UNIVERSITY OF CAMERINO

Doctoral Thesis

Effect of milk processing and steam frothing conditions on microfoam formation

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Life and Health Sciences

One Health

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XXXIV CICLE

2018/2019 - 2021/2022

CONTENTS

1. ABSTRACT

This study focuses on the evaluation of microfoam produced from processed dairy milk using the steaming function of the espresso machine as a function of temperature (30 – 80°C), milk type (pasteurised or UHT), and whey protein contents. Foam characteristics, i.e., foam stability, viscosity, bubble size distribution noticeably varied depending on milk processing type and steam heating temperature. Foams made from pasteurised whole milk tend to be more stable. Bubbles in pasteurised milk foam were better distributed, especially at the temperature of 60°C. It was observed that the optimal final temperature of steam frothing was in the range of 50°C to 60°C. The results of sensory tests show that consumers differentiated between the aroma and flavour of pasteurised and UHT milk, describing the degree of this difference from moderate to strong.

The technique of freeze-distilling (FD) milk was first used at the World Barista Championship 2017. This method is mainly used to intensify the flavour of milk by reducing the amount of water in the milk. The analysis of freeze-distilled milk samples demonstrated the increase in protein and lactose concentration in both pasteurized and UHT milk samples. Particle size distribution was not significantly affected by freezing of milk samples within 24 h with the subsequent thawing. Freeze-distillation also showed no significant effect on milk foaming properties in terms of microfoam expansion, stability, and overrun. The results of the triangle test showed that panellists could distinguish between cappuccino samples made with regular

and freeze-distilled milk for both pasteurized and UHT milk types. Assessors described freeze-distilled samples as *sweeter*, more *intense*, and *rich*.

2. LIST OF ACRONYMS

HQ milk – high-quality pasteurised whole dairy milk
UHT milk – ultra-high temperature processed whole dairy milk
HQ SS – pasteurised semi-skimmed dairy milk
HQ LF – high-quality pasteurised whole lactose-free dairy milk
UHT LF – ultra-high temperature processed whole lactose-free dairy milk
FD – freeze-distilled
MR – Maillard reaction
β-LG – β-lactoglobulin
α-LA – α-lactalbumin
CN – casein

3. INTRODUCTION

The global coffee industry instantly changes, but espresso-based dairy drinks, such as cappuccinos and lattes, remain at the top of consumer's preferences (Czarniecka-Skubina, Pielak, Sałek, Korzeniowska-Ginter, & Owczarek, 2021). The compound annual growth rate of the global coffee market is forecast to grow at an annual rate of 7.64% from 2022 to 2025 and 6.79% in Europe. It was also expected that 81% of total spendings in the Coffee segment will be in coffeeshops, restaurants and other out-of-home places (Statista, 2022).
Continued consumer interest in cappuccino-like beverages means that milk foam made with steam-injection equipment, i.e., traditional coffee machines, needs to be studied more closely. In addition, the evolving field of specialty coffee and professional global barista championships require a deep understanding of the chemical composition of milk in terms of its foaming properties to help competitors achieve stable and predictable results.
Frothing milk with steam in a coffee machine is mainly used in cafes to make cappuccino-like drinks. Steam-injection frothing is a non-isothermal method in which the steam flow is incorporated into the milk volume to obtain air bubbles for frothing while heating (Jimenez-Junca, Sher, Gumy, & Niranjan, 2015).

To standardise and improve the quality of espresso-based dairy drinks, the global Specialty Coffee Association (Coffee Skills Program, SCA) has provided the community with detailed protocols on how to prepare so-called *microfoam* (Fig. 1). According to these protocols, all beverages prepared during championships and training must meet certain parameters for the quality of the milk foam. According to the SCA Coffee Skills Program, a milk foam in the *excellent* category should be smooth, glossy, and moist, with no visible bubbles, and the height of the *microfoam* should not exceed 20 mm. If the foam is uneven, dry and dull, it will not be accepted by the examiner. In addition, foams with smaller bubble size are more likely to be positively described by consumers in terms of *elasticity*, *smoothness* and *fineness* (Hatakeyama et al, 2018).

a **b**

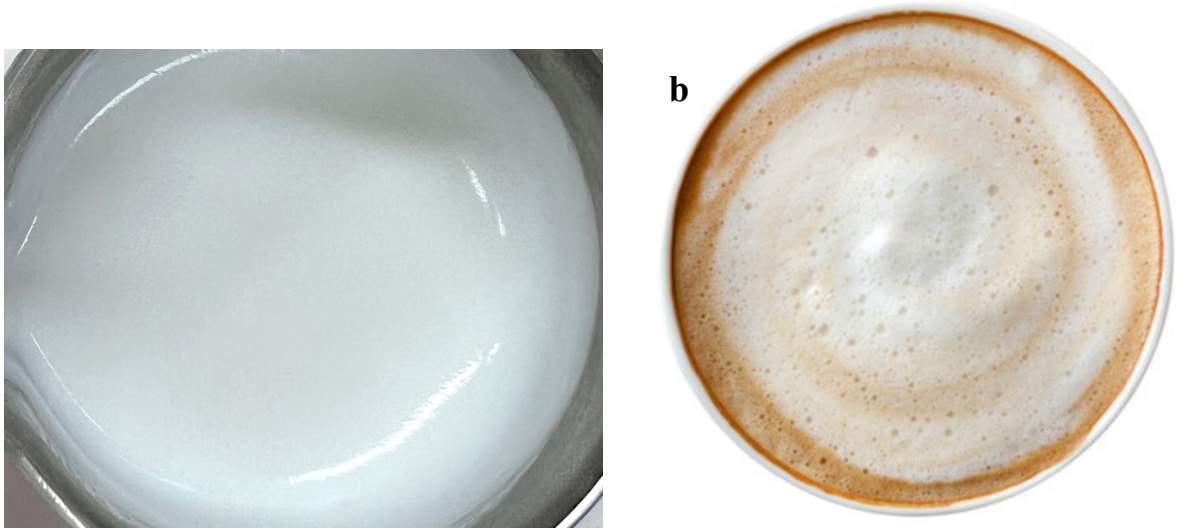


Fig. 1. The surface of milk a) *microfoam* and b) regular foam.

To ensure milk foam stability, surfactants should be present in emulsion to lower the surface tension between phases, i.e., between gas-liquid or between two immiscible liquids. In milk, proteins play the role of surfactants. They form a film around the air bubbles and thus prevent their rapid coalescence. The amphiphilic nature of milk whey proteins (accounting for about 20% of the total protein content), mainly β-lactoglobulin (predominant milk serum protein), α-lactalbumin, immunoglobulins and serum lactalbumin, as well as caseins (accounting for about 80% of the total protein content) and caseinates makes them good natural surfactants. Studies show that protein monomers and oligomers, i.e., nonmicellar casein or native serum proteins, are mainly found at the air-serum interface of dairy whole milk foam, whereas direct adsorption of casein micelles at the interface was not observed (Borcherding, Hoffmann,

Lorenzen, & Schrader, 2008a). Considering this, in the present research the *microfoam* properties in relation to whey protein content has been evaluated.

Previous studies (Dandigunta, Karthick, Chattopadhyay, & Dhoble, 2021; Ho, Dhungana, Bhandari, & Bansal, 2020; Martínez-Padilla, García-Mena, Casas-Alencáster, & Sosa-Herrera, 2014; Oetjen, Bilke-Krause, Madani, & Willers, 2014; Kamath, Huppertz, Houlihan, & Deeth, 2008) extensively evaluated milk foam as a function of milk composition, processing method, and foaming method. However, the design of foaming apparatus greatly varies in different studies and frequently the pre-heating or one final temperature obtained by the steam-treatment was studied. Meanwhile, to the best of our knowledge, there are only a few research papers that focus on change in foam, particularly *microfoam*, properties as a function of milk processing method in combination with steam-heating temperature and serum protein content.

The technique of freeze-distilling (FD) milk was first used at the World Barista Championship 2017. The “Barista magazine” which is a popular and influential media in the coffee industry describes the flavour of freeze-distilled milk as “super creamy, with a texture and a sweetness that” (Nanetti, 2021). This method is mainly used to intensify the flavour of milk by reducing the amount of water in the milk. Freeze-distilled milk, also known as fractional freezing, is a straightforward process. To do this, one should take a pack of milk, freeze it completely, then place it in a cold environment to prevent microbial growth and melt it until part of the bottle's contents, i.e., half, have melted. After that, FD milk can be used for espresso-based milk beverages preparation.

There are several undesirable processes that might occur during freezing/thawing period including: the destruction of fat globules due to the formation if ice crystals that can cause the destabilisation of milk after thawing; loss of the structure of milk casein micelles due to the degradation of milk protein and fat; aggregation of CN micelles after the prolonged storage (> 3 months); degradation of lactose (Yu, Qiao, Zhang, Yan, Li, & Liu, 2021). The stability of human milk after freezing and thawing has been extensively studied (Abranches, Soares, Junior, & Moreira, 2014; Vieira, Soares, Pimenta, Abranches, & Moreira, 2011; Zhang, Qu, Huppertz, Liu, Sun, & Zhou, 2022). However, the stability of cow's milk has not been adequately described.

3.1 Milk composition

Milk is a complex heterogeneous mixture that consists of several main groups of components: proteins, lipids, carbohydrates, minerals, and vitamins. Among those groups, there are present in milk other various compounds like amino acids, growth factors, peptides, nucleotides, cytokines, enzymes, and polyamides (Haug, Høstmark, & Harstad, 2007). The typical ratio of principal components in dairy milk is: 3.4% of proteins (caseins sized from 300 to 500 nm, and whey (sized from 4 to 6 nm), 4.9% of fat present in form of milk fat globules (MFG) measuring from 0.1 to 15 μm in diameter and surrounded by the milk fat globule membrane (MFGM), 4.1% of carbohydrates, mainly lactose – principal milk sugar, and 0.7% of minerals (Mehta, 2015). The milk composition widely varies among mammal species.

3.1.1 Milk proteins

Milk proteins represent one of the largest contributions of milk to human nutrition, and more than 200 types of proteins have been characterised in dairy milk. About 95 % of the nitrogen in milk is present in the form of proteins (Mehta, 2015). There are two main groups of proteins found in milk: caseins (constitute about 80% of total protein content) that consist of four principal molecules, i.e., α_{S1}, α_{S2}, β-, and κ-casein, and whey proteins (about 20% of total protein content), mainly β-lactoglobulin A and B, α-lactalbumin, immunoglobulins, and serum lactalbumin. Milk proteins are fundamentally different in their structure and consequently properties, for example, in their solubility at different pH levels.

Caseins are insoluble in weakly acid solutions and precipitate at pH lower than 4.6 (natural cow milk pH level is 6.6-6.8) whilst whey proteins remain soluble.

In contrast to whey proteins, caseins are not globular proteins, lacking of organised and stable tertiary structure. They are typical rheomorphic phosphoproteins present in milk in the form of casein micelles (CM) – large aggregates that consist of caseins (about 94% of casein micelles dry matter) and calcium phosphate (approx. 6% of CMs). There are also found traces of citrate and magnesium within the micelles (Casanova, Nascimento, Silva, de Carvalho, & Gaucheron, 2021). Molecules of κ-casein are mainly found on the surface of micelles, their hydrophilic C-termini provide the repulsion of casein micelles thus preventing them from the aggregation (Belitz, Grosch, & Schieberle, 2009).

In contrast to the caseins, whey, or serum proteins, are globular proteins with highly-organized tertiary and secondary structures. In dairy milk β-lactoglobulin (18.3 kDa) is the most predominant serum protein and consists more than 50% of total whey protein fraction. The molecule of β-lactoglobulin consists of 162 amino acids, and the sequence includes 5 cysteine residues (one of which Cys^{121} is in the free state) that are capable to form disulphide bonds. Initially, those residues are buried within the protein structure. When protein denatures, for example during the heat treatment of milk, thiol groups (–SH groups) become exposed on the surface of the protein molecule and can build bonds with other molecules, especially with α-lactalbumins and/or κ-caseins. Via the formation of the S-S bridges, –SH groups can participate in protein dimerization (Farkye and Shan, 2015; Belitz, Grosch, & Schieberle, 2009).

Whey protein α-lactalbumin is the smallest (14.2 kDa) of the major whey proteins. It plays an important role in the biosynthesis of lactose. In the Golgi apparatus of the mammary epithelial cell, α-lactalbumin is the regulating component of the lactose synthase complex. Its function is to transfer galactose from uridine diphosphate galactose, which is an intermediate in the production of polysaccharides, to glucose. The molecule of α-lactalbumin is shorter and contains 123 amino acids in the sequence and 8 cysteine residues. Under mild denaturing conditions α-lactalbumin forms molten globule state (Edwards, & Jameson, 2014). This intermediate state of the protein is a compact, unfolded molecule with a high number of secondary structural elements as in the native state and a highly disordered tertiary structure (Alaei, Ashengroph, & Moosavi-Movahedi, 2021). In this state, the protein unfolds more easily than the native protein at the air-water interface (Huppertz, 2010).

3.1.2 Milk sugar

Lactose, or O-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose, is the main carbohydrate in milk. It is a unique sugar produced in the mammary gland of mammals. In some aspects, lactose resembles other reducing sugars, for example as a reactant in the Maillard reaction (Hettinga, 2019). The Maillard reaction impacts the flavour of milk and, consequently, the perception of the final product, i.e., cappuccino or latte, by a final consumer.

Structurally, lactose is a disaccharide consisting of two units: glucose and galactose linked with β (1→4) glycosidic bond, and the molecule has hemiacetal structure (Singh, Rao, Singh,

Sharma, & Arora, 2021). These sugar units present in a six-ring configuration (Fig. 2). Lactose molecule occurs two isomers: α- and β-lactose. The lactose configuration is determined by the position of the hydroxyl group on the C1-atom of the glucose. The configuration of lactose determines the properties of the molecule, i.e., its s crystallisation and solubility behaviour. During the process called mutarotation the ring structure enables the interchange between α- and β-anomers. The equilibrium between two anomers depends on temperature, pH, and concentration (Wong, & Hartel, 2014).

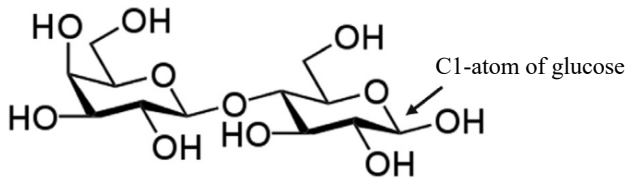


Fig. 2. The chemical structure of the lactose molecule (From: Hettinga, 2019).

The glucose unit of lactose can be hydrolysed by water, resulting in an open chain structure. In solution, lactose is in equilibrium between an open and a closed structure. This explains why lactose is a reducing sugar: the lactose molecule has a reactive carbonyl group when it is in an open structure.

The open chain structure plays an important role from chemical point of view because it is highly reactive, especially in the Maillard reaction. The increase in temperature or the decrease of pH lead to an increase in the open-chain structure (Walstra, Wouters, & Geurts, 2006).

As for the importance of the lactose content for the end consumer, it is crucial for the perception of quality, as lactose influences the sensory properties of milk. Lactose influences the taste and flavour of milk in two ways: it provides the natural sweetness of milk and is one of the main reactants of the Maillard reactions, which leads to the formation of new flavours and aromas. As for the degree of natural sweetness of lactose, it is relatively mild compared to other carbohydrates. For example, glucose and galactose, which form lactose, are sweeter than lactose itself. This makes lactose-free milk sweeter than regular milk with full lactose content (Hettinga, 2019).

3.1.3 Milk fats

The fat in milk is present as an oil-in-water emulsion in form of globules. The average diameter of the globules is 2-4 μm. The globules are stabilised by a phospholipid-protein membrane formed mainly by the apical membrane of the mammalian cell. Because of the difference in density between aqueous phase and fat globules the emulsion tends to separate. According to Mehta (2015), fats are what mostly impacts the mouthfeel of milk, i.e., its smooth, soft, and rich taste properties, moreover, they are carriers of fat-soluble vitamins, i.e. A, D, E, K vitamins.

The homogenisation of raw milk helps to prevent it from creaming and coalescence of fat by reducing the fat globule size. Typically, a two-stage homogenisation is used in the dairy industry. Raw milk preheated to 40-50°C passes through the first-stage valve at 10-30 MPa to break up the fat globules and then through the second-stage valve at 3-5 MPa to separate the resulting agglomerates. The homogenisation of the milk leads to major physical changes in the size of the fat globules and the structure of the fat globule membrane (MFGM). The MFGM is a thin layer of a surface-active membrane, which consists mainly of phospholipids, cholesterol, lipoproteins, glycoproteins and proteins (Kontkanen et al., 2011). During homogenisation, the composition of the MFGM noticeably changes due to the increase in the surface area of the fat globules. The newly formed surfaces of the fat globules are covered by the micelle complex of caseins and whey proteins or only by the caseins (either semi-intact micelles or micelle fragments), depending on whether homogenisation is performed before or after heat treatment (Ho, Dhungana, Bhandari, & Bansal, 2020). MFGM plays the role of an emulsifier, preventing coalescence and flocculation of fat globules and protecting fats from enzymatic activity (Singh, 2006), i.e. lipoprotein lipase activity, which initiates lipolysis of triglycerides, leading to the formation of partial glycerides and free fatty acids (FFA). Lipolysis can occur naturally in raw milk because lipase is involved in the synthesis of triglycerides in the mammary gland. However, lipolysis can occur in raw milk for various reasons, such as: late stage of lactation, mastitis, poor quality of cow feed. In addition, mechanical action during processing, i.e. homogenisation, pumping or mechanical agitation, can lead to MFGM degradation and lipolysis. Lipolysis is considered an undesirable process as it not only leads to the formation of off-flavours in the milk, but also reduces the foaming ability of the milk by increasing the concentration of FFA (> 2 mmoL L^{-1}), mono- and diglycerides, as these have surface-active properties (Timlin et al., 2021). The fat globule membrane can be easily damaged by agitation, heating, or ageing, resulting in defects such

as hydrolytic rancidity and nonglobular (free) fat (Fox, 2022). Milk fat globules consists of more that 400 fatty acids (FA) which are mainly esterified into the triacylglycerol molecules in an orderly manner. FA composition varies widely from one sample to another and depends on season, stage of lactation, breed, and feed type (Larsen, Andersen, Kaufmann, & Wiking, 2014). Apart from triglycerides, other lipid classes are represented by polar lipids, partial glycerides, and sterols (Lecomte, Bourlieu, & Michalski, 2017). The fatty acids in the milk fat have different unsaturation degrees and chain lengths (Ren, Guo, Teng, & Ma, 2021).

The heterogeneity and complexity of milk fat is reflected in its wide and variable melting range. Milk fat is completely liquid at 40°C and completely solid at -40°C. Between these temperatures, milk fat exists as a mixture of crystals and liquid. The ratio of solid to liquid fats depends on composition, dispersion state and heat treatment (Pilhofer, Lee, McCarthy, Tong, & Bruce German, 1994).

In terms of foam formation and stability, milk fat has both positive and negative effects on foam formation and stability, depending on the physical state and type of foam. The degree of influence of milk fat on the foam properties of milk depends primarily on the fat content in a milk sample and also on the ratio between solid and liquid fat as well as the size of the fat globules. In milk foam, fats play a role as natural surfactants with a low molecular weight. The results of Ho et al. (2022) showed that the size of the fat globules affects the foamability of milk samples. The foamability of milk improved significantly when the fat globule size was less than 2.6 μm, while a decrease in fat globule size had no effect on froth stability, especially in the method of frothing by steam injection. Although fat globule size had no effect on the shape of the air bubbles in the foam, the size of the air bubbles increased with increasing fat globule size. In contrast, a comparison of the foaming properties of milk samples whose fat globule size was reduced by homogenisation showed that the reduction in fat globule size controlled foamability, while the change in fat globule membrane composition determined foam stability.

3.1 Maillard reaction

Maillard reaction (MR) is a predominant chemical reaction of carbohydrates with proteins that results in a formation of specific taste and aroma. The Maillard reaction is a non-

enzymatic browning reaction that consists of several stages and depends on the intensity of heating. Once Maillard reaction begins it continues throughout all storage period (Aalaei, Rayner, & Sjöholm, 2019).

Generally, to initiate an amino group from protein, in milk it is mostly ε-amino group of lysine residue from κ-casein and whey proteins, reacts with a carbonyl group of milk's most predominant sugar – lactose (Campbell & Drake, 2013) with a formation of glycosylamine. Since glycosylamine is unstable it undergoes further rearrangements with a formation of the early Amadori product – lactulosyllysine (Su et al, 2020). On the final stage of MR Amadori product undergoes multiple pathways of the reaction that lead to the development of more than 3500 characteristic desirable, for example, in condensed milk, or undesirable MR volatile compounds (Sunds, Maximilian Rauh, Sørensen, & Larsen, 2018).

Despite its complexity, Maillard reaction occurs in three main stages – early, advanced and late (final). In the early stage, reducing sugar lactose reacts with an amino group of lysine residue with a formation of a Schiff's base. This base is an unstable compound and, consequently, through the Amadori rearrangement, it becomes a protein-bound Amadori product – lactulosyllysine bound to protein, or (1-deoxy-1-amino-) lactulosyllysine (Van Boekel, 1998). According to some researchers, at the end of this stage lysine loses its natural availability (O'Brien, 2009). Fig. 3 describes the pathway of the early MR stage.

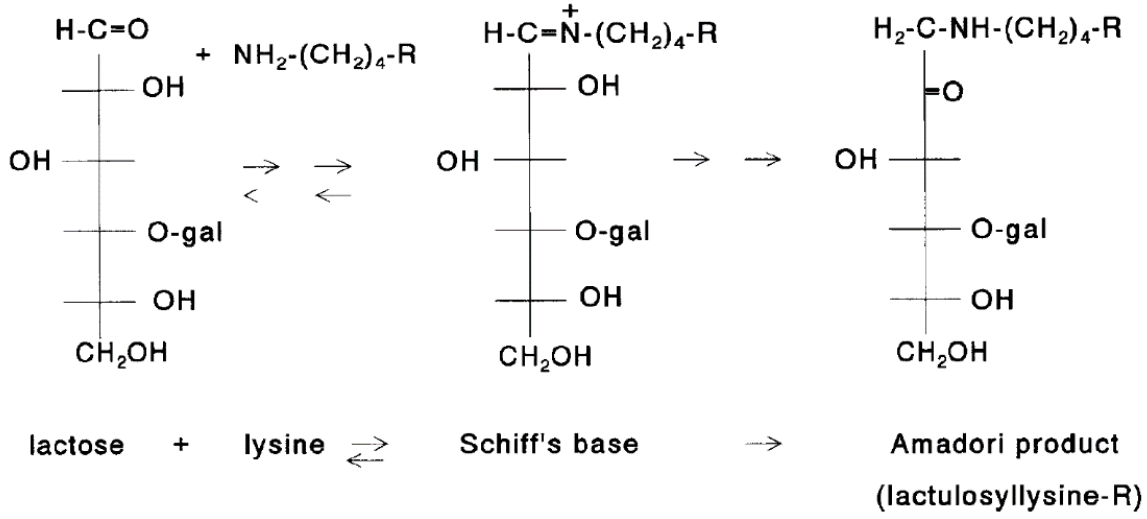


Fig. 3. Early stage of Maillard reaction in milk (Van Boekel, 1998).

