate rheological behaviour. In particular, the model of *Windhab* showed coefficients of determination ranged between 0.80 and 0.99, slightly greater than *Casson* one, being thus the most suitable for these kind of matrix (**Paper V**).

Yield stress and apparent viscosity were evaluated respectively at 5 s^{-1} and 40 s^{-1} (ICA, 2000; Servais 2004). At the same time the *Casson* yield value, *Casson* viscosity, linear yield stress (*Windhab*) and the infinity viscosity (*Windhab*), were extrapolated from the two models applied in order to obtain more detailed information on the rheological properties of white chocolate.

Data analysis proved that the product taken from the refining step (C_{white}), also in this case, has the significantly higher ($p < 0.05$) values of yield stress and viscosity compared to all other samples, highlighting that the amount of stress required to obtain the flow was the highest. This result is probably related with the microstructural results (Ch. 4.3), from which this sample showed a highly aggregated matrix. Sample B_{white} occupy an intermediate position regarding the values of yield stress and viscosity, while samples D_{white} and E_{white} , made up from of less aggregated structures (Ch.4.3), showed statistically lower values than the other samples. In the conching step in fact a further addition of cocoa butter and lecithin was performed. The lubricating effect of these two components affecting the flow behaviour of matrix reduces its viscosity (Dzuy & Boger, 1983; Larsson, 1999; Coussot & Ancy, 1999).

Flow behaviour results of white chocolate were supported by the thixotropy ones. In particular thixotropy measurement are strictly related with the microstructural ones. Thixotropy, representing the energy required to break down the structure that is not recovered during the experimentation period (Roopa & Bhattacharya, 2009), underlines how in very aggregate matrix, this force will be higher and not recovered because of the completely de-structuration of matrix. From the histogram in Figure 23, as expected, samples B_{white} and C_{white} , having the most aggregated structures, show the statistically higher thixotropic values. In agreement with Afoakwa, et al., (2008) the high level of aggregation of the structural matrix undergoes an irreversible break immediately after the removal of the stress. Samples D and E, showed the statistically lower thixotropic values. In agreement with

literature (Afoakwa et al., 2008), in fact, a well-conched and tempered chocolate should not present any thixotropy, and should be characterized by poorly aggregated structures.

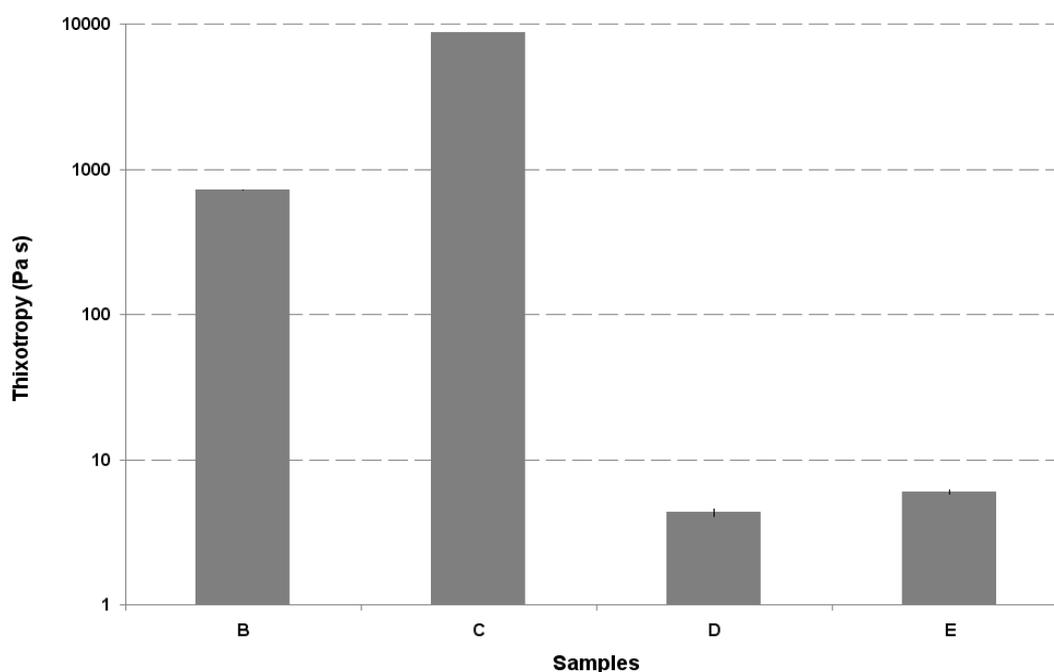


Figure 24. Thixotropy of white chocolate samples (*corresponding to Figure 6 in Paper V*).

Empirical results in terms of firmness, consistency, cohesiveness and index of viscosity obtained by a back extrusion test are shown in Table 13.

Table 13. Textural parameters of white chocolate samples.

Samples	Firmness (N)	Consistency (N s)	Cohesiveness (N)	Viscosity Index (N s)
A _{White}	1.39 ^b	5.65 ^b	6.26 ^a	0.33 ^a
B _{White}	1.03 ^c	6.56 ^c	6.07 ^c	0.51 ^c
C _{White}	8.34 ^d	57.67 ^d	33.08 ^d	5.94 ^d
D _{White}	0.88 ^a	0.62 ^a	2.07 ^a	0.28 ^a
E _{White}	0.92 ^a	0.87 ^a	1.83 ^a	0.18 ^a

^{a-d} values followed by different letters differ significantly at $p < 0.05$ level

Sample C_{white} taken from the refining phase, shows also in this case significantly higher values than the others for all the considered parameters, demonstrating the presence of a compact and very consistent structure.

Samples A_{white} and B_{white} showed intermediate and significantly different values of consistency and cohesiveness index compared to the others. This is probably due to the presence of structures characterized by larger particles size and more empty spaces compared to sample C_{white}. For what concern the hardness and viscosity index, samples A_{white} and B_{white}, showed higher absolute values even if not statistically significant respect samples D_{white} and E_{white} obtained from the last two steps of the manufacture process. D_{white} and E_{white} samples had the lowest values of all the empirical analyzed parameters, confirming the presence of a weakly structure more susceptible to breakage. From the whole analysis of the obtained results, however, it can be stated that the empirical-imitative analysis were not able to discriminate well the structural characteristics of this matrix as a function of process steps.

6 Conclusions

The microstructural studies carried out in this thesis on chocolate products, highlighted interesting findings:

- Each single manufacture process as well as the formulations of cocoa matrices and chocolate type dispersions, have a considerable effect on the microstructure of the suspensions formed.
- Small amount of water in formulations can affects in very strong way the microstructural properties of a food dispersions, increasing the interaction between particles.
- Has been demonstrated that each step of the manufacture process (from mixing to tempering), promoting the reduction of particle size and a different distribution of fat and eventually emulsifiers, involves the breakage and the creation of aggregates in all chocolate products investigated.
- Laser light diffraction and microscopy (optical and electron) techniques, coupled with image analysis, have been very useful in order to discriminate microstructural differences in chocolate samples related to the productive process.

Obtained rheological results showed also important findings:

- Formulation and process affect in considerable way cocoa matrix rheological properties, in terms of flow and oscillatory behaviour.
- Different types and amounts of fats in formulation, involve deep changes in the rheological attribute of the final product, in particular affecting the viscosity of the matrix.
- The manufacture process (from the mixing to the tempering step), involving a reduction in particle size and creating different aggregate structures, induces drastic modifications in the rheological properties of the cocoa matrix.

- Fundamental measurements were very accurate in order to highlight differences between chocolate samples, while empirical ones were not always able to discriminate chocolate samples.

In conclusion, the overall obtained results demonstrate a strictly relationship between microstructural and rheological properties in cocoa and chocolate type matrices, that could be very useful in order to optimize and to improve (through formulation or process) the quality of the final product, mainly in terms of rheological properties, the most critical characteristics for this kind of dispersion products.

7 References

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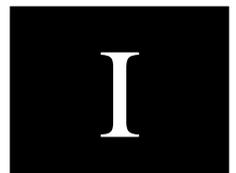
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8 List of Papers

The influence of small amount of water on the microstructural and rheological properties of sugar particle dispersions in cocoa butter



Glicerina V., Tornberg E. and Bergenstål B.

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Manuscript

THE INFLUENCE OF SMALL AMOUNT OF WATER ON THE MICROSTRUCTURAL AND RHEOLOGICAL PROPERTIES OF SUGAR PARTICLE DISPERSIONS IN COCOA BUTTER

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1. Introduction

Particle technology is concerned with the dispersion of particles into different environments (Babini *et al.*, 2005). A very exhaustive example of particles dispersed in a fluid matrix is represented by chocolate. Chocolate can be defined as complex systems made up from different solid particles (sugar, cocoa powder, milk powders, nuts, etc.) dispersed in a continuous fluid (oil) (Afoakwa *et al.*, 2008). The interactions between the suspended particles, and the continuous phase provide information about the existing network and consequently can be associated to the rheological properties and structural characteristics of the final dispersions (Sato *et al.*, 2009). The rheological behaviour of this concentrated suspensions is further influenced by the presence of various additional compounds such as: emulsifiers (Mackie *et al.*, 1989; Johansson & Bergenståhl, 1992c), minor surface-active components present (Kawaguchi *et al.*, 2001) and water (Gaonkar, 1998; Beckett, 2008). In particular water affects stability, quality and physical properties of foods because of the interaction of its molecules with other food constituents (Lewicky, 2004). Range of water concentration in food begins with fraction of 1% and reaches even more than 98%. In fresh products

and liquid food water is contained usually in large amounts, while in baked and sweet products as chocolate the amount of water is around 1-1.5 % (Anese *et al.*, 1996; Ahmed & Hosahalli, 2006).

In products in liquid or viscous state, water involves changes in viscosity and consistency while in solid foods, water affects their response to applied force. The water binding influence of the solid disperse phase or aggregate were no longer a main aspect considered as important for suspension rheological behaviour, overall at low water content, but this does not reflect the reality, (Windhab *et al.*, 2001) and despite of several studies (Mackie *et. Al*, 1989; Hugesolfer, 2000; Beckett, 2008) on the increase of viscosity of chocolate by increasing water content, to our knowledge no basic studies have been carried out on the influence of small amount water on food oil continuous models.

In order to evaluate rheological changes in suspensions and in particular in chocolate products the cup and bob geometry is recommended (ICA, 2000), but in the last years a new kind of geometry has grown in popularity: the vane geometry. This kind of geometry let to measuring the properties of non-Newtonian fluids reducing the slip effects at walls. It also offers a way of introducing the testing element, a thin-bladed vane, into a structured liquid with the minimum amount of disturbance to the sample, making it very suitable for materials such as clay suspensions, chocolate and other gelled liquids (Barnes & Nguyen, 2001). This geometry form a cross shape at the end of a central shaft and can be inserted into the sample with minimal disturbance of the microstructure. The fluid circumscribed by the vane moves as a solid cylindrical body such that slip is effectively eliminated. Inserted into the fluid is an easy and convenient method of performing accurate rheology measurements on such samples (Stokes & Telford, 2004; Baker *et al.*, 2006)

The aim of this work was to investigate the influence of small amounts of water (0.1 % and 0.5% in volume) on the rheological and structural properties of chocolate type dispersion. In order to analyze these relationship, model system were obtained. Rheological properties of different dispersions were evaluated by using a four bladed vane geometry.

2. Materials and methods

2.1 Materials

The materials selected for this study were mainly commercial samples. Dispersion samples were produced by using: cocoa butter, obtained from an Italian confectionary factory, icing sugar purchase at local market and distilled water. The choice to use refined sugar instead of the crystallized one, was strictly related with its small size. Small particles size in fact present higher specific surface area, involving more contact points between them (Afoakwa et al., 2009). The reduction of the particle diameter causes an increase of the particle number, parallel to an increase of contact points between particle-particle. A mixture design was used to obtain different experimental dispersions. In order to simplify the experimental design, the water amount added was kept fixed at the two different values of 0.1 and 0.5, in this way only the amounts of sugar and cocoa butter were two experimental variables were changed to differentiate the formulations. To differentiate the samples, a simplex centroid design (Ruguo, 1999) was applied and ten different samples were obtained (Table 1)

SAMPLES	COCOA BUTTER	SUGAR	WATER
	(ϕ)	(ϕ)	(ϕ)
A1	55	45	0.1
A2	55	45	0.5
B1	52	48	0.1
B2	52	48	0.5
C1	48	52	0.1
C2	48	52	0.5
D1	45	55	0.1
D2	45	55	0.5
E1	40	60	0.1
E2	40	60	0.5

2.2 Methods

Dispersions described in this paper were realized under stirring by using an IKA High Viscosity Mixer equipped with a high efficiency paddle assembly (ColeeParmer, Vernon Hills, USA) applying the vacuum. During stirring dispersions were heated at 40 °C by using a magnetic stirrer, in order to maintain cocoa butter in a liquid/viscous state. Before start mixing, ingredients (cocoa butter and sugar) were left overnight in an oven at 100 °C, in this way all water was evaporated and then left 10 minutes in a dryer. The mixing time was around 20 h. With this procedure samples can be prepared up to a content of 60 % of sugar in the fat.

2.3 Rheological measurements

Measurements were carried out at 35 °C using a controlled-stress rheometer (Kinexus Pro, Malvern, UK) equipped with a fourblade vane geometry, in order to reduce the wall slip phenomenon.

The vane was 21 mm in diameter and 60 mm in height, and was placed in a cup 27 mm in diameter. Special care was taken to minimize air inclusions in the sample.

The rheological behaviour of model suspension was analyzed in steady state conditions, flow tests and creep - recovery ones were performed on each samples, increasing the shear stress from 1 to 100 Pa.

Samples were allowed to equilibrate for 5 minutes at the set temperature before the tests were performed.

2.4. Microstructure analysis

Samples microstructure was observed using a polarized light microscope (Olympus Optical, Tokyo, Japan) at 10x of magnification, taking 10 micrographs for each samples. One drop of dispersion (previously diluted with hexane) was placed on a glass slide and covered with a cover slip carefully placed over the sample, parallel to the plane of the slide and centred to ensure sample thickness was

uniform. Micrographs were captured using a digital camera (Model 2.1 Rev 1; Polaroid Corporation, NY, USA) and observed using Image J (v. 1.38).

2.5. Determination of particle size distribution

Measurement of PSD was made by using a Mastersizer, Micro Laser Diffraction Particle Size 2000 (Malvern Instrument Ltd., Malvern, UK) equipped with a Hydro 2000 SM (B) for paraffin oil (RI 1.450). About 0.3 g of sample was dispersed in oil until an obscuration of 16.85%, following the user guide of the instrument. Speed of stirring was maintained at 1900 rpm for all the measurements.

Size distribution was determined as relative volume of particles in “*size bands*” and presented as size distribution curve and statistics (Malvern Mastersizer Micro Software v. 5.60). PSD parameters obtained were: specific surface area, the largest particle size (D_{90}), mean particle volume (D_{50}), the smallest particle size (D_{10}), Sauter mean diameter ($D[3,2]$) and Weight mean particle diameter ($D[4,3]$).

The last two parameters (Sauter mean diameter and weight mean particle diameter) are the most commonly used to characterize PS and represent respectively: the diameter of a sphere with the equivalent surface to volume ratio of all the particles in the size distribution (1)

$$d_{32} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad (1)$$

and the diameter of a sphere having the average weight of all the particles in the size distribution (2) (Black et al., 1996).

$$d_{43} = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \quad (2)$$

3. Results and discussion

3.1. Fundamental rheological properties

3.1.1. *Effect of the concentration on the apparent yield value*

In Fig. 1 are reported the flow curves of the sugar in oil dispersions with 0.1 % of water, obtained increasing the shear stress from 1 to 100 Pa. In each graph, dispersions were characterized by a constant amount of water but different volume fraction of sucrose, from 45 to 60 %.

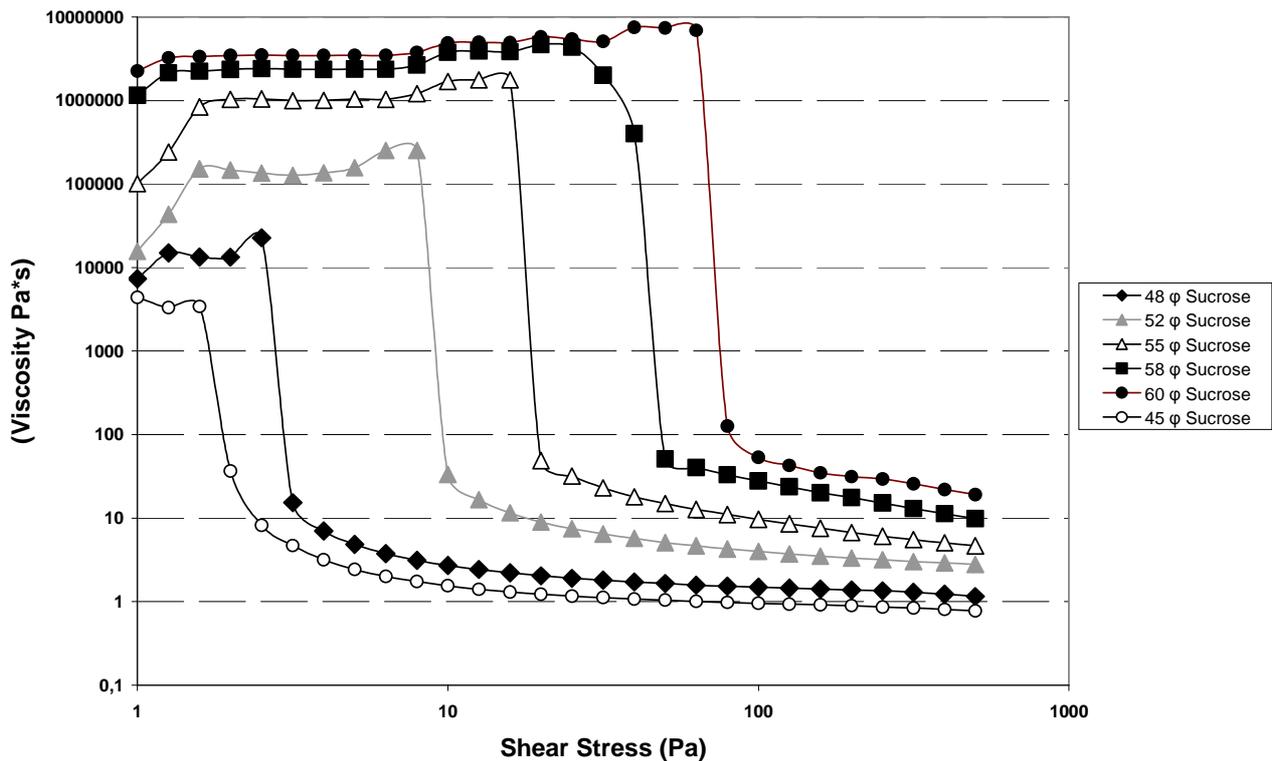


Fig. 1. Changes of viscosity (Pa*s) of sugar in oil dispersions with 0.1 % of water.

All samples exhibited a typical non Newtonian behaviour characterized by dependence of viscosity upon of flow conditions, such as geometry, shear rate or stress applied (Chabbra, 2006). In particular apparent viscosity of these products decreased increasing the shear rate, indicating pseudoplasticity, i.e. shear thinning behaviour (Rao, 1999). This behaviour can be explained in general, by the structural breakdown of the molecules due to the hydrodynamic forces generated and to the increased alignment of the constituent molecules (Izidoro et al., 2008). In highly

concentrated suspensions, as model samples examined in this study, the interaction between particles dominates over the hydrodynamic forces and the material exhibits an apparent yield stress as shown in Fig.1. The apparent yield stress is defined as the minimum stress required by a material to initiate flow (Bayod et al., 2008) and it's considered as the result of the particle–particle interactions (both of hydrodynamic and surface force) and from particle structure (Johansson and Bergenståhl, 1992 b; Macosko, 1994). Yield value is usually determined from the flow curve, in particular the critical stress of the onset of the shear thinning region is commonly used to characterize it (Bayod et al. 2008). The increasing of the sucrose volume fraction involve an increasing of the yield stress values for all dispersion samples, according with the studies of Den Ouden & Van Vliet (1997) and Dzuy & Boger (1983); that evidence a relationship between yield value and particle concentrations. Plotting the sugar concentration as a function of the yield values, (Fig. 2) an exponential correlation was observed with high coefficient of determination ($R^2= 0.99$).

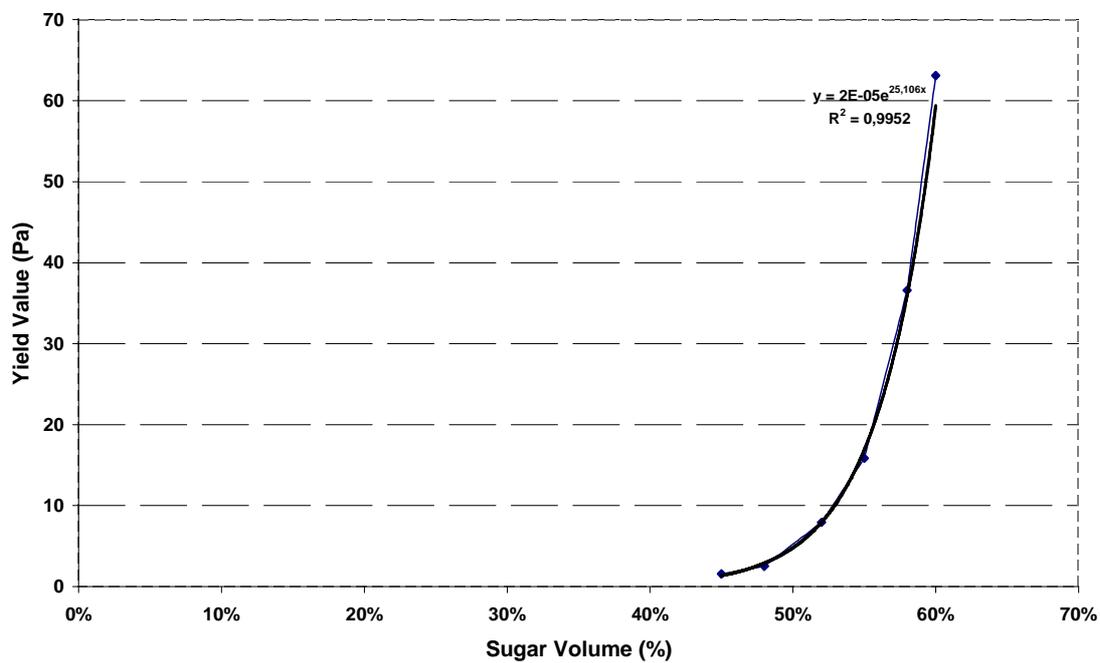


Fig. 2. Correlation between yield value and sugar Volume.

3.1.2 Characterization of the creep- recover properties of dispersions subjected to different levels of stress

Some of the most widely used analyses to evaluate the structure of samples are creep and recovery tests. These consist of deforming the system for a pre-established period of time, applying a constant shear stress and measuring the deformation as a function of time (creep). This is followed by recovery of the structure, obtained cancelling the applied stress (Steffe, 1996).

In this study the evolution of the applied and removed stress was evaluated at the same time of the increasing of the shear stress from 1 to 100 Pa, in order to obtain more information about structure response of the samples to the stress applied .

The results obtained for the evolution of the shear rate as a function of time are shown in Fig. 3.

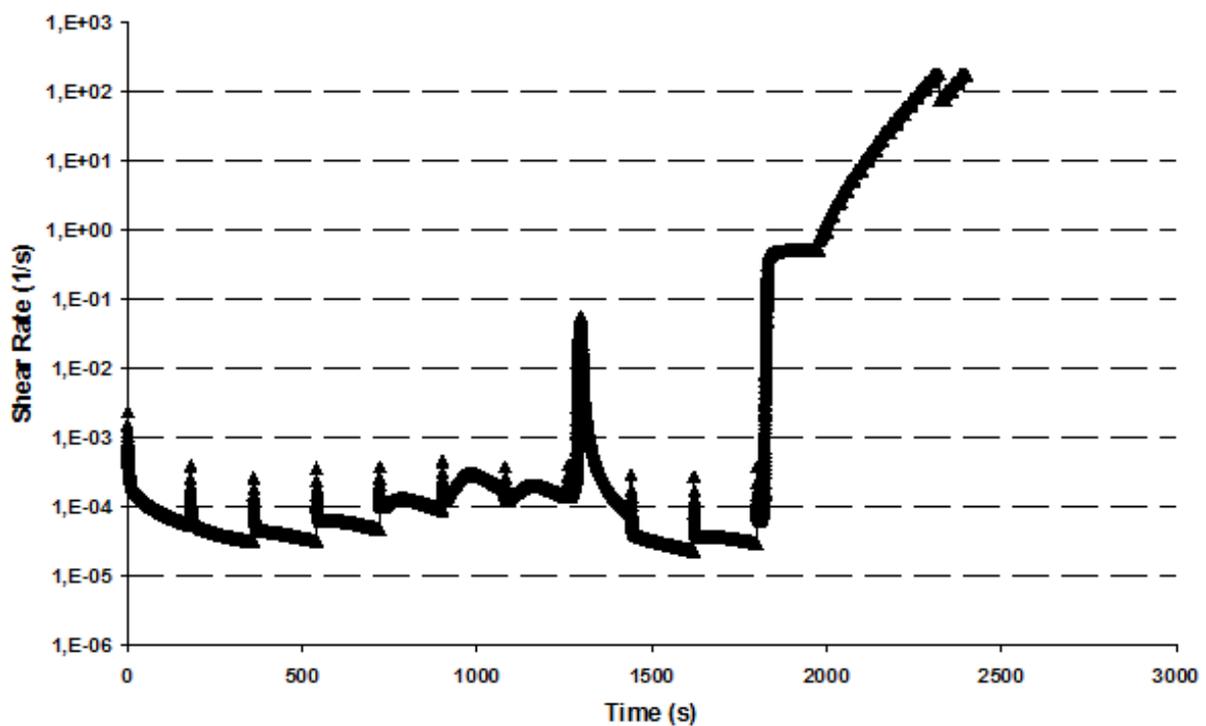


Fig. 3. Creep test of sugar dispersion obtained increasing the shear stress from 1 to 100 Pa.

In general, trend show the same behaviour for all dispersions samples. When the applied shear stress is below a certain stress value, samples recover the initial structure immediately after stress removing, indicating that a solid-like behaviour is predominant. If the stress is above a particular

limit, the sample start to flow exhibiting a liquid-like behaviour (Baldino et al. 2010), showing the presence of a yield stress as previously observed from flow tests. For all samples the flowing limits were evaluated for shear rates comprised between $0,3299 > \dot{\gamma} < 1,01$. In particular shear rate and time needful to obtain the breakage of the structure seem to increase from sample with the lowest amount of sugar until to sample with the highest volume of sugar, probably related with the arise of the solid volume fraction of samples, as noted previously with flow measurement

3.1.3 Effect of the small amount of water on the apparent yield value

The principal aim of this study was to evaluate the influence of small amount of water on the rheological properties of samples expressed in terms of yield value. Figure 4 represents the yield stresses of dispersions at the different sugar and water concentrations.

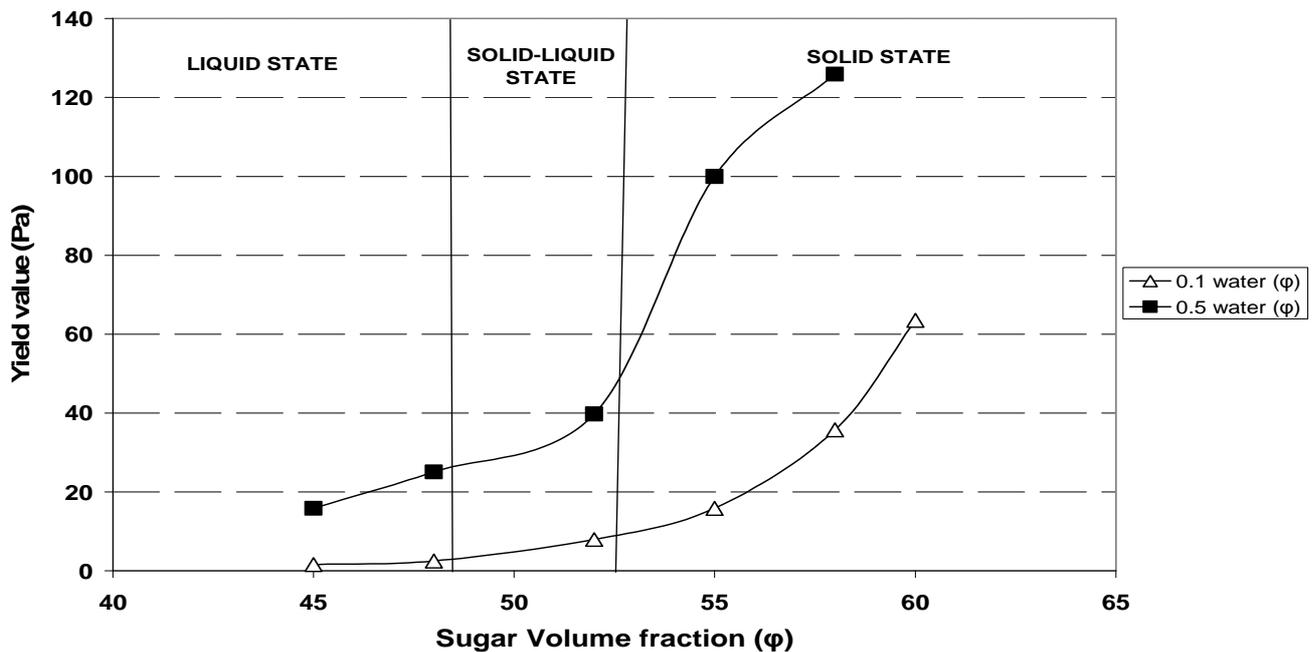


Fig. 4. Diagramme of state of sugar model dispersions obtained at different sugar and water amounts.

In particular the influence of small amount of water on the rheological properties of dispersions was highlighted. In this figure, it can be observed that data for each sugar and water concentration have

well defined points in the curves. As the concentration of sugar and water increases, three viscosity and yield stress regions may be identified. According with the model suggest by Goodwin (2004) it's possible to divide graph samples, showing the sugar volume fraction versus yield value into three main regions, depending on their state of aggregation, obtaining a volume state diagram (liquid, solid-liquid and solid). The first region which represents the low concentration suspensions (from 45 % to 48% ϕ) of sugar behave like Newtonian fluids showing low viscosities and low yield values. The second region that corresponds to the intermediate level of concentration (from 48 % to 52% ϕ of sugar) exhibit slight viscosities with a shear thinning behaviour and intermediate yield values. Above this concentration range, there is the region of highly concentrated samples corresponding to values of sugar $>52\%$ ϕ . The behaviour of the curves in this region may suggest the formation of "structured" aggregates with a strong affinity between the particles. As a consequence, the fluidity of the samples is reduced and this involves an increase the yield values and a consequent drastically increase of the viscosity of samples.

In particular graph showed as the presence of a higher amount of water 0.5ϕ involve a change a in the state of diagramme of the sugar shifting the curve to higher values of yield stress.

When the water content is increased, even if in very low amount, the interaction between crystal change, influenced in strong manner the yield value of dispersions. These results according with Johansson & Bergenståhl, 1992 c, could be interpreted as the effect of the water adsorption onto sugar. Aggregation between sugar particles become more stronger, probably due to the formation of water bridges, involving an increasing of the yield value of the experimental dispersions. From curves was possible to highlighting how the effect of small amount of water on the yield values of dispersions has an impact much higher compared to the sugar one.

With 0.5 % of water a 4th degree polynomial correlation was observed between different yield values with coefficient of regression ($R^2= 1$)

3.2 Microstructural properties of model systems

Light polarized microscopy was used to characterise the variations in sugar crystalline network and particle-particle interaction varying sugar (from 45 to 60 ϕ) and water volume fraction.

Micrographs (Figs 5a,b–6 a,b) showed clear and wide variations in microstructure among samples with different formulations.

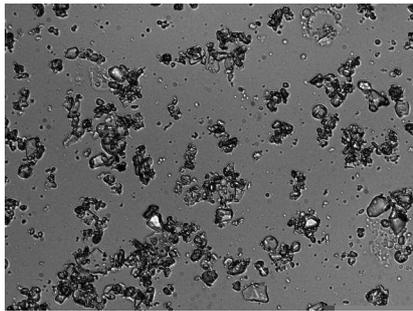


Fig. 5a. Micrograph of model dispersion obtained with 55 ϕ of sugar and 0.1 ϕ of water

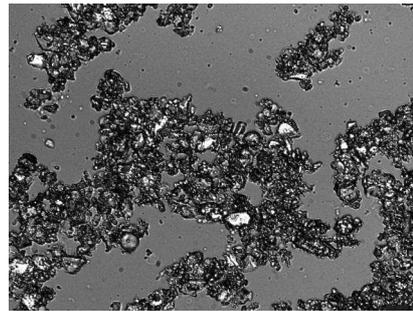


Fig. 5b. Micrograph of model dispersion obtained with 55 ϕ of sugar and 0.5 ϕ of water

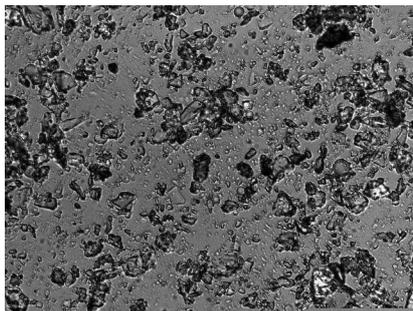


Fig. 6a. Micrograph of model dispersion obtained with 58 ϕ of sugar and 0.1 ϕ of water

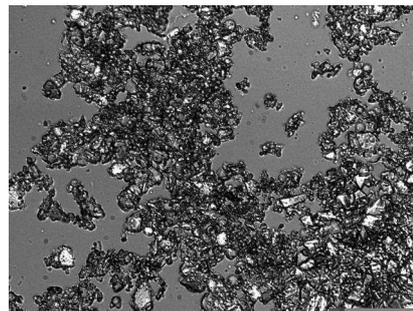


Fig. 6b. Micrograph of model dispersion obtained with 58 ϕ of sugar and 0.5 ϕ of water

In particular four pictures are shown, in order to highlight the influence of sugar and water increase on the structural properties of dispersions. In samples containing the lowest amount of icing sugar and water, weak sugar crystalline networks and reduced particle–particle interactions were observed compared with samples made up from higher concentration of sugar.

Increasing the solid content from 45 to 60 with 0.1 ϕ of water an increase in the aggregation was noted, this is probably due to the increase of the volume fraction of particles that involve an increase in the contact point between particles. Servais et al. (2004) noted that yield stress depended

on amount of small particles (specific surface area) and interactions, and originated in mechanical (friction) and chemical interactions between particles. Prasad et al. (2003) concluded that yield value was determined by inter-particle contacts. Increasing the water amount from 0.1 to 0.5 ϕ for all sugar concentration, these interactions between particles become more strong as shown by pictures. According with the studies of Lewicky (2004), can be noted how, raising water content, the macromolecules of sugar acquire structure, due to their interactions with water molecules and these changing in structure affect strongly the rheological properties of final dispersion.

3.3 Particle size distribution

In Fig. 7 the PSD of the different dispersions are shown. All samples exhibit a wide bimodal size distribution.

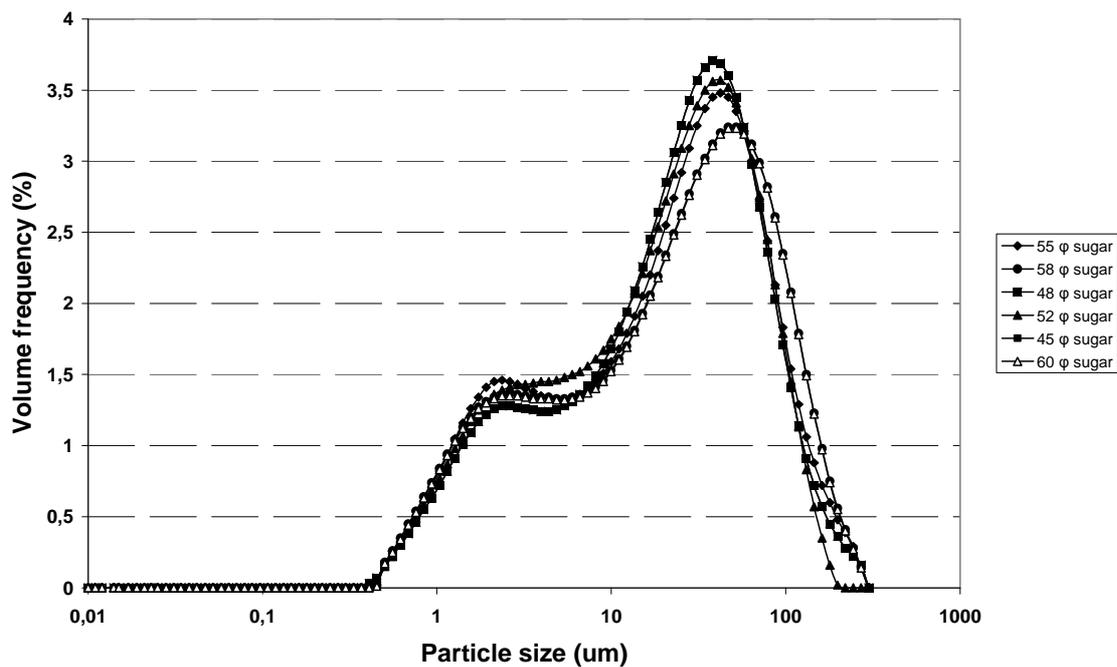


Fig. 7. Particle size distribution of sugar model dispersion with different sucrose concentrations.

Two main peaks were observed, one at about 50 μm and the other at about 3 μm . Not clear differences were evaluated between samples, comparing the two main areas under the PSD curves of this graphs.

However, the PSD expressed as area-based diameter or Sauter diameter (d_{32}), that take in account smaller particles, indicate some interesting differences between samples. Plotting the area-based diameter as the sugar volume fraction, as shown in Fig.8 an inverse relationship between them was noted.

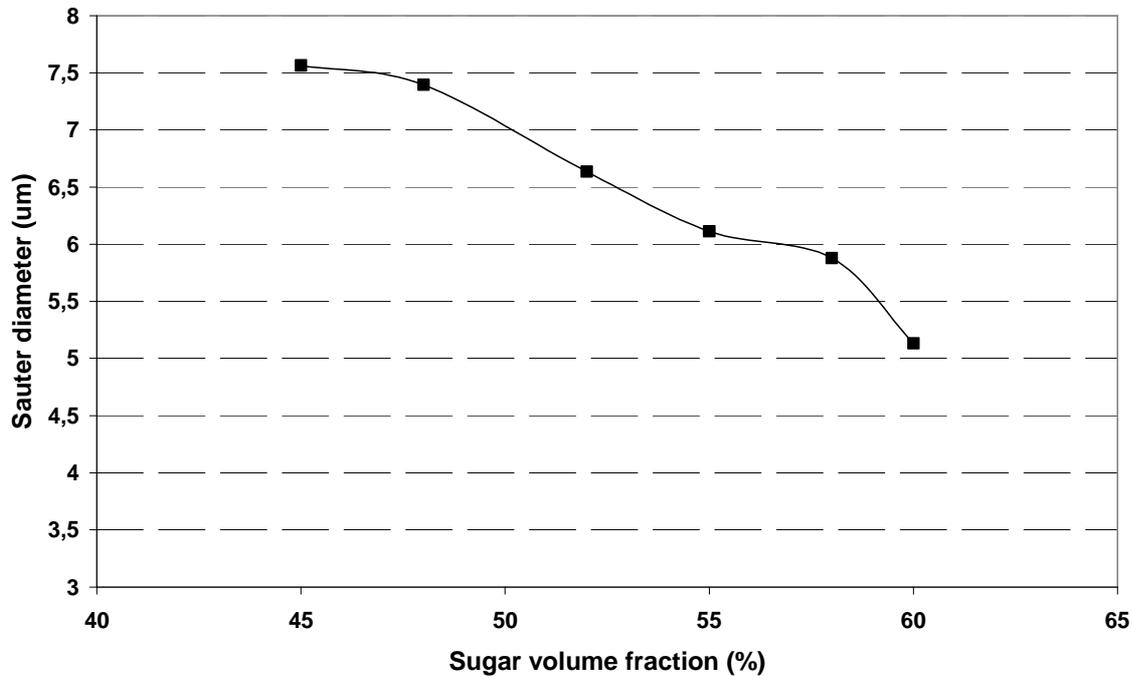


Fig. 8. Relationship between Sauter Diameter and sugar volume fraction.

The increasing of the sucrose volume involved a reduction of d_{32} , indicating the presence of particles with small diameters and more specific surface area that according with the studies of Beckett (1999) and Sokmen & Gunes, (2006) are inversely correlated with component PSD. The increase of specific area diameter resulted in flocculation and agglomeration of particles that restrict mobility and compartmentalisation of the matrix. These results in according with the ones obtained from the rheological and microstructural analysis highlight how the increase in volume sugar fraction involve an increase in the aggregation of particles and in the rheological values.

4. Conclusions

In this paper, a rheological characterization of the sugar- cocoa butter dispersion as a function of the concentration and the humidity, was evaluated, taking into account the time-dependent rheological properties.

The presence of an apparent yield value was noted, probably related to the disruption of the network structure, resulting from attractive particle– particle interactions. From results of rheological measurements, was possible to observe a direct proportionality between the minimum stress required to obtain the flow and the concentration of sucrose present in the dispersions. A strictly and more strong dependency highlighted from rheological analysis, was the one between the apparent yield stress and the water amount of the system. These rheological results were supported from the microstructural ones that showed an increase in the state of aggregation of particles with the raising of sugar and water amount. Water presence even, in very small amount has confirmed to be an important factor able to affect both network strength and the rheological properties of food dispersions.

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The influence of process steps on microstructural, rheological and thermal properties of dark chocolate.



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THE INFLUENCE OF PROCESS STEPS ON MICROSTRUCTURAL, RHEOLOGICAL AND THERMAL PROPERTIES OF DARK CHOCOLATE UTICAJ PROCESNIH KORAKA NA MIKROSTRUKTURNE, REOLOŠKE I TERMIČKE OSOBINE TAMNE ČOKOLADE

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ABSTRACT

The aim of this research was to investigate the influence of different process steps on microstructural, rheological and calorimetric properties of dark chocolate. Samples were obtained at each phase of the manufacturing process: mixing, pre-refining, refining, conching and tempering. Microstructural properties, fundamental rheological parameters (yield stress, apparent viscosity, G' and G'') and thermal ones (Tonset, Tend and ΔH) were evaluated by using an environmental scanning electron microscope (ESEM), a controlled strain – stress rheometer and a differential scanning calorimetry (DSC) respectively. ESEM analysis revealed an increase of the aggregation and contact point between particles from mixing to refining step, that underwent a drastic raising of all considered rheological (yield stress, apparent viscosity, G' , G'') and thermal parameters (Tonset, Tend, ΔH). Samples obtained from the conching and tempering steps were characterized by less dense aggregate structure and lower particle–particle interactions, due to the adding of further cocoa butter and lecithin. The addition of fat and lecithin in fact covering the sugar and cocoa particles, reduced interactions and caused a decrease in all rheological and thermal parameters.

Keywords: dark chocolate, process steps, microstructure rheological properties, thermal properties.

REZIME

Cilj ovog istraživanja bio je da se ispita uticaj različitih procesnih koraka na mikrostrukturne, reološke i kalorimetrijske osobine crne čokolade. Uzorci su uzeti u svakoj fazi proizvodnog procesa: mešanje, pre-prerada, prerada, končiranje i kaljenje. Mikrostrukturne osobine, osnovni reološki parametri (napon, viskoznost, G' i G'') i termičke osobine (Tonset, Tend i ΔH) ocenjeni su korišćenjem elektronskog mikroskopa (ESEM), kontrolisani reometarom i diferencijalnim kalorimetrom (DSC). ESEM analiza pokazala je povećanje agregacije i kontakt tačka između čestica od mešanja do pripreme, koje su pretrpele drastičan porast svih razmatranih reoloških (napon, viskoznost, G' , G'') i termičkih parametara (Tonset, težje, ΔH). Uzorke iz procesa končiranja i kaljenja karakterisale su niža gustina i niža čestica-čestica interakcija, zbog dodavanja kakao butera i lecitina. Dodavanjem masti i lecitina u stvari pokriva šećer i kakao čestice, smanjuje interakcije i izazva pad svih reoloških i termičkih parametara.

Ključne reči: crna čokolada, procesni koraci, mikrostrukturna reološka svojstva, termička svojstva.

INTRODUCTION

Dark chocolate can be defined as a concentrated suspension made up of solid particles (sugar and ground cocoa particles) dispersed in a Newtonian fluid, generally cocoa butter (Afoakwa *et al.*, 2008a). The physico-chemical and microstructural properties of chocolate depend on many factors besides the ingredients and their proportions. The different process steps (mixing, pre-refining, refining, conching and tempering) of chocolate manufacture and the different adopted process parameters can affect chocolate properties which, in turn, determine the behaviour and the characteristics of the final product. The processing of foods, dark chocolate in particular, brings several changes in their microstructure. During chocolate manufacturing, mixtures of sugar, cocoa and fat are heated, cooled, pressurized and refined (Beckett, 2008). These steps break agglomerates and distribute lipid and lecithin-coated particles through the continuous phase, modifying the microstructure of final chocolate (Afoakwa *et al.*, 2009a). Since the macroscopic properties of food, as rheological and thermal attributes are strongly determined by the microstructure, an evaluation of the product microstructure is a necessary prerequisite for understanding its macroscopic properties (Bayod, 2008). In particular rheology properties are important because related both to the efficiency of the main steps in the process (mixing, pumping, transportation, etc.) (Servais *et al.*, 2002), and to the final quality of product (Ahmed & Ramaswamy, 2006). For this reason the knowledge of the relations between microstructure, rheological and thermal properties could be very useful in order to optimize the final properties of choco-

late. Several authors (Servais *et al.*, 2004; Afoakwa *et al.*, 2008a; 2008b; Baldino *et al.*, 2010; De Graef *et al.*, 2011; Efraim *et al.*, 2011; Fernandez *et al.*, in press) have investigated the microstructural and physico-chemical properties of dark chocolate and in particular the rheological and thermal ones. Despite of the interesting obtained results, in all the previous cited works only the characteristics of finished product was taken into account. In our opinion each manufacture step (mixing, pre-refining, refining and conching) cause drastic modification in the product microstructure influencing its final properties. Understanding how the single process step can affect the microstructural properties of dark chocolate, could be very useful in order to manipulate and optimize the process efficiency and mainly to predict and improve quality of final product. For this purpose in the present work the microstructural, rheological and thermal properties of dark chocolate were evaluated throughout the manufacturing process.

MATERIAL AND METHOD

Dark chocolate samples were produced in an Italian confectionery factory using an industrial plant (Buhler, Malmo, Sweden) provided of mixer, pre-refiner, refiner, conching and tempering machine, equipped to produce 6000 Kg of chocolate at every production cycle. Dark chocolate production was made up by different steps: mixing, pre-refining, refining, conching, tempering, demoulding and packing. The formulation employed for the recipe was: cocoa liquor (53%), sugar (39.52%), cocoa butter (7% added during the conching step), soy lecithin (0.3%), sodium carbonate (0.15%) and vanilla extract (0.03%). Experimen-

tal samples were taken after each production phase: mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E). Samples were stored in plastic bucket (1 Kg capacity) at room temperature until the analytical determinations. Before performing each analysis the samples were melted in a microwave at 150 W for 25 minutes. The melting parameters were chosen after preliminary experiments in order to avoid changes in the chocolate properties.

Microstructure analysis

Samples microstructure was observed using an environmental scanning electron microscope ESEM (Evo 50 EP, Zeiss, Germany) equipped with a microprobe (EDS Mod. 350, Oxford Instrument, UK). The detector used was a backscatter electron detector (QBSE) that provided good compositional contrast imaging at 20 kV and in low vacuum mode with 100 Pascal at 500x magnification, taking 10 micrographs for each samples. These parameters were chosen after preliminary trials and according to *Dahlenborg et al. (2010)*, considering that they cause minimal damage of the chocolate surface and in order to optimize the imaging quality. The acquired images were subsequently elaborated using the software Image Pro-plus 6.0 (Media Cybernetics Inc Bethesda, USA).

Fundamental rheological properties

Measurements were carried out at 40 °C using a controlled stress-strain rheometer (MCR 300, Physica/ Anton Paar, Ostfildern, Germany) equipped with a system of coaxial cylinders (CC27). The rheological behaviour of dark chocolate was analyzed in steady state and dynamic conditions. In steady state conditions, after a pre-shearing of 500 s at 5 s⁻¹, viscosity was measured increasing shear rate from 2 to 50 s⁻¹ within 180 s, taking 18 points measurements (*ICA, 2000*). The yield stress (Pa) and the apparent viscosity (Pa s) were obtained according with *ICA (2000)* and *Servais et al., (2004)* evaluating the values of stress respectively at shear rates of 5 and 40 s⁻¹. In dynamic conditions, oscillatory tests were performed to investigate the viscoelastic properties of samples and to evaluate the storage (G') and the loss (G'') modulus. In order to identify the linear viscoelastic range (LVR), in which the viscoelastic properties are independent from the stress conditions, stress sweep tests were applied. Frequency sweep tests were carried out in the viscoelastic linear region at the constant deformation amplitudes of 0.007%, previously evaluated with the stress sweep test, in the range from 100 to 1 Hz.

Thermal properties

Melting properties of dark chocolate were evaluated by using a differential scanning calorimeter (Pyris DSC Series 6, Perkin Elmer Corporation, Wellesley, USA). Adopting the method reported by *Afoakwa et al., (2009b)* DSC was calibrated by using indium (melting T 156.60 °C, ΔH 28.71 J/g) and tin (melting T 231.93 °C, ΔH 60.46 J/g) at a scan rate of 10 °C/min using an aluminium pan as reference. Samples (5 mg) were loaded into 40 ml capacity pans with holes and sealed using a sample press. Pans were heated at 10 °C/min from 15 to 200 °C in a N₂ stream. Onset temperature (T_{onset}), end temperature (T_{end}) and enthalpy of melting (ΔH) were calculated for each peak present in the thermogram obtained (*Gloria and Sievert, 2001*).

Statistical analyses

Analyses of variance (ANOVA) and the test of mean comparison according to Fisher least significant difference (LSD) were applied. Level of significance was P ≤ 0.05.

RESULTS AND DISCUSSION

In Figure 1 (a, b, c, d, e) the micrographs related to the samples A, B, C, D, E obtained by ESEM are shown.

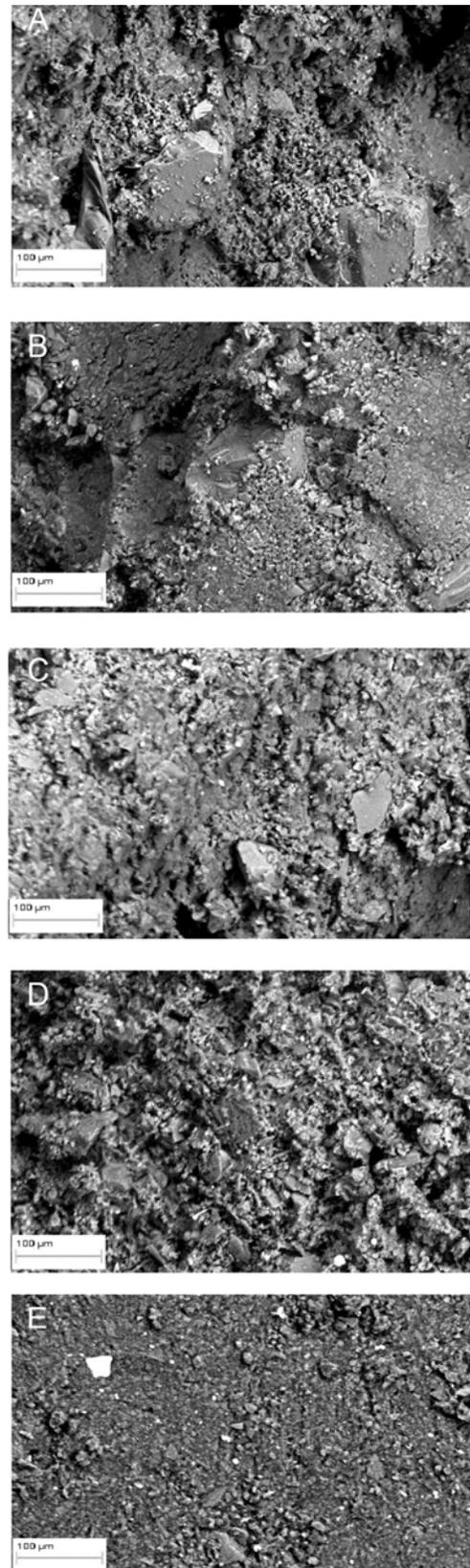


Fig. 1. Micrographs of dark chocolate after different processing steps: (A) Mixing, (B) Pre-refining, (C) Refining, (D) Conching and (E) Tempering. Scale (a) -(e) 100μm

Electronic microscopy was used to characterize the variations, during the different processing steps, in sugar crystalline network, particle-particle interaction and particle-fat phase behaviour of chocolate samples. Microstructural examination showed a clear decrease of samples particle size from the mixing step (A) to the pre-refining one (B), until the refining phase (C) (Figure 2. a, b, c). The reduction of the particle diameter causes an increase of the particle number, parallel to an increase of contact points between particle-particle, due to chemical and mechanical interactions, according to *Servais et al., (2004)* and *Afoakwa et al., (2009a)*. The increase of particle interactions from sample A to C caused a reduction of the particles mobility due to their high aggregation. The result is a fully dense and packed suspension in which small particles fill spaces between large ones (*Bayod, 2008*). Samples D and E (Figure 2 d, e), having particles with smaller size diameter, were constituted by a less dense sugar crystalline network, highlighted by a larger number of void spaces. This could be related both to the further addition of cocoa butter during the conching step that, (*Beckett, 2000; Afoakwa, 2009a*), wets the suspension filling the voids within the crystal network and opens the structure, and also to the addition of lecithin. Lecithin migrates to sugar/fat interfaces and coats sugar crystals, reducing particle-particle interactions and scattering crystals in the fat phase (; *Dhonsi and Stapley, 2006; Beckett, 2008*). In Figure 2 the flow curves of the dark chocolate samples, obtained increasing the shear rate from 2 to 50 s⁻¹ are reported.

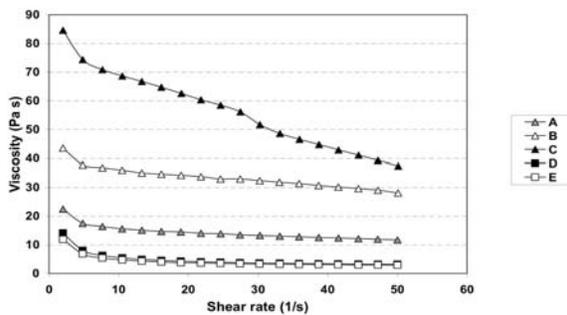


Fig. 2. Changes of viscosity (Pa s) of dark chocolate samples, during mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) steps

All samples exhibited a typical non Newtonian behaviour, characterized by dependence of viscosity upon of flow conditions (*Chhabra, 2006*). In particular, apparent viscosity of these products decreases with the increase of the shear rate, indicating pseudoplasticity. As illustrated by Figure 2, sample C presents the highest values of viscosity with initial values ranging between 80 and 90 Pa s, followed by samples B with initial viscosity values between 40 and 50 Pa s and sample A with values between 20 and 30. The lowest viscosity values belong to samples D and E obtained from the last two steps of the manufacture process. In order to better explain the rheological values obtained by the flow curves, in Table 1 are shown the values of yield stress and apparent viscosity.