In the advanced stage, Amadori product undergoes multiple transformations that strictly depend on the pH level of the system. Those transformations are mainly degradation of Amadori product via its enol forms through acid hydrolysis, oxidative cleavage, or boiling in

oxalic or acetic acid. In general, Amadori product breaks down into multiple fission products of the (1-deoxy-1-amino-) lactulosyllysine (Kumar, Raghavendra, Tokas, Singal, 2017). Low molecular fission products deliver a specific flavour to milk (Van Boekel, 1998).

In the late, or final, stage, sugar fragments and amino compounds are transformed into brown pigments – melanoidins (high-molecular weight nitrogenous compounds) and milk proteins polymerize through the condensation reaction.

Changes in milk aroma induced by the heating are mainly influenced by carbonyl and sulphur flavour compounds. The "off-cooked" flavour appears due to the sulphur volatile compounds. "Stale", or paper-like flavours, are promoted by aldehyde compounds. Dimethyl trisulfide leads to the formation of cabbage-like aroma (Zabbia, Buys, & De Kock, 2012).

Obviously, MR is not the only reaction that forms new flavours in food, but its products are somehow involved in other reactions that lead to the formation of new flavours. The products of MR become precursors for Strecker degradation of amino acids. Even the initial products of MR, i.e., 3-deoxyosones, cause Strecker degradation (Rizzi, 1999). As a result of Strecker degradation, amino acids are decarboxylated and turn into Strecker aldehydes with a side chain. In milk and dairy products, these aldehydes have been identified: 2-methylbutanal and isobutanal in UHT milk, 3-methylbutanal or isovaleraldehyde in pasteurised and sterilised milk (Aalaei, Rayner, & Sjöholm, 2019).

In the case of lactose-free milk produced by the use of β-galactosidase enzyme (lactase), it should be noted that the glucose formed during hydrolysis is more reactive than lactose on MR. Therefore, the visible effect of browning and the change in sensory properties may occur more quickly. There are several ways to heat milk: before or after lactase treatment. If the milk is heated before the lactase treatment, the visible effects of MR are reduced. However, the hygienic requirements are higher and traces of lactase in the final product can lead to the formation of undesirable flavours due to protein degradation. Heating after lactase treatment, the traditional way of processing, inactivates the lactase but with the consequence of extensive MR (Hettinga, 2019).

3.2 Lactose-free milk

In order to diversify the diet of lactose-intolerant people wide number of ways to produce lactose-free dairy foods is available now and the technology of lactose removing from regular cow milk improves all the time. These technologies allow creating a large market of

commercially available lactose-free foods. In the 1980s Valio Company started to research chromatographic separation of lactose from whey to increase the yield of its lactose manufacturing operation. It was investigated that this technology allowed separating lactose from skim milk. In 2001 first lactose-free milk obtained by the chromatographic separation was launched (Tossavainen, 2003). The method used before to separate lactose from milk was called ultrafiltration and it removed salts from the protein fraction at the same time. Chromatographic techniques can be also used at this purpose (ion-exchange chromatographyhydrophobic interaction chromatography and size-exclusion chromatography). In manufacturing lactose-free foods traditionally more than one chromatographic method is used at the same time. Lactose forms lactose-cations compounds that can be separated by a strong cation exchange resin.

Modern technology for producing lactose-free foods is based on primary filtration of regular dairy milk to remove half of the lactose content from the milk and the addition of enzymes to remove the rest of the lactose from the milk.

Before packaging, lactose-free milk (LFM) goes through the same processes as normal milk, e.g., pasteurisation or the most common UHT processing.

As mentioned above, the process of dairy milk hydrolysis involves Maillard reaction and the quality of the lactose-free product can be affected by reactions between proteins and reducing sugars. LFM contains glucose and galactose, which are more reactive in the Maillard reaction than lactose as a disaccharide. This reaction in milk may be a cause of reduction in the nutritional value of milk protein, poor taste and browning.

Reactions between protein and reducing sugars can affect LFM by inappropriate, i.e. high, storage temperature. It was reported that the addition of enzymes after heat treatment, can significantly reduce Maillard reactions during heat treatment and browning of LFM (Harju, Kallioinen, & Tossavainen, 2012).

The removal of side activities, e.g. protease, from the lactose helps to improve the taste of LFM.

3.3 Industrial heat-treatment of milk

Food thermal processing is used for ages to prevent pathogenic microbial growth with subsequent product spoilage. This results in an extension of a food product shelf life.

Raw milk must be subjected to heat processing before selling. The most common milk treatments are pasteurization, mainly high-temperature, short-time, or HTST (85°C for 2–3 seconds), and ultra-high temperature (UHT) treatment (135-150°C for 1-10 seconds, depending on method), in combination with aseptic packaging. There is a key difference in the efficacy of the two methods. The pasteurization destroys pathogens that do not form spores as well as psychrotrophic spoilage bacteria, e.g., *Pseudomonas* species, but it does not affect bacterial spores of thermoduric vegetative bacteria, e.g., *B. circulans* and *B. cereus* (Gopal, Hill, Ross, Beresford, Fenelon, & Cotter, 2015). UHT treatment, in turn, results in deactivation of all non-spore-forming microorganisms and all bacterial spores besides heat-resistant ones (Deeth, 2017).

Although heating prevents milk from spoilage and ensures its microbiological safety, it also causes many chemical changes of milk components. Intense temperatures provoke milk whey proteins denaturation; the exposure of reactive thiol groups on the surface of protein molecules that leads to the exchange reaction between β-lactoglobulin and κ-casein modifying the initial structure and properties of both types of proteins; changes in milk fat globule membrane (MGFM) that alters the cream separation ability of fat globules; Maillard reaction between sugars, mainly lactose, and protein amino groups; degradation of some vitamins, e.g., a highly thermally sensitive vitamin C, as well as folic acid, vitamins B6, B12 and B1. The UHT treatment is not as severe as the sterilization process. However, it is enough for 10-30% loss in above-mentioned vitamins (Belitz, Grosch, & Schieberle, 2009). In addition, studies of UHT milk samples processed in different ways, i.e. directly or indirectly heated, showed that the concentration of furosine, lactulose, galactosyl-β-pyranone and lysinoalanine was increased. From the results, the researchers concluded that furosine and galactosyl-β-pyranone (the product of the advanced Maillard reaction) are markers of milk overheating or adulteration (Ritota, Di Costanzo, Mattera, & Manzi, 2017). Furosine itself is a product of the hydrolysis of ε-N-(2-furoyl-methyl)-l-lysine, which is an Amadori product, i.e. the product formed in the initial phase of the Maillard reaction. The measurement of furosine is now widely used to evaluate and control Maillard reactions in food (Wang, Zhang, Wen, Zheng, Wang, 2020).

Thus, heating affects and changes all the main components of milk - proteins, fats and carbohydrates, as well as the sensory properties of milk.

Despite these factors, manufacturers must ensure the microbiological safety of raw milk and dairy products. Using an appropriate combination of time and temperature treatment of the milk can ensure not only microbiological safety, but also the sensory quality of the milk and high nutritional properties.

3.4 Milk protein denaturation

Milk proteins are sensitive to changes in some parameters in the environment of the emulsion, i.e. pH, temperature, increase of the interface, addition of organic solvents. These changes lead to either a reversible or irreversible change in the tertiary structure of a protein. The above-mentioned treatment thus cleaves the hydrogen bridges, hydrophobic or ionic within the protein structure.

Denaturation can be reversible if there is a denaturing agent that stabilises the peptide chain in its unfolded state, and the native conformation can be reconstituted after the agent is removed. When the unfolded peptide chain is stabilised by interaction with other chains, irreversible denaturation occurs. This occurs due and after the exposure of reactive thiol groups, which are initially hidden in the protein structure. They are involved in the formation of new disulphide bonds within the molecule, which leads to irreversible denaturation (Belitz, Grosch, & Schieberle, 2009).

As heating progresses, the protein molecules unfold, destroying the secondary and tertiary structures and exposing the sulfhydryl (or thiol or –SH) groups. Free –SH (FSH), also known as reactive –SH, are the –SH groups found on the surface of protein molecules. Since FSH is a measure of both native and heat-exposed –SH groups, it can be considered a measure of protein denaturation and thus an indicator of the functional capacity of the denatured protein (Stănciuc, Dumitraşcu, Ardelean, Stanciu, & Râpeanu, 2012). The exposed hydrophobic groups cause rapid hydrophobically associated aggregation of globular protein. Those aggregates are then rapidly transformed into S–S bonded aggregates once the temperature exceeds 75 °C in case of β-LG (Wijayanti, Bansal, & Deeth, 2014). The involvement of exposed thiol groups in the formation of disulphide bonds can also lead to irreversible denaturation of proteins.

The pH value also changes the structure of the proteins. At neutral pH, the interactions of free thiol residues with disulphide bonds lead to the formation of new irreversible intra- and inter-

protein disulphide bonds, resulting in the formation of relatively small aggregates between caseins and globular proteins of the milk. However, after the addition of the bacterial culture to the milk, i.e., in the process of yoghurt making, the pH decreases to 4.5. This reduces the negative charge of the particles in the milk, leading to a decrease in electrostatic repulsion between the particles. This causes the formation of larger aggregates and enables the formation of a protein network or gel (Famelart, Le, Croguennec, & Rousseau, 2013). Fig. 4 demonstrates the interaction between partially denatured under the heat-treatment serum proteins and κ-casein on the surface of casein micelles.

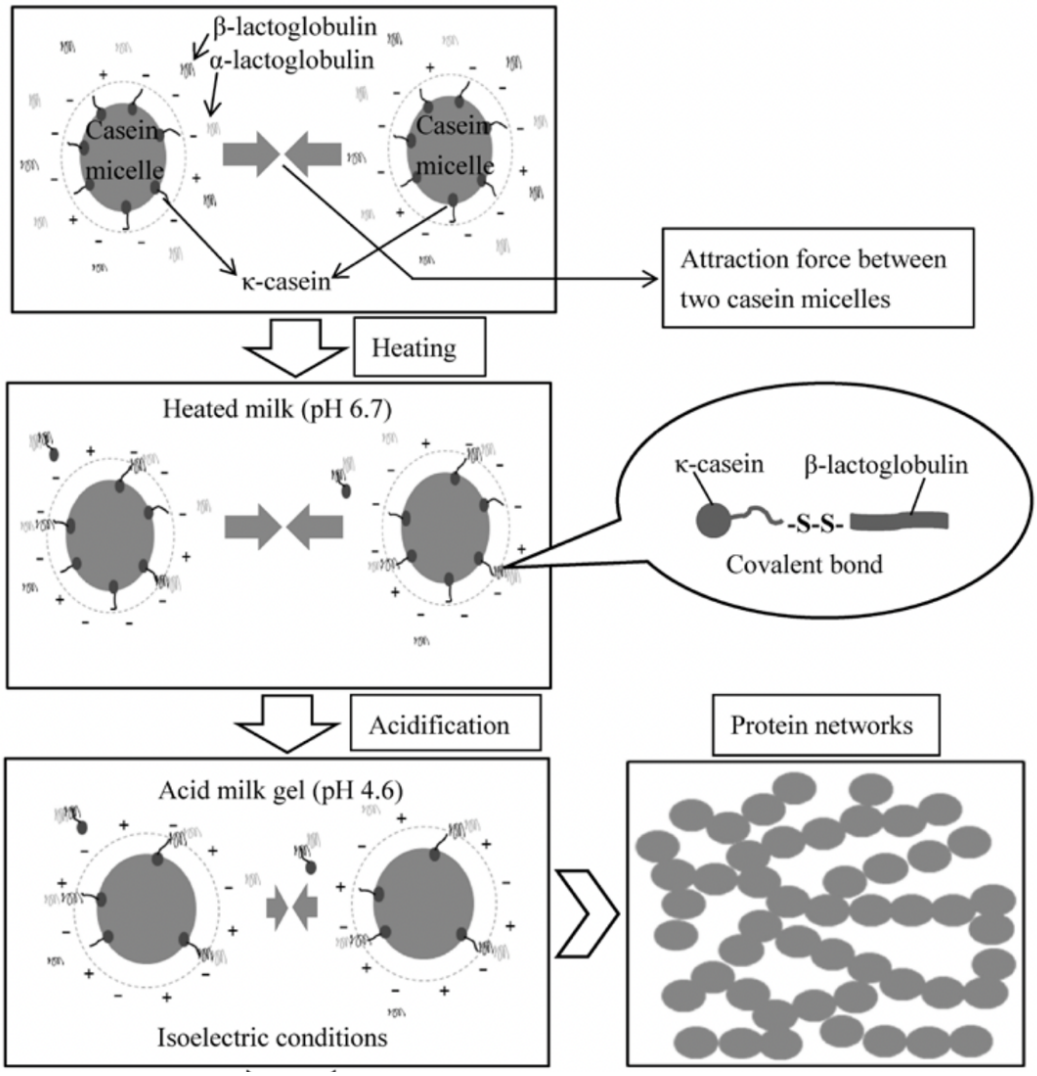


Fig. 4. The schematic representation of thermal and acid-induced denaturation of milk whey proteins and caseins with the subsequent aggregation (From: Mahomud, Katsuno, & Nishizu, 2017)

Thermal denaturation of the major serum proteins in milk, β-lactoglobulin and α-lactalbumin, is well studied. This process begins with industrial heat treatment and continues with subsequent heating of the milk, e.g. during steam frothing for the preparation of espresso-

based milk drinks. Whey proteins are sensitive to thermal treatment, especially because of the disulphide bonds present in their structure, which are easily broken, causing the serum proteins to lose their original structure. Thermal denaturation of whey proteins leads to cross-linking, unfolding, oxidation, aggregation, some changes in digestibility, Maillard reaction, as well as loss of protein bioactivity (Zhang, Zhou, Zhang, & Zhou, 2021).

Fig. 5 summarises the most common processes that occur in the protein molecule during the denaturation process.

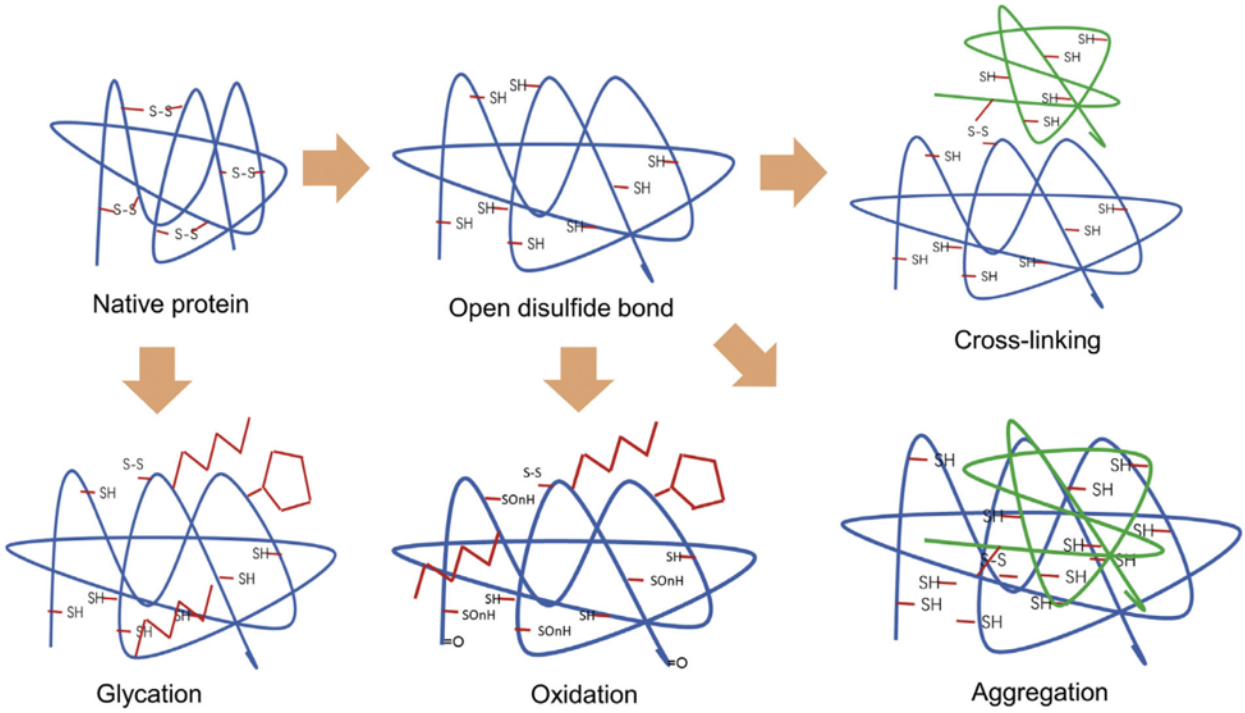


Fig. 5. The denaturation of milk whey proteins including the common effects of the process on the molecular structure of protein (From: Zhang, Zhou, Zhang, & Zhou, 2021).

As far as the physicochemical changes of the whey proteins are concerned, especially the sensory and physical properties, the denaturation caused by the heat treatment affects the appearance of the milk by increasing its whiteness. In addition, heat treatment leads to the formation of undesirable flavours through the formation of volatile sulphur compounds. As for the foaming properties of whey proteins, it has been studied that even moderate heating to 60°C negatively affects their solubility and foaming properties (Teo, Goh, Wen, Oey, Ko, Kwak, & Lee, 2016).

In general, serum proteins begin to denature at temperatures above 60°C. For example, pure α-lactalbumin denatures at an average temperature of 63.7°C. At temperatures above 80°C, α-lactalbumin denatures and begins to bind to other proteins, making the denaturation process

irreversible (Akkerman, Rauh, Christensen, Johansen, Hammershøj, & Larsen, 2016). For β-lactoglobulin, denaturation starts at 70°C and the pH of milk (6.7-6.9), but with prolonged heating (> 30 minutes) irreversible processes occur within the protein structure (Krämer, Torreggiani, & Davies, 2017). Furthermore, heating bovine lactoferrin at temperatures above 70°C leads to denaturation of about 50% of the protein. Heating to 90°C for 15 s leads to complete denaturation of the lactoferrin molecule (Zhang, Zhou, Zhang, & Zhou, 2021).

In summary, increasing the temperature up to 65°C leads to unfolding of the original structure of the globular protein. At this point, the process is reversible, but once the temperature exceeds 70°C, an irreversible aggregation process occurs, generally caused by an S–S or –SH exchange (Qian, Sun, Cao, Tuo, Jiang, & Mu, 2017).

The amphiphilic nature makes proteins good natural surface-active compounds that can stabilise air bubbles in milk foam. As thermal denaturation alters the original structure of globular proteins (Fig. 5), their functional properties are also affected. For example, when hydrogen radicals attack the amino acid residues during the oxidation process and convert them into carbonyl groups, hydrophobic amino acids are exposed on the surface of the protein. This increases the surface hydrophobicity of the protein. However, as heating progresses, the proteins begin to aggregate by forming cross-links, which leads to a decrease in the hydrophobicity of the protein (Feng et al., 2015). In addition, the involvement of milk serum proteins in the Maillard reaction leads to blocking of amino acids, resulting in an increase in the net negative charge and overall hydrophobicity of the protein (Wang & Ismail, 2012). Thus, the formation of a stable foam depends on the temperature of the heat treatment of the milk and the denaturation process should be taken into account to achieve the best stability of the foam. Fig. 6 shows the role of proteins in the formation of foam, particularly in milk.

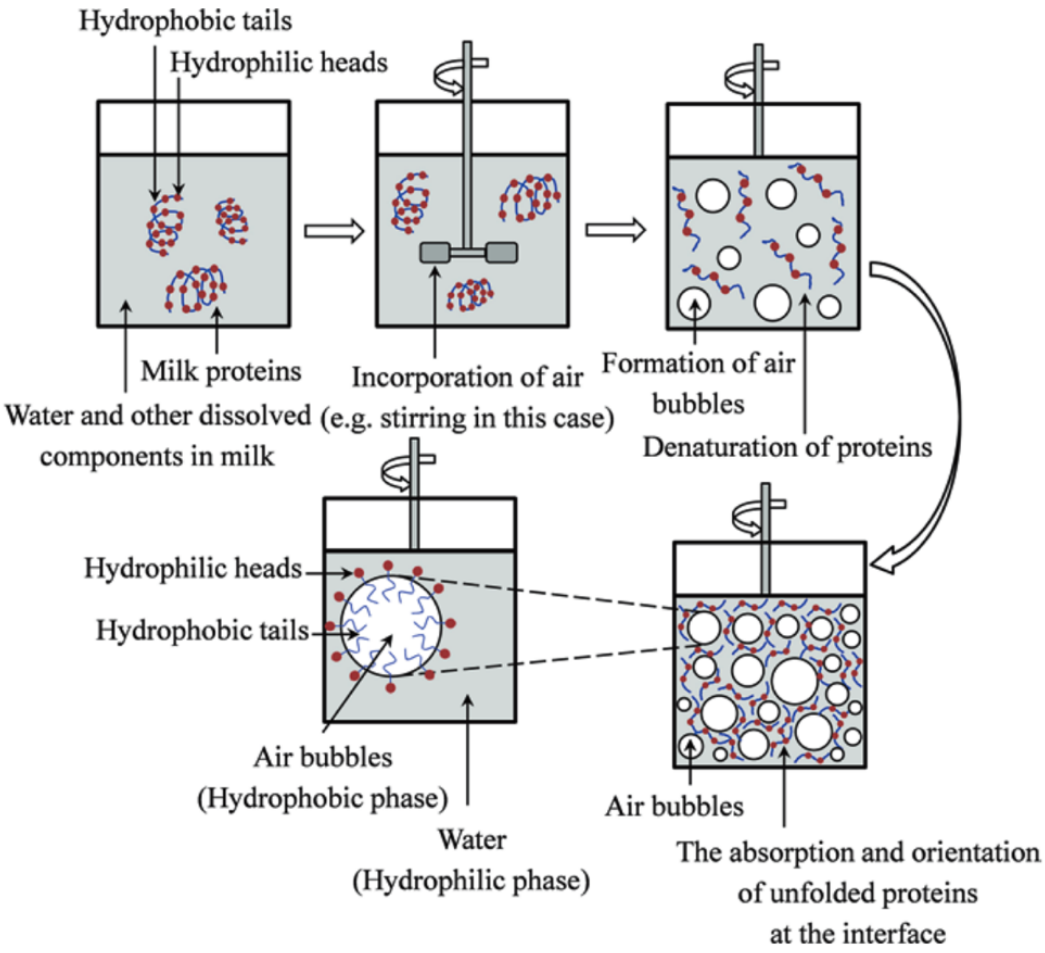


Fig. 6. Illustration of the role of proteins in foam formation (From: Ho, Bhandari, Bansal, 2020).

3.5 Freezing of milk

Freezing can be used to preserve and store milk and for barista purposes, as described in section 3. For this purpose, the milk is completely frozen and then partially thawed.

The key point of freezing is the incomplete inhibition of the biochemical and physicochemical reactions in the food matrix. Freezing slows down these reactions but does not stop them permanently. Therefore, some deteriorative reactions continue in a food during storage: non-enzymatic and enzymatic changes (Sun, 2012).

There are several ways to improve the quality of a frozen product by controlling the freezing process and the storage and preparation before and after freezing.

The speed of freezing plays a crucial role. Fast freezing is usually preferable to slow freezing because the crystallisation of the water is slow in the slower cooling process, resulting in the formation of larger ice crystals. Slow freezing leads to damage of the cell membrane and

shrinkage of the cells. In the case of slow freezing, irreversible damage to the cell structure can occur, leading to an accumulation of water in the cell structure.

Milk and dairy products usually have a relatively high water content in their structure (from 87 to 91%), so freezing dairy products during the freezing process causes many significant physical changes. For example, freezing by slow cooling damages the fat globules and leads to the formation of an undesirable flavour in the final product compared to fast freezing (Tavman & Yilmaz, 2017).