Rheological considered values showed a significantly increase from sample A to sample C. This could be explained considered results obtained from the microstructural examination. The passage from the mixing steps through the pre-refining (B) and refining (C) ones involve a reduction in the particle size that causing an increase in the contact points between them, forms very aggregate structures. According with *Bayod, 2008*; in these conditions samples need of major amount of stress to break them

and initiate to flow. Samples D and E present the lowest values of viscosity parameters probably related with their less aggregate packing structure network. In these steps, in fact the addition of lecithin and further cocoa butter, because of their lubricating action, reduced the particle-particle interactions, increasing their mobility that involved a reduction of viscosity (*Vernier, 1998*). These results are supported by the studies of *Coussot and Ancey, (1999)* and *Larsoon, (1999)* that noticed a high dependence of yield stress and apparent viscosity on particles size and their interaction.

Table 1. Yield stress (Pa) and apparent viscosity (Pa s) of dark chocolate samples

Samples	Yield stress (Pa)	Apparent Viscosity (Pa s)
A	84.50 ^b	586.67 ^b
B	182.33 ^c	1406.70 ^c
C	358.67 ^d	1880.00 ^d
D	38.77 ^a	161.67 ^a
E	33.07 ^a	147.33 ^a

a-d values in the same column followed by different letters differ significantly ($p < 0.05$).

Stress sweep tests were performed in order to identify the linear viscoelastic region (LVR), in which properties of material are independent from the stress conditions. The results (data not shown) showed a narrow viscoelastic region, low intensity stresses can destroy the structure of system.

Results of frequency sweep test in terms of storage and loss modulus, are reported in Figures 3 a, b.

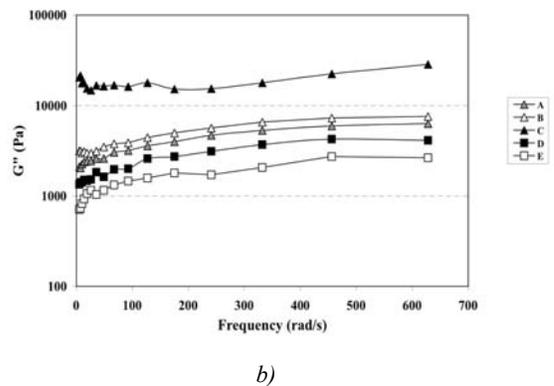
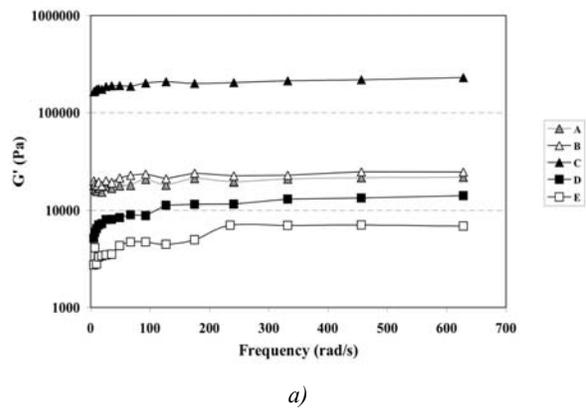


Fig. 3. Storage (a) and loss (b) modulus of dark chocolate samples.

Storage modulus values (G') are higher than loss modulus (G'') ones for all samples, indicating that all dark chocolate samples had a solid, elastic-like behaviour. This suggests that under

non destructive conditions the elasticity has a predominant effect on viscosity (Peressini et al., 2006). Samples B and C showed the highest G' values, due to their aggregate structure according with ESEM results. Sample A, showed viscoelastic properties with intermediate values of G' and G'' inside the clusters B-C and D-E. Some authors (Johansson and Bergensthal, 1992) observed that a high value of G' is related to a high level of interactive forces between particles; this confirms the high amount of stress required by samples B and C to start flow (Figure 1). The lowest parameters of G' and G'' were found for the samples D and E, constituted by a weakly structured system, due to the lubricating and emulsifier effect of fat and lecithin. These results are in agreement with the studies of Johansson and Bergensthal (1992), that highlighted how the effect of emulsifiers on the sugar particles, reducing the changing in the interaction particles and in the network structure ones, involves a decrease of the elastic component G' . In Table 2 are reported the values of T onset, T end and ΔH of dark chocolate samples obtained by heating all chocolate samples from 15 to 200 °C.

Table 2. Melting properties by DSC measurements of dark chocolate samples after mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) steps

Samples	FAT			SUGAR		
	T onset (°C)	T end (°C)	ΔH (J/g)	T onset (°C)	T end (°C)	ΔH (J/g)
A	24.323 ^a	34.011 ^a	38.960 ^a	174.89 ^a	189.98 ^a	30.015 ^a
B	30.614 ^c	36.657 ^c	45.378 ^b	176.36 ^a	190.79 ^a	46.841 ^b
C	30.538 ^c	37.222 ^c	51.464 ^c	180.97 ^b	192.30 ^b	48.954 ^c
D	26.011 ^b	35.059 ^b	37.696 ^a	175.72 ^a	189.68 ^a	29.229 ^a
E	25.667 ^b	35.344 ^b	37.769 ^a	174.73 ^a	190.13 ^a	28.685 ^a

a-d values in the same column followed by different letters differ significantly ($p < 0.05$)

It is known that Tonset corresponds to the temperature at which a specific crystal form starts to melt; T end represents the temperature of the complete melting and ΔH the amount of energy required to complete the liquefaction (Afoakwa et al., 2008b).

Statistically differences between all samples were highlighted regarding Tonset and Tend of fat (cocoa butter) melting. An increase in the Tonset, Tend and ΔH was noted from sample A to C. Samples B and C, constituted by a very aggregate structure, as shown previously, probably needed higher temperature to start and finish their fat melting than sample A. In this sample the absence of an aggregate structure and the presence of large not refined particles, provides less resistance to breakage and melting and are probably the cause of the lowest obtained values of fat T onset and T end. A significantly decrease of these parameters was instead noted in samples D and E, due to the emulsification effect of cocoa butter and lecithin. As far as the sugar melting is concerned, only sample C showed Tonset and Tend values significantly different from those of the others samples.

The ΔH values of both fat and sugar, were higher in samples B and C compared to the other dark chocolate samples, confirming an higher request of energy in order to complete the sugar and fat melting, due to the existence of very consistent structures (Afoakwa et al., 2009b).

CONCLUSION

ESEM analysis was very useful in order to discriminate the differences existing at microstructural level between dark chocolate samples, highlighting the increase of small particles number from the mixing to the refining step, that involves a reduction of the particles mobility due to their high aggregation. Microstructural results were strictly related to the rheological and thermal ones, in fact from the mixing to the refining step there was a drastic increase of all considered rheological (yield stress, apparent viscosity, G' , G'') and thermal parameters (Tonset, Tend, ΔH). Obtained results show that the knowledge of the changes occurring in the product matrix at each manufacturing stage could be very useful in order to optimize the manufacture process efficiency and to improve the quality of final product.

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The influence of different process steps on particle size, microstructure and appearance of dark chocolate.



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THE INFLUENCE OF DIFFERENT PROCESS STEPS ON PARTICLE SIZE,
MICROSTRUCTURE AND APPEARANCE OF DARK CHOCOLATE

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Abstract

The effect of different process steps on microstructural and visual properties of dark chocolate was studied. Samples were obtained at each phase of the manufacture process: mixing, pre-refining, refining, conching and tempering. A laser light diffraction technique and ESEM were used to study the particle size distribution (PSD) and to analyze modifications in the network structure. Moreover colorimetric analyses (L^* , h° and C^*) were performed on all samples. Each step influenced in stronger way the microstructural characteristic of products and above all the PSD. Sauter diameter ($D [3.2]$) decreased from 5.44 μm of mixed chocolate sample to 3.83 μm $D [3.2]$ of the refined one. ESEM analysis also revealed wide variations in the network structure of samples during the process, with an increase of the aggregation and contact point between particles from mixing to refining step. Samples obtained from the conching and tempering were characterized by small PS, and a less dense aggregate structure. From colour results, samples with the finest particles, having larger specific surface areas and the smallest diameter, appeared lighter and more saturated than those with coarse particles.

Keywords: Chocolate; Manufacture steps; Microstructure; Particle size distribution; ESEM; Appearance.

Practical Applications

Dispersions quality is affected by particles characteristics and from the process.

Influence of single steps on structural properties are useful, to improve the rheological ones.

ESEM and laser diffraction are suitable techniques to study changes in microstructure

Introduction

Dark chocolate can be defined as a concentrated suspension made up of solid particles (sugar and ground cocoa particles) dispersed in a Newtonian fluid, generally cocoa butter (Afoakwa and others 2008). Quality and stability of final products are affected by a number of factors, such as: particle size (PS), particle size distribution (PSD) and interaction between them and are strictly related to the raw materials but also to the manufacture process (Servais and others 2002; Granger and others 2005; Sato and others 2009; Baldino and others 2010). The physico-chemical and microstructural properties of chocolate depend on many factors besides the ingredients and their proportions. The different process steps (mixing, pre-refining, refining, conching and tempering) of chocolate manufacture and the different adopted process parameters can affect chocolate properties which, in turn, determine the behaviour and the characteristics of the final product. An effective control of the technological parameters is required in order to achieve a constant and desirable quality of the final product (Muller–Fischer and others 2005, Baixauli and others 2007). The processing of foods and in particular of dark chocolate, brings several changes in their microstructure. During chocolate manufacturing, mixtures of sugar, cocoa and fat are heated, cooled, pressurized and refined (Beckett 2000). These steps not only affect PS reduction, but also break agglomerates and distribute lipid and lecithin-coated particles through the continuous phase, modifying the microstructure of final chocolate (Afoakwa and others 2009). The interactions between the suspended particles and the continuous phase provide information about the existing network and consequently can be associated to the properties and characteristics of the dispersions (Sato and others 2009). Since macroscopic properties of foods, as the appearance attributes and colour are strongly determined by the microstructure of the food material, evaluation of the microstructure of food and its components is a necessary prerequisite for understanding its macroscopic properties (Aguilera and Stanley 1999).

Environmental scanning electron microscopy (ESEM) is one of the most suitable technique used to investigate microstructure of foods and to highlight the changing in the different structures obtained during the manufacturing process (Kalab and others 1996). ESEM in fact is a technique producing high resolution images of chocolate specimen due to the way in which the image is created; ESEM images have a characteristic three-dimensional appearance and let to investigate the structure of a sample (Dahlenborg and others 2010). Laser diffraction analytical method is also a very spread technique used for particle size analysis, based on the laser light scattered from particles. This method has the advantage of high speed, good reliability and high reproducibility (Ma and others 2000).

Afoakwa and co-workers (2008, 2009) used this technique obtaining good results in order to study some PSD aspects of dark chocolate.

Appearance of chocolate, a macroscopic properties, summarized as: gloss (strictly dependent from the chroma values), lightness, shininess and translucency, since must respond to consumer's acquired expectations, becomes a key attribute in customer choice and acceptability of final product (Beckett 2003; Whitefield 2005; Briones and others 2006). These colour parameters are psychophysical phenomena associated with the way in which light is reflected, absorbed and scattered by a surface. In general the colour characteristics can be natural in origin, or the result of manufacture process (Saluena and Gamasa, 2012). In the case of dark chocolate because of the complex matrix, appearance is strictly dependent on the process steps and mostly related with the PSD.

To our knowledge no papers are available about the influence of single process steps on dark chocolate microstructural and appearance properties. Most of the literature (Servais and others 2004; Afoakwa and others 2008, 2009; Baldino and others 2010; Efraim and others 2011) has focused on the study of these characteristics after the final tempering process step.

In our opinion, in order to improve the rheological properties of final product, it would be interesting to study in dept the evolution of these important quality characteristics during the

different process phases (mixing, pre- refining, refining, conching and tempering). For this purpose in the present work the microstructural properties of dark chocolate were evaluated during all manufacturing process of dark chocolate.

Materials and Methods

Materials

Dark chocolate samples were produced in an Italian confectionery factory using an industrial plant (Buhler, Malmo, Sweden) provided of mixer, pre-refiner, refiner, conching and tempering machine, equipped to produce 6000 Kg of chocolate at every production cycle. Dark chocolate production was made up by different steps as shown in detailed way in Fig. 1.

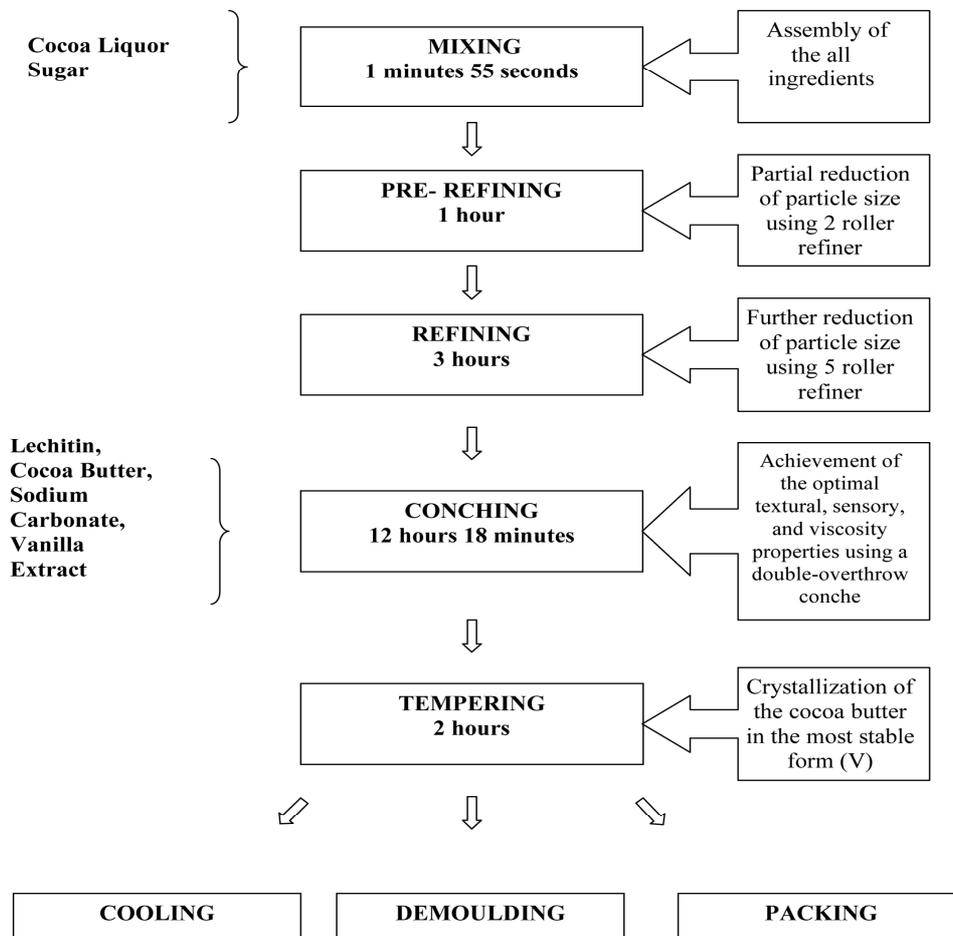


Fig. 1. Scheme of chocolate manufacturing process (adapted from Babin, H. 2005).

The formulation employed for the recipe was: cocoa liquor (53%), sugar (39.52%), cocoa butter (7% added during the conching step), soy lecithin (0.3%), sodium carbonate (0.15%) and vanilla extract (0.03%). Experimental samples were taken after each production phase: mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E). Samples were stored in plastic bucket (1 Kg capacity) at room temperature until the analytical determinations. Before performing each analysis the samples were melted in a microwave (Stortz and Marangoni, 2013), at 150 watt for 25 minutes. The melting parameters were chosen after preliminary experiments in order to minimize changes in the chocolate properties.

Methods

Determination of particle size distribution

Measurement of PSD was made by using a Mastersizer, Micro Laser Diffraction Particle Size 2000 (Malvern Instrument Ltd., Malvern, UK) equipped with a Hydro 2000 SM (B) for paraffin oil (RI 1.450). About 0.3 g of dark chocolate was dispersed in oil until an obscuration of 16.85%, following the user guide of the instrument. Speed of stirring was maintained at 1900 rpm for all the measurements.

Size distribution was determined as relative volume of particles in “*size bands*” and presented as size distribution curve and statistics (Malvern Mastersizer Micro Software v. 5.60). PSD parameters obtained were: specific surface area, the largest particle size (D_{90}), mean particle volume (D_{50}), the smallest particle size (D_{10}), Sauter mean diameter ($D[3,2]$) and Weight mean particle diameter ($D[4,3]$).

The last two parameters (Sauter mean diameter and weight mean particle diameter) are the most commonly used to characterize PS and represent respectively: the diameter of a sphere with the equivalent surface to volume ratio of all the particles in the size distribution (1)

$$d_{32} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad (1)$$

and the diameter of a sphere having the average weight of all the particles in the size distribution (2) (Black and others 1996).

$$d_{43} = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \quad (2)$$

Microstructure analysis

Samples microstructure was observed using an environmental scanning electron microscope ESEM (Evo 50 EP, Zeiss, Germany) equipped with a microprobe (EDS Mod. 350, Oxford Instrument, UK). The detector used was a backscatter electron detector (QBSE) that provided good compositional contrast imaging at 20 kV and in low vacuum mode with 100 Pascal at 500x magnification, taking 10 micrographs for each samples. These parameters were chosen after preliminary trials and according to Dahlenborg and others (2010), considering that they cause minimal damage of the chocolate surface and in order to optimize the imaging quality. With this kind of instrument ESEM, samples are not coated and images are more dependent on sample rather than coating characteristics, thus the true structure can be analyzed (Rousseau 2007). The acquired images were subsequently elaborated using the software Image Pro-plus 6.0 (Media Cybernetics Inc Bethesda, USA).

Colorimetric measurements

Colour of samples was measured using a colour spectrophotometer mod. Colorflex (Hunterlab, USA) equipped with a sample holder (diameter 64 mm). Colour was measured using the CIE L*a*b* scale and illuminant D65. The instrument was calibrated with a white tile (L* = 98.03, a* = -0.23, b* = 2.05) and the calibration was also validated with green standard tile (L* = 53.14, a* = -26.23, b* = 12.01) before the measurements.

Numerical values of a* and b* were converted into hue angle (h°) and Chroma (C*) that represent

the hue and the saturation index (McGuire, 1992): $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$, $h^\circ = [\arctang(b^*/a^*)/2\pi]^* 360$.

Each analysis was executed in triplicate.

Statistical analyses

Analyses of variance (ANOVA) and the test of mean comparison according to Fisher Least Significant Difference (LSD) were applied on all obtained data. Level of significance was $P \leq 0.05$.

The statistical software used was STATISTICA, version 8.0. (StatSoft, Tulsa, Oklahom).

Results and discussion

Particle size distribution

In Table 1 are reported the results of the particle size distribution.

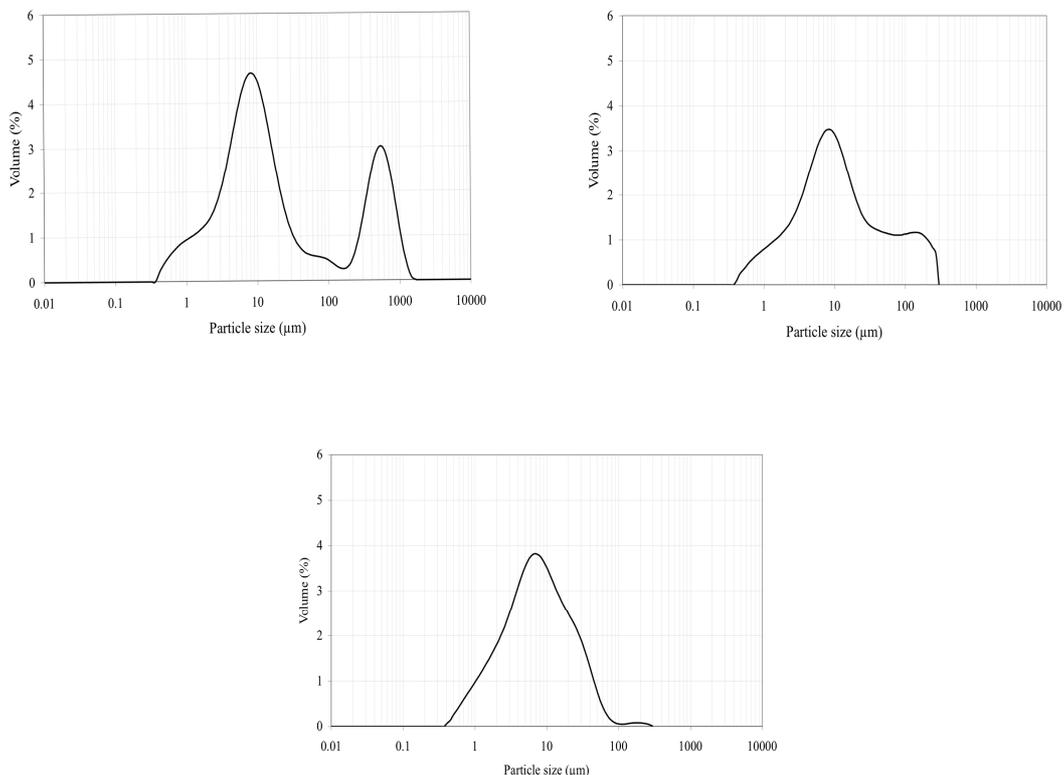
Table 1. Particle size distribution of the dark chocolate.

Samples	D(v,0.9)* (μm)	D(v,0.1)* (μm)	D(v,0.5)* (μm)	D[3,2]* (μm)	D[4,3]* (μm)	Specific surface area ($\text{m}^2 \text{g}^{-1}$)
A	621.19 ^a	2.18 ^a	11.64 ^a	5.44 ^a	155.36 ^a	1.10 ^e
B	129.96 ^b	1.93 ^b	10.69 ^b	4.98 ^b	41.85 ^b	1.20 ^d
C	31.28 ^c	1.68 ^c	7.58 ^c	4.13 ^c	14.65 ^c	1.45 ^c
D	25.63 ^c	1.63 ^d	7.12 ^d	3.95 ^d	10.85 ^c	1.52 ^b
E	27.10 ^c	1.56 ^e	6.91 ^e	3.83 ^e	11.09 ^c	1.57 ^a

* D(v, 0.9), D(v, 0.1), D(v,0.5), D[3,2] and D[4,3] respectively represent 90 %, 10%, 50%, Sauter mean diameter and Weight mean diameter of all particles finer than this size.

a-d values in the same column followed by different letters differ significantly at $p < 0.05$ level.

Wide differences in PSD were highlighted comparing results of different samples. The volume-based diameter ($D[4,3]$) is mainly determined by the large particles present in the suspension, while the area-based diameter ($D[3,2]$) takes into account also small particles. Small particles are important in determining the physico-chemical and textural properties of the material because they fill spaces between larger particles and contribute to the network structure of the suspension (Bayod 2008). A statistically significant decrease in D_{10} , D_{50} , $D[3,2]$ values was obtained from sample A to sample E with a simultaneous increase of the specific surface area. These results are in agreement with those of Beckett (2000) and Sokmen and Gunes (2006) that stated an inverse correlation between specific surface area and the others PSD parameters in chocolate. They also indicate that process steps from mixing to tempering involve a drastic reduction in particle size (from $5.44 \mu\text{m}$ to $3.83 \mu\text{m}$ of Sauter diameter) with a probable increase of the particles number and a concurrent increase of points of contact. No statistically differences were found from samples C to E for what concern $D[4,3]$ and D_{90} parameters. In Fig. 2 (a, b, c, d, e) the volume curves of particle size distributions of different samples are shown.



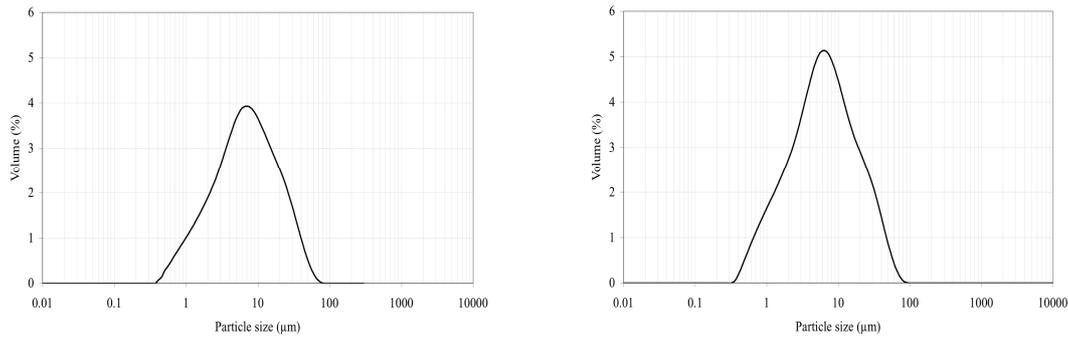


Fig. 2. Particle size distribution of dark chocolate after: (a) Mixing, (b) Pre-refining, (c) Refining, (d) Conching and (e) Tempering steps.

It can be observed a narrow bimodal distribution of samples A (Fig. 2 a), a wide bimodal distribution of sample B (Fig. 2b) and a narrow unimodal distribution of samples C, D and E (Fig. 2 c, d, e). These results mean the influence of different steps, reducing the particle size, involve a change in the particle distribution that moves from a bimodal trend to an unimodal one. Moreover from a graphical point of view different heights in the curves were highlighted. From sample A to B a drastic reduction in the height of the second peak (in a range from 100 to 1000 μm) was observed, this confirms the decrease in particle size values previously evaluated. Another increase in the curves peak height was noted from sample C to E (from 3.8 to 6 %) in the range between 1 and 100 μm . This trend, that involves an increase of the volume occupied by small particles, confirms the reduction of particle size observed from refined to tempered samples.

According to Beckett (2000) D_{90} and specific surface area are the two key parameters to assess PSD during chocolate manufacture. Large particles diameters determine chocolate coarseness influencing textural characteristics of final product, while specific surface area is related to the presence of fat that influence flow properties. For this reason, the control and the optimization of manufacture process becomes a nodal point in order to improve the final properties of dark chocolate.

Microstructural properties of dark chocolate

In Fig. 3 (a, b, c, d, e) the micrographs relating to the samples A, B, C, D, E obtained by ESEM are shown.

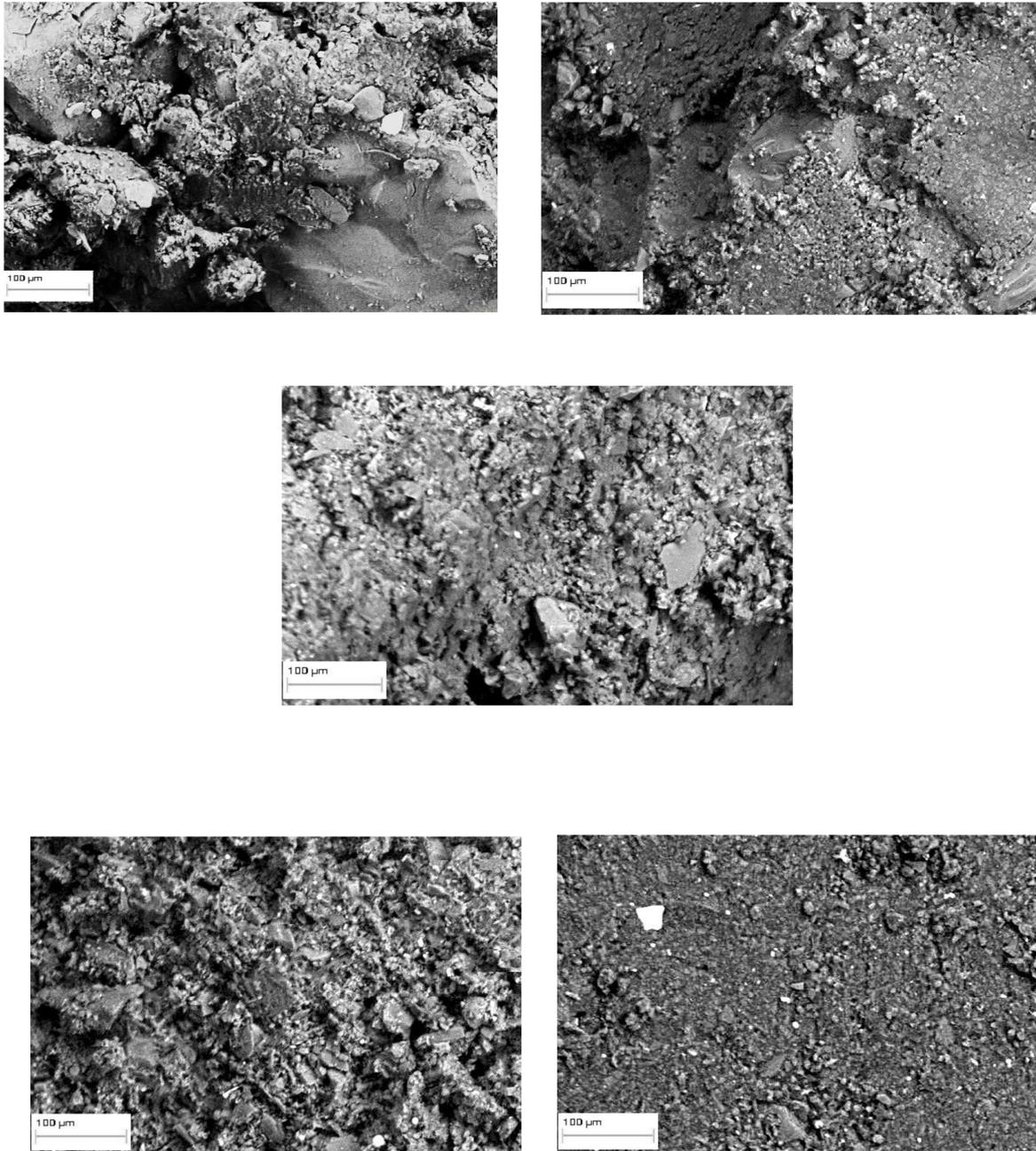


Fig.3. Micrographs of dark chocolate after different processing steps: (a) Mixing, (b) Pre-refining, (c) Refining, (d) Conching and (e) Tempering.

Microscopy was used to characterize the variations, during the different processing steps, in sugar crystalline network, particle-particle interaction and particle-fat phase behaviour of chocolate samples. The micrographs showed a strong heterogeneity in size and microstructure between samples. Microstructural examination, carried out with the image analysis (Table 2) showed a decrease of samples PS from the mixing step (A) to the tempering phase (E), in agreement with PSD analysis results.

Table2. Microstructural analysis of the dark chocolate

Samples	<i>Particle size</i> (<i>Feret diameter</i>)	<i>Number of</i> <i>particles/area</i>	<i>Area occupied by</i> <i>particles (%)</i>
A	466.85 ^a ± 1.99	263 ^c ± 1.52	54
B	105.26 ^b ± 1.90	367 ^b ± 2.00	65
C	29.34 ^c ± 1.93	460 ^a ± 3.05	85
D	22.72 ^c ± 2.70	521 ^a ± 2.00	40
E	19.32 ^c ± 2.72	532 ^a ± 2.65	42

a-c values in the same column followed by different letters differ significantly at $p < 0.05$ level.

The absolute values of PS evaluated with the image analysis were lower compared to the ones obtained with the laser light diffraction technique. According to the studies of Pieri and others 2006 the distribution of disc-shaped particles, (not spherical as in the case of a chocolate matrix), obtained with a laser light diffraction technique shifts the particles size towards larger fractions, respect to the ESEM results. The reduction of the particle diameter caused an increase of the particle number, parallel to an increase of contact points between particle-particle (Glicerina and others 2013), due to chemical and mechanical interactions (Servais and others 2004; Afoakwa and others 2009).

The percentage of the relative areas occupied by particles, increased from the mixing step (A) to the refining one (C) and then decreased in the samples D and E. In such samples, which are derived by the steps of conching and tempering, the presence of smallest particles is parallel to the presence of a lower packed structure in which particles are more separated.

The increase of particle interactions and specific surface area from sample A to C caused a reduction of the particles mobility due to their high aggregation. The result is a fully dense and packed suspension in which small particles fill spaces between large ones (Bayod 2008).

Samples D and E (Fig. 3 d, e), having particles with small size diameter, were constituted by a less dense sugar crystalline network, highlighted by a larger number of void spaces. This could be related both to the further addition of cocoa butter during the conching step that, in agreement with Beckett (2000) and Afoakwa (2009), wets the suspension filling the voids within the crystal network and opens the structure, and also to the addition of lecithin. In fact lecithin migrates to sugar/ fat interfaces and coats sugar crystals, reducing particle-particle interactions and scattering crystals in the fat phase (Beckett 2000; Dhonsi and Stapley 2006). The micrographs of sample E seems to be apparently different from that of sample D. This is due to the effect of tempering step, that improves the sample surface aspect making it more shining and smooth.

Colorimetric measurements

The lightness (L^*) hue angle (h°) and chroma (C^*) values of chocolate samples are shown in Fig. 4, 5 and 6 respectively.

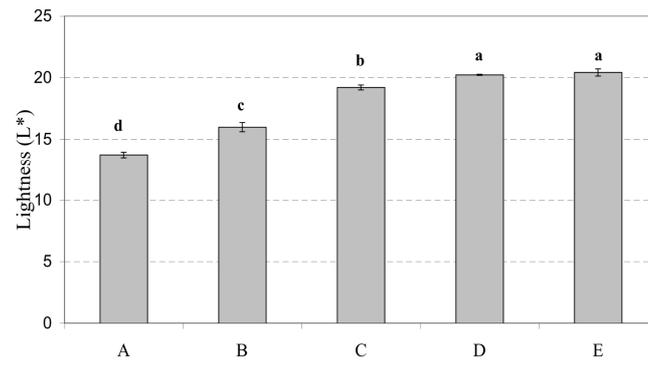


Fig. 4. Lightness (L*) colour parameter of dark chocolate samples.

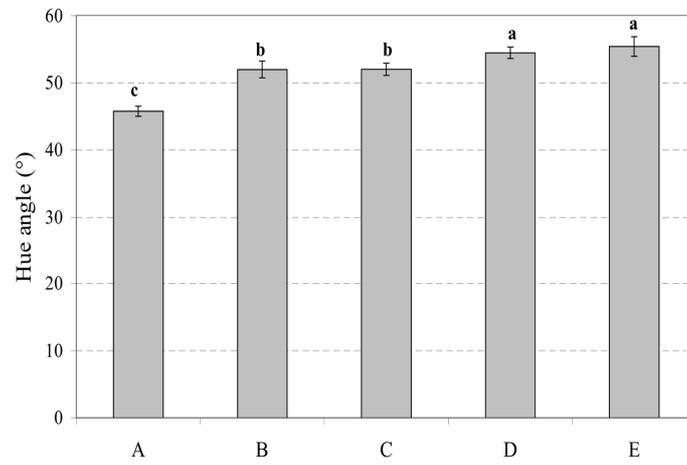


Fig. 5. Hue angle (h°) colour parameter of dark chocolate samples.

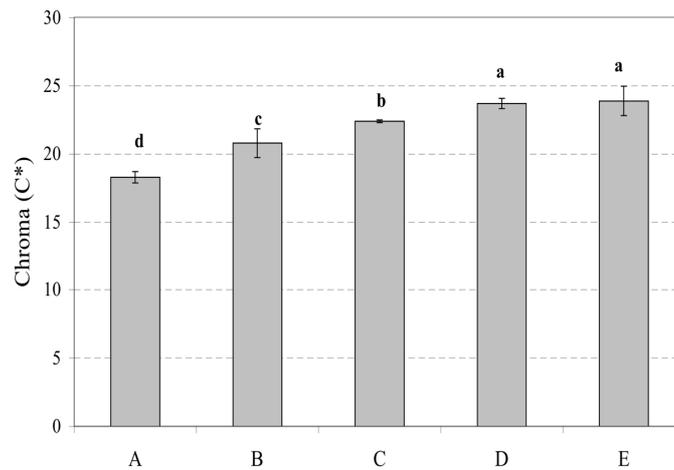


Fig. 6. Chroma (C*) colour parameter of dark chocolate samples.

Samples D and E, characterized by smaller particles of the other samples, presented the highest significantly values of lightness, hue angle and chroma. B and C chocolate samples, with the most aggregated structures, showed intermediate values for all considered colour parameters, while sample A, with the less dense and homogenous network, due to particles with the highest size, showed the lowest significantly values of colour parameters. The highest values of L*, h° and C* were recorded with the decrease of the particle size in the final samples (C, D, E). In particular, the addition of cocoa butter during conching step (D), determining an inherent crystalline network, scatters light further increasing all colour parameter values. Results obtained can be explained, according to the studies of Sagufy and Graf (1991), Hutchings (1994), and Afoakwa (2008), by the inverse relationship that exists between scattering light factors and particles size. Dark chocolate samples with the finest particles have a large specific surface area and small diameter that tend to scatter more light, appearing lighter and more saturated compared to samples with coarse particles.

Conclusions

Both ESEM and the laser diffraction methods were very useful and accurate in order to discriminate the differences existing at microstructural level between dark chocolate samples during processing steps.

ESEM provided detailed information about the different network between sugar particles in chocolate samples, highlighting the presence of more or less aggregate structures due to the particles-particles interaction during chocolate process. Laser diffraction technique, on the other hand, was very appropriate for measuring PSD evolution and providing results in form of number rather than images to let a more rapid comparison of samples.

Overall obtained results evidenced the increase of small particles number and specific surface area, in the chocolate samples from the mixing to the refining step, that involved a reduction of the particles mobility (Glicerina and others 2013), due to their high aggregation. The result was a microstructure evolution until a fully dense and packed suspension of refined sample, in which the smallest particles fill spaces between the largest ones. Moreover, during process, a reduction in the network density was noted after the addition of fat and lecithin, even if in presence of small particles. From obtained data it is possible to state as an extended and rigorous microstructural evaluation of this kind of samples is feasible using both Micro Laser Diffraction and ESEM analytical techniques.

Control and optimization of the technological parameters of the manufacture process (in terms of microstructure and PSD) show to be a critical point in order to obtain desirable attributes and characteristics in the final product.

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Effect of manufacturing process on the microstructural
and rheological properties of milk chocolate

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IV

**EFFET OF MANUFACTURING PROCESS ON THE MICROSTRUCTURAL AND
RHEOLOGICAL PROPERTIES OF MILK CHOCOLATE**

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Abstract

The effect of different process steps on microstructural, rheological and visual properties of milk chocolate was studied. Each process step affects the microstructural characteristics of milk chocolate, involving modifications on its macroscopic properties, such as rheological attributes. Milk chocolate samples were obtained at each phase of the manufacture process: mixing, pre-refining, refining, conching and tempering. Microstructural properties (network structure and particle size) and rheological parameters (yield stress, viscosity, thixotropy, G' and G'') were evaluated by using respectively an environmental scanning electron microscope (ESEM), and a controlled strain–stress rheometer. Colorimetric analyses (L^* , h° and C^*) were also performed. ESEM analysis revealed important changes in the network structure during process, with a reduction in particle size and an increase in the voids between aggregates, from the mixing to the refining step. Moreover, an increase of all rheological analyzed parameters from mixed sample to the refined one was found. Samples obtained from the conching and tempering steps were characterized by the lowest statistically significantly values of all rheological parameters. This could be related to the changes in the structure aggregation evidenced by ESEM analysis. From colour results, the samples with the finest particles appeared lighter and more saturated than those with coarse particles.

Keywords: Milk Chocolate; Manufacture steps; Microstructure; Rheology; Appearance.

1. Introduction

Milk chocolate is a complex rheological system having solid particles (cocoa, milk powder and sugar) dispersed in cocoa butter, which represent the fat phase (Pajin et al., 2013). Milk powder is one of the main ingredient of milk chocolate (being used at about 20% w/w in the formulation); this ingredient affects the sensory characteristics of the final product, the processing behaviour and the rheological properties of the fluid chocolate mass (Franke & Heilzmann, 2008; Taylor et al., 2009). The determination of the rheological properties of chocolate is important during manufacturing processes in order to obtain high quality products with well-defined characteristics (Gonçalves & Lannes, 2010). The rheological characteristics of milk chocolate (pseudoplastic flow, yield stress, viscosity, thixotropy and viscoelasticity) are in fact influenced by formulation (amount of fat, amount and type of emulsifiers) as well as by processing steps (mixing, pre-refining, refining, conching and tempering) (Tscheuschner & Wunsche, 1979; Vavreck, 2004; Schantz & Rohm, 2005). The processing of milk chocolate involves, during each single step (mixing, pre-refining, refining, conching and tempering), modifications in its final quality and attributes, influencing in a strong way the microstructure of the product (aggregation, de-aggregation, reduction of particle size, immobilization of cocoa butter, etc.) (Afoakwa et al., 2009a; Aguilera et al., 2000). In particular, milk powder with its own physical characteristics and inner porosity may have a significant impact on the chocolate processing conditions and on the physical and organoleptic properties of the final product (Liang & Hartel, 2004).

To our knowledge no papers are available in literature regarding the influence of the single process step on microstructural, rheological and appearance properties of milk chocolate.

In our opinion, in order to improve the final quality of milk chocolate it would be interesting to study in depth the evolution of these important quality characteristics during the different process phases (mixing, pre- refining, refining, conching and tempering). For this purpose in the present

work the influence of each process phase on microstructural, rheological and colorimetric properties of milk chocolate were evaluated during the overall manufacturing process.

2. Materials and methods

2.1. Materials

Milk chocolate samples were produced in an Italian confectionery factory by using an industrial plant (Buhler, Malmo, Sweden) provided of mixer, pre-refiner, refiner, conching and tempering machine, and equipped to produce 6000 Kg of chocolate at every production cycle. Milk chocolate production was made up by different steps as shown in Fig. 1.

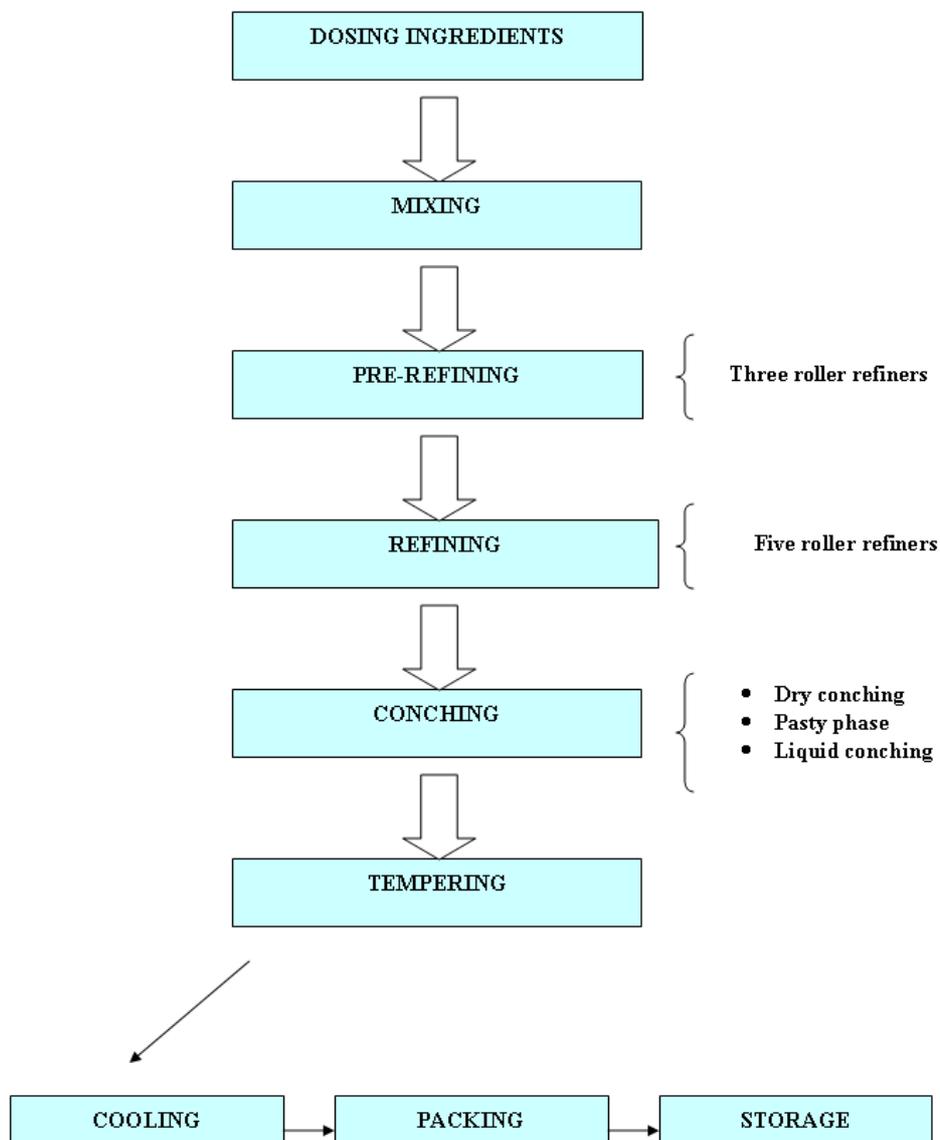


Fig. 1. Scheme of chocolate manufacturing process (adapted from Babin, H. 2005).

The ingredients used in the chocolate formulation were: sugar (47%), cocoa butter (25%), whole milk powder (21%) and cocoa liquor (18%). The experimental samples were taken after each production phase: mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E). Samples were stored in plastic bucket (1 Kg capacity) at room temperature until the analytical determinations. Before performing the analysis the samples were melted in a microwave (Stortz and Marangoni, 2013) at 150 watt for 25 minutes. The melting parameters were chosen after preliminary experiments in order to minimize changes in the chocolate properties.

2.2. Methods

2.2.1. Microstructure analysis

Samples microstructure was observed using an environmental scanning electron microscope ESEM (Evo 50 EP, Zeiss, Germany) equipped with a microprobe (EDS Mod. 350, Oxford Instrument, UK). The detector used was a backscatter electron detector (QBSE) that provided good compositional contrast imaging at 20 kV and in low vacuum mode with 100 Pascal at 500x magnification. These parameters were chosen after preliminary trials and according to Dahlenborg et al. (2010), in order to cause minimal damage on the chocolate surface and in order to optimize the images quality. By using this kind of instrument ESEM, samples are not coated and the images are more dependent on sample rather than coating characteristics, in this way the true structure can be analyzed (Rousseau, 2007). Ten micrographs for each chocolate sample were taken. The acquired images were subsequently elaborated using the software Image Pro-plus 6.0 (Media Cybernetics Inc Bethesda, USA).

2.2.2. Fundamental properties

Measurements were carried out at 40°C using a controlled strain-stress rheometer (MCR 300, Physica/ Anton Paar, Ostfildern, Germany) equipped respectively with a bob and cup geometry and with a plate-plate system to perform analysis in steady state conditions and the dynamic tests

respectively. In steady state conditions, after a pre-shearing of 500 s at 2 s^{-1} , viscosity was measured as function of increasing shear rate from 2 to 50 s^{-1} (ramp up) within 180 s, then decreasing from 50 to 2 (ramp down), within each ramp 18 measurements were taken (ICA, 2000).

Chocolate rheological flow curves are usually fitted (Afoakwa et.al., 2008, 2009b; Taylor et al., 2009) by using the Casson model, that is a well-known rheological model to describe the non-Newtonian flow behaviour of fluids with a yield stress (Joye, 2003). In particular, some fluid products, like chocolate, are well described by this model because of their non linear yield-stress-pseudoplastic nature. According to Chevalley (1991) curve points represent a case for a better fit to chocolate data, if the exponent is taken as 0.6 rather than 0.5.

For this reason, in this study the obtained flow curves were evaluated and fitted according to the rheological model of Casson, modified by Chevalley (1991), in order to obtain a better fit of the chocolate samples. The model used is represented in the following equation (1):

$$\tau^{0.6} = \tau_0^{0.6} + n_{PL} y^{0.6} \quad (1)$$

where τ_0 is the yield stress and η_{PL} is the so-called “plastic viscosity”. In order to measure the goodness of fit, the determination coefficient (R^2) was determined. The yield stress and the apparent viscosity were obtained according to Afoakwa, Paterson, & Fowler (2008) and ICA (2000), evaluating the shear stress respectively at 5 and 40 s^{-1} . In particular, the apparent viscosity evaluated at the shear stress of 40 s^{-1} according to Do, Hargreaves, Wolf, & Mitchell (2007), reflects the microstructure of the sample taking into account the presence of aggregates.

The samples thixotropy was evaluated according to Servais, Ranch, & Roberts, (2004), from the difference between viscosity measured at 40 s^{-1} during ramp up and ramp down. The thixotropy values represent in very close way the value of the hysteresis area between the viscosity curves during the ramp up and the ramp down. The loop area designates the energy required to break down the structure not recovered during the experimentation period (Roopa & Bhattacharya, 2009) and represents the rate of the internal breakdown of matrix (Dolz, González, Delegido, Hernández, & Pellicer, 2000).

In dynamic conditions, oscillatory tests by using a plate-plate geometry were performed in order to investigate the viscoelastic properties of samples and to evaluate the storage (G') and the loss (G'') modulus. In order to identify the linear viscoelastic range (LVR), in which the viscoelastic properties are independent from the stress conditions, strain sweep tests were applied. Frequency sweep tests were carried out in the viscoelastic linear region at the constant deformation amplitudes of 0.12%, previously evaluated with the strain sweep test, in the range from 1 to 100 Hz.

2.2.3. Colorimetric measurements

Colour of chocolate samples was measured using a colour spectrophotometer mod. Colorflex (Hunterlab, USA), equipped with a sample holder (diameter 64 mm). Colour was measured in the CIE $L^*a^*b^*$ scale using the D65 illuminant. The instrument was calibrated with a white tile ($L^* = 98.03$, $a^* = -0.23$, $b^* = 2.05$) and the calibration was also validated with a green standard tile ($L^* = 53.14$, $a^* = -26.23$, $b^* = 12.01$) before the measurements.

Numerical values of a^* and b^* were converted into hue angle (h°) and Chroma (C^*) that represent the hue and the saturation index: $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$, $h^\circ = [\arctang(b^*/a^*)/2\pi] * 360$ (Mc Guire, 1992).

2.3. Statistical analyses

All the analysis were carried out in triplicate for each chocolate sample.

Analyses of variance (ANOVA) and the test of mean comparison according to Fisher Least Significant Difference (LSD) were conducted on all obtained data. Level of significance was $P \leq 0.05$.

The statistical software used was STATISTICA, version 8.0. (StatSoft, Tulsa, Oklahom).

3. Results and discussion

3.1. Microstructural properties of milk chocolate

In Fig. 2 (a, b, c, d, e) micrographs of milk chocolate samples obtained by ESEM analysis are shown.

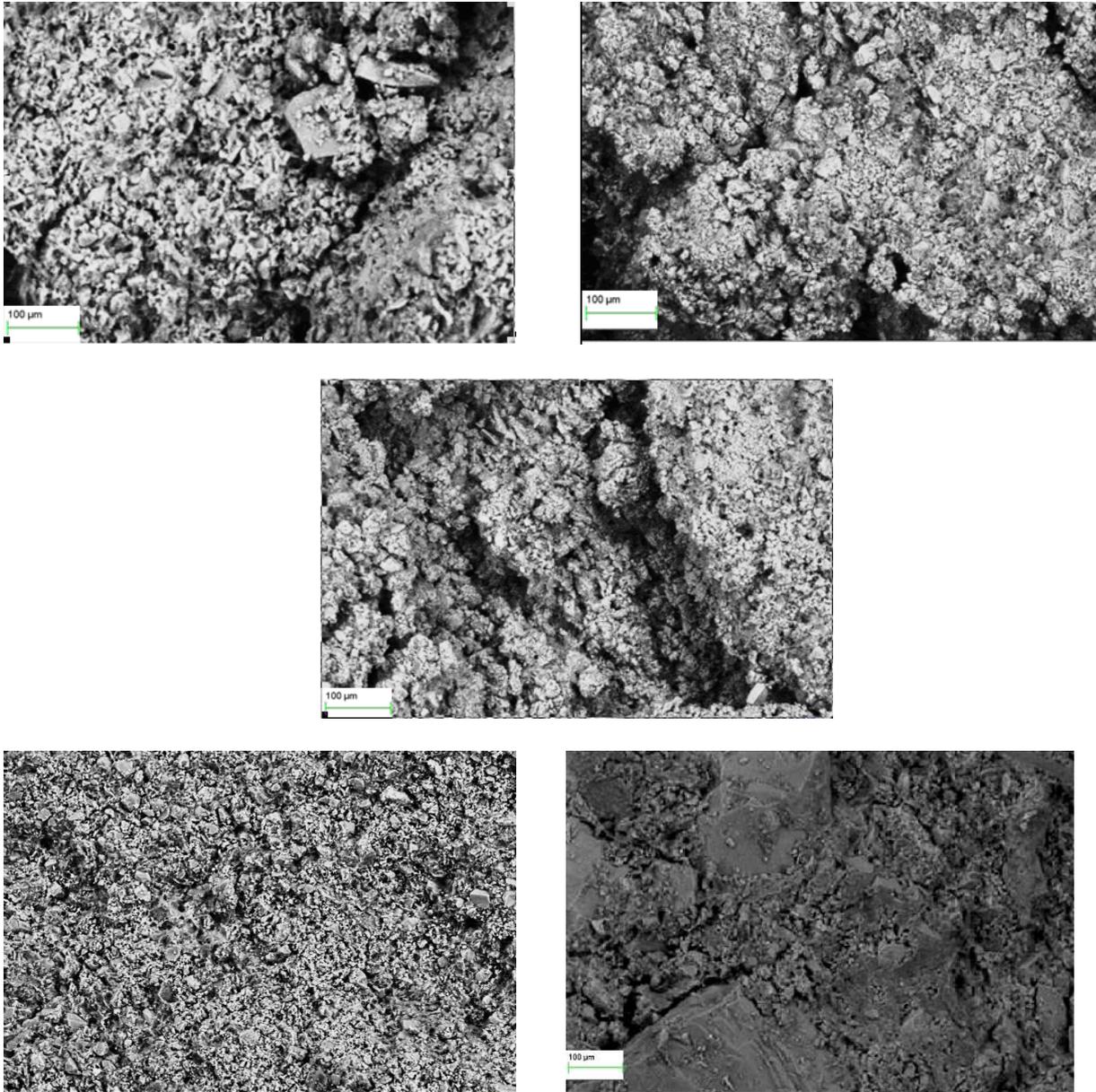


Fig.2. Micrographs of milk chocolate after different processing steps: (a) Mixing, (b) Pre-refining, (c) Refining, (d) Conching and (e) Tempering.

ESEM was employed in order to evaluate all the microstructural modifications occurred on chocolate samples during the different process steps, concerning sugar crystalline networks,

particle-particle interactions, presence of voids and particle-fat behaviour (Afoakwa, Paterson, Fowler, & Vieira, 2009). Microstructure examination, carried out with the image analysis (Table 1), highlighted different structures and particle diameters between samples obtained from the manufacturing steps.

Table1. Microstructural analysis of the milk chocolate

Samples	Particle size (Ferret diameter)
A	103±2.57
B	67±3.54
C	29±2.37
D	22±2.56
E	17.91±3.75

ESEM micrographs showed a decrease in the particle size from sample A to C (Table 1), parallel to an increase in the presence of large voids between aggregates (Fig. 2 a, b, c). The reduction of the particles diameter causes an increase in the particles number, parallel to an increase in the contact points between them, due to chemical and mechanical interactions (Afoakwa, Paterson, Fowler, & Vieira, 2009; Servais, Ranch, & Roberts, 2004). The increase of particle interactions from sample A to C, due to the raise of their specific surface area, involves a reduction of the particles mobility, due to their high aggregation (Bayod, 2008). On the other side, the presence of large voids between aggregates (filled with cocoa butter) involves an immobilization of a part of cocoa butter that can not contribute to the continuous fluid phase flow. According with the studies of Windhab (2000), the immobilized fluid fraction in the particle aggregates can be considered as an increase of solid volume, as explained in the following equation:

$$\phi_{eff} = \phi_s + \phi_{sif} + \phi_{vif} + \phi_{hifi} \quad (2)$$

Where ϕ_s = is the volume occupied by solid particles, ϕ_{sif} = is the volume of the fluid immobilized by surface, ϕ_{vij} = is the volume of fluid immobilized in particle cavities and into inner voids in particle aggregates and ϕ_{hif} = is the part of fluid immobilized when particles or aggregates move within the continuous phase such as in rotation.