The water phases during freezing are generally divided into two groups, the free water and the bound water. The bound water remains liquid at low temperatures. The product is usually damaged when the bound water freezes in the food matrix and it is also responsible for some harmful reactions, e.g. enzymatic, during storage.

There are several processes that destabilise the food system that are associated with freezing, including: fat emulsion disruption, development of undesirable flavours, growth of pathogenic bacteria during the thawing process and protein flocculation. The severity of these processes depends on the duration of frozen storage and the final storage temperature (Bottiroli, Zhang, Aprea, Fogliano, Hettinga, & Gasperi, 2020).

As mentioned in section 3.1.4, the lipids in milk are in the form of an emulsion, which is stabilised by the MFGM surrounding the fat globules. The MGFM is sensitive to the freezing process and its damage can lead to fat separation. This problem could be solved by controlling the cooling speed. Slow freezing is more likely to damage the MFGM leading to the fat separation. Moreover, damage to the MFGM makes the fat globules susceptible to oxidation processes leading to the formation of undesirable flavours (Zhang, Mustafa, Ng-Kwai-Hang, & Zhao, 2006). Homogenisation and the addition of sugar before freezing can help to mitigate this side effect and lead to a more stable emulsion after thawing.

As for milk proteins, caseins are the most sensitive to freezing. According to Tavman & Yilmaz (2017), casein micelles can lose their stability and precipitate after thawing.

Generally, the precipitation of casein micelles after thawing makes a dairy product thicker and there might appear a layer of casein floccules on the walls of the package.

Importantly, caseins need weeks in the frozen state before they become insoluble (Bottiroli, Zhang, Aprea, Fogliano, Hettinga, & Gasperi, 2020). More, casein flocculation is reversible if the product is properly shaken after thawing and have not been stored for a long time or at temperatures lower than 23°C (Goff & Sahagian, 1996).

The main milk sugar, lactose, is responsible for about 55% of the freezing point depression. Although fast freezing has been shown to be preferable for milk fat stability, some studies (Tavman & Yilmaz, 2017) have shown that slow freezing is preferable for protein stability due to the lactose nucleation process. Koschak et al (1981) reported that lactose hydrolysis can also prevent protein destabilisation during milk freezing. The soluble sugars, whose content is increased by the conversion of lactose, delay the precipitation of milk proteins so that they remain stable for a longer period during frozen storage.

3.6 Milk frothing and foam formation and structure

A foam is usually defined as a two-phase system in which the gas bubbles are dispersed in a continuous emulsified phase. The formation of milk foams, or milk frothing, is a multi-stage process that requires simultaneous presence of gas, liquid and solid components, and surfactants to prevent newly formed bubbles from aggregation and collapsing. The foamability and foam stability of an emulsion strongly depends on the surface tension of this emulsion. The higher the surface tension, the lower the ability of an emulsion to form foam and stabilise it (Huppertz, 2010).

Milk frothing generally consists of two main steps: incorporating air and stabilising the air bubbles. There are also different techniques of frothing, including: gas, steam or air injection, mechanical agitation, and supersaturation.

During the steam, gas or air injection frothing, gas or air (cold or hot) are injected directly to the milk through small holes in the foaming apparatus. The foam properties in this case are determined by the duration of aeration, the gas flow rate, the size of the holes and the product temperature.

In case of foaming by mechanical agitation the foam will be a result of mixing of the product with the gas with an input of mechanical energy. The foam properties are mainly influenced by the intensity and duration of stirring and the product temperature.

During the supersaturation process the gas is dissolved in a liquid phase under pressure with the subsequent release of pressure (Ho, Bhandari, Bansal, 2020).

During frothing, the natural surfactants contained in the milk begin to adsorb at the interface between the air and the serum after the introduction of air (during steam frothing), reducing the surface tension and thus the energy required to form the bubble interfaces (Ritacco, 2020).

This improves foam formation and foam stability, as the reduction in surface tension promotes the formation of an interfacial film, which in turn promotes foam formation (Walstra, 1989). Milk proteins dissolve well in water and due to their amphiphilic nature and ability to reorient both hydrophobic and hydrophilic groups at the air-serum interface, they adsorb rapidly to the surface of air bubbles lowering the surface tension and preventing bubble coalescence. At the interface, the proteins unfold and rearrange the polar and nonpolar groups towards the aqueous and non-aqueous phases, respectively. This results in various interactions, mainly through hydrophobic and electrostatic interactions and hydrogen bonds between the unfolded proteins. As a result, a strong, viscous and elastic interfacial film is formed with the ability to stabilise air bubbles (Huppertz, 2010; Ho, Bhandari, Bansal, 2020).

Importantly, surface tension alone does not explain foam stability (Langevin, 2008). Surfactants also provide certain properties to interfaces, such as surface viscoelasticity, which helps stabilising the liquid film against rupture. The presence of surfactant molecules reduces surface tension and also contributes to the kinetic stabilisation of the system by slowing down the three main dynamic processes that drive liquid foams to their ultimate end including: drainage, or dehydration, coarsening and coalescence.

In foam, the air bubbles are separated by liquid films, named lamellae. The point where the lamellae meet is called the plateau border. Fig. 7 illustrates the typical structure of liquid foams. At the plateau border the pressure (P_P) is lower than the pressure along the thin liquid film region (P_L) due to the curvilinear shape of the air-serum interface. Due to this pressure difference, the fluid moves towards the plateau border.

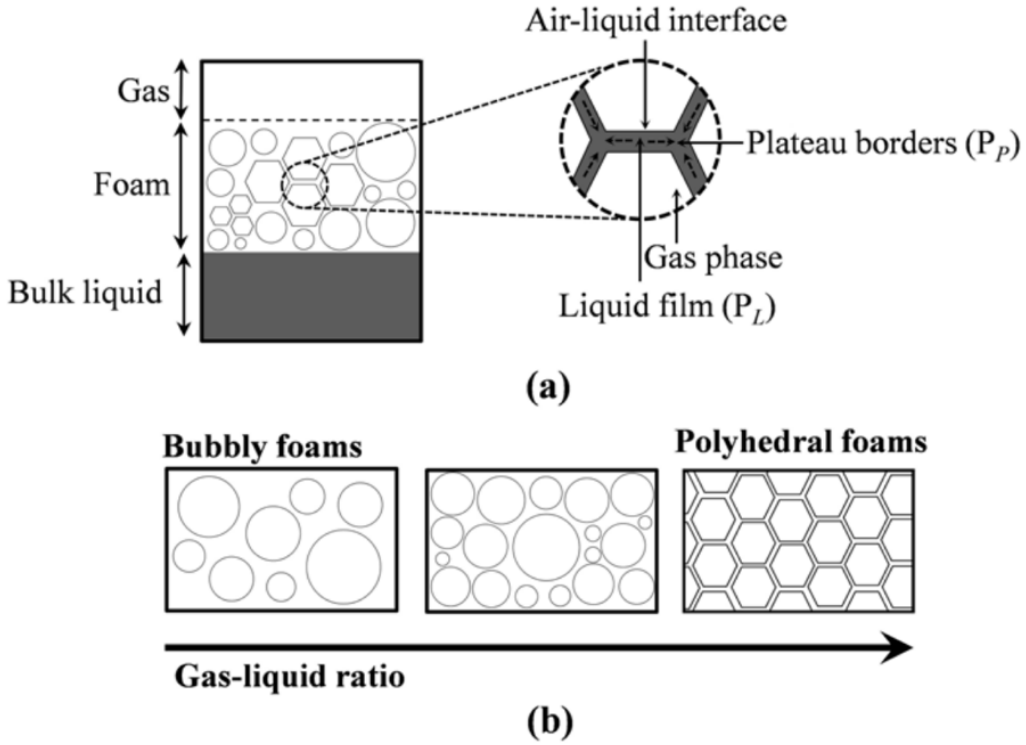


Fig. 7. a) Typical structure of foam and b) types of foam. Dotted arrows indicate the direction of movement of liquid film (From: Ho, Bhandari, Bansal, 2020).

There are two main types of foam: bubbly and polyhedral. The overall structure of the foam and the shape of the bubbles depend on the relative content of liquid and gas. When the liquid content is high, the bubbles are spherical, but as the liquid content decreases, the bubbles take on a polyhedral shape because they cluster together and press against each other as the liquid drains away and the films become thinner (Ritacco, 2020; Venkatachalam, John, & Kuppuswamy, 2015).

In the case of milk foam, the spherical foams are usually found in the initial phase of frothing, when the number of air bubbles is low and the liquid fraction is large. However, when processes of air bubbles destabilisation occur, i.e. the drainage of the liquid film and coalescence of the air bubbles, the air bubbles come into contact with each other. As a result, the air bubbles are gradually deformed into a polyhedral shape because of the difference of buoyancy forces of the air bubbles (Huppertz, 2010).

There are three main processes that lead to the destabilisation and, consequently, destruction of foams including: *drainage of liquid film from the foam*, *coalescence of air bubbles*, and *disproportionation of gas bubbles*, or *Ostwald ripening*.

During the process of *drainage of liquid film from the foam*, also known as creaming of air bubbles, the air bubbles move upwards as the liquid flows to the edges of the plateau, from where it drains through these channels into the mass of liquid. This movement occurs due to the difference in density between the air bubbles and the surrounding liquid, and due to the pressure differences at the plateau boundary and along the thin liquid film areas.
As a result, the liquid film between the air bubbles becomes thinner and the air bubbles start to move. Eventually, the bubbles coalesce and collapse because the liquid film between them ruptures.
The coalescence of air bubbles, or the merging of bubbles, results from the collapse of liquid films between the air bubbles and the subsequent formation of larger bubbles. As a result of coalescence, the number of smaller bubbles decreases and the number of large bubbles in the foam increases.
Ostwald ripening or the *disproportionation of gas bubbles* can be defined as the diffusion of gas from the small bubbles to the large ones through the continuous phase.
This diffusion of gas occurs between the difference in internal pressure between large and small bubbles. The internal pressure in larger bubbles is lower than in smaller bubbles because of the higher Laplace pressure difference across the curved surface of an air bubble. As the bubbles with the significantly different internal pressure approach each other, the air diffuses from the small bubbles into the larger ones. This causes the larger bubbles to become larger and the small bubbles to disappear. This leads to destabilisation and collapse of the foam (Huppertz, 2010; Ho, Bhandari, Bansal, 2020).

3.7 Lipid oxidation

Oxidation of lipids is one of the most important factors affecting the shelf life of food, as it leads to chemical changes that affect taste and cause food spoilage. This process is considered one of the main factors in the loss of milk quality during processing and storage.
Dairy milk, as a complex food matrix, can undergo lipolysis (section 3.1.3) and oxidation due to industrial heat treatment and storage. This is due to the presence of omega-6 linolenic acid and omega-3 alpha-linolenic acid in its composition, which are susceptible to oxidation due to the presence of multiple double bonds (Dias et al., 2020).
Lipid peroxidation is the breakdown of lipids due to oxidative damage, making their assessment a useful marker of oxidative stress. Indeed, polyunsaturated lipids containing carbon-carbon double

bonds in the lipid bilayers of the membrane are susceptible to oxidative attack, typically by reactive oxygen species (ROS), leading to the formation of end products such as malondialdehyde (MDA) (Custodio-Mendoza, Caamaño-Fernandez, Lage, Almeida, Lorenzo, Carro, 2022). "Reactive oxygen species" (ROS) can be broadly defined as a set of derivatives of molecular oxygen that occur as a normal feature of aerobic life (Sies & Jones, 2020).

MDA is formed from polyunsaturated fatty acids when a carbon-carbon double bond is attacked by a free radical, leading to the formation of an unsaturated lipid radical with the release of H_2O. Further O_2 deposition leads to the formation of peroxyl radicals and lipid hydroperoxides. The peroxyl radical can develop cyclisation thanks to its cis-double bond, which is homoallylic to the peroxyl group. The intermediate free radicals formed after cyclisation can cyclise again to form cycling endoperoxides, and they are cleaved to generate MDA (Fig. 8) (Mas-Bargues, Escrivá, Dromant, Borrás, & Viña, 2021). The measurement of MDA content in milk is directly proportional to the extent of lipid peroxidation.

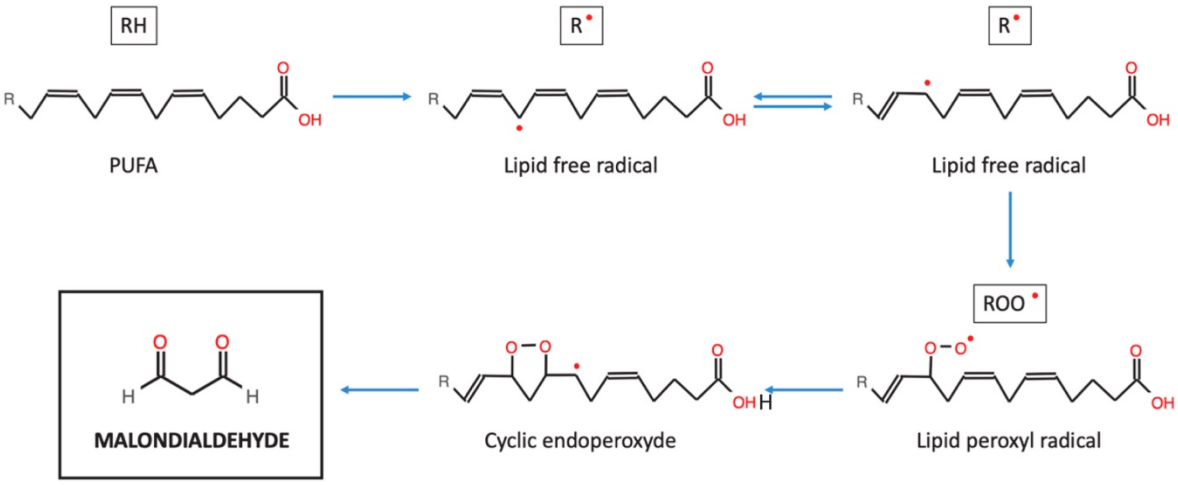


Figure 8. Lipid peroxidation and formation of malondialdehyde (MDA). (From Mas-Bargues et al., 2021)

The studies conducted by Santini showed that cappuccino samples prepared with pasteurised milk (HQ) had a higher MDA content compared to HQ milk samples within the whole temperature range (60°C - 80°C), suggesting that the lipid peroxidation process is faster in cappuccino than in milk without coffee (Santini, 2022).

Oxidation of lipids is another source of new aroma compounds. Volatiles such as pentanal and hexanal – products of lipid oxidation – are formed in milk during storage and are therefore

an important marker for the freshness of UHT milk, as it has a longer shelf life (Chávez-Servín, Castellote, & López-Sabater, 2008).

Lipid oxidation comprises several stages: the initial stage, in which alkyl and peroxy radicals of the lipid are formed from the polyunsaturated fatty acids; in the next step of the reaction, the compounds formed in the previous step react with the fatty acids to form hydroperoxides; these hydroperoxides are further converted into epoxy, hydroxy, dihydroxy, trihydroxy or ketone fatty acid metabolites known as oxylipins; further decomposition of the oxylipins via β-scission leads to the formation of secondary products, which are mostly volatiles, i.e. alcohols, ketones, alkenals, short-chain aldehydes (Yang, Schmelzer, Georgi, Hammock, 2009). The methyl ketones and the aldehydes are responsible for the rancid flavour, which is also described as tallowy, coconut-like or cardboard-like (Kilic-Akyilmaz, Ozer, Bulat, & Topcu, 2022).

4. AIM OF THE RESEARCH PROJECT

The current project was performed in cooperation with the Research and Innovation Coffee Hub (RICH) and aimed to evaluate which type of milk, including freeze-distilled samples, is more suitable for frothing with the steam nozzle of the traditional espresso machine. The technique of freeze-distilling (FD) milk was first used at the World Barista Championship 2017. This method was mainly used to intensify the flavour of milk by reducing the amount of water in the milk.

The aim of comparing of differently heat-treated milk types was to evaluate which type of milk was more suitable for frothing by specifically investigating the chemical and physical properties of the *microfoams* produced from the different types of processed milk treated with the steamer of a professional coffee machine at different temperatures (30 – 80°C in increments of 10°C). A sensory panel was also conducted to evaluate whether the difference between pasteurised and UHT milk could be detected by untrained sensory judges and evaluate personal preferences of milk type of assessors. Studies of FD milk aimed to compare foaming properties of milk samples before and after freezing/thawing process as well as to evaluate possible sensory differences between non-treated and FD milk.

To this end, the main objectives of the project were:

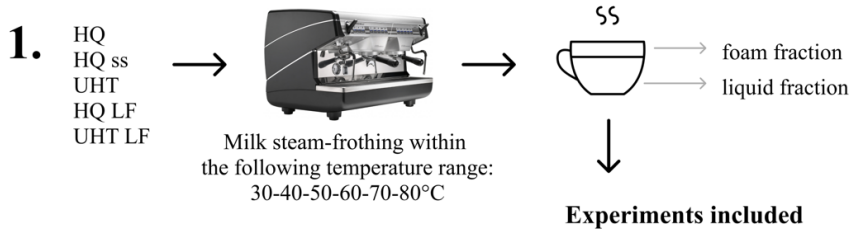
- Analysis of the physical properties of the *microfoam* produced from the different types of processed milk treated with the steamer of a professional coffee machine Nuova Simonelli Appia II at different temperatures (30 – 80°C in steps of 10°C) were studied including: stability of milk *microfoam*; viscosity of milk *microfoam*; analysis of bubble size distribution; measurement of extension and stability of milk *microfoam* as a function of temperature, protein content and milk type, i.e. pasteurised or UHT processed.

- Studies of the chemical properties of milk samples: protein and lactose content; measurement of thiol groups to assess the degree of denaturation of serum proteins; particle size distribution of regular and freeze-distilled milk samples.

- The analysis of the chemical properties of the milk and the physical properties of the milk *microfoam* was followed by the sensory analysis of the milk samples to see if the sensory testers could distinguish between pasteurised and UHT and between regular and freeze-distilled milk samples.

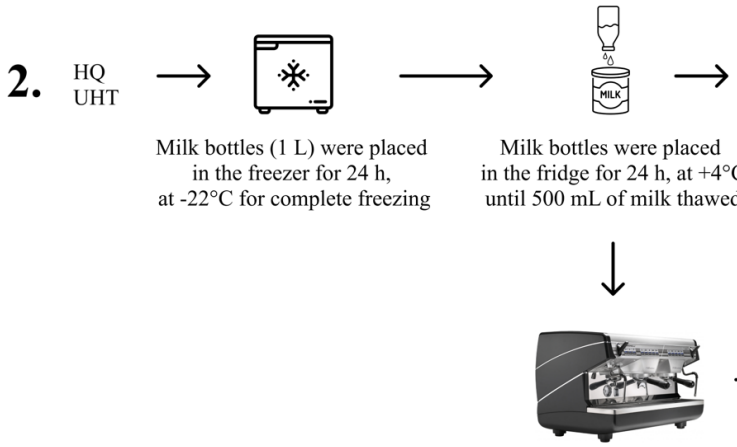
The outcome of the project was a versatile analysis of milk *microfoam* to achieve more stable frothing results for coffee professionals and a deeper understanding of how the type of milk processing, including freeze-distillation, depends on the physical properties of the *microfoam*.

5. EXPERIMENTAL DESIGN



Experiments included following measurements:

- Protein content measurement (Bradford method, SDS-PAGE)
- Determination of thiol groups (Ellman's method)
- Foam stability analysis
- Foam viscosity measurements
- Analysis of bubble size distribution in foam
- Sensory discrimination tests



Experiments on cold milk included following measurements:

- Protein content (Bradford method, SDS-PAGE)
- Lactose content (UV-method)
- Particle size distribution (Mastersizer)

Experiments on frothed milk included following measurements:

- Foam viscosity
- Foam extension and stability
- Sensory discrimination tests

List of acronyms:

HQ – high-quality pasteurised full-lactose whole dairy milk

UHT – ultra-hight temperature treated full-lactose whole dairy milk

HQ ss – pasteurised full-lactose semi-skimmed dairy milk

HQ LF – pasteurised lactose-free whole dairy milk

UHT LF – ultra-hight temperature treated lactose-free whole dairy milk

FD – freeze-distilled dairy milk

6. MATERIALS AND METHODS

Cow milk was purchased from a local supermarket. The commercial milk types investigated are shown in Table 1 together with the corresponding proximate composition.

Table 1. Commercial milk types investigated and proximate composition (grams per 100 mL) described in the information sheet provided by manufacturers.

Milk type	Fats	Proteins	Sugars
Pasteurised whole high-quality (HQ)	3.6	3.2	5.0
Ultra-high temperature whole (UHT)	3.6	3,1	4.8
Microfiltered pasteurised whole without lactose (HQ LF)	3.0	3.2	4.9[a]
UHT whole without lactose (UHT LF)	3.6	3.2	4.9[a]
Pasteurised semi-skimmed (HQ SS)	1.6	3.2	4.9

[a] From which lactose is less than 0.1 g per 100 ml

Bio-Rad protein assay dye reagent concentrate was obtained from Bio-Rad Laboratories (USA). Tris base, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent) and acetic acid were purchased from Sigma-Aldrich Company (U.K.).

The equipment for milk heating, coffee beans grinding, and espresso preparation was obtained from the Nuova Simonelli SpA (Belforte del Chienti, Italy): Appia II semi-automatic two group espresso machines; Mythos Plus on demand espresso grinder.

6.1. Milk foam preparation

Milk with a volume of 150 mL and an initial temperature of 8±1°C was heated with the steam nozzle (steamer) of the coffee machine in a metal jug (350 mL). Milk frothing consisted of two steps: First, the nozzle was immersed in milk and milk was heated to 35±3°C; then the steamer was lifted to the surface of the milk to introduce air, and milk was steamed until it reached the required temperature within the range of 30°C to 80°C in increments of 10°C.

The duration of frothing depends on the desired final temperature of milk and averages 20 seconds. The jug was kept in the refrigerator before frothing milk to ensure temperature uniformity across samples.
All foams met the requirements for *microfoam*, i.e., they were finely textured, had a glossy surface, and consisted of microscopic uniform bubbles.

6.2. Milk freeze-distillation

For freeze-distillation, 1 L of whole pasteurised (HQ) or whole ultra-hight temperature treated (UHT) milk was placed in the freezer at -22°C for 24 h for the complete freezing. After that milk was thawed at 4°C until half (500 mL) of the initial volume was melted to the glass bottle. All samples were analysed at the same day after thawing and stored at 4°C.
Studies of freeze-distilled milk were performed at the Aristotle University of Thessaloniki (Greece), School of Food Chemistry and Technology, in cooperation with prof. Adamantini Paraskevopoulou in terms of the exchange research activity.

6.3. Milk protein content and determination of reactive thiol groups

To measure the whey protein content, caseins were precipitated from milk as follows: each milk sample was heated with a steamer at a temperature range of 30°C to 90°C in increments of 10°C. Then, 10% acetic acid (v/v) was added to 30 mL of each milk until the pH reached 4.6. The samples were then centrifuged at 3000 g for 20 minutes at 5°C. On the supernatant the total whey protein content was determined according to the method of Bradford (Bradford, 1976) using a UV-VIS spectrophotometer UV-2450 (Shimadzu Corporation, Japan), whereas thiol groups (expressed as cysteine) were determined by the modified Ellman method (Ellman, 1959), following the protocol by Cosio and co-workers (Cosio, Mannino, & Buratti, 2000). The content of free thiol groups was approximated according to the calibration curve that was constructed using N-acetyl-L-cysteine (98%, AlfaAesar by ThermoFisher, Germany) in the concentration range from 1 to 100 μM. The normalized free thiol group concentration was expressed in nmol mg^{-1} and calculated by the division of the thiol concentration (nmol mL^{-1}) by the protein concentration (mg mL^{-1}).