For this reason in order to know the effective solid content in a dispersion, all the parameters presents in the equation (2) must be taken into account. In particular, the cocoa butter immobilized in large voids can have a significant impact on the rheological behaviour of the milk chocolate system (Windhab, 2000).

The micrographs of Fig. 2 (d, e), related to the samples after conching and tempering steps, show a further reduction in the particle size coupled to a reduction of the larger voids between aggregates, that leads to a reduction of the fluid immobilization. In the conching step a destruction of the previous obtained agglomerates and a re-distribution of cocoa butter between particles was noted, according to Attaie, Breitschuh, Braun, & Windhab, (2003). Cocoa butter in fact, due to its free-moving lubricating plastic flow, coats particles and reduces forces and aggregation between solid particles (Beckett, 2000), thus improving their mobility.

3.2. Fundamental rheological properties

In Fig. 3 the flow curves of the milk chocolate samples, obtained increasing the shear rate from 2 to 50 s^{-1} , are reported.

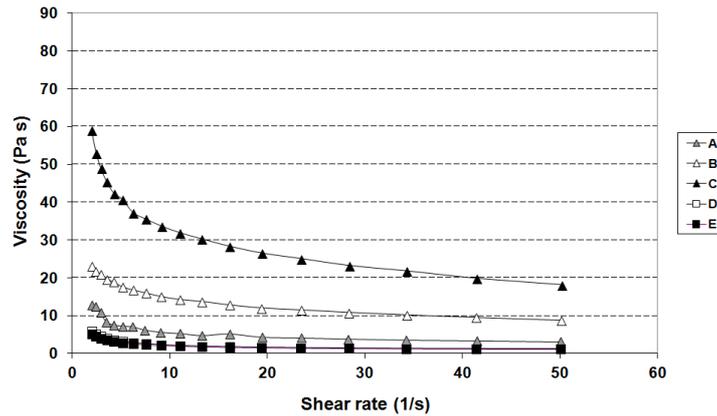


Fig. 3. Changes of apparent viscosity (Pa s) of milk chocolate samples, during mixing (A), prerefining (B), refining (C), conching (D) and tempering (E) steps.

The apparent viscosity (η) against shear rate ($\dot{\gamma}$) was used to represent the rheological behaviour of milk chocolate; it is evident that the apparent viscosity decreases increasing the shear rate, which proves the pseudoplastic or shear thinning nature of chocolate.

According to Juszczak, Witczak, Fortuna & Banys, (2004) this behaviour can be attributed to the breakdown of the inner structure dispersions, in fact the increase of shear rate causes the drop in viscosity of the molecules orientating along the flow lines.

As illustrated in Fig. 3, sample C had the highest viscosity with initial values ranging around 60 Pa s, followed by sample B with initial viscosity values between 20 and 30 Pa s and sample A with values between 10 and 20 Pa s. D and E samples, obtained from the last two steps of the manufacture process, had the lowest viscosity values, ranging from 0 to 10 Pa s.

In order to better explain the rheological values obtained by the flow curves, the *Casson yield value* and the *Casson plastic viscosity* parameters were calculated applying the Casson model modified by Chevalley (1991), moreover yield stress and apparent viscosity values were obtained according to Afoakwa, Paterson, & Fowler (2008) and ICA (2000). All these data are reported in Table 2 for each chocolate sample.

Table.2. Casson yield values, Casson Plastic Viscosity, Yield stress and Apparent Viscosity of milk Chocolate samples.

Samples	<i>Casson Yield value</i>	<i>Casson Plastic Viscosity</i>	Yield stress	Apparent Viscosity
	(Pa)	(Pa*s)	(Pa)	(Pa*s)
A	6.82 ^b	4.38 ^b	37.10 ^b	3.84 ^b
B	11.97 ^c	7.82 ^c	91.10 ^c	10.84 ^c
C	35.70 ^d	15.36 ^d	209.33 ^d	23.23 ^d
D	2.75 ^a	1.55 ^a	16.93 ^a	1.53 ^a
E	1.95 ^a	0.21 ^a	14.56 ^a	1.32 ^a

a-d values in the same column followed by different letters differ significantly at $p < 0.05$ level.

All data were well fitted by the *Casson* model, providing high determination coefficients (R^2) that varied from 0.75 to 0.99. A significantly increase in both *Casson* obtained parameters was highlighted from sample A to C. This could be attributed to the increase of the contact point between particles, that need of a major amount of stress to initiate the flow, and to the presence of large void spaces that immobilized cocoa butter between aggregates. In this state the fat can not contribute to the flow as lubricant (Franke & Heinzelmann, 2008). Samples D and E were characterized by the lowest and significantly similar values of both *Casson* parameters. In particular, the obtained values of plastic viscosity are in agreement with the results of Wichchukit, Mccarthy & Mccarthy (2004), that showed that *Casson viscosity* of milk chocolate with 20% of cocoa butter, ranged from 7 to 48 Pa s and led to decrease with the adding of lubricant. In the

samples studied in this research work the highest value of *Casson* viscosity was lower (25.7 Pa s), than the one obtained in the study of Wichchukit, Mccarthy & Mccarthy (2004), probably due to a higher amount of cocoa butter used in formulation (25%), that caused a greater lubricating effect and a reduction of particle–particle interactions (Vernier, 1998).

The yield stress and apparent viscosity parameters, exhibited the same trends of the *Casson yield value* and of the *Casson Plastic Viscosity* in milk chocolate samples. According to the studies of Do, Hargreaves, Wolf, & Mitchell (2007) in fact an increase in the apparent viscosity, as from sample A to C, also in this case indicates an higher degree of particles aggregation, while a decrease of this parameter, as for samples D and E, underlines a lower degree of interactions, as confirmed by microstructural analysis results.

Thixotropy results are shown in Fig. 4.

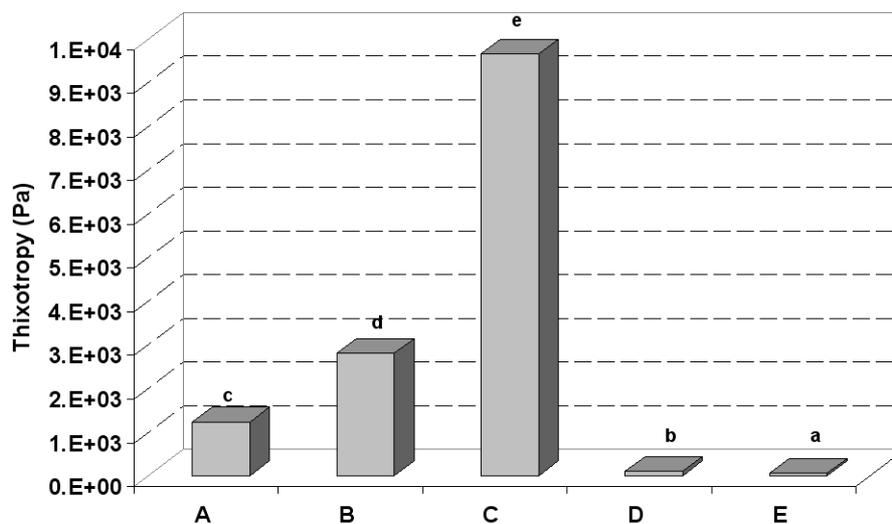


Fig. 4. Thixotropy of milk chocolate samples.

It is possible to notice how C and B samples, that had the most aggregate structure, presented also the significantly highest thixotropy values, related to a more damaged structure after removing the stress. This result according to Afoakwa, Paterson, & Fowler, (2008) could be attributed to the high aggregation of the particulate system and to an elevate number of interactions between particles. Sample A was characterized by an intermediate thixotropic value, between B-C and D-E ones,

strictly related with the results obtained from microstructural examination, that reflects the presence of coarse particles and a weak solid structure compared to B and C samples. The lowest significantly values of thixotropy were showed by chocolate samples D and E. According with literature (Afoakwa, Paterson, & Fowler, 2008) in fact, a well conched and tempered chocolate should not be thixotropic and hence should not have a very aggregate structure.

The results of frequency sweep test in terms of storage and loss modulus, evaluated respectively at a frequency of 1 Hz, are reported in Table 3.

Table 3. Storage and loss modulus of milk chocolate samples evaluated at 1 Hz.

Samples	G' (Pa)	G'' (Pa)
A	8416 ^b	1281 ^b
B	13673 ^c	2357 ^c
C	72746 ^d	16873 ^d
D	3983 ^a	807 ^a
E	2873 ^a	798 ^a

a-d values in the same column followed by different letters differ significantly ($p < 0.05$).

The response of all samples to the imposed deformation is the stored potential energy, characterized by the predominance of the elastic modulus (G') over the viscous one (G'') (Ahmed & Ramaswamy, 2006). B and C samples were characterized by a relative more elastic structure compared to that of the other samples (A, D and E). As reported in previous studies (Johansson & Bergensthåll, 1992; Glicerina et al., 2013) high values of G' are related to a high level of interactive forces between particles; this confirms the high amount of stress necessary to B and C samples to start flow.

The significantly lowest values of G' and G'' were found for the samples D and E, constituted by a weakly structure.

3.3. Colorimetric measurements

The lightness (L^*) and hue angle (h°) values of A – E milk chocolate samples are shown in Fig. 5.

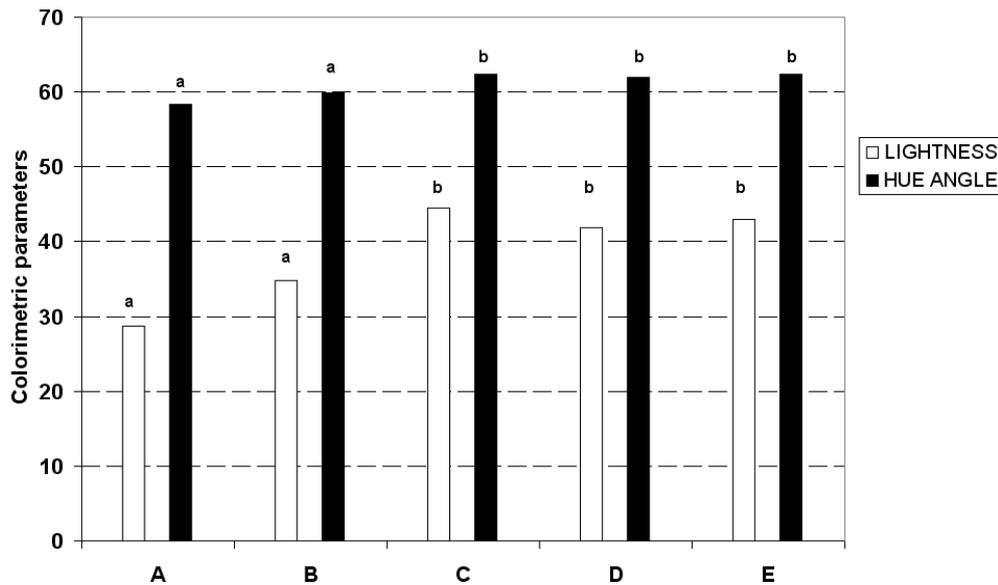


Fig. 5. Lightness (L^*) and hue angle (h°) colorimetric parameters of milk chocolate samples.

A similar trend of lightness and hue angle values was observed in all samples. A and B samples, characterised by coarser particles, had the lowest significantly values of both colour parameters. As known (Voltz and Beckett, 1997; Afoakwa et al., 2008), the human eye detects colour according to how the light is reflected from the surface, thus the size of the both non-fat solid and crystalline fat particles affects the colour of chocolate. In particular, in a dense packed medium, light scattering factors are inversely related with particle diameters (Sagufy & Graf, 1991; Afoakwa, Paterson, Fowler & Vieira, 2008), for this C, D and E samples, having finer particles and a large specific surface area, tended to scatter more light, appearing lighter than A and B samples, that had larger

particles. At the same time the highest hue angle values were found in C, D and E samples, that had a more yellowish-brown hue than A and B ones.

4. Conclusions

The modifications in the microstructure of milk chocolate during the different processing steps involve deep changes in the rheological and colorimetric parameters of product.

In particular, the decrease in particle size detected from sample A to C, simultaneously to an increase in the void spaces that immobilize cocoa butter, involves an increase in all rheological analyzed parameters. The re-distribution of cocoa butter during the conching step, led to a decrease in all rheological values in D and E samples, probably because of the reduction in particle-particle interactions due to the cocoa butter that, wrapping particles, reduces forces between them. At the same time, colorimetric characteristics were also affected by the different microstructure of samples.

From results obtained in this work it can be concluded that the knowledge of the influence of process parameters on the milk chocolate microstructure becomes very important in order to modify, improve and/or optimize the rheological and colorimetric properties of final product.

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Microstructural and rheological properties
of white chocolate during process

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**MICROSTRUCTURAL AND RHEOLOGICAL PROPERTIES OF WHITE CHOCOLATE
DURING PROCESSING**

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Abstract

The relationships between the microstructural and rheological properties of white chocolate were studied during the main steps (mixing, pre-refining, refining, conching and tempering) of the manufacture process. Laser light diffraction and a light microscope techniques coupled with image analysis were used to study the particle size distribution (PSD) and the modifications in the network structure of samples. At the same time rheological parameters in terms of yields stress, apparent viscosity and thixotropy were evaluated by using a stress-strain controlled rheometer. *Casson* and *Windhab* rheological models, widely used for dark and milk chocolate matrices, were used in order to better explain the rheological values obtained by the flow curves.. Particles analysis revealed significant changes in the network structure during process. In particular, a reduction in particle size and an increase in the matrix aggregation and of all rheological analyzed parameters, from mixed sample to the refined one were found. The white chocolate samples obtained from the conching and tempering steps were characterized by the lowest significantly values of all rheological parameters and had a weak structure. Differences between samples were well discriminated by both the rheological models applied.

1. Introduction

Chocolate can be defined as a concentrated suspension made up of solid particles (sugar and ground cocoa particles) dispersed in a Newtonian fluid, generally cocoa butter (Afoakwa et al. 2008). In particular, the white chocolate is the product free of coloring matters, obtained from cocoa butter, milk or milk products and sugars; it contains not less than 20 % cocoa butter and 14 % milk solids, obtained by partial or total dehydration of whole, partially or completely skimmed milk; cream, partly or wholly dehydrated; butter or milk fat, the latter must be present in an amount at least equal to 3.5 % (2000/36/EC).

Rheological characteristics are the most important attributes for chocolate matrix, affecting properties such as viscosity, consistency and mouth feel, that influenced in strong way the quality and stability of final products. The flow behaviour of chocolate is determined by its composition and above all by its manufacture process (Servais et al. 2002; Granger et al. 2005; Sato et al. 2009; Baldino et al. 2010). The manufacture process of chocolate is quite complex and is divided into several stages such as: mixing, pre - refining and refining, conching and tempering. Each single step, influencing in strong way the microstructure of the product (aggregation, de-aggregation, reduction of particles size, etc) involves modifications in its final rheological attributes and quality (Afoakwa et al. 2009; Aguilera 2005).

Despite several studies on dark and milk chocolate (Afoakwa et al. 2009; Baldino et al. 2010, Glicerina et al. 2013a; Glicerina et al. 2013b), to our knowledge no basic rheologic and microstructural studies are presents in literature on white chocolate. Several rheological models were elaborated and applied in order to predict and better study the rheological behaviour of dark and milk chocolate type dispersions (Hugelshofer 2003), but no information on their application on white chocolate matrix were highlighted.

In this study, the influence of different step of the manufacture process on the microstructural and rheological properties of white chocolate samples was evaluated. Two different rheological models, the *Casson* and the *Windhab* one, widely used for dark and milk chocolate formulations, were compared, in order to assess the most accurate in predicting the rheological behaviour of white chocolate.

2. Materials and methods

2.1. Materials

White chocolate samples were obtained by an Italian confectionery factory by using an industrial plant (Buhler, Malmo, Sweden) provided of mixer, pre-refiner, refiner, conching and tempering machines, and equipped to produce 6000 Kg of chocolate at every production cycle. White chocolate production was made up by different steps as shown in Fig. 1.

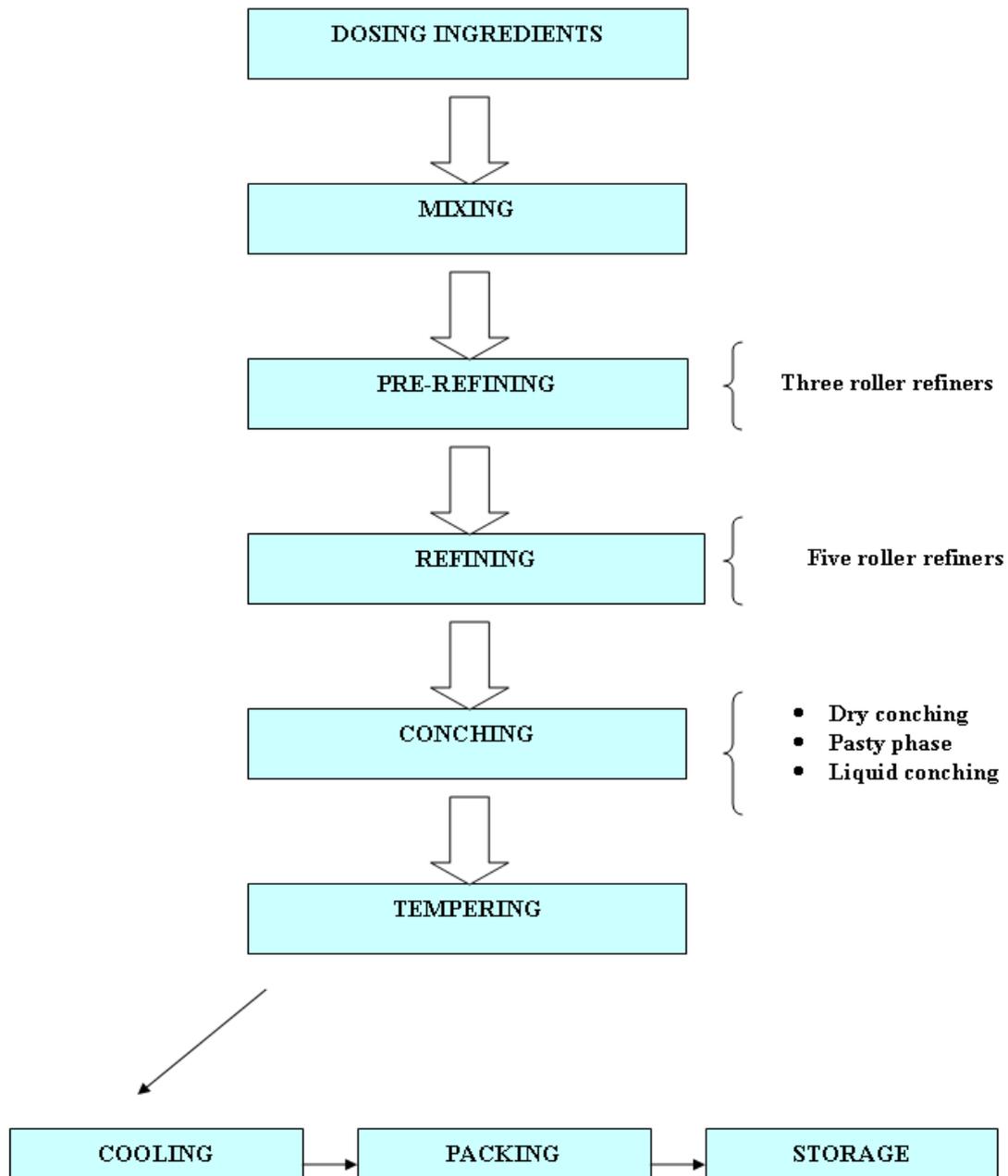


Figure 1. Scheme of chocolate manufacturing process (Adapted from Afoakwa et al. 2007).

The recipe of white chocolate was: sugar (47%), cocoa butter (31%) (in part added during the mixing step and the remaining during the conching step), whole milk powder (21.5%) and lecithin (0.5%). Experimental samples were taken after each production phase: mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E). Samples were stored in plastic bucket (1 Kg capacity) at room temperature until the analytical determinations. Before performing the analysis the samples were melted in a microwave (Stortz and Marangoni 2013) at 150 watt for 25 minutes and further subjected to an ultrasonic bath treatment at 50 °C for 20 minutes, in order to fluidify the product (Afoakwa et al. 2007). The melting parameters were chosen after preliminary experiments in order to minimize changes in the chocolate properties.

2.2. Methods

2.2.1. Determination of particle size distribution (PSD)

Measurement of PSD was made by using a Mastersizer, Micro Laser Diffraction Particle Size 3000 (Malvern Instrument Ltd., Malvern, UK) equipped with a Hydro 2000 SM for iso-propanol (RI 1.38). About 0.2 g of white chocolate was dispersed in oil until an obscuration of 17.5%, following the user guide of the instrument. Speed of stirring was maintained at 1530 rpm for all the measurements.

Size distribution was determined as relative volume of particles in “*size bands*” and presented as size distribution curve and statistics (Malvern Mastersizer Micro Software, 3000). PSD parameters obtained were: specific surface area, the largest particle size (D_{90}), mean particle volume (D_{50}), Sauter mean diameter ($D[3,2]$), that takes into account the particles with the smallest sizes, and Weight mean particle diameter ($D[4,3]$), mainly determined by the presence of large particles in suspension.

The last two parameters (Sauter mean diameter and weight mean particle diameter) are the most commonly used to characterize PS and represent respectively: the diameter of a sphere with equivalent surface to volume ratio of all the particles in the size distribution (1):

$$d_{32} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad (1)$$

and the diameter of a sphere having the average weight of all the particles in the size distribution (2) (Black et al. 1996):

$$d_{43} = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \quad (2)$$

2.2.2. Microstructure analysis

Samples microstructure was observed using a light microscope (Olympus Optical, Tokyo, Japan) at 10x of magnification, taking 10 micrographs for each samples. One drop of dispersion (previously diluted with hexane) was placed on a glass slide and covered with a cover slip carefully placed over the sample, parallel to the plane of the slide and centred to ensure sample thickness was uniform. Micrographs were captured using a digital camera (Model 2.1 Rev 1; Polaroid Corporation, NY, USA). The acquired images were subsequently elaborated using the software Image Pro-plus 6.0 (Media Cybernetics Inc Bethesda, USA).

2.2.3. Fundamental properties

Measurements were carried out at 40 °C using a controlled strain-stress rheometer (MCR 100, Physica/ Anton Paar, Ostfildern, Germany) equipped respectively with a bob and cup geometry. In steady state conditions, after a pre-shearing of 500 s at 2 s⁻¹, viscosity was measured as function of

increasing shear rate from 2 to 50 s⁻¹ (ramp up) within 180 s, then decreasing from 50 to 2 s⁻¹ (ramp down); within each ramp 18 measurements were taken (ICA 2000).

In this study the obtained flow curves were evaluated and fitted according to two rheological models: the *Casson* (ICA 1973) and the *Windhab* (IOCCC 2000) one.

The *Casson* model used is represented in the following equation (3) as:

$$\tau^{0.6} = \tau_0^{0.6} + \eta_{PL} y^{0.6} \quad (3)$$

where τ_0 is the yield stress and η_{PL} is the so-called “plastic viscosity”.

The model of *Casson* was originally introduced for suspension of pigments and describes the rheological behaviour of viscoplastic fluids (Bolenz and Tischer 2013). In the 1973 the ICA recommended the use of the *Casson* model for shear rates between 5 and 60 s⁻¹ (Ludger and Teixeira 2007). The *Casson* model is then the most known and used to study the rheological behaviour of cocoa dispersions; however, according to Weipert et al. (1993), sometimes this model does not reflect in accurate way the physical properties of chocolate as its rheological properties do not fit exactly to the *Casson* equation. For this reason further models were developed (e.g. *Windhab* one).

The *Windhab* model has been recommended (Ludger and Teixeira 2007) for shear rates in the range between 2 and 50 s⁻¹ at 40 °C (4):

$$\tau = \tau_0 + \eta_{\infty} * \gamma + (\tau_1 - \tau_0) (1 - e^{-\gamma./\gamma.*}) \quad (4)$$

This model assumes that when liquid chocolate is put under shear, there is a change in structure of the molten chocolate. This can be observed by noting a decrease in viscosity from an initial value (structure of no shear) to a steady state value. Here, the ordering forces (“building structure”) and disordering forces (“breaking down structure“) are in equilibrium, and the chocolate shows an

equilibrium viscosity. When the shear stress is increased further, an equilibrium viscosity is reached which no longer decreases any further, and a final viscosity η_{∞} is attained. In the region of this final viscosity η_{∞} there is a straight line with a constant slope in the flow curve. This straight line can be extrapolated back to the point of zero shear rate in order to find the intercept. This intercept would give the parameter τ_1 which is a hypothetical yield stress (Ludger and Teixeira 2007) (Fig. 2).

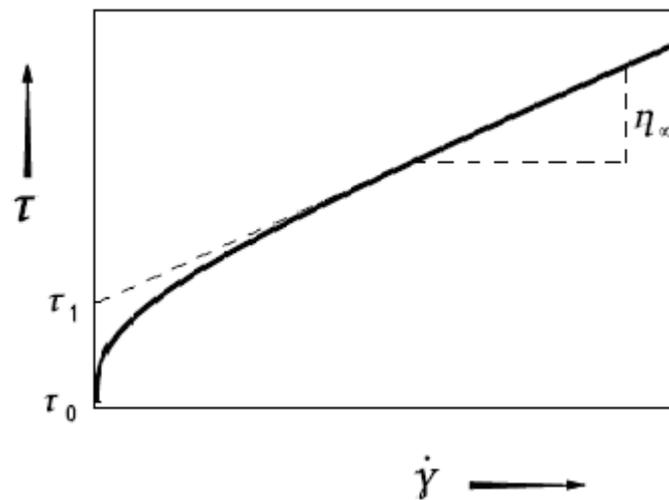


Figure 2. Schematic diagramme of the *Windhab* model (Ludger and Teixeira 2007).

In order to find the point on the curve where the final viscosity η_{∞} is reached, from the *Windhab* model can be obtained a second parameter $\tau^* = \tau(\dot{\gamma}^*)$ which indicates that the shear-induced loss of structure is at a maximum when $\dot{\gamma} = \dot{\gamma}^*$ is reached.

In order to measure the goodness of fit, the determination coefficient (R^2) was determined. The yield stress and the apparent viscosity were obtained according with Afoakwa et al. (2008) and ICA (2000), evaluating the shear stress respectively at 5 and 40 s^{-1} . In particular, the apparent viscosity evaluated at the shear stress of 40 s^{-1} , according with Do et al. (2007), reflects the microstructure of the sample, taking into account the presence of aggregates.

Thixotropy, instead, was evaluated, according with Servais et al. (2004), from the difference between viscosity measured at 40 s^{-1} during ramp up and ramp down. The thixotropy values

represent in very close way the value of the hysteresis area between the viscosity curves during the ramp up and the ramp down. The loop area designates the energy required to break down the structure that is not recovered during the experimentation period (Roopa and Bhattacharya 2009) and represents the rate of the internal breakdown of matrix (Dolz et al. 2000).

2.3. Statistical analyses

The results are reported as average of at least three determinations for each sample.

Analyses of variance (ANOVA) and the test of mean comparison, according to Fisher Least Significant Difference (LSD), were applied on all obtained data. Level of significance was $P \leq 0.05$.

The statistical software used was STATISTICA, version 8.0. (StatSoft, Tulsa, Oklahom).

3. Results and discussion

3.1 Particle size distribution

In Table 1 the results of the particle size distribution of white chocolate samples during process steps are reported.

Table 1. Particle size distribution and specific surface area of white chocolate samples.

Samples	D(v,0.9)* (μm)	D(v,0.5)* (μm)	D[3,2]* (μm)	D[4,3]* (μm)	Specific surface area ($\text{m}^2 \text{g}^{-1}$)
A	280 ^a	94.5 ^a	18.6 ^a	122 ^a	0.50 ^a
B	143 ^b	39.6 ^b	12.4 ^b	59 ^b	1.32 ^b
C	47.7 ^c	13.6 ^c	6.67 ^c	34.4 ^c	2.21 ^c
D	44.1 ^c	13.4 ^c	5.82 ^c	22.1 ^c	2.61 ^c
E	34.4 ^c	12.5 ^c	5.44 ^c	16.2 ^c	1.99 ^c

D(v,0.9), D(v,0.5), D[3,2] and D[4,3] respectively represent 90%, 50%, Sauter mean diameters and the weight mean diameter

Large differences in PSD were obtained between chocolate samples. A reduction in all PS diameter measures was recorded from sample A to E with a simultaneous increase in the specific surface area; substantial and significantly different data were obtained only from sample A to C.