6.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the Laemmli (1970), on HQ milk and UHT milk. The three thermal treatments were: not heated (NT) and "heated" up to 50 and 80°C respectively by the steamer. After thermal treatment, milk was put in an ice bath for 5 min and then centrifuged at 27,000 g for 15 min. Subsequently, caseins were precipitated by adding 10% acetic acid until pH 4.6 was reached and then centrifuged at 27,000g for 15 min. The supernatant was recovered and 30 µg of each sample was loaded in the electrophoretic gel. Electrophoresis was carried out at 4°C with a constant voltage of 200 V, under reducing conditions using a 15% acrylamide-bis-acrylamide solution and the Mini Protean III device (Bio-Rad, gel size 7 x 8 cm x 0.75 mm). Proteins were stained by Coomassie Blue (0.1% Coomassie Brilliant Blue R250 in 50% methanol and 10% acetic acid). The markers used were Bio-Rad molecular weight standards, low range (phosphorylase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45.0 kDa; carbonic anhydrase, 31.0 kDa; soybean trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa).

Other samples such as pasteurised (HQ), ultra-high temperature (UHT), freeze-distilled pasteurised (HQ FD), and freeze-distilled ultra-high temperature (UHT FD) milk samples were subjected to a different electrophoresis analysis, using a 12% Bis-Tris polyacrylamide gel in the presence of 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (Thermo Fisher Scientific, USA). The electrophoresis was carried out at 4°C with a constant voltage of 200 V, under reducing conditions and the Mini Protean III device (Bio-Rad, gel size 7 x 8 cm x 0.75 mm). BlueStar Prestained Protein Marker (Nippon Genetics Europe GMBH, Germany) were used as protein markers covering molecular weights from 10 to 170 kDa.

Obtained gel was destained in the solution consisting of 50% methanol, 10% acetic acid. The gel was left with the solution until the moment when the bands were visible and the blue background was gone.

In all cases, the destained gel was scanned at 600 dpi and subjected to image analysis using PDQuest software (Version 7.1.1; Bio-Rad Laboratories, Hercules, CA), according to the protocol provided by the manufacturer in order to calculate the molecular mass and the normalized quantity of each protein band in the gel.

6.5. Lactose content measurement

The quantitative determination of lactose was performed using the Lactose/D-Galactose kit (Boehringer Mannheim / R-Biopharm, Darmstadt) following the instruction in the datasheet. The protocol was based on the principle that at pH 6.6 in the presence of the enzyme β-galactosidase and water lactose was hydrolysed to D-glucose and D-galactose. In presence of the enzyme β-galactose dehydrogenase (Gal-DH) at pH 8.6 D-galactose was oxidized by nicotinamide-adenine dinucleotide (NAD) to D-galactonic acid. The amount of NADH formed on the previous step was stoichiometric to the amount of lactose, and D-galactose, respectively.

For protein precipitation, 2 g of milk was poured into a 100 mL volumetric flask and diluted with 20 mL of water. Then 1.0 mL of 3M trichloroacetic acid was added. The solution was incubated for 10 min, centrifuged at 3000 g for 15 min at 5 °C, neutralised with 1M NaOH, filled up to 100 mL with water, and filtered with Whatman filter to get a clear solution for the assay.

To prepare samples for lactose measurement, 100 µL of a solution containing citrate buffer of pH 6.6, NAD, and magnesium sulphate, dissolved in ultrapure water was mixed with 25 µL of a β-galactosidase suspension (100 U) and 50 µL of deproteinised milk sample. The mixture was vortexed incubated for 20 min at 25 °C. After the incubation, 500 µL of potassium diphosphate buffer pH 8.6 and 950 µL of ultrapure water was added to the solution. The mixture was vortexed, incubated for 2 min at 25°C, and the absorbance (A1) of the solution was measured at 340 nm using UV-Vis spectrophotometer (Hitachi U-2000, Japan). Then 25 µL of 40 U galactose dehydrogenase suspension was added, the mixture was vortexed and incubated for 15 min at 25°C. After the incubation, the absorbance (A2) was measured. The blank solution was prepared according to the same protocol, without adding the milk sample.

Lactose content in milk samples was calculated according to the following equations:

1. $\Delta A_{lactose} = (A2 - A1)_{sample} - (A2 - A1)_{blank}$

2. $c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A_{lactose} [g\ L^{-1}]$,

where V is the final volume (mL), v is the sample volume (mL), MW is the molecular weight of the substance assayed (g mol^{-1}), d is the light path (cm), ε is the extinction coefficient of NADH at 340 nm and equals 6.3 L mol^{-1} cm^{-1}.

6.6. HPLC-VWD analysis of espresso samples

To measure foam stability 100% roasted Arabica coffee beans were used to make the cappuccino. The espresso blend consisted of beans from Brazil (Mundo Novo, natural), India (Plantation, S795 & Kent, fully washed) and Ethiopia (Yirgacheffe, Heirloom, fully washed). The content of caffeine, trigonelline and chlorogenic acid (3-O-caffeoylquinic acid (3-CQA), 5-O-caffeoylquinic acid (5-CQA), 3,5-dicaffeoylquinic acid (3,5-diCQA) in the brewed espresso was measured by a HPLC-VWD using a Gemini C18 110A analytical column for separation (250 × 3 mm I.D., 5 μm, Phenomenex, Cheshire, U.K), following the protocol described by Khamitova et al (2020). This analysis was performed in collaboration with Dr. Massimo Ricciutelli (HPLC-MS Laboratory, University of Camerino).

6.7. Foam decrease rate

To prepare cappuccino, 150 mL of each type of milk was heated to the following temperatures using the steamer of the coffee maker: 30-40-50-60-70-80°C. The initial temperature of milk was 8±1°C.

The frothed milk was carefully poured into a glass cup for espresso (Section 6.6). The cup was then placed in front of a fixed camera, and a ruler was placed next to the cup. The camera took pictures at fixed times: 30 seconds, 1 minute, 1 minute 30 seconds, 2, 4, 5, 6, 8, 10, 13, 16, 20, 25, 30, 35, 40, and 45 minutes. The images obtained were analysed using Image-Pro Plus software (version 5.1, Media Cybernetics, Inc., USA). Three measurements of foam height were taken for each time. Foam stability was expressed in values of foam decrease rate (FDR). The FDR value was considered as a slope (the change in log(Y) when the X changes by 1.0) that quantifies the steepness of the line created for each temperature of steam heating and each milk type. For that a straight line was fitted on a semi-log graph, where X axis is linear and Y axis is logarithmic using the Prism 9 Software. The FDR value is inversely related to the foam stability, i.e., the higher the FDR value, the less stable the milk foam.

The FDR measurements were performed in the presence of espresso, i.e., in the form of cappuccino, and in this analysis the *microfoams* were evaluated in static mode for the first time and were expected to most closely resemble the real conditions of cappuccino preparation. This experimental setup could help in further studies on the dependence of espresso coffee with different composition on the FDR of the *microfoam*.

6.8. Foam viscosity

Milk *microfoams* prepared at six temperatures (30, 40, 50, 60, 70, 80°C) were carefully transferred with a spoon to a rheometer plate 30 seconds after foaming. Viscosity tests were carried out using a rotary rheometer (Kinexus Lab+, Malvern Instruments Limited, U.K.). Experimental parameters included: plate geometry PU20 SR3863 SS: PL61 ST S2711 SS; gap 2 mm, shear stress rate from 0.01 s^{-1} to 0.1 s^{-1}; 10 samples per decade; test duration 1.5 min.

The viscosity of freeze-distilled milk samples was analysed at 50, 60, and 70°C respectively using the HAAKE Viscotester iQ (ThermoFisher Scientific, USA). For the analysis following experimental parameters were used: 25°C; cylindric rotor CC25 DIN/Ti; 15 samples per decade; test duration 150 s; shear stress rate from 0.01 s^{-1} to 0.2 s^{-1}.

To quantify the characteristic numerical parameters, all flow curves were analysed using the power law model, which is a general mathematical model that describes most flow behaviours and represents the relationship between the shear rate and the shear stress according to the following equation:

$$\sigma = K\gamma^n,$$

where σ is the shear stress, K is the consistency factor corresponding to the viscosity of the sample, γ is the shear rate, and n is the flow behaviour index.

6.9. Foam expansion, stability, and overrun

The comparison of foaming properties (expansion, stability and overrun) of regular and freeze-distilled milk samples was carried out at three temperatures most commonly used for

the preparation of espresso-based milk drinks: 50, 60 and 70°C, respectively. The milk sample (200 mL) was poured into a 350 mL stainless steel milk-frothing jug at 5 °C and then frothed using the steam nozzle of the espresso machine according to the protocol described in section 6.1. The average foaming time depended on the final temperature of the milk and was 10-15 s on average. After frothing, milk *microfoams* were immediately poured into a graduated cylinder and foam and liquid volume straight after poring and after 15 min were recorded. The experiments were performed at 25°C.

Foam stability (% FS), foam expansion (% FE), and overrun (%) were calculated according to the following equations respectively:

$$\%\, FS = \frac{foam\ volume\ after\ 15\ \text{min}\ \ (mL)}{initial\ foam\ volume\ (mL)} \times 100$$

$$\%\, FE = \frac{initial\ foam\ volume\ (mL)}{initial\ liquid\ volume\ (mL)} \times 100$$

$$\%\, Overrun = \left(\frac{mass_{liquid} - mass_{foam}}{mass_{foam}}\right) \times 100$$

6.10. Foam strength

Microfoam strength was analysed using the TA-XT2 Texture analyser (Stable Microsystems, Godalming, UK). A compression test was performed using the 25 mm aluminium cylinder probe. Test speed was set at 1.0 mm s^{-1} with the distance of 25 mm. The maximum force (g) was calculated from the resulting force–time curves.

6.11. Bubble size distribution in foam

To measure bubble size distribution, milk samples were foamed and a glass was carefully dipped into the milk foam and wiped from one side. Images of the milk froths were taken using the 4x/0.10 objective of the Leica DM2500 microscope with a built-in DFC320 digital camera (Germany) with the following image parameters: Exposure 3.91 ms; Saturation 1.0; Range 0.40; Gain 1.2x. Photographs were taken at 5 random locations on a glass 30 seconds after foaming and then after 10 minutes of the experiment.

The images were then analysed using ImageJ software (version 2.1.0, National Institute of Health, USA). For each image, a fixed central region was evaluated to avoid interference from uneven side bubbles. For this purpose, an optimised Hough Circle Transform plugin was implemented:

```
#@ImagePlus image
#@Double hough_threshold (value=0.55)

// Contrast
run("Enhance Local Contrast (CLAHE)", "blocksize=127 histogram=256 maximum=3
mask=*None* fast_(less_accurate)");

//Crop edges, keep only center of image
makeRectangle(306, 250, 1664, 1030);
run("Crop");
// Some housekeeping
original = getTitle();
close("\\Others");
roiManager("Reset");

// Preprocessing make 32 bit, flatten and subtract BG
run("32-bit");
run("Median...", "radius=2");
run("Duplicate...", "title=BG");
run("Gaussian Blur...", "sigma=10");
imageCalculator("Divide create 32-bit", original,"BG");
rename("Hough");

// Apply laplacian to enhance edges
run("FeatureJ Laplacian", "compute smoothing=3");

// Auto-threshold and over-estimate particles
```

```
setAutoThreshold("Huang dark");

// Remove particles smaller than 100px2
run("Analyze Particles...", "size=100-Infinity pixel show=Masks");

// Erode slightly to stop hough from finding too many circles
run("Erode");

// suggestion: Speed up, use a smaller image
//run("Scale...", "x=0.5 y=0.5 width=512 height=512 interpolation=Bilinear create");

// Use a Hough transform on the mask to find likely candidates.
run("Hough Circle Transform","minRadius=13, maxRadius=61, inc=2, minCircles=1,
maxCircles=1000, threshold="+hough_threshold+", resolution=500, ratio=1.0,
bandwidth=10, local_radius=10, reduce show_mask show_scores results_table");

// BUG: ImageJ does not wait for the Hough plugin to finish, so we have to check and wait
until it is done.
// we do this by checking the existence of the 'Score map' image
done = false;
while (!done) {
        done = isOpen("Score map");
        wait(500);
}

// Merge all images for easy viewing
selectImage(original);

// Need to make all 32-bit to match score image
run("32-bit");

selectImage("Mask of Hough Laplacian");
```

```
run("32-bit");

// Give nice lookup table
selectImage("Score map");
resetMinAndMax();
run("mpl-viridis");

// Merge and display as stack
run("Merge Channels...", "c1=["+original+"] c2=[Mask of Hough Laplacian] c3=[Score
map] create keep");
Stack.setDisplayMode("color");
rename(original+"_Results");

close("\\Others");
```

After the implementation of the plugin described above, results table contained number of circles and sizes. The plugin described above helped to overcome some limitations in evaluation of bubble size distribution including: low contrast of inner part of the bubbles, overlapping of bubbles, and interfering smaller bubbles laying on the lower layers of foam.

6.12. Particle size distribution

The particle size measurement of milk samples was performed according to the method of Ransmark (Ransmark, Svensson, Svedberg, Göransson, & Skoglund, 2019). According to the methodology, milk was diluted 1:1 v/v with redistilled water and added to the dispersant (water). The particle size distribution of samples was measured using a laser diffraction particle size analyser Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). The absorption index was set at 0.001, the refractive index (RI) was set at 1.46 for milk for the red laser (632.8 nm), and 1.47 for blue laser (470 nm). The RI of water for the red laser was set to 1.33 (632.8 nm). Dispersant temperature was 20°C ± 2°C The laser obscuration was adjusted at approx. 10%. The surface-weighted mean diameter D[3,2] and the volume-weighted mean diameter D[4,3] were determined to characterise the size of particles.

The Malvern MasterSizer 2000 “general purpose” analysis model based on volume distributions was used to evaluate data. Triplicate measurements were performed for each sample.

According to the methodology suggested by Ransmark et al., (2019) samples were also analysed with a casein micelle dissociating agent (CMDA, 3.125 g of Tween 20 and 9.375 g of EDTA in 200 mL of deionised water.). To dissociate casein micelles 2 mL of milk was mixed with 2 mL of redistilled water and 2 mL of CMDA. The mixture was heated to 40°C and the pH was adjusted to 10 with 1M NaOH.

6.13. Sensory discrimination tests

Thirty-one untrained panellists performed two sensory discrimination tests: the Triangle Test (ISO 4120:2021) and the Duo-Trio Test (ISO 10399:2017). The panel for the triangle test consisted of three samples with three-digit random codes. Two samples were always identical and one was different. Participants were asked to identify the different sample, describe the intensity of the difference from mild to extreme, indicate some characteristics of the different sample (in free form), and note which sample they preferred most: the different one or the two identical samples. The milk samples for the sensory tests (triangle and duo-trio tests) were heated to 55±1°C using the steamer of the coffee machine and then randomly served to the testers in identical 200 mL ceramic cups. Each examiner stayed in a personal area separated from the others and provided by the red light to avoid differentiation based on the colour of the milk sample. Panellists were asked to evaluate both the aroma and taste of the milk samples using the cupping spoon. Between each sample, they were provided with filtered water to rinse their mouths.

Questionnaires (Fig.9a) were evaluated for the first part of the test when the panellists were asked to choose the different sample. However, for the analysis of characteristics and the degree of difference between samples only correct answers were considered.

a Triangle test scoresheet

Name_____

Date_____

Product_____ _____

Two of these three samples are identical, the third is different. Please analyze aroma first and then taste a sample. Then mark the odd sample.

1. Taste the samples in the order indicated and identify the odd sample.

Code:

521 [ ]
450 [ ]
764 [ ]

2. Indicate the degree of difference between the duplicate samples and the odd sample. If possible, please describe the difference between samples.

Slight [ ]
Moderate [ ]
Much [ ]
Extreme [ ]

Please in own words describe the difference (if possible):

_____
_____
_____

3. Which sample do you prefer better?

Odd sample_____
Duplicates_____

b Paired comparison test scoresheet

Name_____
Date_____
Product_____

You are presented with two sets of pairs of coded samples and a Control Sample. Please taster the Control Sample first. Then taste other samples and indicate which of samples has same taste of Control Sample.

Please analyse aroma first (smell) and then taste the sample (mouth).

You should cross the sample identical to the Control Sample.

Code:

Pair 1.	904 [ ]	260 [ ]
Pair 2.	417 [ ]	587 [ ]

Fig. 9. The example of the questionnaire for a) the triangle test, b) the duo-trio test.

For the duo-trio test, panellists were provided with two randomly coded (3-digit) pairs of samples and a control sample. Panellists were asked to taste the control sample first and then find the control sample within each pair. Answers were recorded in the separated questioner for the duo-trio test (Fig.9b).

The triangle test for the freeze-distilled milk samples were performed among 40 panellists using the identical procedure as for regular milk samples. Panellists were asked to find the different sample within three identical cappuccino samples. The cappuccino for the triangle test were made according to the following procedure: three shots of espresso (15 mL; blended freshly roasted coffee beans by Arabica varieties) and milk were prepared simultaneously and immediately served to the assessors together with a glass of filtered water.
The samples for the triangle tests were coded according to the Table 2. The questionnaire for the triangle test with the freeze-distilled milk samples was identical to one presented at the Fig.9a.

Table 2. Design of the sensory discrimination test (triangle test).

AAB	BBA	BAA	AAB
BAA	AAB	AAB	BBA
BAB	ABA	BAB	AAB
ABA	BAB	ABA	BAA
ABB	BBA	BAB	ABA
BAA	AAB	ABA	BAB
AAB	BBA	BAB	ABB
BBA	AAB	ABB	BAA
ABA	BBA	BAA	ABA
BAB	BAA	BBA	BAB

6.14. Statistical analysis

The results were analysed by Prism 9 Software (version 9.2; GraphPad Software, LLC, USA). The statistical difference between the samples were investigated by the analysis of variance (ANOVA). Samples were considered as statistically different when p-values did not exceed

0.05. All experiments were performed in triplicates. Results of sensory discrimination tests were analysed by XLSTAT software (Addinsoft, France).

7. RESULTS AND DISCUSSION

7.1. Milk protein content and determination of reactive thiol groups

Formation and stability of milk foams depend on surfactants, i.e., proteins, and foaming temperature. According to the previous studies (Borcherding, Hoffmann, Lorenzen, & Schrader, 2008a; Brooker, Anderson, & Andrews, 1986), caseins are more thermally stable than whey proteins and mainly nonmicellar caseins and native whey proteins are adsorbed on the surface of the air-serum interface. At the same time, the partial denaturation of whey proteins at temperatures above 60°C (Anema, & Li, 2000) seems to weaken the foaming properties of milk (Borcherding, Lorenzen, Hoffmann, & Schrader, 2008b). For this reason, caseins were precipitated and the amount of serum proteins in pasteurised and UHT milk was measured to analyse the dependence of industrial heat treatment on whey protein content and consequently on milk foaming properties.

The measurement of the total protein content of milk was consistent with the information on the packages and averaged 31.74±0.76 mg mL^{-1} for pasteurised milk with lactose (HQ), 32.40±2.08 mg mL^{-1} for UHT milk with lactose, 33.22±0.61 mg mL^{-1} for pasteurised lactose-free milk (HQ LF), 32.51±1.09 mg mL^{-1} for UHT lactose-free milk (UHT LF), and 32.68±0.87 mg mL^{-1} for pasteurised semi-skimmed milk with lactose (HQ SS).

Table 3 shows the whey protein content of the different types of milk at increased temperature and shows that there was no significant difference between full-lactose and lactose-free samples. Based on that data, in this section we consider only the results of HQ and UHT with lactose, since the presence or absence of lactose did not significantly affect the protein content and, consequently, the foamability.

Table 3. Experimental data on whey protein content in mg ml^{-1} measured by the Bradford method in pasteurised whole milk with lactose (HQ), UHT whole milk with lactose (UHT), pasteurised whole milk without lactose (HQ LF), UHT whole milk without lactose (UHT LF), semi-skimmed pasteurised milk with lactose (HQ ss). Results are mean values and standard deviations of three replicates

Temperature (°C)	HQ (whey proteins, mg ml^{-1})	UHT (whey proteins, mg ml^{-1})	HQ LF (whey proteins, mg ml^{-1})	UHT LF (whey proteins, mg ml^{-1})	HQ ss (whey proteins, mg ml^{-1})
NH	6.63±2.43	1.38±0.30	6.51±1.32	1.29±0.26	6.32±1.24
30	6.66±1.04	1.46±0.35	6.32±1.56	1.18±0.19	6.43±1.17
40	7.37±1.11	1.40±0.57	6.87±1.29	1.22±0.32	6.98±1.02
50	7.55±1.46	1.37±0.37	7.01±1.74	1.31±0.24	7.20±1.28
60	7.55±1.92	1.34±0.35	7.16±1.02	1.30±0.21	7.22±1.39
70	7.43±1.09	1.33±0.45	6.98±1.27	1.28±0.34	7.08±1.44
80	6.15±1.30	1.30±0.45	6.07±1.15	1.12±0.27	6.01±1.12
90	4.67±1.52	1.09±0.34	5.21±1.10	0.96±0.18	5.10±0.99

Whey protein concentration in supernatants (obtained as described in the section 6.3) of pasteurised milk was significantly higher ($P < 0.05$) than in UHT samples (Table 3). This result supports the idea (Qi, Ren, Xiao, & Tomasula, 2015) that UHT treatment noticeably affects the protein structure and functional properties of milk whey proteins. A significant difference between whey protein content in pasteurised and UHT milk supernatants was mainly associated with the industrial treatment. Temperature plays an important role in the foaming properties of milk and alters the native protein conformation. Whey proteins begin to denature at high temperatures, leading to some reversible and irreversible changes in whey protein structure and nutritional properties, as well as the formation of new complexes with κ-casein on the surface of the micelles at pH less than 6.7, i.e. at a natural dairy milk pH and below (Dumpler, Huppertz, & Kulozik, 2020). The formation of these complexes leads to some changes in the physical properties of milk, i.e., changes in milk turbidity and colour, and increased viscosity. This might also affect the foamability of dairy milk.

A study by Kamath and co-authors (Kamath, Huppertz, Houlihan, & Deeth, 2008) suggests that foam produced from UHT milk is less stable than that produced from pasteurised milk. However, even strong heating (up to 90°C) with the steamer did not change the amount of serum proteins in milk supernatant, especially in UHT milk, and we suggest that this might happen because of the short treatment time (approx. 20 seconds, depending on the desired final temperature and the type of steam nozzle).

To approximate the quantity of each protein the 15% SDS-PAGE was made (Fig. 10). The decrease in the concentration of whey proteins observed in the HQ milk and even more in the UHT milk could be related to the decrease of whey proteins that occurs after the steamer treatment at high temperatures (50°C and 80°C) if compared to milk samples not subjected to the steamer injection (HQNH and UHTNH, Fig. 10).