These results are in agreement with those of Beckett (2010) and Sokmen and Gunes (2006) that stated the presence of an inverse correlation between the specific surface area and the other parameters related to the distribution of particles in cocoa matrix. Largest particles affect the textural properties of the final product while the specific surface area is related to the requirement of fat to obtain desirable flow properties.

Results obtained from the PSD analysis underlines as each single process step involves drastic changes in PSD of white chocolate matrix, also if the major changes in the microstructure of the samples were occurred from the mixing to the refining step, as also graphically shown in curves of Figures 3 a, b, c, d, e.

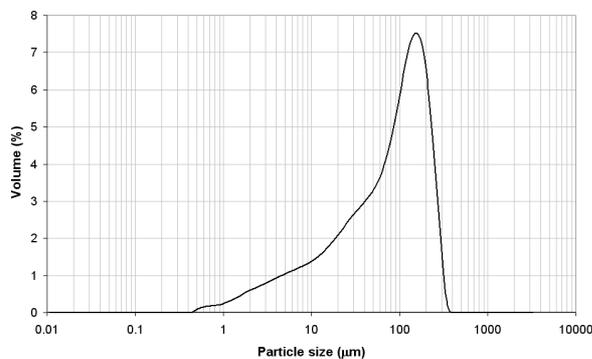


Figure 3 a. Particle size distribution of mixed white chocolate sample.

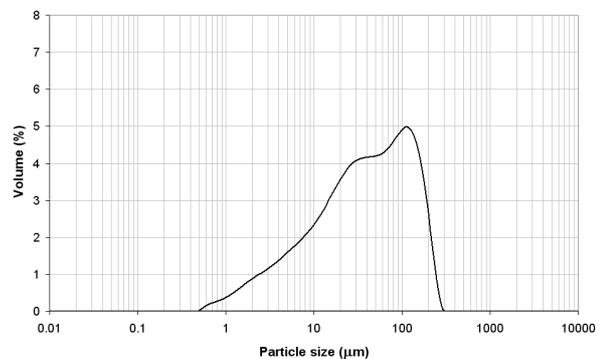


Figure 3 b. Particle size distribution of refined white chocolate sample

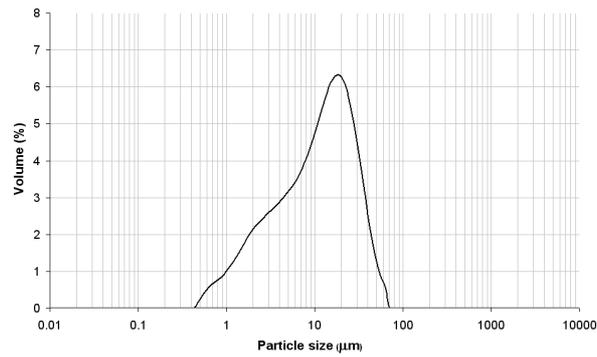


Figure 3 c. Particle size distribution of refined white chocolate sample.

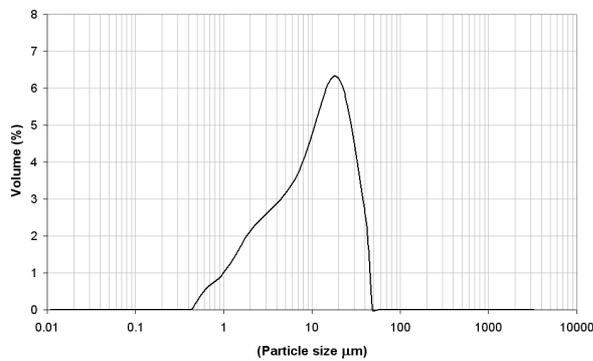


Figure 3 d. Particle size distribution of conched white chocolate sample.

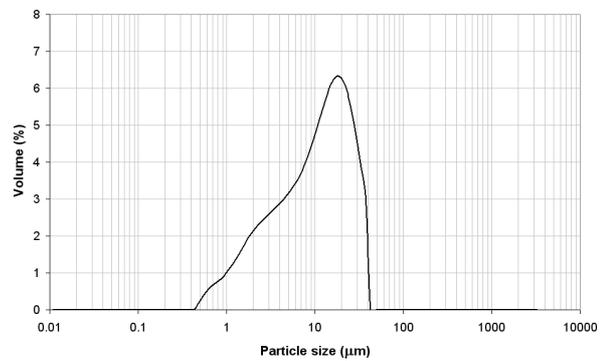


Figure 3 e. Particle size distribution of tempered white chocolate sample.

From figures, it can be observe an unimodal PS distribution in sample A (Fig. 3 a), shifted until the highest particles size values (around 130 μm), a wide bimodal PS distribution of sample B (Fig. 3b), with the presence of a second peak shifted toward PS lower, and a narrow unimodal distribution in C, D and E samples (Fig. 3 c, d, e).

3.2 Microstructural properties of white chocolate

Micrographs of white chocolate samples were obtained by using a light microscope. White chocolate in fact, thanks to its optical properties in terms of lightness, due to the absence of cocoa particles, can be easily observed by using this kind of microscope.

Figures 4 a, b, c, d, e show the images of A, B, C, D and E white chocolate samples acquired at a resolution of 10 x.

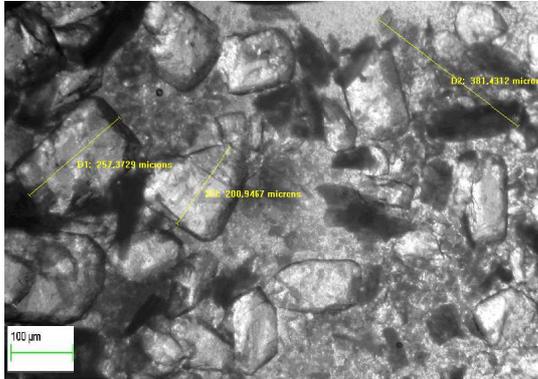


Figure 4 a. Light micrograph of mixed white chocolate sample (A)

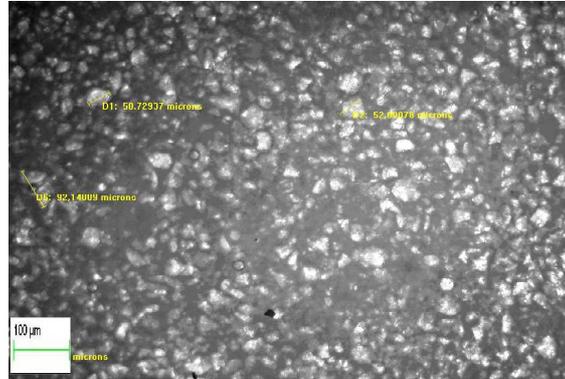


Figure 4 b. Light micrograph of pre-refined white chocolate sample (B).

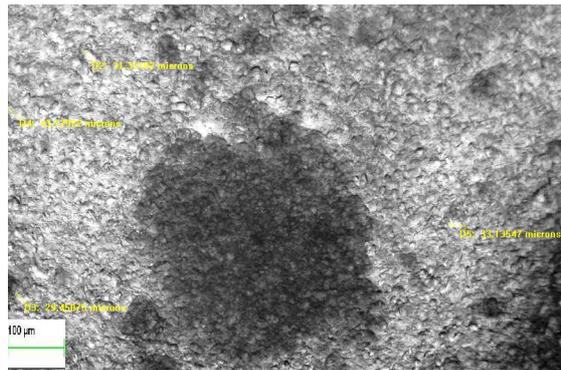


Figure 4 c. Light micrograph of refined white chocolate sample (C).

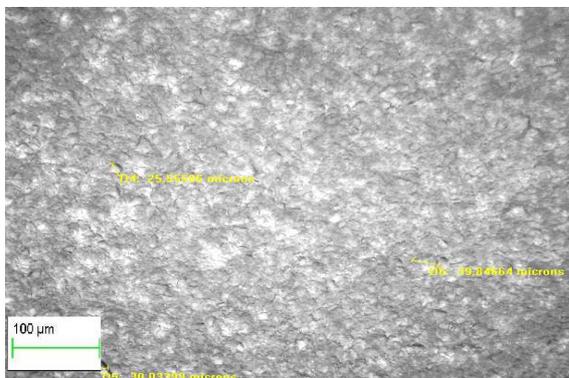


Figure 4 d. Light micrograph of conched white chocolate sample (D).

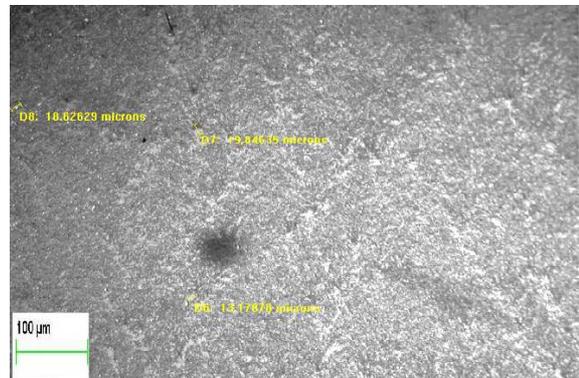


Figure 4 e. Light micrograph of tempered white chocolate sample (E).

In Table 2 the results of the microstructural analysis, obtained by evaluating the particles Feret's diameter, defined as the distance between two tangent lines to the two itself opposite sides (Allen 1997), are reported .

Table 2. Microstructural analysis of the white chocolate samples.

Samples	Feret diameter (μm)
A	320.00 ^a \pm 11.87
B	75.00 ^b \pm 15.87
C	33.00 ^c \pm 3.15
D	26.00 ^c \pm 2.34
E	15.65 ^c \pm 3.75

^{a-c} values in the column followed by different letters differ significantly at $p < 0.05$ level

Microstructural analysis confirmed PSD results obtained with the laser light diffraction technique. A reduction in particle size was observed from the mixing step (sample A) to the refining one (sample C). As shown in the related micrographs the the reduction of the particle diameter caused an increase of the particle number, parallel to an increase of contact points between particles (Glicerina et al. 2013b), due to chemical and mechanical interactions (Servais et al. 2004; Afoakwa et al. 2009). In white chocolate the interactions between the matrix components are particular evident in sample C, taken from the refining step. From the analysis of Figure 4 c, is in fact possible to detect the presence of a large black area in the middle of the image, due to the aggregation of particles in suspension, that involves a lower diffusion of the light.

Samples obtained from the conching (D) and tempering (E) phases revealed a further reduction in particle size (Table 2), parallel to a reduction in particle-particle interactions (Figures 4 d, e) . Consequently, the structure of these samples appears to be less packed and probably more fluid than in the previous samples (A, B ,C). This is probably due, both to the effect of the latter process stages and in particular to the further addition of cocoa butter and lecithin during the conching step.

Cocoa butter wraps the individual particles in the matrix, filling the empty spaces between them, and thus giving rise to a less aggregated and compact structure (Vernier 1998). Moreover, lecithin migrates to the sugar/fat interface and coats sugar crystals, reducing particle-particle interactions and the dispersed crystals in the fat phase (Dhonsi and Stapley 2006).

3.3. Fundamental rheological properties

In Fig. 5 the flow curves of the white chocolate samples, obtained increasing the shear rate from 2 to 50 s^{-1} , are reported.

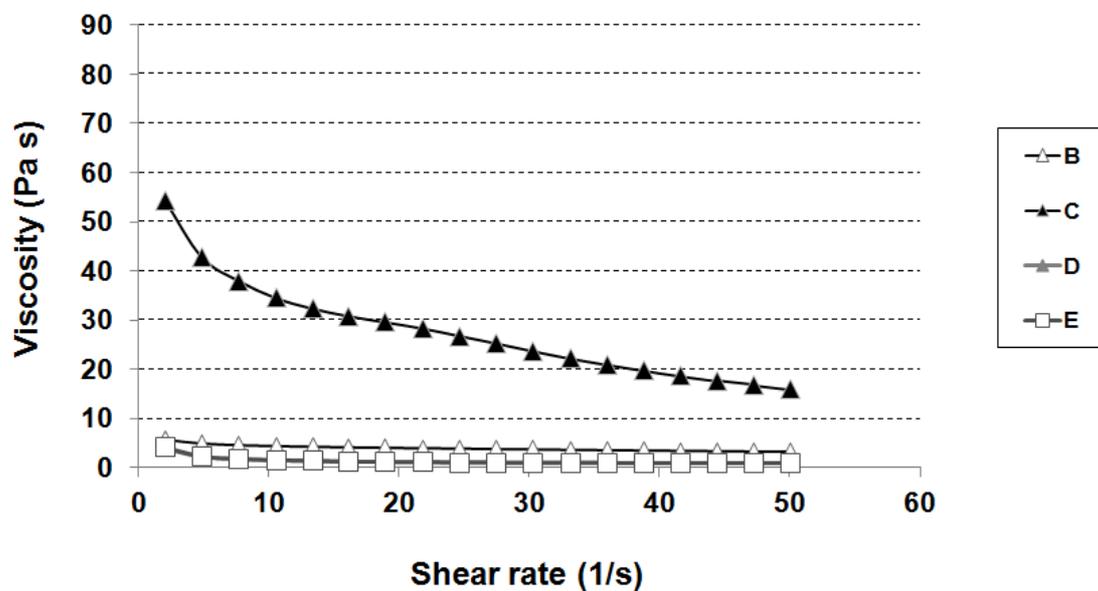


Figure 5. Flow curves of white chocolate samples

Due to the presence of sugar particles with large size and the to the presence of a completely unstructured matrix in the mixed sample A, it was not possible to perform rheological analysis on its, even after treatment in microwave.

All samples exhibited a typical non-Newtonian behaviour, characterized by the viscosity dependence on the flow conditions (Chabbra 2006). In particular, the apparent viscosity of these products decreased with the increase of the shear rate, indicating pseudoplasticity. This behaviour

can be explained by the structural breakdown of the molecules due to the generated hydrodynamic forces and to the increased alignment of the same molecules (Izidoro et al. 2008). As illustrated by Fig. 5, sample C presents the highest viscosity values with an initial data around 55 Pa s, it is followed by samples B with an initial viscosity value of about 6 Pa s. The lowest viscosity values, between 0 and 5 Pa s, were recorded for D and E samples, obtained from the last two process steps. In order to better explain the rheological values obtained by the flow curves, the *Casson yield value*, the *Casson plastic viscosity*, the *Windhab* linear yield stress and the *Windhab* infinity viscosity parameters were calculated applying respectively the Casson and the *Windhab* models. Moreover, yield stress and apparent viscosity values were obtained according with Afoakwa et al. (2008) and ICA (2000). All these data are reported in Table 3.

Both applied models showed high coefficient of determination (R^2) ranged between 0.75 and 0.99. In particular, the *Windhab* model determined R^2 between 0.80 and 0.99, slightly higher than the *Casson* one, thus describing in more accurate way the rheological behaviour of white chocolate matrix.

Table 3. Yield stress, Apparent Viscosity, Windhab Linear yield stress, Windhab Infinity viscosity, Casson Yield value, Casson Plastic viscosity, of white chocolate samples

Samples	Yield stress (Pa)	Apparent Viscosity (Pa s)	Windhab Linear yield stress (Pa)	Windhab Infinity viscosity (Pa s)	Casson Yield value (Pa)	Casson Plastic viscosity (Pa s)
B	23.42 ^b	3.63 ^b	24.15 ^b	2.67 ^b	2.45 ^b	2.56 ^b
C	206.76 ^a	23.80 ^a	254.32 ^a	10.12 ^a	47.21 ^a	9.79 ^a
D	10.31 ^c	0.93 ^c	7.75 ^c	0.67 ^c	5.11 ^b	0.53 ^c
E	11.15 ^c	0.98 ^c	8.25 ^c	0.70 ^c	6.00 ^b	0.59 ^c

^{a-c} values in the same column followed by different letters differ significantly at $p < 0.05$ level

Data analysis showed that the product taken from the refining step (C) presents the significantly highest ($p < 0.05$) values of yield stress and viscosity compared to all other samples, highlighting that the amount of stress required to obtain the flow was the highest. This result is related with those of microstructural analysis, from which this sample showed a highly aggregated matrix (Afoakwa et al. 2009). Sample B showed intermediate values of yield stress and viscosity between the other samples, while D and E samples, that had the less aggregated structures presented yield stress and viscosity values statistically lower than the other samples. The lubricating effect of cocoa butter and lecithin, added in the conching phase, affects the flow behaviour of matrix, reducing its viscosity. These results are supported by the studies of Dzuy and Boger (1983), Coussot and Ancy (1999) and Larsson (1999) that noticed a high dependence of yield stress and apparent viscosity on the particles size and their interactions.

Also for what concern *yield value (Casson)*, *linear yield stress (Windhab)*, *plastic viscosity (Casson)* and the *infinity viscosity (Windhab)* samples C, obtained from the refining step, showed the highest values. D and E samples had the lowest of these parameters and not significantly different between them.

Thixotropy of white chocolate samples are reported in Figure 6. Thixotropy representing the energy required to break down the structure that is not recovered during the experimentation period (Roopa and Bhattacharya 2009), underlines how in a very aggregate matrix, this force will be high and not recovered because of the completely de-structuration of matrix. Moreover thixotropy is strictly related with the microstructural characteristics of food matrix. As expected, samples B and C (Figure 6), having the most aggregated structures, had the statistically highest thixotropic values. These values are due to the increased damage of the structure, highlighted immediately after the stress removal, which can be attributed (Afoakwa et al. 2008) to a high level of aggregation of the structural matrix, which undergoes an irreversible break. Samples D and E, showed the lowest thixotropic values, confirming that a well-conched and tempered chocolate should not present any thixotropy and should be characterized by poorly aggregated structures (Afoakwa et al. 2008).

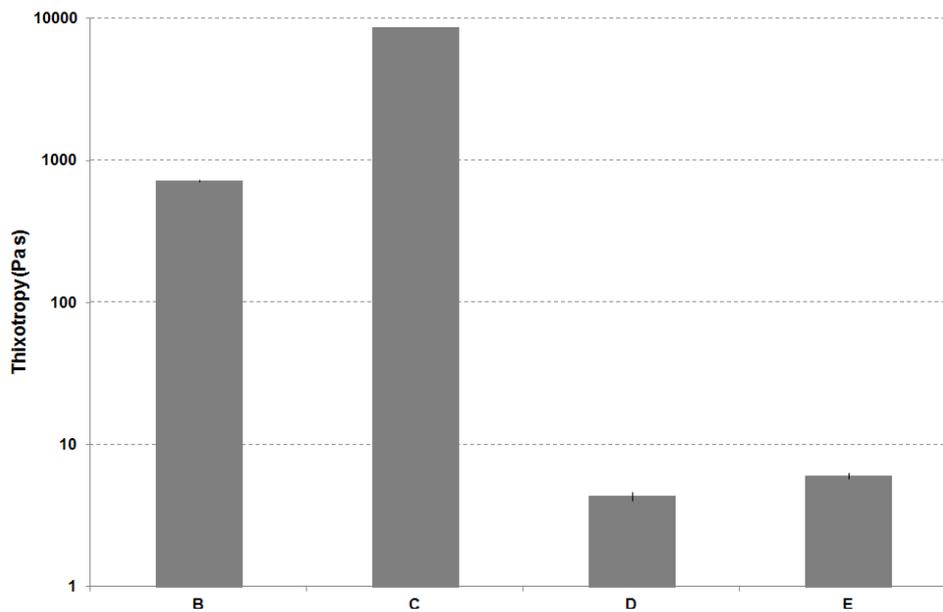


Figure 6. Thixotropy of white chocolate samples.

4. Conclusions

The results obtained in this research confirm that white chocolate exhibits a complex rheological behaviour, strictly related to its microstructural properties. Microstructural analysis is a fundamental parameter in order to study the modifications of the rheological properties of the white chocolate during through the manufacture process. All used analytical techniques (Laser light diffraction, light microscopy and rheology in steady state conditions) were very accurate in order to discriminate white chocolate samples on the basis of process phases which were subjected. Between the rheological models normally used to predict and well study the rheological behaviour of chocolate matrices, the *Windhab* one showed to be the more suitable in order to describe the flow behaviour of white chocolate products.

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Rheological characteristics of nut creams realized
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RHEOLOGICAL CHARACTERISTICS OF NUT CREAMS REALIZED WITH DIFFERENT TYPES AND AMOUNTS OF FATS

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ABSTRACT

The effects of different types (fractionated palm oil and hydrogenated fats – HFs) and amounts of fats on the rheological and textural properties of laboratory made nut creams were evaluated. Samples were produced in a roller ball mill pilot plant, and the color, rheological and textural characteristics were evaluated. Experimental creams were compared with two commercial ones. The highest amount of HFs led to an increase of consistency, viscosity and elasticity of creams involving a reduction in colorimetric parameters compared to ones with or without a low level of HFs. Finally, the potential interchangeability between fundamental and empirical measurements was demonstrated.

PRACTICAL APPLICATIONS

Quality and stability of nut creams are affected by a number of factors, such as types and different amounts of fats. The study of the influence of the impact of different types and amounts of fats is important for manufacturing improvements in order to optimize the different nut cream formulations and the final rheological quality of products. This study may provide a useful guide for producers in order to let them to discriminate and choose a right fat composition of nut creams on the basis of their overall rheological characteristics, assessed by empirical and/or fundamental measurements.

INTRODUCTION

Nut creams can be defined as a complex system made up of different solid particles (sugar, cocoa powder, milk whey, milk powder, nuts, etc.) dispersed in a continuous fluid (oil) (Afoakwa *et al.* 2008b). To study them, an extended and rigorous characterization using detailed methodological advancements is important in order to predict and evaluate their properties and relate them to quality characteristics and performances (cream stability, creaminess and processability) (Peressini *et al.* 2006).

The final quality and characteristics of these products arise not only from the manufacturing process through the steps of mixing, refining, conching and cooling but also from the formulation that becomes a key parameter in the study of the rheological behavior of similar products (Fernandes *et al.* 2013). The type, quality and quantity of

each ingredient used in a recipe, particularly fats, affect the creams' rheological and textural properties and also the flow parameters (Fang and Zhang 1997). Firmness, consistency, cohesiveness and viscosity, and yield stress are useful parameters in order to evaluate the degree of consistency and spreadability of creams. Fat ingredients are complex mixtures consisting of a large number of fatty acids assembled with glycerol into different molecular structures known as glycerides. The type and positional distribution of fatty acids control the physical properties of the fats. According to Larsson and Quinn (1994) and Ribeiro *et al.* (2009), a high amount of saturated fatty acids contained in a sample (i.e., the hydrogenated fats) strongly influences its consistency, increasing it. In fact, the studies of Ribeiro *et al.* (2009) showed a significant ($P < 0.05$) and linear relationship between the consistency and the SFC in different fat mixture samples.

Nut creams are examples of food products that contain significant amounts of fats, and thus, the sensory attributes of creams, such as bite firmness (brittle solid), melting properties (rough quickly melting), stickiness (not sticky very sticky) and smoothness (very grainy very fine grinding), are dependent on the mechanical strength of the fat crystal network. A fat crystal network is the product of an aggregation process of molecules into crystals and of crystals into larger clusters, until a space-filling three-dimensional network is formed (Awad and Marangoni 2006).

The study of nut cream rheology is important because it is related to the performances of the main steps in the process (mixing, pumping, transportation, etc.) (Servais *et al.* 2002) and also to the final quality and the sensorial characteristics of products such as cohesiveness, consistency and spreadability. During a rheological characterization of this kind of product, viscosity and flow properties are the most analyzed parameters. Moreover, because of the complex rheological behavior of these materials, other structural properties must be investigated by using dynamic tests. These measurements, in the linear viscoelastic region (LVR), are independent of the strain or stress applied but are only related to the structure of the product (Baldino *et al.* 2010).

References in literature to the rheological characterization of nut and cocoa creams (Bravin *et al.* 2004; Peressini *et al.* 2006) are scarce. Moreover, to our knowledge, no papers are available about the influence of various types of fats in formulation on the creams' rheological properties. Since, as previously reported, rheological properties are fundamental parameters for the characterization of nut creams and the used ingredients can affect them in a significant manner, an evaluation of the impact of different types and amounts of fats is important in order to optimize the different formulations.

The aim of this study was to investigate the influence of different formulations, obtained by varying the type and amount of fats, on the rheological and textural properties of laboratory made nut creams. The experimental nut creams were compared with two commercial ones produced by Italian confectionery factories.

MATERIALS AND METHODS

Materials

The type of fats used in the cream formulations were fractionated palm oil and hydrogenated fats (HFs), supplied by a local confectionery factory. Some physicochemical properties of these ingredients, reported in the product specifications, are shown in Table 1.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF FATS USED IN THE EXPERIMENTAL CREAM FORMULATIONS

Specific values of fats	Palm oil	HF
Peroxide value (meqO ₂ /kg oil)	2.0	1.0
Moisture (%)	0.10	0.10
Solid fat content* (%)	1.5	86
Free fatty acid (as oleic acid) (%)	0.15	0.10

* Evaluated by pulse-nuclear magnetic resonance at 25°C.
HF, hydrogenated fat.

A mixture design was used to obtain different experimental creams. The experimental variables used to differentiate the cream formulations were as follows: type of fat (fractionated palm oil and an HF) and their quantities. To differentiate the samples, a simplex centroid design (Ruguo 1999) was applied and five different cream samples were obtained (Table 2). The amount of fats was always 20% of the whole formulation. All the other ingredients, supplied by a confectionery factory, and amounts were held constant using the following proportions: sugar (45.55%), commercial nut paste (13%), milk whey (10%), cocoa powder (6.5%), milk powder (4%), nut flavor (0.15%), soy lecithin (0.4%) and stabilizer (0.4%). Two commercial nut creams produced by Italian confectionery factories were used as controls. These samples were indicated as (X1) the medium-standard quality and (X2) the market leader. From the product label, the ingredient composition for X1 cream is as follows: sugar, vegetal and hydrogenated fats (5%), nut paste (13%), cocoa powder, skimmed milk powder (4%), milk whey, lactose, soy lecithin, natural extracts; for X2 cream: sugar, vegetal fats, nuts (13%), cocoa powder, skimmed milk powder (5%), milk whey, soy lecithin, natural extracts.

Three replicates were obtained for each experimental cream; for the commercial nut creams, three specimens from different lots were used.

Formulation

Experimental creams were produced in a roller ball mill pilot plant (ME-MRC, ME.TRA, Verona, Italy), equipped to produce 4 kg of mixture, by mixing all the ingredients and

TABLE 2. MIXTURE DESIGN USED FOR THE FAT FORMULATION IN CREAM SAMPLES

Cream samples	Palm oil (%)	HF (%)
A	100	0
B	75	25
C	50	50
D	25	75
E	0	100

HF, hydrogenated fat.

50% of lecithin for 15 min at 40C. In the same machine, the ingredients were refined and conched for a further 120 min at 40C in order to reduce particle size and viscosity of the cream and to remove any bad flavor and unpleasant taste. After conching, the remaining lecithin was added and mixed for a further 15 min at 40C to improve the viscosity. In the final step, the product was cooled for 10 min at 37C.

The creams were stored in plastic buckets (5 kg capacity) at room temperature (22 ± 1 C) for 7 days before performing analyses.

Colorimetric Measurements

The color of samples was measured using a color spectrophotometer model Colorflex (Hunterlab, Reston, VA) equipped with a sample holder (diameter 64 mm). The color was measured using the CIE $L^*a^*b^*$ scale and illuminant D65. The instrument was calibrated with a white tile ($L^* = 98.03$, $a^* = -0.23$, $b^* = 2.05$), and the calibration was also validated with a green standard tile ($L^* = 53.14$, $a^* = -26.23$, $b^* = 12.01$) before taking the measurements.

Numerical values of a^* and b^* were converted into hue angle (h°) and chroma (C^*) that represent the hue and the saturation index: $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$, $h^\circ = [\arctan(b^*/a^*)/2 \pi]^* 360$ (McGuire 1992).