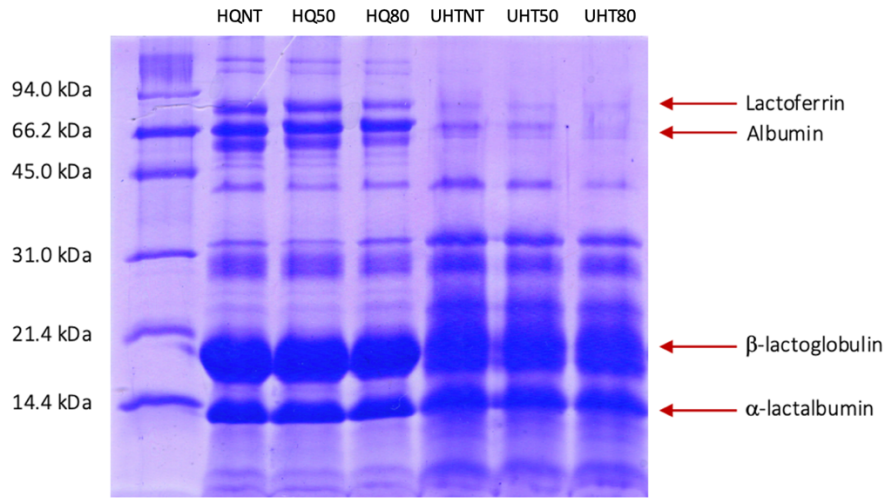


Fig. 10. 15% SDS-PAGE of supernatants from whole pasteurised (HQ) and whole UHT milk obtained after casein precipitation. Before casein precipitation milk samples were subjected to stem injection up to 50 °C and 80 °C by (HQ50; HQ80; UHT50; UHT80). St Bio-Rad low-molecular-weight standard. HQNH: pasteurised milk not heated, UHTNH: UHT milk not heated. The arrows indicate the lactoferrin (84.0 kDa), albumin (68.5 kDa), b-lactoglobulin (18.4 kDa) and a-lactalbumin (13.7 kDa).

Before casein precipitation, milk samples were heated up to 50°C and 80°C by the steamer. The main whey proteins identified by the electrophoresis gel were: lactoferrin (84.0 kDa), albumin (68.5 kDa), α-lactalbumin (13.7 kDa), and β-lactoglobulin (18.3 kDa). The normalised quantity of each protein, determined by the PDQuest software, is shown in Fig.10 where, as expected, it is possible to notice the higher concentration of lactoferrin and albumin in HQ milk with respect to the UHT milk ($P < 0.005$ and $P < 0.001$ respectively). Furthermore, in the HQ milk sample, the steamer treatment up to 80°C led to a significant decrement of lactoferrin, and a slight but not significant decrease of β-lactoglobulin, while the UHT milk samples the heated up to 80°C with the steamer caused the drastic reduction of lactoferrin and albumin, a β-lactoglobulin decrease and a significant decrement of α-lactalbumin occurs (Fig. 11).

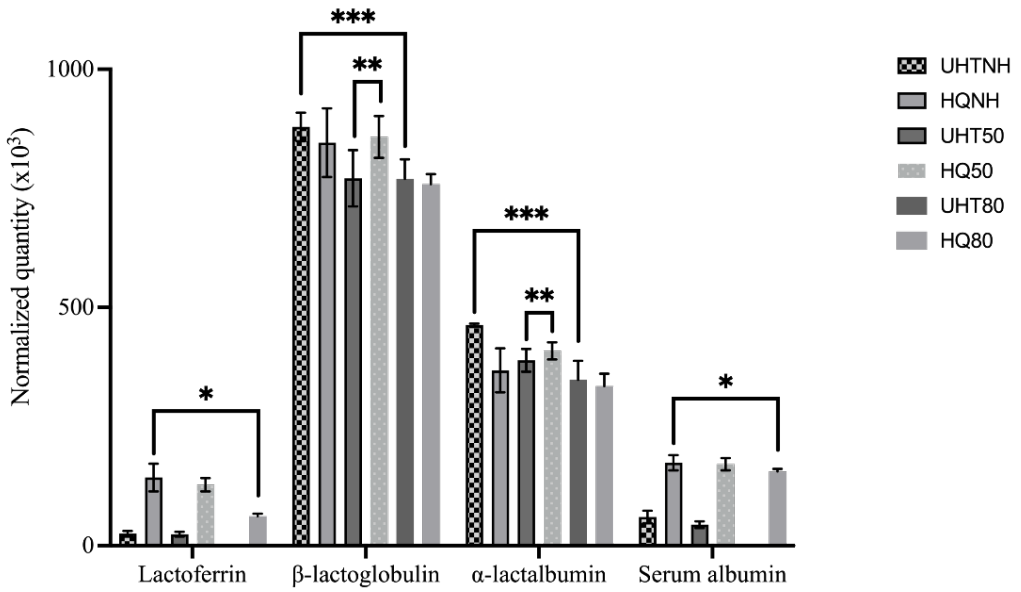


Fig. 11. Quantitative analysis of whey proteins from HQ and UHT milk performed by PDQuest software on a 15% SDS-PAGE gel loaded with the following samples: HQNH, HQ50, HQ80 (whole pasteurised milk not treated, heated up to 50 °C and 80 °C, respectively); UHTNT, UHT50, UHT80 (UHT milk not treated, heated up to 50 °C and 80 °C, respectively). All milk samples were heated by the steamer as discussed in the Materials and Methods section. The electrophoretic analyses were performed in triplicate. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001.

The analysis of protein content (total) of freeze-distilled (section 6.2) non-heated milk samples also has been performed. The results of the experiment showed the significant increase in concentration of proteins in all samples and averaged 32.18±0.23 mg mL^{-1} for pasteurised whole milk with lactose (HQ), 23.92±1.10 mg mL^{-1} for whole UHT milk with lactose (UHT), 50.91±0.55 mg mL^{-1} for freeze-distilled pasteurised whole milk with lactose (HQ FD), and 37.55±1.10 mg mL^{-1} for freeze-distilled whole UHT milk with lactose (UHT FD). The total protein content was analysed because the casein fraction in milk is considered particularly sensitive to freezing because it leads to the flocculation of caseins. However, studies have shown that the flocculation of caseins, which leads to insolubility of casein, only occurs after several weeks of freezing milk (Bottiroli, Zhang, Aprea, Fogliano, Hettinga, & Gasperi, 2020).

The SDS-PAGE with the subsequent PDQuest analysis of the protein fraction of regular and freeze-distilled milk samples showed the increase of α-casein in HQ FD and UHT FD compared to the regular HQ and UHT milk samples, respectively, and the β-casein concentration in the UHT FD sample compared to the UHT milk (Figures 12, 13). In addition, the concentration of α-lactalbumin was increased in the freeze-distilled pasteurised milk samples compared to the regular pasteurised milk samples. The concentration of serum

albumin, κ-casein and β-lactoglobulin was not significantly affected by freeze-distillation. It was suggested that the increase in protein concentration might be related to the removal of some of the water from the milk, but not to protein aggregation due to freezing was observed, as the particle size distribution in regular and freeze-distilled milk samples was identical.

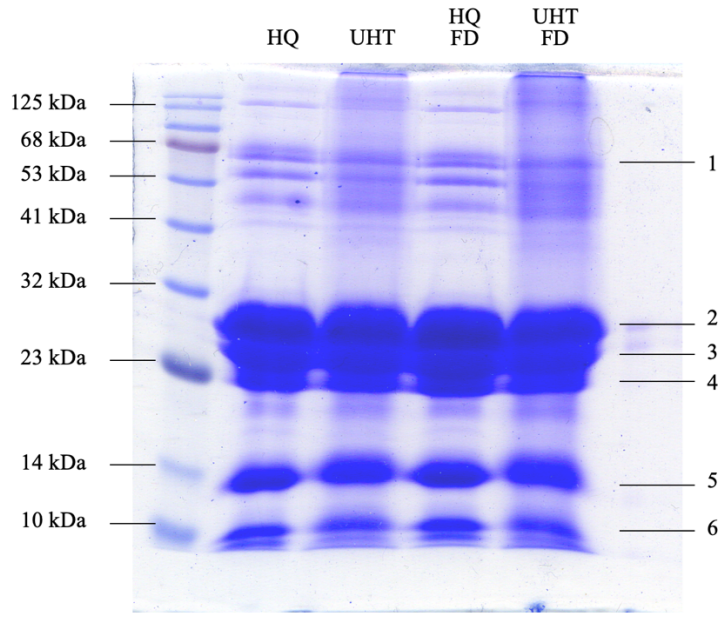


Fig. 12. 12% SDS-PAGE of pasteurised (HQ), ultra-high temperature (UHT), freeze-distilled pasteurised (HQ FD), and freeze-distilled ultra-high temperature (UHT FD) treated milk samples. St BlueStar Prestained Protein Marker. The lines on the right side of the image indicate following proteins: 1 – albumin (67 kDa), 2 – α-casein (23.6 – 25.4 kDa), 3 – β-casein (23.6 kDa), 4 – κ-casein (19.0 kDa), 5 – β-lactoglobulin (18.4 kDa), 6 – α-lactalbumin (13.7 kDa). The expected molecular weight of proteins is indicated in brackets. Protein bands were identified by comparing the gel with another gel already reported in the literature by Vincent, Elkins, Condina, Ezernieks, & Rochfort (2016).

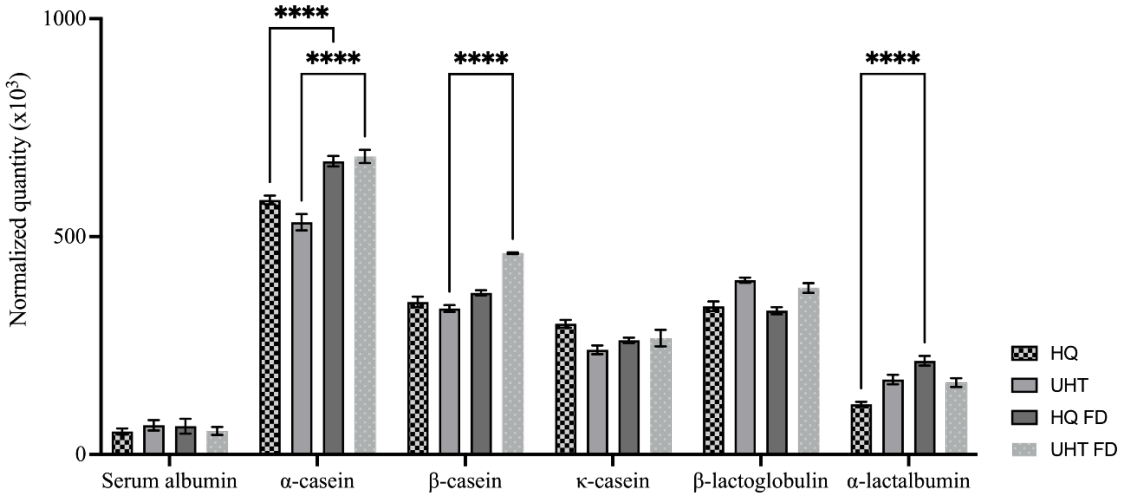


Fig. 13. Quantitative analysis of whey proteins from HQ and UHT milk performed by PDQuest software on a 12% SDS-PAGE gel loaded with the following samples: whole pasteurised (HQ), whole ultra-high temperature treated (UHT), freeze-distilled whole pasteurised (HQ FD), and whole ultra-high temperature treated (UHT FD) milk. The electrophoretic analyses were performed in duplicate. ****P < 0.001.

The measurement of free thiol groups was performed to assess the degree of denaturation of the whey proteins after the treatment with the steamer at increasing temperature and to possibly link this to the character and stability of the milk *microfoam*. The molecule of β-lactoglobulin contains 5 cysteine residues (one of which, Cys^{121}, is in the free state) that can form disulphide bonds (Belitz, Grosch, & Schieberle, 2009). Originally, these residues are hidden in the protein structure. When the protein denatures, for example during heat treatment of milk (at temperatures above 40°C), hydrophobic thiol groups (–SH) are exposed on the surface of the protein molecule and can form bonds with other molecules (Farkye & Shan, 2015; Belitz, Grosch, & Schieberle, 2009).

Fig. 14 illustrates significantly higher ($P < 0.05$) quantity of free thiols in UHT milk samples in comparison to pasteurised milk samples (HQ) expressed as normalised quantity of FSH in nmol mg^{-1}.

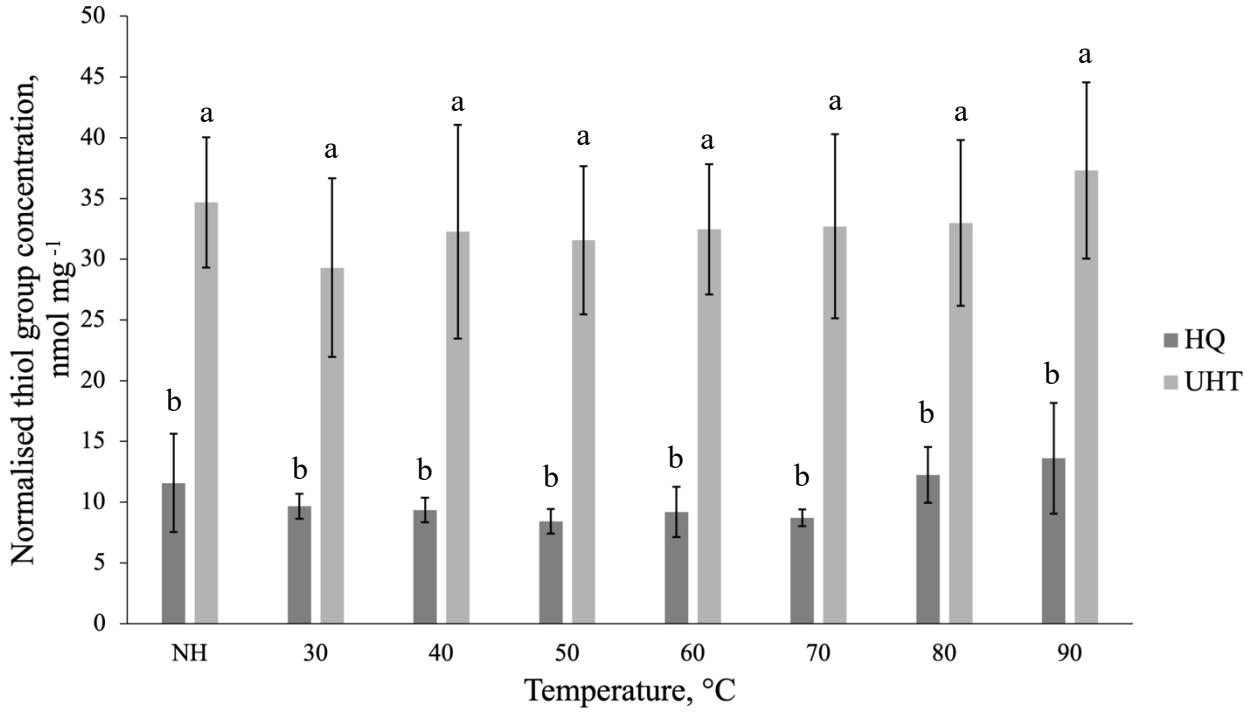


Fig. 14. Normalised quantity of free thiol groups in whole pasteurised (HQ) and whole UHT milk at different temperatures. Results represented are mean values of three repetitions and standard deviations. Letters above the bars highlight significantly different values. Values with the same superscript letter do not differ significantly ($p > 0.05$).

Summarising the data on the whey protein content and the concentration of free thiol groups in the milk samples, there was a significant difference in the serum protein and FSH-group

content between pasteurised (HQ) and UHT milk. It is noticeable that the content of free thiol groups, normalised to the protein content, calculated at each injection temperature, is higher in the UHT milk than in the pasteurised milk. In the UHT milk, the proteins are indeed more denatured than in the HQ milk and the thiol groups are therefore more exposed. Since higher temperatures are applied during UHT treatment, the degree of denatured serum proteins was higher in UHT milk, which is also evident in the results of SDS-PAGE (Figures 10, 11). Although denaturation of whey proteins increases the viscosity of milk, foaming with steam dilutes milk with water, which may prevent the formation of more stable foams at higher temperatures (70°C and above).

7.2. Lactose content measurement

Lactose measurement was made to evaluate the effect of freeze-distillation of milk on the content of this disaccharide. The concentration of lactose was measured in non-treated milk samples as well as in both fractions of freeze-distilled milk: thawed part used for future analysis of microfoam and the residual liquid. Table 4 summarises the data on lactose concentration in different milk samples.

Table 4. The concentration of lactose in g L^{-1} in whole pasteurised milk with lactose (HQ), freeze-distilled whole pasteurised milk with lactose (HQ FD), the residual liquid after freeze-distillation of pasteurised milk (HQ FD res.), whole ultra-high temperature treated milk with lactose (UHT), freeze-distilled whole ultra-high temperature treated milk with lactose (UHT FD), and the residual liquid after freeze-distillation of UHT milk (UHT FD res.).
Results represented are mean values of three repetitions and standard deviations. Values with the same superscript letter do not differ significantly ($p < 0.05$).

	Milk type					
	HQ	HQ FD	HQ FD res.	UHT	UHT FD	UHT FD res.
Lactose content, g L^{-1}	44.07 ± 1.25^{a}	71.50 ± 1.05^{b}	14.23 ± 0.5^{d}	45.50 ± 0.75^{a}	74.37 ± 0.74^{c}	11.83 ± 0.65^{d}

The comparison of the lactose content in non-heated milk samples showed that the amount of lactose in FD milk samples is approx. three times higher than in regular milk considering the fact that part of the lactose (Table 4, HQ FD res. and UHT FD res. respectively) remains in the liquid fraction which was not used for foaming. That correlates with the answers of

assessors who participated in the sensory panel. The majority of panellists noted the freeze-distilled milk samples as *sweeter* than the regular ones.

7.3. Foam decrease rate

According to the approach used in this study, *microfoam* stability, represented as foam decrease rate (FDR), depends on the final temperature of milk heating.

It is also important to point out the average composition of the espresso coffee used for the FDR measurements as it might affect the foam behaviour. For this purpose, the HPLC-VWD method showed that it contained chlorogenic acid in the form of 3-CQA, 790.9 mg L^{-1} (SD 24.4), 5-CQA, 1432.7 mg L^{-1} (SD 40.4), 3,5-diCQA 223 mg L^{-1} (SD 34.5); caffeine, 4544.1 mg L^{-1} (SD 179.6); trigonelline, 2946.5 mg L^{-1} (SD 187.8) and nicotinic acid, 106.61 mg L^{-1} (SD 8.9). Reactions between milk and coffee compounds vary depending on the coffee type, origin, process, and variety. For example, milk proteins might interact with coffee compounds with the subsequent effect on milk foaming properties since proteins play a role of natural emulsifiers in foam formation (Rashidinejad et al., 2021). Studies reported that milk protein associate with plant-derived, e.g. form coffee, polyphenolic compounds. Those interactions might affect the functionality of milk proteins and, consequently, the FDR as one of the parameters if milk foam stability (Rashidinejad, Birch, Hindmarsh, & Everett, 2017).

The experiment lasted 45 minutes, but a significant difference in behaviour was observed only during first 13 minutes of observation, therefore the results shown in Fig. 15 (a and b) reported the dependence of the foam decrease rate on temperature during 13 minutes of experiment in different types of milk.

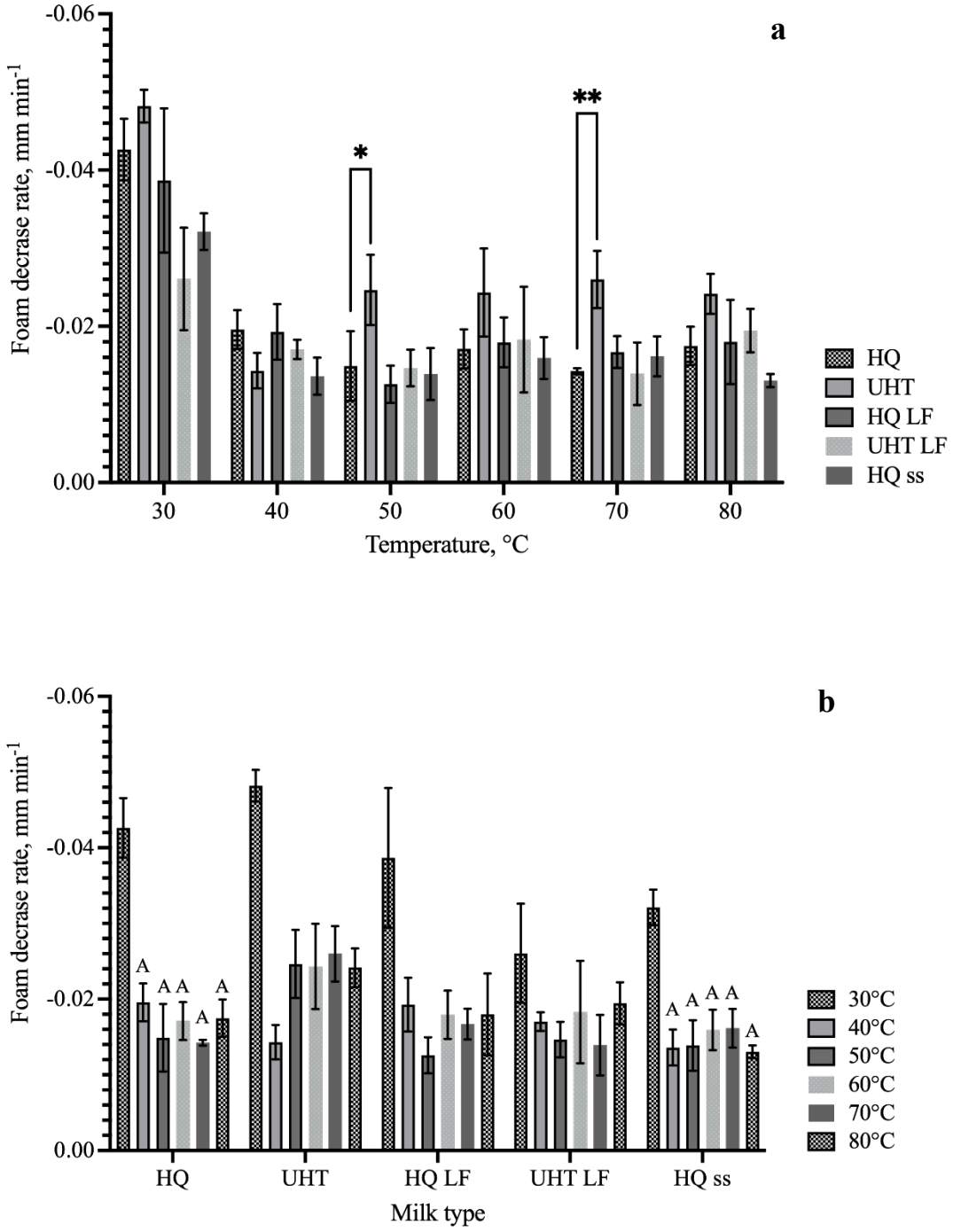


Fig. 15. The dependence of a) foam decrease rate of whole pasteurised (HQ), whole UHT, whole lactose-free pasteurised (HQ LF), whole UHT lactose free (UHT LF), and pasteurised semi-skimmed (HQ ss) milk samples and b) milk type on temperature during first 13 minutes of experiment. Results shown are means of three replicates and standard deviations. Letters above the bars highlight statistically similar values.

Detailed analysis of the data showed that the FDR of UHT milk was significantly higher from that of pasteurised milk at all temperatures ($P < 0.05$). Moreover, according to this experiment, UHT milk forms the least stable *microfoam* of all tested milk types. Lactose-free milk, both UHT and pasteurised, did not show any specific behaviour in terms of foam

stability, since it contains the same number of serum proteins as regular cow milk (Table 3). The difference in stability within each type of milk was significant throughout the temperature range only between the *microfoam* produced at 30°C and all other temperatures within the range (40 – 80°C) (Fig. 14b). At 30°C, all foams showed the least stability.

It was also noticed that the initial foam height affects the FDR. The higher the foam is (> 20 mm), the faster it collapses ($P < 0.05$).

The experiment on foam stability showed the higher rate of decrease, i.e. faster breakdown of foam, for UHT milk samples within the whole temperature range (Fig. 15a, b). This was related to the lower serum protein content (on average 1.33 mg/ml for UHT milk as opposed to 6.75 for pasteurised milk samples), the higher degree of protein aggregation expressed by free thiol content (Fig.14), and the potential association of denatured whey proteins with casein micelles in UHT milk.

Comparing the semi-skimmed (HQ ss) with whole milk foam samples (HQ), it was noticed that they have similar stability. Milk fat directly affects the stability of milk foams (Kamath, Webb, & Deeth, 2011). At lower temperatures (up to 40°C), milk fat is present in milk in both melted and solid forms, making the fats more susceptible to coalescence. It was observed that the difference in FDR at 40°C disappears when all milk fats melt and are only in liquid form. The melting points of triglycerides vary from -40°C to 72°C, but the general melting point of milk fat is 37°C – 40°C, as triacylglycerols with lower melting points dissolve triacylglycerols with higher melting points (McCarthy, 2006). This could be confirmed by the observation that semi-skimmed pasteurised cow milk exhibited lower FDR than whole milk samples at low temperatures (up to 40°C) (Fig. 15a, b). At all other conditions, semi-skimmed milk showed no significant difference in terms of FDR.

In summary, the FDR measurements show that microfoam from UHT whole milk is less stable over the entire temperature range compared to pasteurised milk samples. In addition to the difference in the FDR of *microfoams* discussed above, it was noticed that the initial foam height might affect the stability of foam. Higher foams might be formed due to the inappropriate foaming technique leading to the incorporation of large bubbles into milk. This usually happens when the steam nozzle has not been immersed enough into milk. The faster collapse of higher foams may be due to the heterogeneous distribution of temperature within the foam, i.e., it is lower at the surface than in the lower layer. In addition, the larger difference in the internal pressure of the differently sized air bubbles leads to faster coalescence.

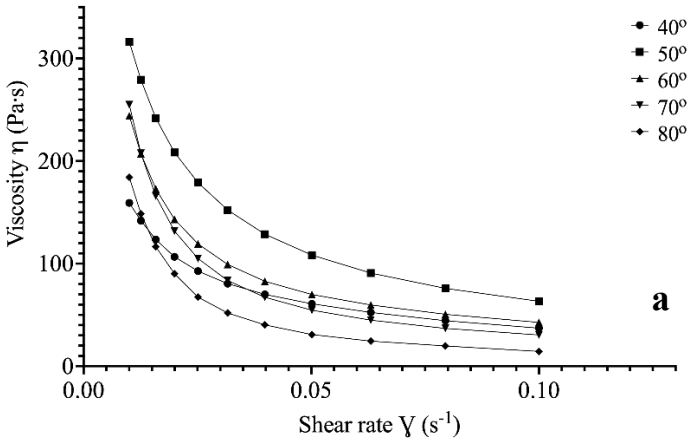
7.4. Foam viscosity

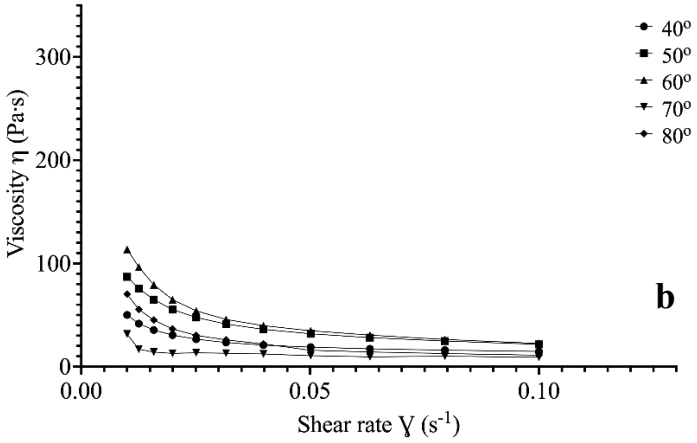
Although previous studies (Jimenez-Junca, Gumy, Sher, & Niranjan, 2011; Silva et al, 2008) have adequately described the rheological properties of milk foams formed by steam injection, in this work this aspect was deepened for *microfoams*.

As noted by Silva et al. (2008), the viscosity of milk foams depends strongly on the frothing temperature. For this reason, viscosity measurements were performed on *microfoams* prepared at different frothing temperatures (30, 40, 50, 60, 70, 80 °C). According to Fig. 16 a-e, the *microfoam* prepared from pasteurised milk is more viscous than that prepared from UHT milk at all temperatures. The highest viscosity values were observed at 50°C and 60°C. *Microfoams* from lactose-free milk, both pasteurised and UHT, were not significantly different from the samples with full lactose content. In agreement with Kamath et al. (2008), the peak of *microfoam* stability is reached at 65°C and thereafter foamability decreases at high temperatures, with viscosity values decreasing at temperatures of 70°C and higher for all milk types.

Compared to regular milk foam, *microfoams* are about 3 times less viscous (Jimenez-Junca, Gumy, Sher, & Niranjan, 2011). In addition, microfoams tend to thin immediately after being placed on the rheometer plate and reach the plateau at a shear rate of 0.5 s-1. This makes them more suitable for pouring and latte-art techniques. However, due to their lower viscosity and rapid thinning, they tend to disintegrate more quickly with increasing shear stress.

Increasing the temperature decreases the viscosity of milk (Fig.16a-e), thus facilitating the migration of milk proteins to the air-serum interface. In addition, the decrease in milk viscosity may promote the drainage of liquid from the foam. This is an expected process as the drainage of the liquid fraction in foams under gravity creates a vertical gradient in the gas volume fraction (Völp, Kagerbauer, Engmann, Gunes, Gehin-Delval, & Willenbacher, 2021).


40º
50º
60º
70º
80º
Viscosity η (Pa·s)
Shear rate γ̇ (s-1)
a


40º
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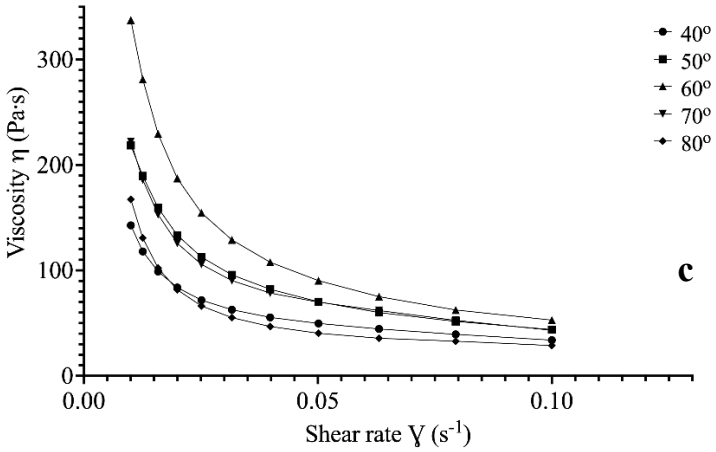
Viscosity η (Pa·s)
Shear rate γ̇ (s-1)
b


40º
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Viscosity η (Pa·s)
Shear rate γ̇ (s-1)
c

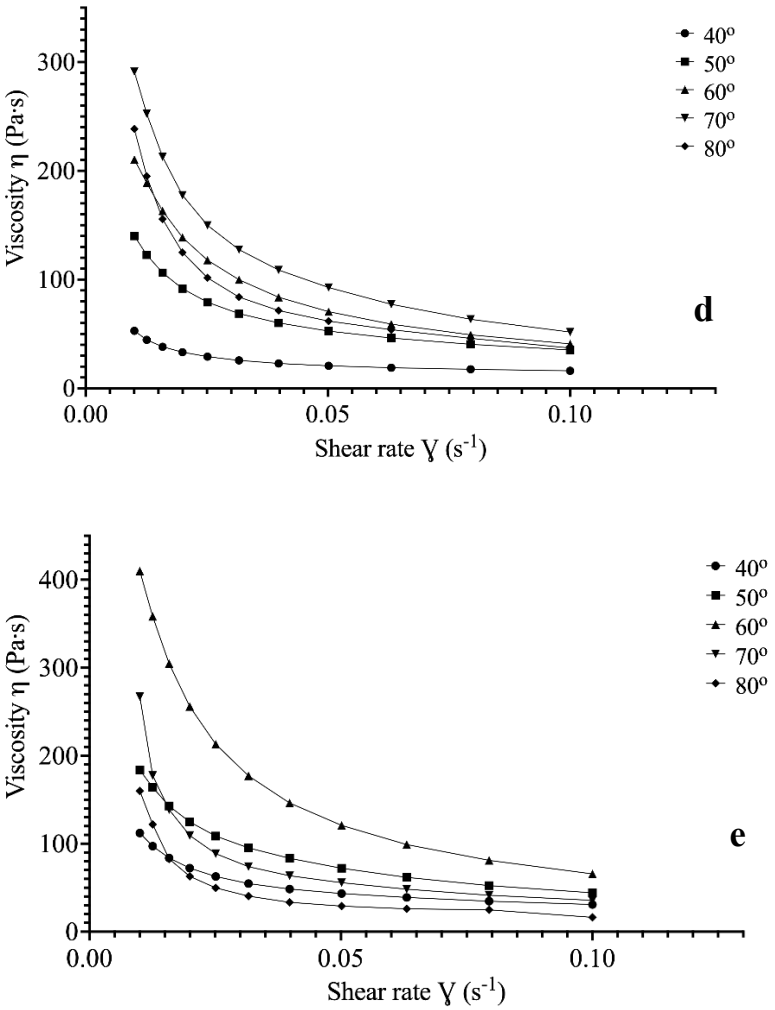


Fig. 16. The apparent viscosity (η) of foams made from a) whole pasteurised milk with lactose (HQ), b) whole UHT milk with lactose (UHT), c) whole pasteurised milk without lactose (HQ LF), d) whole UHT milk without lactose (UHT LF), e) semi-skimmed pasteurised milk with lactose (HQ SS) as functions of the shear rate (γ̇) at 25ºC. Plate geometry PU20 SR3863 SS: PL61 ST S2711 SS; gap 2 mm, shear stress rate from 0.01 s^{-1} to 0.1 s^{-1}; 10 samples per decade; test duration 1.5 min. Results represented are mean values of three repetitions, error bars were omitted to improve clarity.

Viscosity tests were performed as this is the most common rheological analysis for characterising low viscosity samples such as milk foams, measuring both the viscosity of the system and the flow properties. The obtained flow curves show the shear stress/shear rate dependence for the different milk types at 60°C and 50°C (Fig. 17).

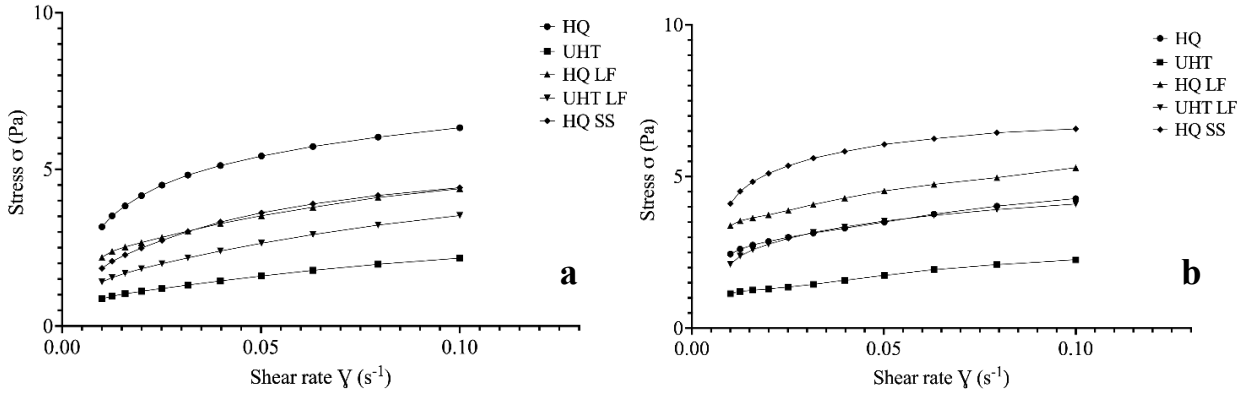


Fig. 17. Flow curves for all studied milk types including whole pasteurised (HQ), whole UHT, whole lactose-free pasteurised (HQ LF), whole UHT lactose free (UHT LF), and semi-skimmed pasteurised (HQ ss) milk samples at temperatures a) 50°C and b) 60°C. Plate geometry PU20 SR3863 SS: PL61 ST S2711 SS; gap 2 mm, shear stress rate from 0.01 s^{-1} to 0.1 s^{-1}; 10 samples per decade; test duration 1.5 min. Results represented are mean values of three repetitions, error bars were omitted to improve clarity.

The analysis of the *microfoams* have shown that they exhibit non-Newtonian shear-thinning behaviour and can be considered as pseudoplastic fluids (Fig. 17).

Difference in foam viscosities at different temperatures might be caused by several reasons including: bubble quantity and distribution, decreased viscosity of milk facilitating the migration of protein molecules to the air-serum interface, difference in speed of drainage (Huppertz, 2010). Foams with more uniform bubble size distribution appeared to be more stable and viscous, i.e., at 50 and 60°C.

The results of power law analysis (Table 5) numerically support the observations of the dependence of the stress from the shear rate. Since milk *microfoams* demonstrate pseudoplastic behaviour (Fig. 17) the power law index is below 1. There was a decrease in viscosity with the increase of the temperature from 40°C to 80°C that starts at higher temperatures after 70°C. The viscosity of milk *microfoams* made at 30°C could not be determined because of their rapid destruction.

Table 5. Effect of temperature on power law viscosity calculated from the power law equation fit of experimental data in the shear rate range of 0.01 to 0.1 s^{-1} for milk foams from pasteurised (HQ), UHT whole milk, lactose-free pasteurised (HQ LF), lactose-free UHT whole milk (UHT LF), and semi-skimmed pasteurised (HQ ss) milk samples. Figures are means of three replicates and standard deviation

	Power law viscosity (Pa*s)

°C	HQ	UHT	HQ LF	UHT LF	HQ SS
40	8.92±3.48	4.75±2.90	9.08±3.30	7.02±1.76	8.87±2.75
50	12.47±1.11	5.52±0.35	8.83±3.38	8.94±0.53	10.75±2.61
60	7.80±0.64	5.08±2.07	8.20±2.60	8.23±1.12	10.46±3.30
70	3.63±1.64	9.83±4.07	8.18±1.94	8.84±0.59	5.45±0.03
80	1.16±0.21	0.16±0.11	6.26±2.74	2.77±1.24	3.07±1.33

The excessive, i.e. visible, drainage of liquid from foams was observed at foams prepared at higher temperatures > 70 °C. The amount of water incorporated is highly dependent on the coffee machine and steam parameters. Frothing milk with steam in the Nuova Simonelli Appia II coffee machine resulted in the following increase in volume at the end of frothing (1 mL = 1 g), as summarised in Table 6.

Table 6. The volume increase in ml after steaming the milk to the appropriate temperature. The percentage of volume increase over the initial volume is given in parentheses. The frothing of the milk was done with the coffee machine Nuova Simonelli Appia II.

Temperature (°C)	Increase in volume (ml)
30	7.5 (5%)
40	10.5 (7%)
50	14 (9.3%)
60	17 (11.3%)
70	20.5 (13.7%)
80	22.5 (15%)

At temperatures above 70°C liquid drainage was observed for all milk types (Fig. 18). The liquid drainage may be caused by the self-association of caseins and the formation of whey-casein complexes at higher temperatures (more than 65°C according to the study of Kamath et al. (2011). That potentially lead to the formation of a more viscous foam. However, the

foam cannot be considered microfoam as it loses moisture and is not accepted according to the "moist" parameter of the foam rating.

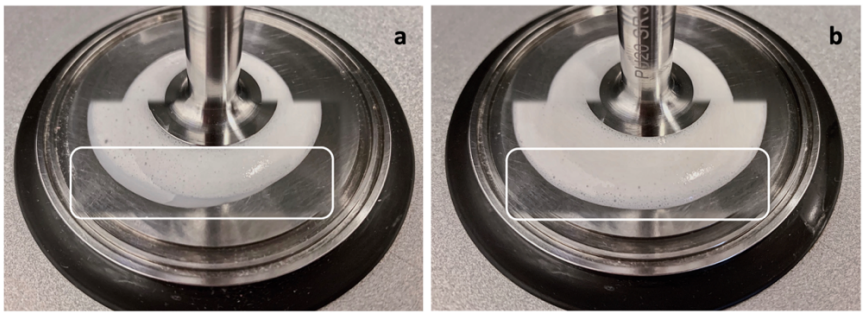


Fig. 18. Images of milk foams made after 10 minutes of experiment from a) whole pasteurised milk (HQ) at temperatures > 70°C b) whole pasteurised milk (HQ) at temperatures < 70°C demonstrating the liquid drainage at different temperatures.

Observations showed that all milk types behaved similarly, and both pasteurised and UHT milk *microfoams* prepared at higher temperatures (> 70°C) tended to lose liquid from the structure more rapidly than at lower temperatures (< 70°C). Interestingly, this does not correlate with the fact that the higher the degree of denaturation of the whey proteins, the higher the viscosity of milk, and consequently the water holding capacity of the foams increases (Bals & Kulozik, 2003). This can be explained by the fact that milk in this study was foamed with water-saturated steam, which leads to dilution of milk with water, thus decreasing the viscosity of milk at higher temperatures and the protein concentration.
The comparison of the behaviour of *microfoams* made from regular and freeze-distilled milk samples (Fig. 19) showed that they have similar viscosity within the whole temperature range (50°C, 60°C, and 70°C).

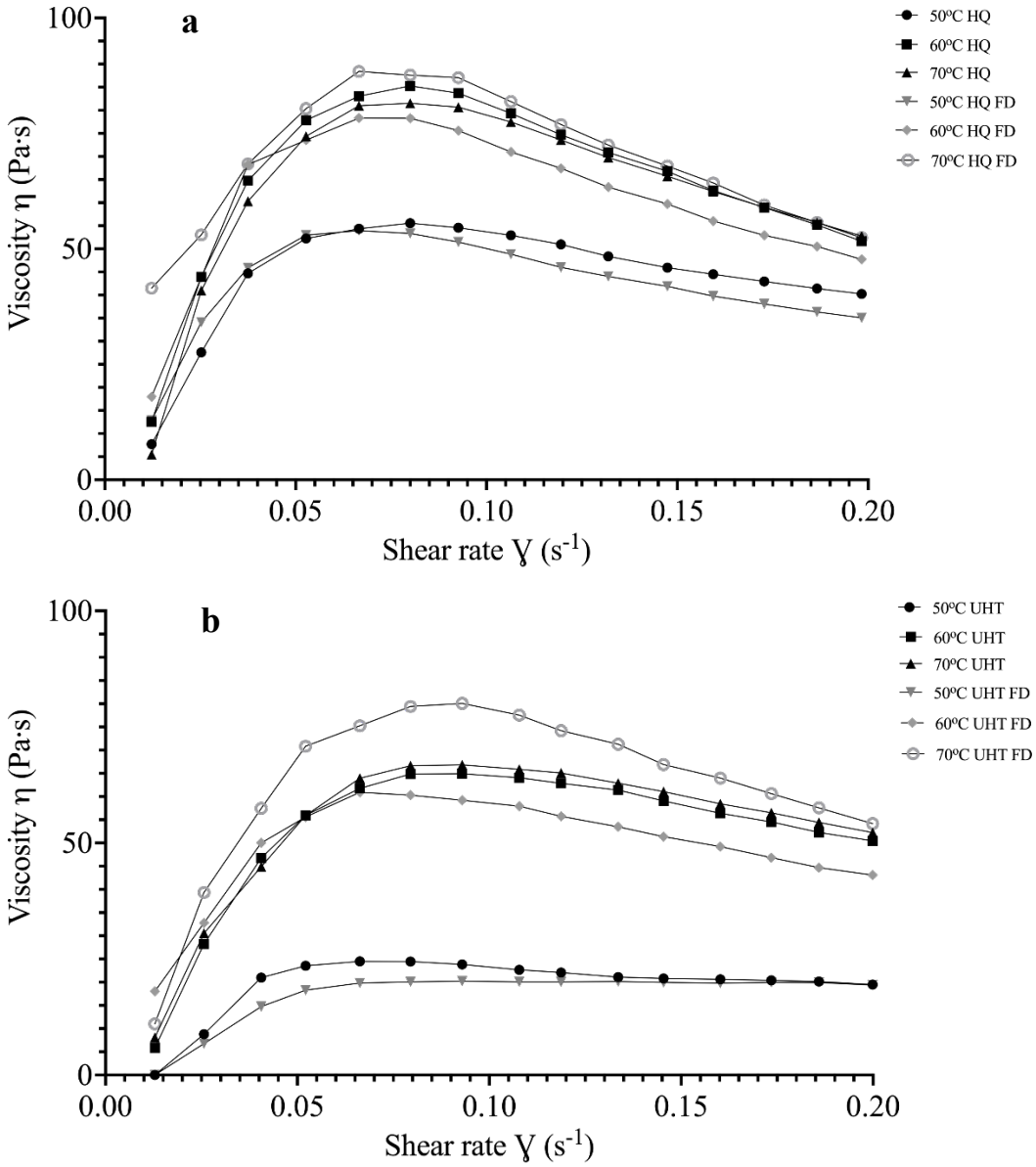


Fig. 19. The apparent viscosity (η) of microfoams made from a) whole pasteurised milk with lactose (HQ), freeze-distilled whole pasteurised milk with lactose (HQ FD), b) whole ultra-high temperature treated milk with lactose (UHT), freeze-distilled whole ultra-high temperature treated milk with lactose (UHT FD) as functions of the shear rate (γ̇) during 10 minutes of experiment at 25 °C. Cylindric rotor CC25 DIN/Ti; 15 samples per decade; test duration 150 s; shear stress rate from 0.01 s^{-1} to 0.2 s^{-1}. Results represented are mean values of three repetitions, error bars were omitted to improve clarity.

As discussed above, milk microfoams show shear-thinning behaviour, so the viscosity of milk microfoams becomes lower at higher shear rates. Freeze-distillation did not seem to significantly affect the foaming properties of the milk. This is also consistent with the results of particle size distribution measurement, as the viscosity of milk foam demonstrated the dependence on the solids content of the milk (Ho, Le, Yan, Bhandari, & Bansal, 2018). Data obtained with MasterSizer (section 7.8) showed no significant difference in particle

size distribution between regular and freeze-distilled milk samples. Furthermore, the increased viscosity of freeze-distilled milk (Fig. 20) did not seem to affect the viscosity of the microfoams produced from these samples. However, it could contribute to increasing the stability of microfoams made from pasteurised milk, as the higher viscosity of the milk was associated with better stability of the milk foam (Martínez-Padilla, García-Rivera, Romero-Arreola, & Casas-Alencáster, 2015).