Static and Dynamic Rheological Measurements

Measurements were carried out at 25C using a controlled-stress rheometer (MCR 300, Physica/Anton Paar, Ostfildern, Germany) provided of a geometrical rough plate. The plates were 35 mm in diameter and the gap was 1 mm. The rheological behavior of nut creams was analyzed in static state and under dynamic conditions. In steady-state conditions, using flow tests, viscosity was measured, increasing the shear rate from 0.02 to 20 s⁻¹, taking 30 measurement points, in accordance with the previous studies of Peressini *et al.* (2006). Before starting measurements, each sample was stabilized in the rheometer for 5 min at 25C by using a TEK 150P circulating bath (Physica/Anton Paar).

Nut cream is not a simple Newtonian product; in fact, its viscosity is not independent of shear rate or shear stress. In this study, the obtained flow curves were evaluated and fitted according to the rheological model of Ostwald, commonly referred to as the Power Law model (Holdsworth 1993; Hugelsofer 2000). The Power Law model is represented in the following as

$$\sigma = K\dot{\gamma}^n \quad (1)$$

where σ is the shear stress (Pa), K is the consistency index (Pa·sⁿ), $\dot{\gamma}$ is the shear rate (1/s) and n is the dimensionless

flow behavior index. In order to measure the goodness of fit, the regression coefficient (R^2) was determined. Using the Power Law model, an important classification of products is possible: for values of n from 0.1 to 0.95, the products are known as pseudoplastic and are characterized by a decrease in apparent viscosity with increasing shear rate, i.e., shear thinning. For n values in the range of 0.95–1.05, the behavior is essentially Newtonian, ideally $n = 1$. For values of n greater than 1.05, the products are referred to as dilatants, i.e., shear thickening.

In dynamic conditions, oscillatory tests were performed at 25C in order to investigate the viscoelastic properties of samples and to evaluate the storage (G') and the loss (G'') modulus. In order to identify the LVR, in which the viscoelastic properties are independent of the stress conditions, stress sweep tests were applied, at a constant frequency of 1 Hz and with a stress in the range from 10 to 100 Pa. Frequency sweep tests were carried out in the viscoelastic linear region, for all samples, at a shear stress of 34.2 Pa, previously evaluated with the stress sweep test, in the range from 0.06 to 628 rad/s.

Texture Analysis

A TA.HDi 500 Texture Analyzer (Stable Micro System, Vienna Court, U.K.) was employed to investigate the textural properties of nut creams. All measurements were performed using a back extrusion test. The test was carried out in a back extrusion container (50 mm in diameter), 75% filled with the sample, using a disk (35 mm) attached to an extension bar, with a load cell of 50 kg. The parameters used were as follows: a pretest speed of 1 mm/s, a test speed of 1 mm/s, a post-test speed of 1 mm/s and a distance of 30 mm.

The “peak” or maximum force is taken as a measurement of firmness (N); the higher the value, the firmer is the sample. The area under the curve up to this point is taken as a measurement of consistency (N·s), so the higher the value, the thicker the sample consistency. The negative region of the graph, drawn on probe return, is the result of the weight of the sample that is lifted primarily on the upper surface of the disc on return, i.e., due to back extrusion, and hence gives an indication of the viscosity (resistance to flow off the disc). The maximum negative force is taken as an indication of the cohesiveness (N) of the sample, so the more negative the value, the more “cohesive” is the sample. The area of the negative region under the curve is an indication of viscosity index (N·s) of the sample and may be referred to as the “work of cohesion” (Angioloni and Collar 2009).

All the analytical determinations were made at least in triplicate for each subsamples.

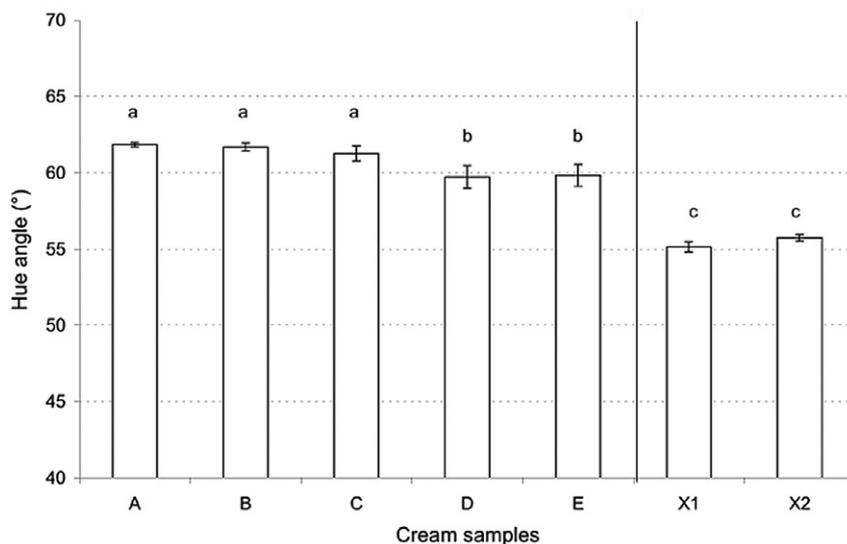


FIG. 1. HUE ANGLE (H°) COLOR PARAMETER OF NUT CREAM SAMPLES

Within each sample, means with different letters differ significantly from each other ($P < 0.05$).

Fundamental and empirical rheological tests were carried out only on A, B, C, X1 and X2 samples, being D and E creams extremely consistent and too viscous to conduct textural or oscillatory measurements; this was probably due to the high amount of HF in their formulations.

Statistical Analyses

Analyses of variance and the test of mean comparison using Fisher's least significant difference were applied. The level of significance was at $P \leq 0.05$.

Correlation analysis at 95% significance was carried out to evaluate the relationships between rheological and textural properties of cream samples. The statistical software used was STATISTICA (StatSoft, Tulsa, OK), version 8.0.

RESULTS AND DISCUSSION

Color

The hue angle and chroma results of cream samples are shown in Figs. 1 and 2, respectively. The A, B and C cream samples with the lowest quantity of HF showed the highest hue angle values (over 60°), statistically different from D and E samples. These last two samples did not show statistically different h° values from each other, probably because they had the highest HF quantities. Commercial samples (X1, X2) showed the lowest and not statistically significant h° different values. From these results, it seems that the samples with less HF amount (A, B, C) had a more intense hue than the other experimental samples and were quite

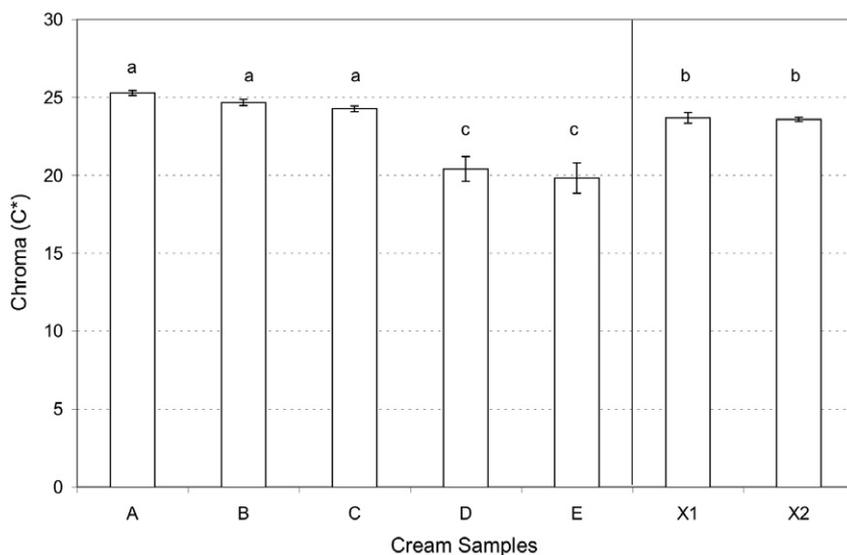


FIG. 2. CHROMA (C^*) COLOR PARAMETER OF NUT CREAM SAMPLES

Within each sample, means with different letters differ significantly from each other ($P < 0.05$).

different from the commercial ones. Also for chroma results (Fig. 2), A, B and C samples showed the highest and statistically different values. The lowest ones were found in D and E cream samples. The most saturated samples were A, B and C, whereas the less saturated were D and E. This means that A, B, C samples had a more intense and saturated color than others. Commercial samples had intermediate chroma values. Among the experimental cream samples, the lowest hue angle and saturation values were found in the samples richer in HF; this is probably due to the bigger crystallinity of this kind of fat, which scatters the light.

Fundamental Rheological Properties: Flow Behavior and Viscoelastic Properties

As known from the literature (Larsson and Quinn 1994; Marangoni and Narine 2002; Braipson-Danthine and Deroanne 2006), there is a direct relationship between solid fat content (SFC), representing the amount of fat crystals in the blends (Habi and Rahimmd 1998) and firmness of food products. The percentage of solid fat measured at 25°C represents the hardness of a specific fat and it is directly proportional to HF content (Piska *et al.* 2006).

In Table 3, the n and K parameters obtained applying the Power Law model on cream samples are reported. The used model fitted well with the flow test data as shown in Fig. 3a,b, giving high determination coefficients (R^2) that varied from 0.75 to 0.99. All samples were characterized by $0.2 < n < 0.43$, typical for a product with shear thinning behavior, well illustrated in Fig. 4, where the apparent viscosity of these products decreased, increasing the shear rate from 0.02 to 20/s, indicating pseudoplasticity. This behavior can be explained by the structural breakdown of the molecules due to the hydrodynamic forces generated and to the increased alignment of the constituent molecules (Izidoro *et al.* 2008).

It is known (Nawar 1985) that the consistency of fats depends on the number, size and type of crystal networks as well as on the proportion of solids in the fat. As reported in

Table 3, B and C samples showed higher values of K index compared with sample A due to a high amount of HF in the formulation. In particular, sample C, realized with the highest amount of HF (50%), showed the significantly highest value of K . The consistency is strictly related to the HF level and SFC, i.e., 86% in this particular kind of fat (Table 1). Sample A had the lowest index of consistency due to its SFC of 1.5% and probably to the absence of HF or hard solid vegetable fats.

The X1 and X2 cream samples had K index intermediate values among those of experimental creams. X1 had only a few amount of HF (5%), while X2 did not have HF, as reported in the label; consequently, their quite high K index values could be attributed to the presence of hard vegetable fats with an SFC composition similar to the HF ones. These results show that samples B and C, obtained with a high amount of HF, are likely characterized by a highly aggregate structure and this is in agreement with the highest viscosity values (Fig. 4) showed by the same samples (Foegeding *et al.* 2011). Moreover, as shown in Fig. 4, the viscosity of the A sample was very similar to that of X1; X2 had an intermediate initial viscosity value (206 Pa s). Sample A, made up of palm oil and a 1.5% SFC, had a lower consistency, a less aggregated and elastic structure than B and C, made with 25 and 50% of HF. X1 commercial sample showed a plastic structure similar to A, while X2 cream presented a structure more consistent and elastic close to that of B sample, probably due to the presence of hard vegetable fats or HF. Stress sweep tests were performed in order to identify the LVR, in which properties of material are independent of the stress conditions. Obtained results (Fig. 5) showed a narrow viscoelastic region; therefore, low intensity stresses could destroy the structure of the system.

Results from frequency sweep test in terms of storage and loss modulus are shown in Figs. 6 and 7, respectively. Storage modulus values (G') were higher than loss modulus (G'') ones for all samples, indicating that all cream samples had a solid, elastic-like behavior. This suggests that under nondestructive conditions, elastic modulus has a predominant effect on the dissipative one.

Samples B and C showed a stronger elastic structure than A, having higher G' values. This trend is probably related to the presence of a strong network of crystal fats. According to Larsson and Quinn (1994), with a low SFC, as in sample A, the fat remains like a viscous liquid and not in an elastic and elastoplastic form. When the SFC increases, the physical interaction among the crystals produces an elastic structure, immobilizing the liquid oil in the crystalline network and giving high values of storage modulus. Some authors noted that a high value of G' is related to a high level of interactive forces between particles (Johansson and Bergenstahl 1992). In this study, G' and K index values had the same trend, higher G' and higher K index.

TABLE 3. FLOW BEHAVIOR DIMENSIONLESS INDEX (n) AND CONSISTENCY INDEX (K) OF NUT CREAM SAMPLES FROM PARAMETERS OF THE POWER LAW MODEL FOR THE NUT CREAMS ANALYZED

Cream samples	K (Pa·s ^{n})	n
A	76.42 ^a	0.34 ^b
B	234.53 ^d	0.20 ^a
C	284.21 ^e	0.20 ^a
X1	124.66 ^b	0.32 ^b
X2	172.19 ^c	0.43 ^b

Mean values followed by the same letter in each column are not significantly different ($P < 0.05$).

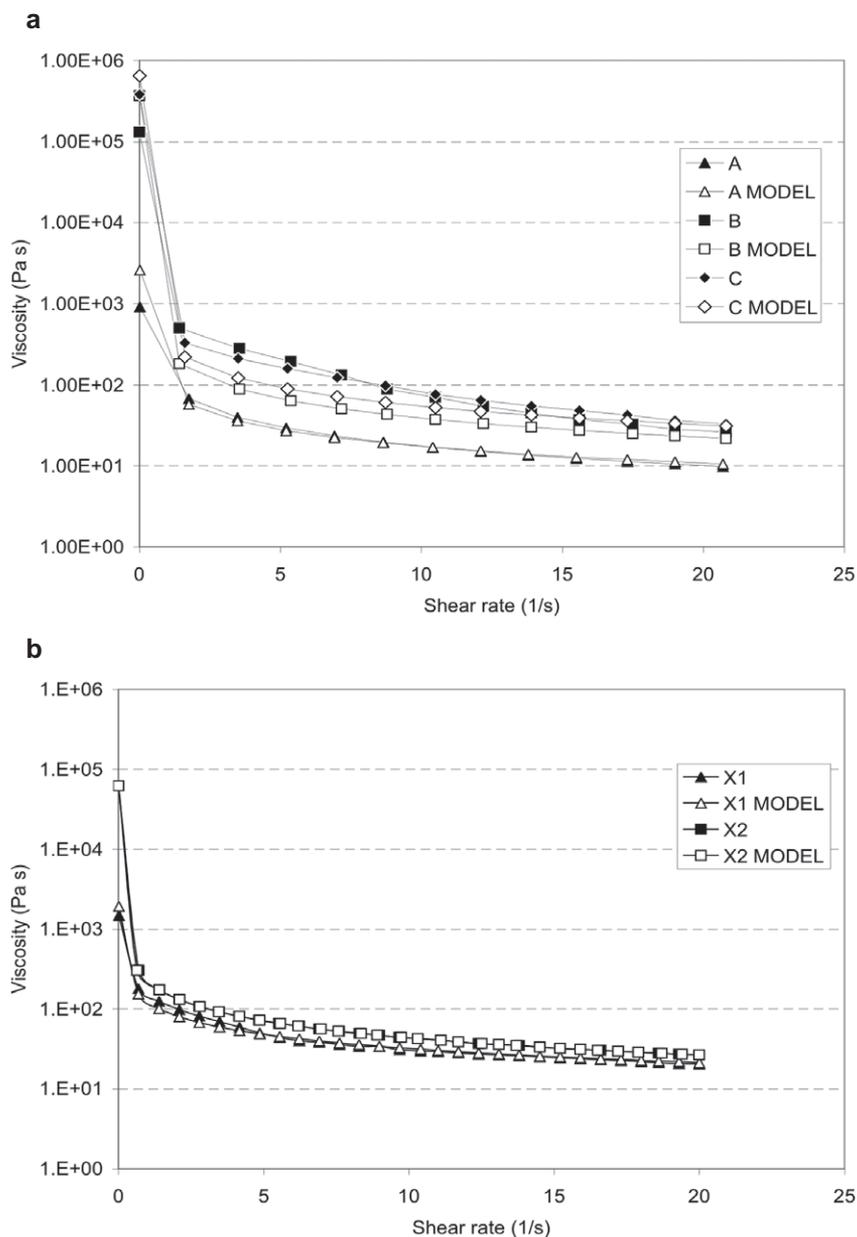


FIG. 3. (A, B) SAMPLES FLOW CURVES (FILLED SYMBOLS) AND USED MODEL FITTED (EMPTY SYMBOLS)

Also in this case, X1 and X2 commercial samples showed loss modulus values intermediate between that of A and B–C creams. This could be related, as reported above, to the presence of hard saturated fats in the formulation. This general trend was similar also for G' values.

Texture Characteristics

Results of cream samples' textural properties are reported in Table 4. The experimental samples (A, B, C) were significantly different for all the considered parameters. Between the experimental creams, sample A showed the lowest values of firmness,

consistency, cohesiveness and viscosity index, probably related to the scarce SFC. B and C cream samples, made up of a higher content of HF than A, were characterized by the highest values of the considered textural parameters.

X1 was significantly different from X2 and not from A for all textural parameters. The similarity between structural properties of X1 and A could be related to the presence of low HF content (5%) in the former and palm oil in the latter. These ingredients could induce a low resistance to compression and decompression, reducing cream samples consistency. Sample X2 showed intermediate values of firmness and cohesiveness between A and B ones. The presence

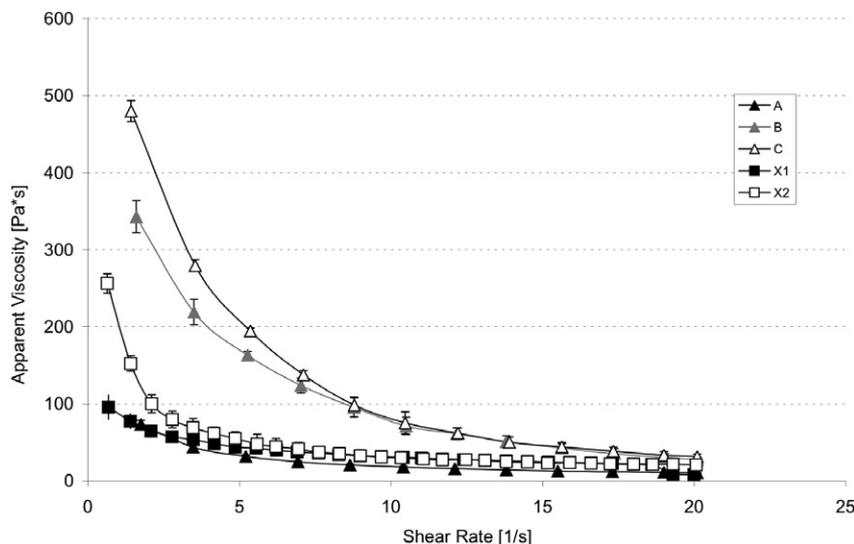


FIG. 4. CHANGES OF APPARENT VISCOSITY (Pa·s) OF NUT CREAM SAMPLES

of hard vegetable fats or HF in X2 and B samples could be the cause of their similar consistency and viscosity index values.

These results are in agreement with those obtained by fundamental rheological measurements.

Relationships between Textural and Rheological Measurements

The results of correlation analysis between textural (firmness, consistency, cohesiveness and viscosity index) and rheological properties (index of consistency, storage and loss modulus) of nut cream samples are reported in Table 5.

High positive and significant correlations between all rheological and textural parameters were found; in fact, the obtained correlation coefficients (*r*) ranged from 0.737 to 0.911. These results, in agreement with those of Afoakwa *et al.* (2008a) and Bourne (2002), suggest that the fundamental rheological parameters are closely related to the empirical ones, being closely linked to the physical structure of cocoa cream products, characterized by the micro- and macrostructural elements, represented by different solid particles dispersed in a Newtonian liquid. The obtained results indicate therefore the usefulness of both methods in studying nut creams' rheological behavior. Nevertheless, empirical parameters are usually used, especially in food industry, to assess food quality more than to give information about food microstructure.

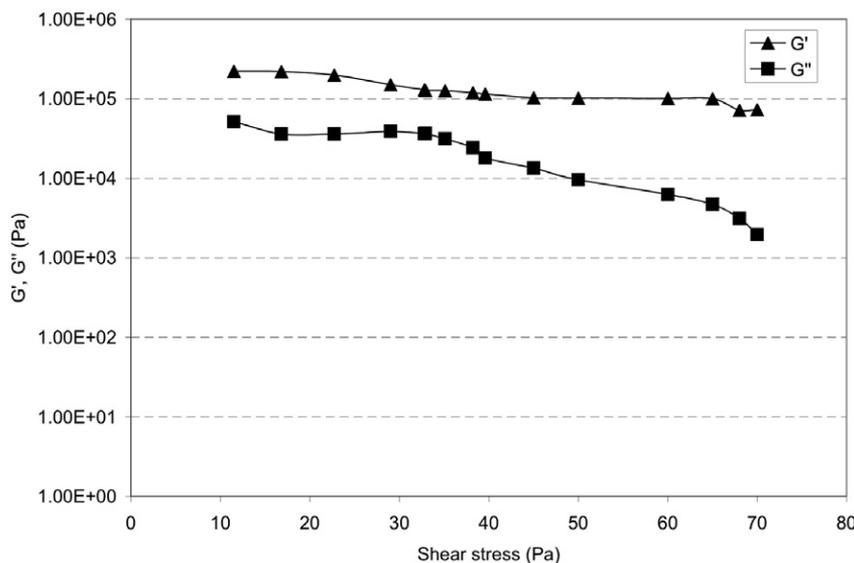


FIG 5. STRESS SWEEP TESTS OF CREAM SAMPLES

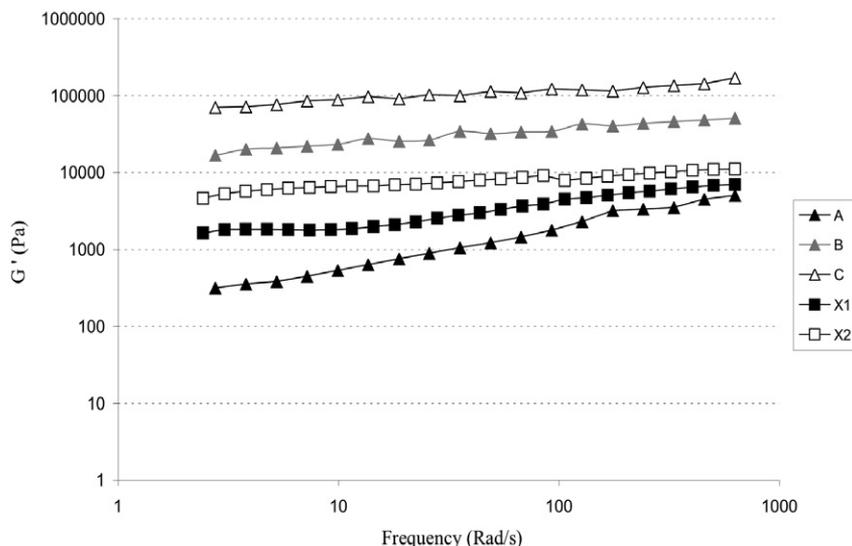


FIG. 6. CHANGES OF STORAGE MODULUS G' (Pa) OF NUT CREAM SAMPLES

CONCLUSIONS

The quality and rheological stability of nut creams are affected by a number of factors, such as the type and differ-

ent amounts of fats. This study may provide a useful guide for producers in order to let them to discriminate and choose a right fat composition of nut creams on the basis of their overall rheological characteristics, assessed by empirical and/or fundamental measurements. The results obtained in this work confirm that the use of high amount of HFs

TABLE 4. TEXTURAL PROPERTIES OF CREAM SAMPLES

Cream samples	Firmness (N)	Consistency (N s)	Cohesiveness (N)	Index of viscosity (N·s)
A	4.70 ^a	82.04 ^a	-3.76 ^a	-53.05 ^a
B	28.98 ^c	427.97 ^b	-24.65 ^c	-321.20 ^b
C	66.46 ^d	994.67 ^c	-47.92 ^d	-590.00 ^c
X1	3.28 ^a	67.88 ^a	-3.05 ^a	-53.70 ^a
X2	19.81 ^b	357.38 ^b	-16.68 ^b	-259.70 ^b

^{a-d} Values in the same column followed by different letters differ significantly at $P < 0.05$ level.

TABLE 5. CORRELATION ANALYSES BETWEEN TEXTURAL AND RHEOLOGICAL PARAMETERS OF NUT CREAM SAMPLES

	Firmness	Consistency	Cohesiveness	Index of viscosity
K	0.796***	0.767***	0.808***	0.737***
G'	0.911***	0.826***	0.880***	0.777***
G''	0.891***	0.793***	0.858***	0.762***

* Significant at $P < 0.05$; ** $P < 0.01$; *** significant at $P < 0.001$.

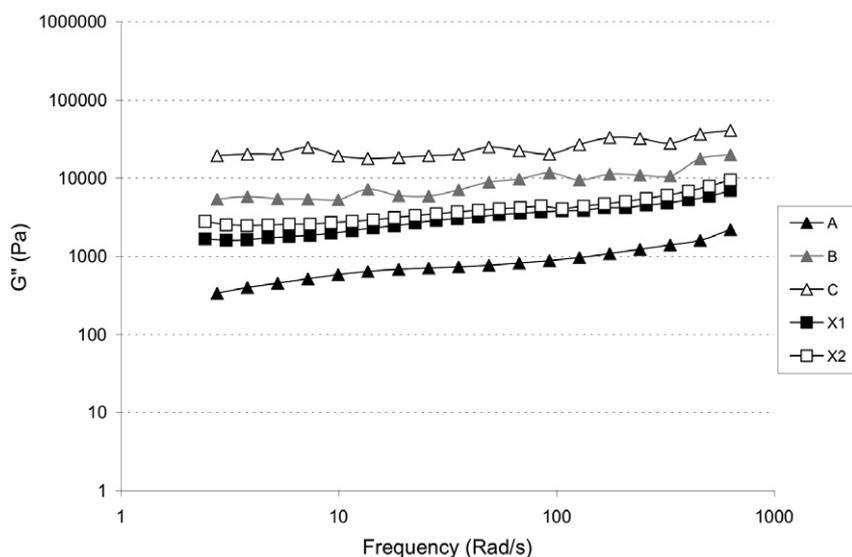


FIG. 7. CHANGES OF LOSS MODULUS G'' (Pa) OF NUT CREAM SAMPLES

and/or hard vegetable fats leads to a strong fat network, which implies more aggregated structure. Between the experimental creams, sample B showed textural properties similar to the X2 commercial one. Because the latter is the market leader in terms of overall quality, it could be reasonable to assume that the rheological characteristics of B cream are the most suitable for commercial applications.

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Rheological, textural and calorimetric modifications of dark chocolate during process



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ABSTRACT

The influence of several process steps on the rheological, textural and calorimetric characteristics of dark chocolate was evaluated. Samples were obtained at each phase of the manufacturing process: mixing, pre-refining, refining, conching and tempering. Rheological fundamental and empirical properties and thermal characteristics were evaluated on samples. Samples showed marked shear thinning behaviour with yield stress strictly related with the different process steps. Fundamental measurements results were well fitted by the power-law model. From the mixing to the refining samples underwent a drastic significantly ($p < 0.05$) increase of all considered fundamental (yield stress, apparent viscosity, G' , G'' and K index), empirical (firmness, consistency, cohesiveness, viscosity index) and thermal parameters (T onset, T end, ΔH). This could be attributed to the reduction of samples particle size that involve an increase of the contact point between them. Subsequently, the addition of fat and lecithin in conching and tempering phase, covering the sugar and cocoa particles, reduced interactions and caused a significantly ($p < 0.05$) decrease in all rheological, textural and thermal parameters.

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1. Introduction

Dark chocolate is a fat – continuous food dispersion, where a continuous network is formed by solid fat, sugar crystals and ground cocoa particles (Johansson and Bergensthal, 1992).