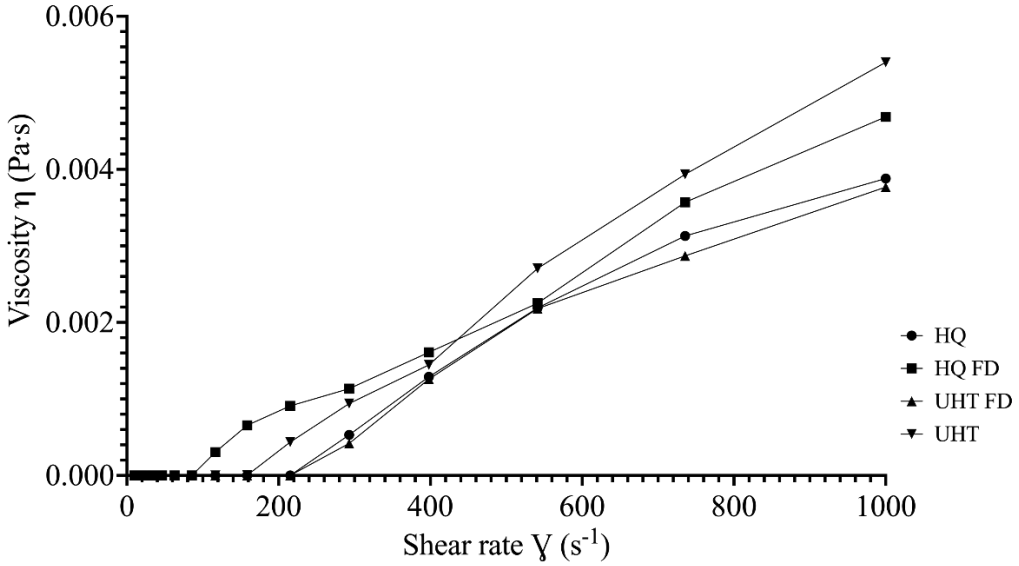


Fig. 20. The apparent viscosity (η) of whole pasteurised milk with lactose (HQ), freeze-distilled whole pasteurised milk with lactose (HQ FD), whole ultra-high temperature treated milk with lactose (UHT), and freeze-distilled whole ultra-high temperature treated milk with lactose (UHT FD) milk samples as functions of the shear rate (Ɣ) during 10 minutes of experiment at 25°C. Cylindric rotor CC25 DIN/Ti; 15 samples per decade; test duration 150 s; shear stress rate from 0.01 s^{-1} to 0.2 s^{-1}. Results represented are mean values of three repetitions, error bars were omitted to improve clarity.

7.5. Foam expansion, stability, and overrun

The comparison of foaming properties of freeze-distilled and regular milk samples was performed. The stability of milk *microfoam* was considered according to several parameters: foam expansion, foam stability, and overrun. The overrun value represents the density of the foam. It therefore refers to the volume of air incorporated in the foam by the steaming device of the espresso machine (Zakidou & Paraskevopoulou, 2020; Huppertz, 2010). The foaming properties of regular and freeze-distilled milk *microfoam* samples are summarised in Table 7.

Table 7. Foaming properties (foam expansion (FE), foam stability (FS), overrun) of regular whole pasteurised dairy milk (HQ), freeze-distilled whole pasteurised dairy milk (HQ FD), regular whole ultra-high temperature treated dairy milk (UHT), and freeze-distilled whole ultra-high temperature treated dairy milk (UHT FD) milk samples heated by the

steamer of the espresso machine up to 50, 60, and 70°C respectively. Results are mean values and standard deviations of three replicates. Letters represent statistically different values; values in the same column with the same superscript letter do not differ significantly ($p < 0.05$). The values were only compared within their groups, i.e. FE (%) was not compared with FS (%) or Overrun (%), so different letters do not mean that these groups are statistically different.

	50°C			60°C			70°C		
	FE (%)	FS (%)	Overrun (%)	FE (%)	FS (%)	Overrun (%)	FE (%)	FS (%)	Overrun (%)
HQ	81±4 [b]	73±4 [a]	259±10 [d]	66±10 [b]	71±4 [a]	238±11 [d]	80±11 [b]	72±7 [a]	335±7 [e]
HQ FD	75±8 [b]	77±3 [a]	263±7 [d]	91±19 [b]	71±4 [a]	250±2 [d]	91±2 [b]	69±5 [a]	295±3 [f]
UHT	74±3 [b]	74±7 [a]	253±1 [d]	84±11 [b]	73±2 [a]	261±3 [d]	100±8 [c]	70±5 [a]	364±21 [g]
UHT FD	75±8 [b]	77±3 [a]	273±3 [d]	91±19 [b]	71±4 [a]	266±2 [d]	91±2 [b]	69±5 [a]	305±6 [f,h]

The comparison of regular and freeze-distilled samples showed that there is no significant difference in foam expansion (FE) and foam stability (FS) between these two groups of samples. However, the value of microfoam overrun is significantly higher for HQ and UHT milk samples is significantly higher than the overrun of FD samples at 70°C. More, overrun of UHT milk samples was significantly higher than that of HQ. Nevertheless, the comparison of overrun of freeze-distilled HQ and UHT milk samples showed similar behaviour within the whole temperature range. However, overrun of FD samples from both HQ and UHT milk is significantly higher at 70°C than at 50°C. Fig. 21 shows the detailed visualisation of the statistical analysis data for the overrun value.

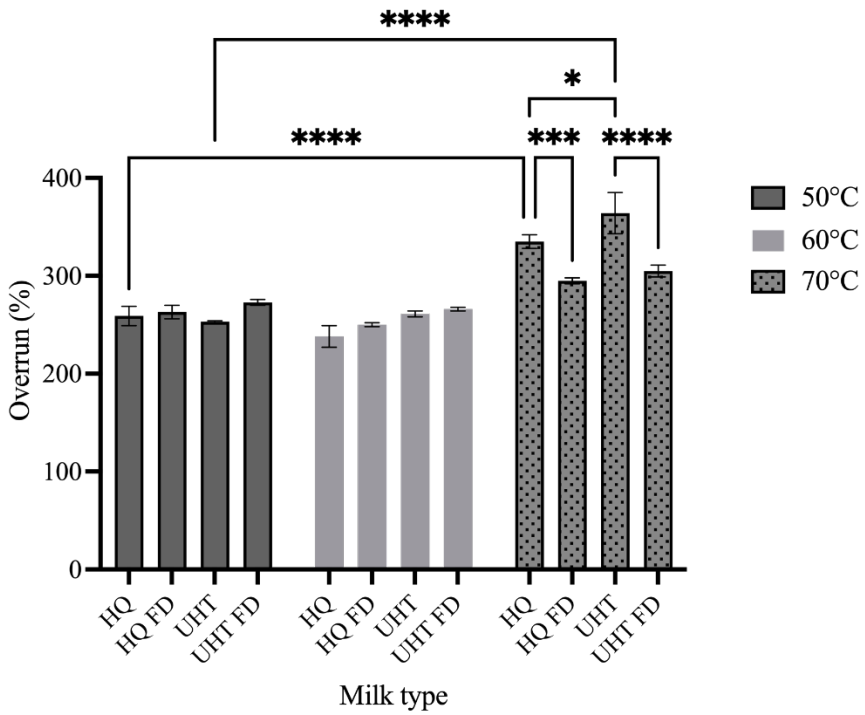


Fig. 21. Overrun of regular whole pasteurised dairy milk (HQ), freeze-distilled whole pasteurised dairy milk (HQ FD), regular whole ultra-high temperature treated dairy milk (UHT), and freeze-distilled whole ultra-high temperature treated dairy milk (UHT FD) milk samples heated by the steamer of the espresso machine up to 50, 60, and 70°C respectively. Results are mean values and standard deviations of three replicates. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001.

The increase in overrun at 70°C reflects the decrease in the density of the microfoam at high temperatures, which correlates with the results of the FDR and foam viscosity measurements. The intense liquid drainage at temperatures above 70°C described in the section 7.4 also correlates with the increased overrun of the microfoam. A lower density of the microfoam could lead to difficulties in the proper pouring of milk and microfoam onto the espresso and in the creation of latte art due to the friability of the microfoam.

7.6. Foam strength

Measuring the strength of the foam, i.e. the resistance of the foam to compression, was considered as one the ways to evaluate foam texture. The results of foam strength measurement on whole pasteurised dairy milk (HQ), freeze-distilled whole pasteurised dairy milk (HQ FD), regular whole ultra-high temperature treated dairy milk (UHT), and freeze-distilled whole ultra-high temperature treated dairy milk (UHT FD), heated by the steamer of the espresso machine up to 50, 60, and 70°C are summarised in Fig. 22.

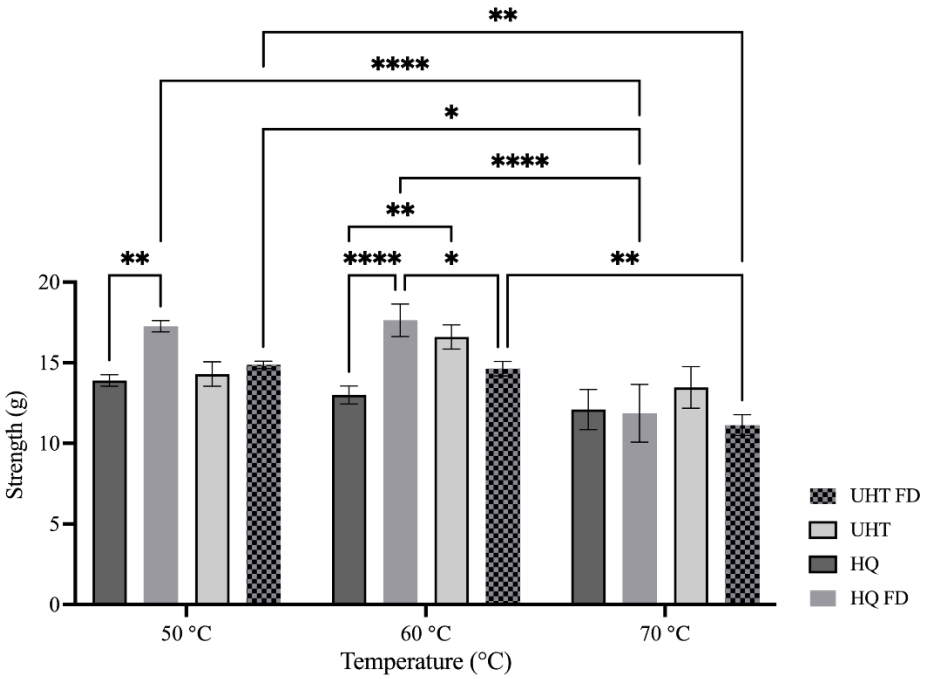
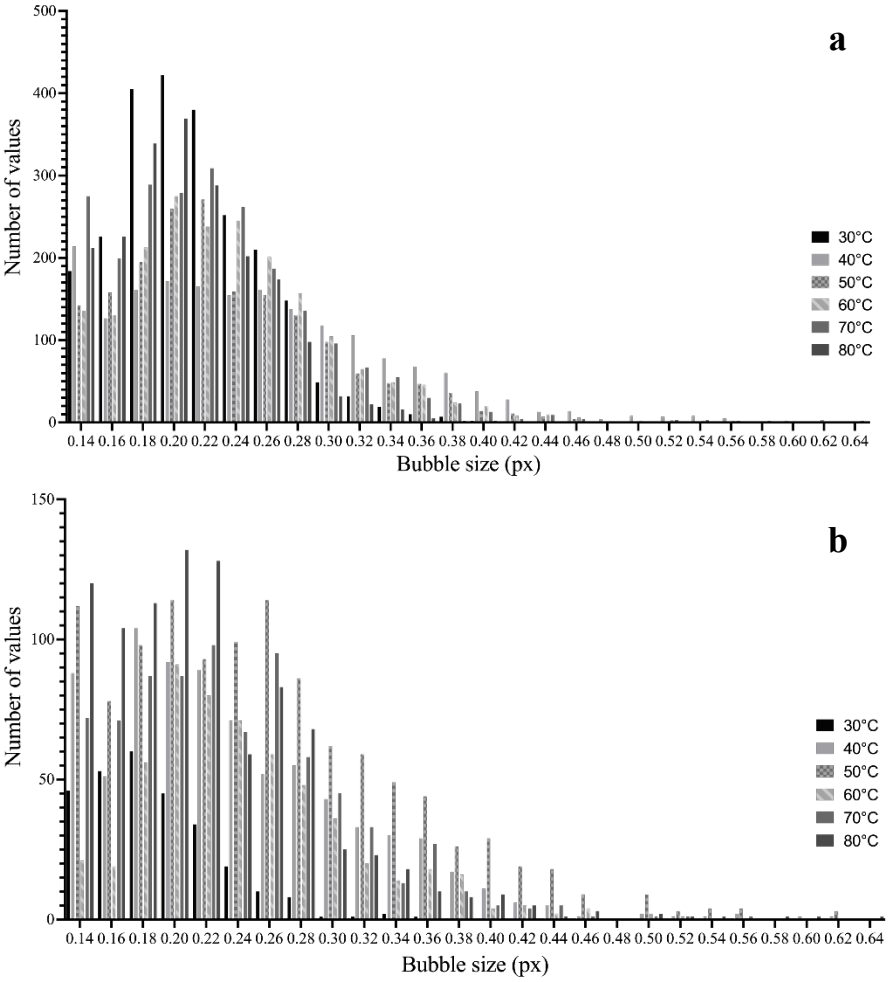


Fig. 22. Foam strength (g) of regular whole pasteurised dairy milk (HQ), freeze-distilled whole pasteurised dairy milk (HQ FD), regular whole ultra-high temperature treated dairy milk (UHT), and freeze-distilled whole ultra-high temperature treated dairy milk (UHT FD) milk samples heated by the steamer of the espresso machine up to 50, 60, and 70°C respectively. Results are mean values and standard deviations of three replicates. *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001.

Microfoams of freeze-distilled HQ and UHT milk samples prepared at 70°C showed significantly lower strength than those prepared at 50°C. However, the strength of microfoams prepared from regular HQ and UHT milk was identical throughout the temperature range from 50 to 70°C. The freeze-distilled HQ samples had higher strength than regular HQ milk at 50 and 60°C, respectively. That might be associated with higher protein content in freeze-distilled milk samples. The amount of protein in FD samples was ~ 1.5 times higher than in regular milk samples (Section 7.1). The strength of microfoam from regular UHT milk is slightly higher than that of HQ over the entire temperature range. At higher temperatures (70°C), all microfoams had similar strength, which was lower than that produced at lower temperatures. This could be related to the denaturation of the proteins and the aggregation processes that occur due to the intensive heat treatment. The results correlate with the results of other studies on the textural properties of the *microfoam* (sections 7.5 and 7.6) and show that freeze-distillation does not affect the ability of the milk sample to form stable and robust microfoams.

7.7. Bubble size distribution in foam

Bubble size distribution has been performed to evaluate the homogeneity of bubble size distribution in milk microfoam because the foam containing differently-sized bubbles disintegrate quicker due to the difference in the inner pressure of air bubbles (section 3.6). The results of microscopic analysis of milk *microfoam* show that higher (> 70°C) foaming temperatures lead to the formation of coarser foams with less uniformed distribution of bubble sizes. This is also true for foams prepared at 30°C. However, these foams are very unstable and decompose much more than all others within 10 minutes of observation (Fig. 23 a-c; b-d).



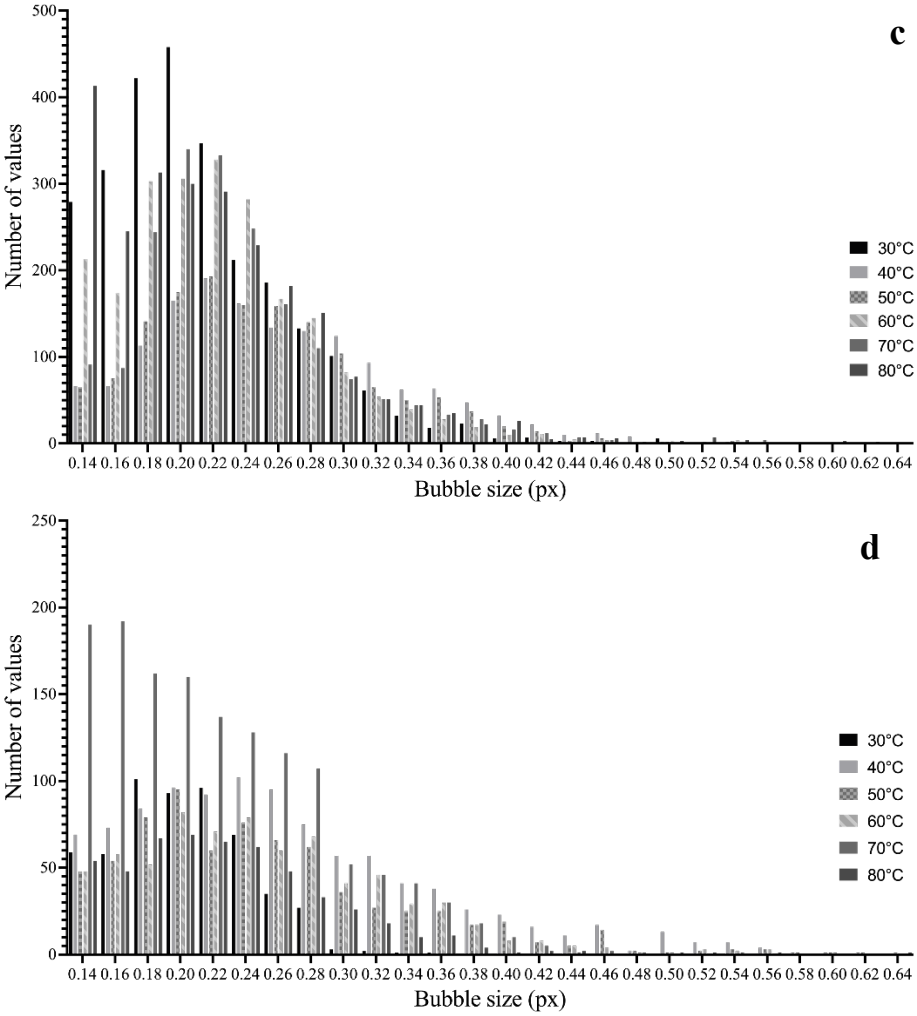


Fig. 23. Bubble size distribution of a) foam made from whole pasteurised milk (HQ) at initial time of 30 seconds after foaming b) foam made from whole UHT milk at initial time of 30 seconds after foaming c) foam made from whole pasteurised milk (HQ) after 10 minutes after foaming d) foam made from whole UHT milk after 10 minutes after foaming. All measurements were performed in triplicates at five random fragments of milk foam dispersed on the microscope glass.

Microscopic analysis of bubble size distribution in *microfoams* showed the higher stability, i.e, more bubbles were observed in foams after 10 min of experiment, of foams made within the range from 40 to 60°C in comparison to 30°C and temperatures above 70°C (Fig. 23). However, the nature of that difference depended on temperature. At 30°C foams collapsed quickly due to the insufficient foaming time and quantity of bubbles formed, and consequently lower viscosity that can prevent drainage, while at higher temperatures foams lost moisture and partially dried up after 10 minutes of observations.

Fig. 24 shows this phenomenon where small bubbles surround the large ones. Well-dispersed foams stabilised by milk proteins can be quite stable during the average consumption time, which is about 10 – 15 minutes.

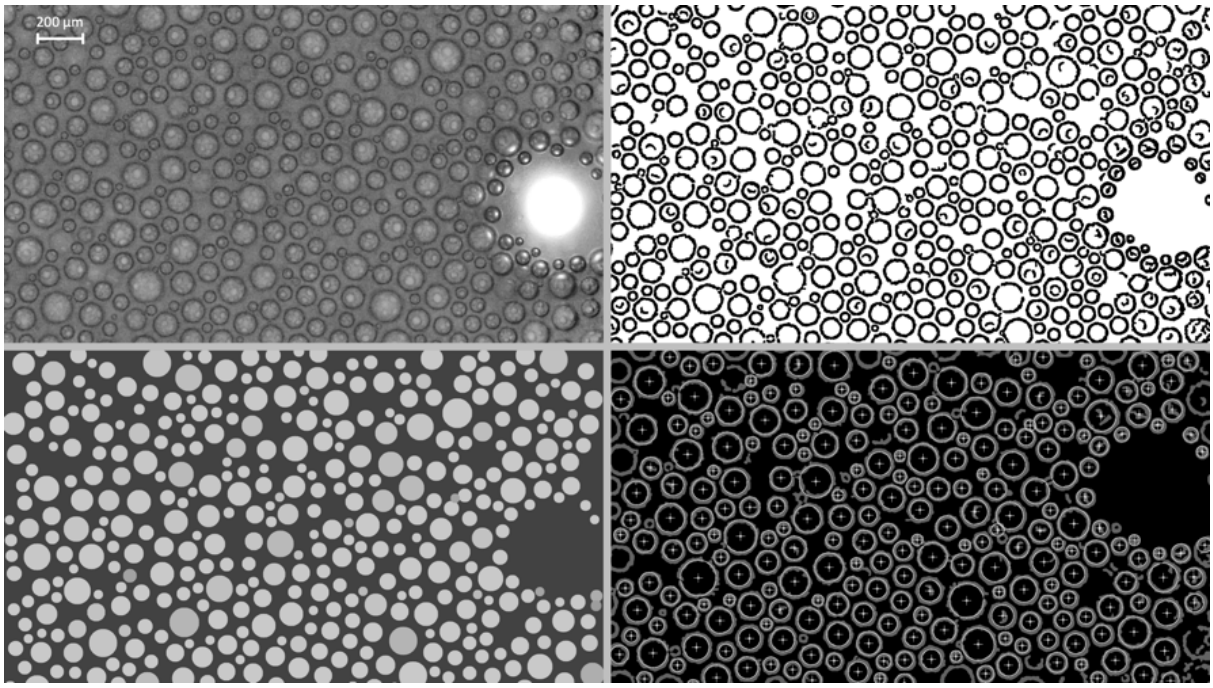


Fig. 24. Steps of image analysis of milk foams made under the microscope using the customised plugin of Fiji ImageJ image analysis: The procedure is based on using a Hough transform on the mask to find likely candidates and starts by enhancing the contrast and cropping the image according to the given parameters, then it applies the Laplacian to enhance the edges, removes particles smaller than 100 px^2, erodes slightly to prevent Hough from finding too many circles. At the end of the run, the plugin presents the results in the form of a table with the number of circles and their size. The foam presented was made from whole UHT milk at 70°C. For each milk type and foaming temperature measurements were performed in triplicates at five different parts of foam dispersed on the microscope glass.

The foam disintegrates when the bubbles approach each other due to the outflow of liquid from the lamellar film, when the film decreases and ruptures, and consequently coalesces (Damodaran, 2006). Natural colloidal systems tend to separate quite quickly as this leads to a minimization of the contact area at the interface and hence the free energy of the system. More stable foams can be formed within the range from 40 to 60°C due to the lower protein denaturation degree at those temperatures (Huppertz, 2010; Kamath, Huppertz, Houlihan, & Deeth, 2008). The drainage of liquid occurs within the whole temperature range, however, as mentioned above, it occurs faster at temperatures above 70°C. More, it was showed that the viscosity of foams at high temperatures is lower than, for example, at 50 – 60°C, thus the upwards movement of spherical air bubbles cannot be sufficiently prevented.

Moreover, foams are generally stable if the bubbles are well distributed and do not have an excessive number of large bubbles (Pugh, 2016). Bubbles of unequal radius have a greater

variance in their internal pressure, and because of the difference in Laplace pressure, smaller bubbles will gather on the surface of larger ones and coalesce more rapidly. This is because of an inverse relationship between droplet size and Laplace pressure (Fox, Ingenpass, & Zachow, 2003). Foams at low and high temperatures demonstrated large variability of bubble sizes that could lead to greater instability of those foams (Fig.23).