Rheological and textural characteristics are very important for fat continuous food, because they affect important properties such as viscosity, consistency and mouth feel. The rheological properties of these foods are complex and depend on many factors such as composition and processing conditions (Baixauli et al., 2007). During dark chocolate processing the ingredients are mixed, pre-refined, refined, conched and tempered in order to obtain desired rheological properties for a final defined product texture (Chevalley, 1999; Servais et al., 2004). The study of chocolate rheology is important, because it is related both to the efficiency of the main steps in the process (mixing, pumping, transportation, etc.) (Servais et al., 2002), and also to the final quality and the sensorial characteristics of product (Ahmed and Ramaswamy, 2006). Chocolate processing techniques are also important since chocolate consumers have well-informed opinions and expectations regarding appropriate texture and its viscosity characteristics (Afoakwa et al., 2008c). From a rheological point of view, dark chocolate

exhibits a complex rheological behaviour, i.e. it is a non-Newtonian, shear-thinning fluid that shows an apparent yield stress (minimum amount of energy to initiate fluid flow) and a plastic viscosity (energy to keep fluid in motion), strictly dependent on manufacture process (Bourne, 2002; Servais et al., 2004; Afoakwa et al., 2008a).

At the same time the characterization of the melting properties of dark chocolate during its manufacture process is very important in order to evaluate the effects of each step on the fat systems behaviour (Foubert et al., 2003; Marangoni and McGauley, 2003). Cocoa butter can crystallize as a function of triglyceride composition (Talbot, 1999) and due to the process parameters into six polymorphic forms (I–VI), where form I is the less stable and V the most desirable form, which can transform to VI, the most stable in storage (Talbot, 1999; Ali et al., 2001). During the manufacture process usually the tempering step is used to obtain the desirable form V with a melting temperature of 32–34 °C, in order to impart the desired glossy appearance, good snap, contraction and enhanced shelf life (Talbot, 1999; Beckett, 2000; Timms, 2003). Poorly tempered chocolate can develop in storage a white or grey surface layer, named fat bloom.

Several authors (Servais et al., 2004; Afoakwa et al., 2008a,b, 2009b; Baldino et al., 2010; De Graef et al., 2011; Efraim et al., 2011; Fernandez et al., in press) have investigated the physico-chemical properties of dark chocolate and in particular the rheological and thermal ones. In particular interesting relationships were highlighted between these product properties and formulation, cooling rate, particle size and fat crystallization kinetics.

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In all the previous cited works, although results obtained were very interesting, they referred only to finished product. Even if it is known (Vavreck, 2004; Schantz and Rohm, 2005 and Afoakwa et al., 2009b) that the formulation and the final tempering process step affect dark chocolate properties, in our opinion each manufacture step (mixing, pre-refining, refining and conching) cause drastic modification in the product influencing its final quality. Understanding how the single process step can affect the final rheological and thermal properties of dark chocolate, could be very useful in order to manipulate and optimize the process efficaciously, and mainly to predict and improve quality of final product. In the present work the rheological fundamental, empirical and thermal properties of dark chocolate were evaluated on samples obtained at each phase of the manufacturing process.

2. Materials and methods

2.1. Materials

Dark chocolate samples were produced in an Italian confectionery factory using an industrial plant (Buhler, Malmo, Sweden) provided of mixer, pre-refiner, refiner, conching and tempering machine, equipped to produce 6000 kg of chocolate at every production cycle. Dark chocolate production was made up by different steps as shown in detailed way in Fig. 1. The formulation employed for the chocolate recipe was: cocoa liquor (38% cocoa butter plus 15% cocoa powder), sugar (39.52%), cocoa butter (7% added during the conching step), soy lecithin (0.3%), sodium carbonate (0.15%) and vanilla extract (0.03%). Experimental samples were taken after

each production phase: mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E), from three production cycles carried out in different days. Therefore three samples for each production phase were obtained. Samples were stored in plastic bucket (1 kg capacity) at room temperature until the analytical determinations. Before performing each analysis the samples were melted in a microwave at 150 W for 25 min. The melting parameters were chosen after preliminary experiments in order to avoid changes in the chocolate properties.

2.2. Methods

2.2.1. Fundamental rheological properties

Measurements were carried out at 40 °C using a controlled stress–strain rheometer (MCR 300, Physica/Anton Paar, Ostfildern, Germany) equipped with a system of coaxial cylinders (CC27). The rheological behaviour of dark chocolate was analyzed in steady state and dynamic conditions. In steady state conditions, after a pre-shearing of 500 s at 5 s⁻¹, viscosity was measured increasing shear rate from 2 to 50 s⁻¹ within 180 s, taking 18 points measurements (ICA, 2000).

In this study the obtained flow curves were evaluated and fitted according to the rheological model of Ostwald, commonly referred to as the Power Law model (Holdsworth, 1993; Hugelshofer, 2000). The Power Law model is represented in the following equation:

$$\sigma = K\dot{\gamma}^n \quad (1)$$

where σ is the shear stress (Pa), K is consistency index (Pa sⁿ), $\dot{\gamma}$ is the shear rate (1/s) and n is the dimensionless flow behaviour index.

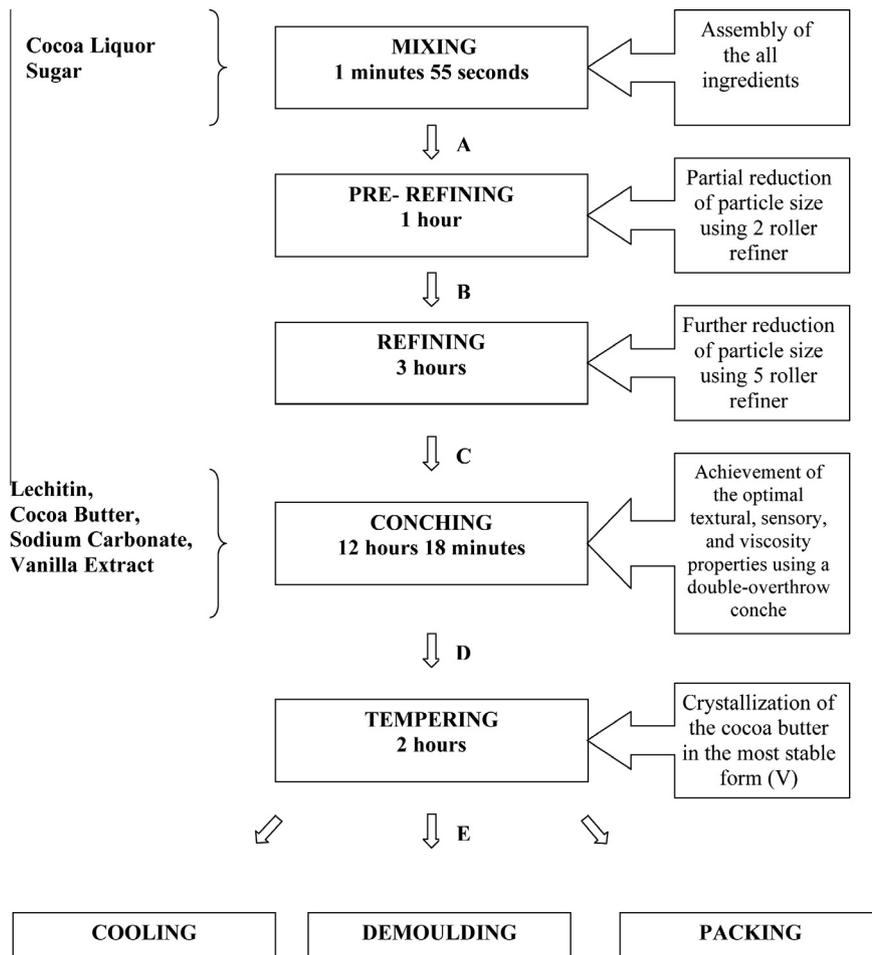


Fig. 1. Scheme of chocolate manufacturing process (adapted from Babin, 2005).

In order to measure the goodness of fit, the regression coefficient (R^2) was determined. Using the Power Law model, one important classification of products is possible: for values of n from 0.1 to 0.95, the products are known as pseudoplastic and are characterized by a decrease in apparent viscosity increasing the shear rate, i.e. shear thinning. For values of $n = 0.95$ – 1.05 , the behaviour is essentially Newtonian, ideally $n = 1$. For values of n greater than 1.05, the products are referred to as dilatants, i.e. shear thickening.

The yield stress (Pa) and the apparent viscosity (Pa s) were obtained according with ICA (2000) and Servais et al. (2004) evaluating the values of stress respectively at shear rates of 5 and 40 s^{-1} .

ICA choose two representative shear rates for yield stress and plastic viscosity that were as far apart as possible and two shear rates that provided high repeatability with low error measured.

In dynamic conditions, oscillatory tests were performed to investigate the viscoelastic properties of samples and to evaluate the storage (G') and the loss (G'') modulus.

In order to identify the linear viscoelastic range (LVR), in which the viscoelastic properties are independent from the stress conditions, stress sweep tests were applied.

Frequency sweep tests were carried out in the viscoelastic linear region at the constant deformation amplitudes of 0.007%, previously evaluated with the stress sweep test, in the range from 100 to 1 Hz.

2.2.2. Empirical properties

A TA.HDi 500 Texture Analyzer (Stable Micro System Vienna Court, England) was employed to investigate the textural properties of chocolate products. All measurements were performed using a back extrusion test. The test was carried out in a back extrusion container (50 mm in diameter), filled for the 75% with the sample, using a disk (35 mm) attached to a extension bar, with a load cell of 250 kg. The parameter used were: a pre test speed of 1 $mm s^{-1}$, a test speed of 1 $mm s^{-1}$, a post test speed of 1 $mm s^{-1}$ and a distance of 30 mm.

The textural properties that let to investigate the structure of a sample were: firmness (N), the point of maximum force, during penetration, evaluated at 30 mm; consistency (N s), the positive area up to the maximum force during probe descent; cohesiveness (N), the peak maximum of the negative region during probe return; the viscosity index (N s), the area of the negative region of the graph during probe return.

2.2.3. Thermal properties

Melting properties of dark chocolate were evaluated by using a differential scanning calorimeter (Pyris DSC Series 6, Perkin Elmer Corporation, Wellesley, USA). Adopting the method reported by Afoakwa et al. (2009b) DSC was calibrated by using indium (melting T 156,60 °C, ΔH 28,71 J/g) and tin (melting T 231,93 °C, ΔH 60,46 J/g) at a scan rate of 10 °C/min using an aluminium pan as reference. Samples (5 mg) were loaded into 40 ml capacity pans with holes and sealed using a sample press. Pans were heated at 10 °C/min from 15 to 200 °C in a N_2 stream. Onset temperature (T_{onset}), end temperature (T_{end}) and enthalpy of melting (ΔH) were calculated for each peak presents in the thermogram obtained (Gloria and Sievert, 2001).

2.3. Statistical analyses

Analyses of variance (ANOVA) and the test of mean comparison according to Fisher least significant difference (LSD) were applied. Level of significance was $P \leq 0.05$.

Correlation matrix comprising regression and correlation analysis at 95% significance were used to evaluate the relationships between rheological and textural properties of dark chocolate samples.

The statistical software used was STATISTICA (StatSoft, Tulsa, Oklahoma), version 8.0.

3. Results and discussion

3.1. Fundamental rheological properties

In Fig. 2 the dark chocolate sample flow curves, obtained increasing the shear rate from 2 to 50 s^{-1} , are reported.

All samples exhibited a typical non Newtonian behaviour characterized by dependence of viscosity upon of flow conditions (Chabbra, 2006). In particular, apparent viscosity of these products decreases with the increase of the shear rate, indicating pseudoplasticity. This behaviour can be explained by the structural breakdown of the molecules due to the hydrodynamic forces generated and to the increased alignment of the constituent molecules (Izidoro et al., 2008). As illustrated by Fig. 2, sample C presents the highest values of viscosity with initial values ranging between 80 and 90 Pa s, followed by samples B with initial viscosity values between 40 and 50 Pa s and sample A with values between 20 and 30. The lowest viscosity values belong to samples D and E obtained from the last two steps of the manufacture process.

In order to better explain the rheological values obtained by the flow curves, in Table 1 are shown the K and n parameters, obtained applying the Power Law model, and the values of yield stress and apparent viscosity.

All samples were well fitted by the Power Law model, provided high correlation coefficients (R^2) varying from 0.75 to 0.99. As shown in Table 1 all dark chocolate samples were characterized by $n < 1$, typical for *shear thinning* behaviour which corroborates the pseudoplasticity of the samples obtained by flow tests. The adimensional n parameters are an index of the transition from one flow regime to another and strictly related to the Reynolds number. Usually Reynolds number tends to increase when n values decrease (Sivakumar et al., 2006).

The K parameter, strictly related with the consistency of a product (Nindo et al., 2007), showed a significantly increase from sample A to sample C, that had the highest K value. The observed increase in the K values from A to C, can be explained considering that after the mixing phase sample A is completely unstructured, compared with samples B and C. The passage through the pre-refining step (B) and refining (C) involve in fact an agglomeration in the structure that suggests how these samples are characterized by higher K values of sample A. Samples D and E present the lowest values of K parameter probably related with their less aggregate packing structure network. In these steps, in fact the addition of lecithin and further cocoa butter, because of lubricating action, reduced the particle–particle interactions, increasing their mobility that involved a reduction of viscosity (Vernier, 1998). These results are supported by the studies of Dzuy and Boger (1983), Coussot and Ancey (1999) and Larsson (1999) that noticed an high dependence of yield stress and apparent viscosity on particles size and their interaction. Even if from a statistically point of view samples A, D and E are not significantly different for K value, they are completely different for the structure. Sample A is in fact characterized by large particles not refined and not aggregated between them, while samples D and E are made up from very small aggregate particles obtained after the pre and refining step, but because of the addition of cocoa butter and lecithin during the conching step a reduction in their aggregation and hence in the K values was observed.

The yield stress and apparent viscosity parameters, exhibited the same trends of the consistency index (K), showing a significantly increase of their values from sample A to C ($P < 0.05$). The highest K values could be attributed to the increase of the contact

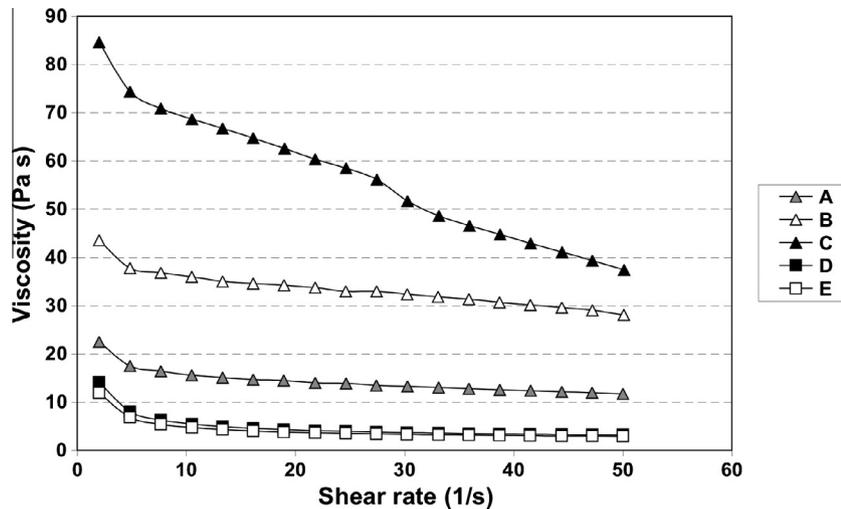


Fig. 2. Changes of apparent viscosity (Pa s) of dark chocolate samples, during mixing (A), prerefining (B), refining (C), conching (D) and tempering (E) steps.

Table 1

Consistency index (K), flow behaviour index (n), yield stress (Pa) and apparent viscosity (Pa s), of dark chocolate samples.

Samples	n	K (Pa s ^{n})	Yield stress (Pa)	Apparent viscosity (Pa s)
A	0.83 ± 0.005 ^a	16.49 ± 1.55 ^a	84.50 ± 5.97 ^b	586.67 ± 6.80 ^b
B	0.86 ± 0.005 ^c	47.89 ± 1.32 ^b	182.33 ± 6.50 ^c	1406.70 ± 5.77 ^c
C	0.76 ± 0.001 ^b	96.51 ± 6.13 ^c	358.67 ± 6.65 ^d	1880.00 ± 5.00 ^d
D	0.58 ± 0.002 ^a	15.20 ± 0.51 ^a	38.77 ± 1.35 ^a	161.67 ± 5.13 ^a
E	0.60 ± 0.0008 ^a	12.69 ± 0.09 ^a	33.07 ± 0.15 ^a	147.33 ± 1.15 ^a

^{a-d} Values in the same column followed by different letters differ significantly at $p < 0.05$ level.

point between particles, that forming spanning stress bearing paths (Afoakwa et al. 2009a) need of a major amount of stress to initiate the flow. Samples D and E were characterized by the lowest and significantly similar values of both parameters.

Stress sweep tests were performed in order to identify the linear viscoelastic region (LVR), in which properties of material are independent from the stress conditions. The results (data not shown) showed a narrow viscoelastic region, low intensity stresses can destroy the structure of system.

Results of frequency sweep test in terms of storage and loss modulus, evaluated respectively at the reference frequency 1 Hz, are reported in Table 2.

G' is an index of the elastic behaviour of a sample, and represents the deformation energy stored in the sample during the shear process. G'' value instead, corresponds to the viscous component of a sample and is a measure of the energy lost from it during the shear process (Angioloni and Collar, 2009). Storage modulus values (G') are higher than loss modulus (G'') ones for all samples, indicating that all dark chocolate samples had a solid, elastic-like behaviour. This suggests that under non destructive conditions the elasticity has a predominant effect on viscosity (Peressini et al., 2006).

Table 2

Storage G' (Pa) and loss G'' (Pa) modulus of dark chocolate samples evaluated at 1 Hz.

Campioni	G'	G''
A	16,346 ± 15 ^b	2046 ± 23 ^b
B	18,593 ± 25 ^c	3161 ± 8 ^c
C	167086 ± 25 ^d	21,473 ± 28 ^d
D	5966 ± 24 ^a	1406 ± 21 ^a
E	4133 ± 20 ^a	1396 ± 21 ^a

^{a-d} Values in the same column followed by different letters differ significantly at $p < 0.05$ level.

Samples B and C showed the highest significantly values of G' compared to the others samples. Sample A, showed viscoelastic properties with intermediate values of G' and G'' inside the clusters B–C and D–E. Some authors (Johansson and Bergensthal, 1992) observed that a high value of G' is related to a high level of interactive forces between particles; this confirms the high amount of stress needed from samples B and C to start flow (Fig. 2).

The significantly lowest parameters of G' and G'' were found for the samples D and E, constituted by a weakly structured system, due to the lubricating and emulsifier effect of fat and lecithin. These results are in agreement with the studies of Johansson and Bergensthal (1992), that highlighted how the effect of emulsifiers on the sugar particles, reducing the changing in the interaction particles and in the network structure ones, involves a decrease of the elastic component G' .

3.2. Empirical properties

The results of textural properties of dark chocolate samples are shown in Table 3.

Textural parameters (firmness, consistency, cohesiveness and index of viscosity) are extremely important for the evaluation of the mechanical properties of chocolate (strictly related to the sensory characteristics during consumption) and also to predict its rheological behaviour during processing (Beckett, 2000; Bourne, 2002). The dark chocolate C and B samples showed for all the considered parameters significantly higher values compared to other samples. These results in agreement with the ones obtained from the fundamental rheological analysis, support the presence of an extremely hard and consistent structure that arise a strong resistance to its compression.

Sample A showed intermediate values of consistency and index of viscosity, between those of samples C–B and D–E; D and E

Table 3
Textural properties of dark chocolate samples.

Samples	Firmness (N)	Consistency (N * s)	Coesiveness (N)	Viscosity index (N * s)
A	54.90 ± 7.72 ^a	498.26 ± 17.91 ^b	36.47 ± 4.94 ^a	414.40 ± 12.82 ^b
B	146.57 ± 8.48 ^b	739.80 ± 12.58 ^c	84.83 ± 9.64 ^b	718.90 ± 15.35 ^c
C	378.82 ± 8.42 ^c	1624.40 ± 19.41 ^d	199.90 ± 10.38 ^c	1106.00 ± 8.23 ^d
D	29.30 ± 6.45 ^a	147.76 ± 9.26 ^a	22.33 ± 4.75 ^a	147.00 ± 9.84 ^a
E	20.10 ± 2.19 ^a	110.14 ± 13.96 ^a	16.07 ± 1.25 ^a	105.07 ± 7.56 ^a

^{a–d} Means values followed by the same letter in each column are not significantly different ($p < 0.05$).

chocolate samples are characterized by the lowest values of all textural parameters, demonstrating the presence of a weak network structure, more sensitive to the breakage.

High, positive and significant correlations between all rheological and textural parameters were found. The results of correlation analysis between textural (firmness, consistency, cohesiveness and viscosity index) and rheological parameters (index of consistency, yield stress, plastic viscosity, storage and loss modulus) of dark chocolate samples are reported in Table 4.

These results suggest that the fundamental rheological parameters (in steady and dynamic conditions) are strictly related to the empirical ones, indicating the usefulness of both methods in studying dark chocolate rheological behaviour.

3.3. Thermal properties

In Fig. 3 the dark chocolate thermograms, obtained heating all chocolate samples from 15 to 200 °C, are reported.

It's known that T onset corresponds to the temperature at which a specific crystal form starts to melt; T end represents the temperature of the complete melting and ΔH the amount of energy required to complete the liquefaction (Afoakwa et al., 2008b).

Results showed that all samples were characterized by two similar and distinct endothermic peaks, the first one in a range between 20 and 38 °C, due to the melting of fat (Afoakwa et al., 2009b) and the second one in a range between 160 and 192 °C that, according with Okuno et al. (2003) and Beckett et al. (2006), represents the endothermic peak of sucrose melting. In particular sample C, as shown by the thermogram, is characterized by two double endothermic peaks, the first one is related to the fat melting and the other one with the sugar melting. The presence of double or multiple peaks is a common phenomenon observed by DSC in polymorphic species (Roy et al., 2007). The double peak presence can be attributed to the existence of different crystal structure with different stability inside of the same compound; this characteristic is called polymorphism (Ling and Spruiell 2006; Pan et al., 2007). Regarding the double peak associated with the fat melting of sample C this is probably due to the simultaneously presence of two of the six polymorphic crystal of the cocoa butter, with different melting point (Roy et al., 2007). A similar explanation could be attributed to the double melting of the sugar particles; in fact, according with Okuno et al. (2003) and Lee and Lin (2007) sugar can exist in two different polymorphic structures: Form I and Form

II with different chemico-physical and hence melting properties strictly dependent on the arrangement of their crystal. Moreover the presence of a solvent such as water play an important role during the process of melting and crystallization of sugar, influencing its melting behaviour (Bhandari and Hartel, 2002; Beckett et al., 2006). During the roller-refining step, sugar particles are heated, cooled and pressurized, this involves an arrangements of molecules present in their crystals. In particular, the fracturing of sugar particles can create surface with extremely high temperature, that are able to absorb the humidity present in the surrounding, modifying sugar structures and their properties (Bouzas and Brown, 1995; Vernier, 1998). Accordingly, the formation of a double peak in sample C could be probably attributed to the presence of some small amorphous sugar fraction (obtained after the absorption of water by crystal particles), instead that to the presence of polymorphic species (Bhandari and Hartel, 2002). It is possible also to exclude that the presence of a double peak could be related to a crystal of different size because, from previously experiments a monomodal distribution of particles was obtained after the refining step.

The values of T onset, T end and ΔH of dark chocolate samples are reported in Table 5.

Statistically significant differences between all samples were highlighted regarding T onset and T end of cocoa butter melting. An increase in the T onset, T end and ΔH was noted from samples A to C. Samples B and C, constituted by a very aggregate structure, as shown previously by the fundamental and the empirical results, probably needed higher temperature to start and finish their fat melting than sample A. In sample A the absence of an aggregate structure and the presence of large not refined particles, provides less resistance to breakage and melting, is probably the cause of the lowest obtained values of T onset and T end. A significantly decrease of these parameters was instead noted in samples D and E, due to the emulsification effect of cocoa butter and lecithin. In fact according, with the studies of Johansson and Bergensthal (1992) and Lonchamp and Hartel (2004), lecithin migrating to sugar/fat interface, coats sugar crystal that promotes the deagglomeration of particles involving a reduction of the temperatures necessary for the complete melting of samples.

The ΔH values of both fat and sugar, were higher in samples B and C compared to the other dark chocolate samples, confirming an higher request of energy in order to complete the sugar and fat melting, due to the existence of very consistent structures (Afoakwa et al., 2009b).

Table 4
Correlation analyses between textural, rheological and power law parameters of dark chocolate samples.

	Yield Stress	Apparent Viscosity	K	G'	G''
Firmness	0.986***	0.896***	0.943***	0.644**	0.689**
Consistency	0.978***	0.889***	0.966***	0.763***	0.796***
Cohesiveness	0.980***	0.887***	0.968***	0.796***	0.831***
Index of Viscosity	0.910***	0.975***	0.820***	0.569*	0.609*

n.s.: not significant.

* Significant at $P < 0.05$.

** $P < 0.01$.

*** Significant at $P < 0.001$.

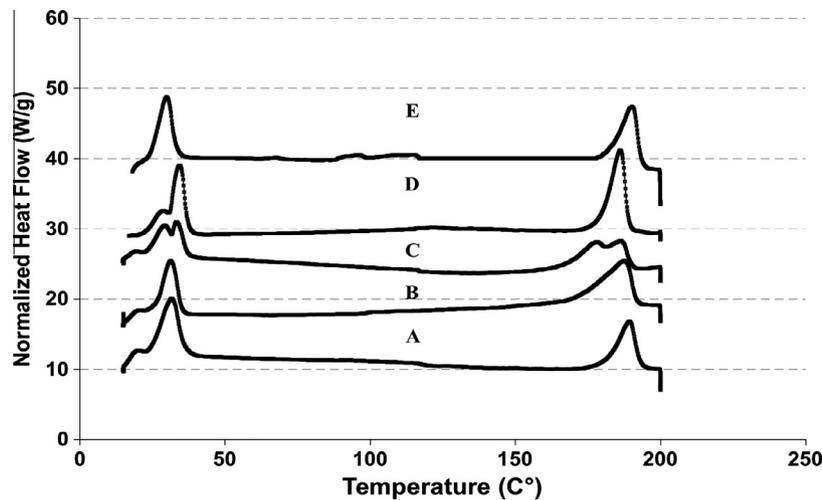


Fig. 3. DSC thermograms of dark chocolate samples.

Table 5

Melting properties of dark chocolate samples after mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) steps.

Samples	FAT			SUGAR		
	T_{onset} (°C)	T_{end} (°C)	ΔH (J/g)	T_{onset} (°C)	T_{end} (°C)	ΔH (J/g)
A	24.32 ± 0.16^a	34.01 ± 0.87^a	38.96 ± 3.10^a	174.89 ± 0.56^a	189.98 ± 0.66^a	30.01 ± 1.63^a
B	30.61 ± 0.66^c	36.65 ± 1.20^c	45.37 ± 1.35^b	176.36 ± 1.71^a	190.79 ± 0.73^a	46.84 ± 0.67^b
C	30.54 ± 0.77^c	37.22 ± 0.18^c	51.46 ± 0.85^c	180.97 ± 0.71^b	192.30 ± 0.40^b	48.95 ± 0.92^c
D	26.01 ± 0.37^b	35.06 ± 0.63^b	37.69 ± 3.5^a	175.72 ± 1.03^a	189.68 ± 0.41^a	29.22 ± 0.72^a
E	25.66 ± 0.13^b	35.34 ± 0.55^b	37.76 ± 2.95^a	174.73 ± 1.11^a	190.13 ± 1.05^a	28.68 ± 1.19^a

^{a-d} Values in the same column followed by different letters differ significantly ($p < 0.05$).

4. Conclusions

Rheological, textural and thermal properties of dark chocolate are strictly related to the different steps of the manufacturing process. From the mixing to the refining step there is a drastic increase of all considered rheological (yield stress, apparent viscosity, G' , G'' and K index), empirical (firmness, consistency, cohesiveness, viscosity index) and thermal parameters (T_{onset} , T_{end} , ΔH). Subsequently, the addition of fat and lecithin in conching and tempering phase, covering the sugar and cocoa particles, reduced interactions and created more void space between them (filled with cocoa butter). This lubricating action involved a decrease in all rheological, textural and thermal parameters at the end of process.

Obtained results show that the knowledge of the changes occurring in the product matrix at every manufacturing stage could be very useful in order to optimize the production efficiency and to improve the quality of final product.

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