As mentioned earlier, to meet the standards for *microfoam*, the milk foam should be well distributed and not contain visible large bubbles. This gives *microfoam* an advantage in stability over "traditional" milk foam for espresso-based drinks. Fig. 25 shows that the air bubbles are more evenly distributed in the foams prepared at 60°C than at 50°C. In addition, UHT milk foams generally have a higher number of air bubbles at 60°C than at 50°C. The higher stability of the milk foams within the selected temperature range was observed at 50°C to 60°C.

As for the semi-skimmed milk, the results showed no significant difference in *microfoam* stability between full and semi-skimmed pasteurised milk.

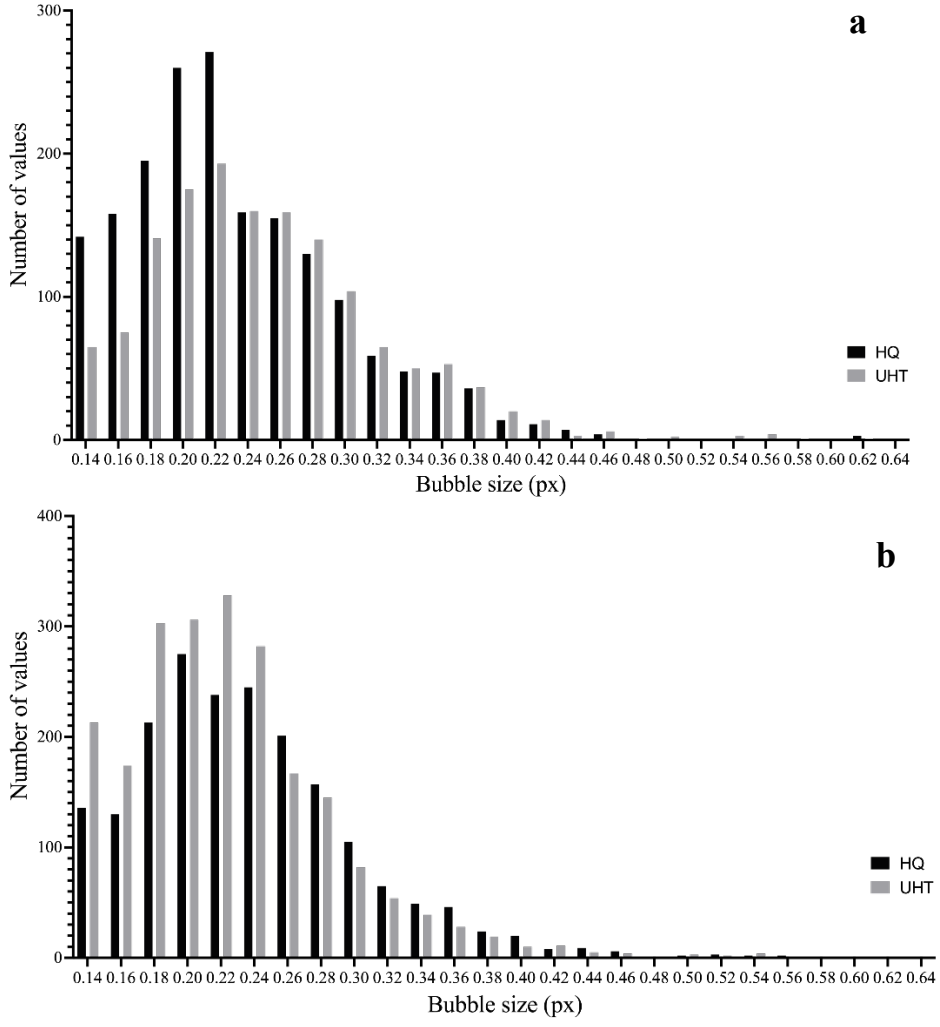


Fig. 25. Bubble size distribution at a) 50°C and b) 60°C for foams made from whole pasteurised (HQ) and whole UHT milk at initial time i.e., in 30 seconds after foaming.

Regarding the effect of fat on foam stability, the semi-skimmed pasteurised milk showed the lowest bubble distribution, which correlates with the data on FDR and viscosity. However, fat in milk affects not only foam formation and stability. Whole milk can be chosen for espresso-based milk beverage preparation because higher fat content in milk affects coffee flavour perception, i.e., it provides more milk flavour and slightly masks some coffee flavour characteristics such as burnt, sour, and roasted (Parat-Wilhelms, Denker, Borcherding, Hoffmann, Luger, & Steinhart, 2005; Itobe, Nishimura, & Kumazawa, 2015). However, if needed, whole milk can be successfully substituted by milk with lower fat content. It was observed that foams made from semi-skimmed milk are even more stable than those made from whole milk. Studies by Kamath et al. (Kamath, Huppertz, Houlihan, & Deeth, 2008) showed that unlike pasteurised homogenised whole milk, foam lamellae around smaller bubbles in skim milk foams remained well defined and no distinct liquid areas were observed on the surface of the foam, indicating that bubble bursting is not common.

7.8. Particle size distribution

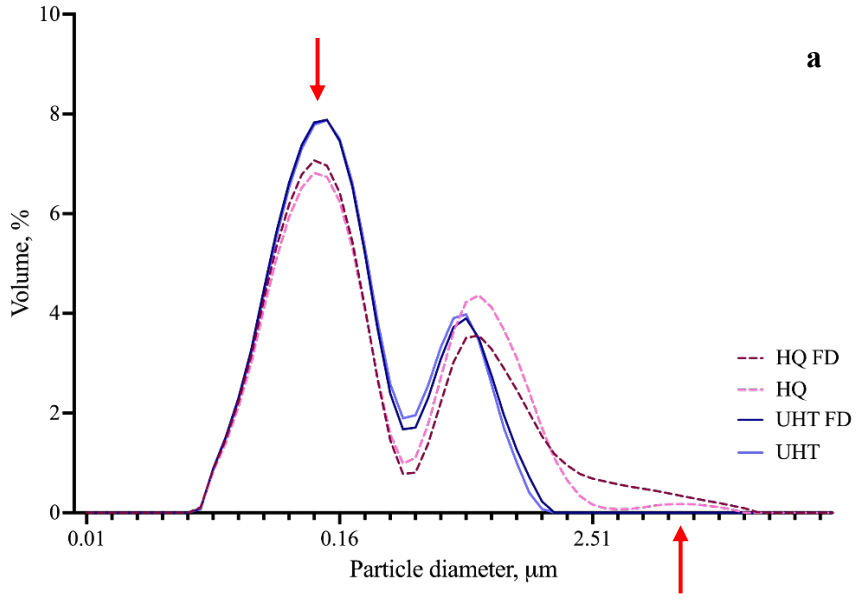
The size of fat globules plays an important role in milk colloidal system stability and might affect foaming properties and foam stability (Zakidou & Paraskevopoulou, 2020). The measurement of particle size distribution in milk samples was performed for pasteurised (HQ), UHT, and freeze-distilled (FD) milk samples. The aim of this analysis was to evaluate the dependence of freeze-distillation on particle size distribution, particularly, fat globules. For that purpose, casein micelles were previously dissociated with a casein micelle dissociating agent (CMDA, section 6.12). The particle size distribution in milk samples with and without CMDA is summarised in Table 8.

Table 8. Particle size distribution in milk samples including: ultra-high temperature treated whole milk (UHT), freeze-distilled UHT (UHT FD), pasteurised whole milk (HQ), freeze-distilled HQ (HQ FD) with casein (with CN) and after the addition of casein micelle dissociating agent (no CN). Results are expressed as the surface-weighted mean diameter (D[3,2]) and volume-weighted mean diameter (D[4,3]) in μm. Figures are means of three replicates and standard deviation.

	D[3,2]		D[4,3]	
	With CN	No CN	With CN	No CN
UHT	0.143±0.002	0.186±0.001	0.282±0.004	0.363±0.001
UHT FD	0.142±0.015	0.168±0.007	0.289±0.001	0.318±0.008
HQ	0.159±0.000	0.184±0.002	0.505±0.011	0.506±0.004
HQ FD	0.155±0.001	0.183±0.003	0.653±0.005	0.679±0.006

The increase of D [3,2] and D [4,3] in case of HQ FD in comparison to the regular HQ milk samples could potentially lead to the deterioration of foaming properties since large droplets can lower foaming ability of milk, especially at low temperatures around 4 °C (Kamath, Huppertz, Houlihan, & Deeth, 2008). However, textural studies demonstrated that freeze-distillation does not significantly alter microfoam texture and stability (sections 7.5 – 7.7). UHT and UHT FD samples did not demonstrate this difference in surface-weighted and volume-weighted mean diameters.

Fig. 26 summarises the data on particle size distribution before and after the addition of CMDA to dissociate CN micelles. The addition of the CMDA decreased the number of particles within the distribution range of 0.02–0.04 μm. This might be associated with the dissociation of CN micelles since their size averages 0.2 μm (Yun, & Imm, 2021). The decrease in the number of larger particles in milk samples might be caused by the dissociation of larger CN aggregates due to the addition of the CMDA (Ransmark, Svensson, Svedberg, Göransson, & Skoglund, 2019).



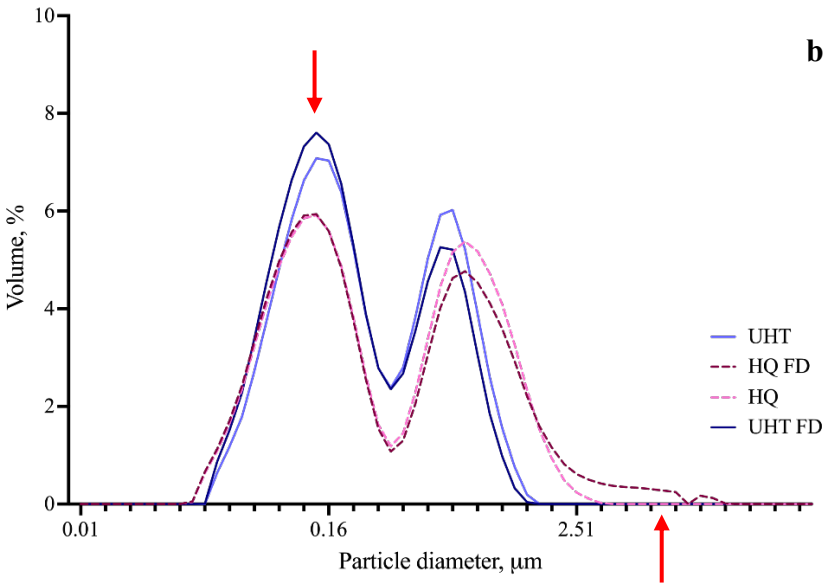


Fig. 26. Particle size distribution in milk samples including: ultra-high temperature treated whole milk (UHT), freeze-distilled UHT (UHT FD), pasteurised whole milk (HQ), freeze-distilled HQ (HQ FD) a) before the addition of casein micelle dissociating agent (CMDA) and b) after the addition of CMDA. Red arrows compare the difference in particle size distribution within the range of 0.02–0.04 µm and of larger particles (> 2.5 µm) to illustrate the effect from the addition of sol A to milk. Measurements were performed in triplicates; error bars were omitted to improve clarity.

7.9. Sensory discrimination tests

To better understand whether there was a noticeable difference in the aroma and taste of pasteurised and UHT milk samples, a sensory analysis test was conducted with 31 (n=31) testers. Lactose-free milk samples were not included in the sensory test because consumer perception of the sweeter taste of lactose-free milk compared to regular cow's milk has been extensively studied (Rizzo, Harwood, & Drake, 2020; Adhikari, Dooley, Chambers, & Bhumiratana, 2010; Palacios, Badran, Spence, Drake, Reisner, & Moskowitz, 2010). Separated triangle tests was performed to compare regular and freeze-distilled (FD) milk samples (pasteurised and UHT) in the form of cappuccino beverage. The sensory panel was conducted with 40 (for pasteurised milk panel) and 41 (for UHT milk panel) (n=40, n=41) assessors.

The first part of the sensory evaluation was conducted using a triangle test, in which a higher proportion of correct choices indicated a detectable difference between two types of milk (pasteurised and UHT). The second part of the sensory analysis test was duo-trio test when panellists were asked to compare two pairs of samples to the control sample. The null hypothesis (H_o) and the alternative hypothesis (H_a) were accepted or rejected.

Table 9 summarises the sensory panel data of the triangle and duo-trio tests. The triangle test showed that 87.1% of the assessors clearly recognized and noted a perceptible difference between pasteurised and UHT milk. The triangle test of regular anf freeze-distilled milk samples showed that 70% of the assessors clearly recognized and noted a perceptible difference between regular and FD pasteurised milk cappuccino samples and 73.2% of the assessors noted a significant difference between regular and FD UHT milk cappuccino samples. Consequently, there was a significant sensory difference ($P < 0.05$) between pasteurised and UHT milk and alternative hypothesis H_a was accepted.

Table 9. Triangle and duo-trio tests of pasteurised (HQ), ultra-hight temperature treated (UHT), and freeze-distilled (FD) milk sensory panel results.

Test	N of answers (correct/total)	% of correct answers	P-value
Triangle			
HQ/UHT	27/31	87	<0.001
HQ/HQ FD	28/40	70	<0.0001
UHT/UHT FD	30/41	73.2	<0.0001
Duo-trio			
Pair 1	27/30	90	<0.0001
Pair 2	24/30	80	0.0004

Table 10 shows that the majority (85.14%) of the assessors rated the difference between two samples as *moderate* to *much* degree. The table 10 also demonstrates that the majority in both pasteurised and UHT milk panels indicated the degree of difference between regular and freeze-distilled samples as *moderate*. No assessors evaluated the difference as *extreme*.

Table 10. The degree of difference between pasteurised (HQ), ultra-hight temperature treated (UHT), and freeze-distilled (FD) milk samples according to the triangle test results.

Description	Number of answers		
	HQ/UHT	HQ/HQ FD	UHT/UHT FD

Slight	2 (7.4%)	9 (32%)	9 (21.9%)
Moderate	11 (40.7%)	11 (39.4%)	15 (36.6%)
Much	12 (44.44%)	8 (28.6%)	7 (17%)
Extreme	2 (7.4%)	0 (0%)	0 (0%)

Further, panellists noted their preferences and a brief description of the samples. The majority (66.7%) of the panellists indicated that they preferred the UHT sample and 33.3% of the testers indicated that they preferred pasteurised milk sample.

According to the analysis of the free-form descriptions written by the judges, the UHT milk sample has a distinct sweet and cooked taste, a more intense aroma, tastes more traditional and had a vanilla flavour compared to the double samples. In contrast, the judges pointed out the metallic taste and aftertaste of the pasteurised milk, as well as a certain flatness compared to the UHT. The word clouds created based on the descriptions are shown in Fig. 27.

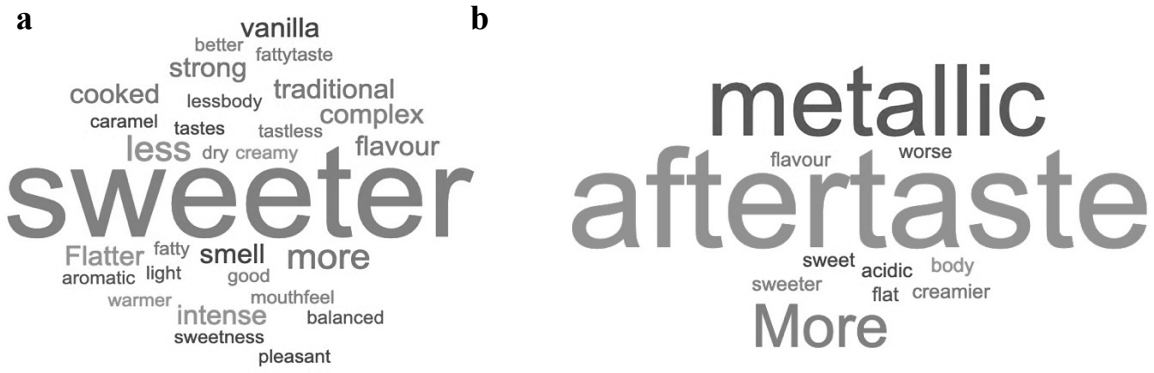


Fig. 27. Word clouds made on a basis of descriptions of a) ultra-hight temperature treated (UHT) and b) pasteurised (HQ) milk sample provided by the participants of the triangle test (n=27).

Fig. 28 illustrates word clouds made based on the free-form descriptions describing regular and freeze-distilled milk samples. According to the questionnaires, cappuccino made on freeze-distilled milk both HQ FD and UHT FD had a distinctly sweeter flavour, creamier texture and more intense aroma. It was also noticed that cappuccino samples prepared on regular milk were described as *bitter* that could be possibly associated with the ability of concentrated freeze-distilled milk to better mask the natural bitterness of the espresso coffee.

Fig. 28. Word clouds made on a basis of descriptions of cappuccino samples prepared with a) pasteurised freeze-distilled (HQ FD), b) regular pasteurised (HQ), c) ultra-hight temperature treated UHT freeze-distilled (UHT FD), and d) regular UHT milk provided by the participants of the triangle test (n=28 and 31 respectively).

Finally, panellists noted their preferences. The slight majority (55.5%) of the panellists indicated that they preferred the freeze-distilled milk sample in the pair of regular and freeze-distilled pasteurised milk (HQ/HQ FD). As for the pair of regular and UHT milk (UHT/UHT FD), the majority (61.3%) of assessors noted that they prefer freeze-distilled sample.

The results of the duo-trio test confirmed H_a, as a significant number of panellists were able to find the control sample in a pair of samples (Table 9). There was a total of 6 incorrect responses in the second pair; 3 of the panellists who chose the incorrect response chose it in both pairs; 2 of the assessors answered correctly in the first pair and incorrectly in the second; none of the assessors who chose the incorrect response in the first pair answered correctly in the second; all the panellists who answered incorrectly in the first pair chose the incorrect response in the second pair. The slight difference in results could be due to sensory fatigue in the judges. Nevertheless, the percentage of correct answers remains significant in both pairs.

The results of sensory discriminations tests showed that panellists can distinguish pasteurised and UHT milk samples (Table 9). Most of the assessors described the UHT samples as sweeter, intense, caramel-like, and cooked. We suggest that characteristics of UHT milk samples described by the assessors mostly relate to the initial stages of the Maillard reaction and generally were positively received.

8. CONCLUSIONS

In this thesis an in-depth analysis of *microfoams* obtained from whole pasteurised (HQ), whole ultra-high temperature treated (UHT), lactose-free whole pasteurised and UHT, pasteurised semi-skimmed, and freeze-distilled (FD) pasteurised and UHT milk samples has been carried out. There was observed a significant difference in the serum protein and SH-group content between pasteurised and UHT milk. It is noticeable that the normalised quantity of free thiol groups is higher in UHT milk than in pasteurised milk in relation to the whey protein content. Since higher temperatures are applied during UHT treatment, the degree of denatured serum proteins was higher in UHT milk. Although denaturation of whey proteins increases the viscosity of milk, foaming with steam dilutes milk with water, which may prevent the formation of more stable foams at higher temperatures (70°C and above). As for freeze-distilled (FD) milk samples, the analysis of the protein fraction of regular and FD milk samples showed the increase of α-casein in HQ FD and UHT FD compared to the regular HQ and UHT milk samples, respectively, and the β-casein concentration in the UHT FD sample compared to the UHT milk. In addition, the concentration of α-lactalbumin was increased in the freeze-distilled pasteurised milk samples compared to the regular pasteurised milk samples. The concentration of serum albumin, κ-casein and β-lactoglobulin was not significantly affected by freeze-distillation. It was suggested that the increase in protein concentration might be related to the removal of some of the water from the milk, but not to protein aggregation due to freezing was observed, as the particle size distribution in regular and freeze-distilled milk samples was identical.

- The comparison of the lactose content in non-heated milk samples showed that the amount of lactose in FD milk samples is approx. three times higher than in regular milk considering the fact that part of the lactose remains in the liquid fraction which was not used for foaming.
- One of the parameters of foam stability is foam decrease rate (FDR). FDR measurements showed that microfoam from UHT whole milk is less stable over the entire temperature range compared to pasteurised milk samples. In addition to the difference in the FDR of *microfoams* discussed above, it was noticed that the initial foam height might affect the stability of foam. Higher foams might be formed due to the inappropriate foaming technique leading to the incorporation of large bubbles into

milk. This usually happens when the steam nozzle has not been immersed enough into milk. The faster collapse of higher foams may be due to the heterogeneous distribution of temperature within the foam, i.e., it is lower at the surface than in the lower layer.

- The analysis of the rheological properties of *microfoams* showed that they exhibit non-Newtonian shear-thinning behaviour and can be considered as pseudoplastic fluids. Difference in foam viscosities at different temperatures might be caused by several reasons including: bubble quantity and distribution, decreased viscosity of milk facilitating the migration of protein molecules to the air-serum interface, difference in speed of drainage. Foams with more uniform bubble size distribution appeared to be more stable and viscous, i.e., at 50 and 60°C. Freeze-distillation did not seem to significantly affect the foaming properties of the milk. This is also consistent with the results of particle size distribution measurement, as the viscosity of milk foam demonstrated the dependence on the solids content of the milk. The increased viscosity of FD milk did not seem to affect the viscosity of the microfoams produced from these samples. However, it could contribute to increasing the stability of microfoams made from pasteurised milk, as the higher viscosity of the milk was associated with better stability of the milk foam.
- The comparison of regular and freeze-distilled samples showed that there is no significant difference in foam expansion and foam stability between these two groups of samples. However, the value of microfoam overrun that represents the density of foam is significantly higher for HQ and UHT milk samples is significantly higher than the overrun of FD samples at 70°C. More, overrun of UHT milk samples was significantly higher than that of HQ. Nevertheless, the comparison of overrun of freeze-distilled HQ and UHT milk samples showed similar behaviour within the whole temperature range. The increase in overrun at 70°C reflects the decrease in the density of the microfoam at high temperatures, which correlates with the results of the FDR and foam viscosity measurements.
- The microscopic analysis of air bubbles within microfoam showed that they are more evenly distributed in the foams prepared at 60°C than at 50°C. In addition, UHT milk foams generally have a higher number of air bubbles at 60°C than at 50°C. The higher stability of the milk foams within the selected temperature range was observed at 50°C

to 60°C. As for the semi-skimmed milk, the results showed no significant difference in *microfoam* stability between full and semi-skimmed pasteurised milk.

- Studies of particle size distribution of regular and FD milk samples showed the increase of D [3,2] and D [4,3] in case of HQ FD in comparison to the regular HQ milk samples that could potentially lead to the deterioration of foaming properties since large droplets can lower foaming ability of milk. However, textural studies demonstrated that freeze-distillation does not significantly alter microfoam texture and stability. UHT and UHT FD samples did not demonstrate this difference in surface-weighted and volume-weighted mean diameters.
- The results of sensory discrimination tests showed a significant number of panellists were able to distinguish between pasteurised and UHT milk samples. Most of the assessors described the UHT samples as *sweeter*, *intense*, *caramel-like*, and *cooked*. It was suggested that characteristics of UHT milk samples described by the assessors mostly relate to the initial stages of the Maillard reaction and generally were positively received.
- The significant number of assessors could also distinguish between regular and freeze-distilled milk samples for both pasteurised and HQ milk type. According to the analysis of the free-form descriptions written by the judges, cappuccino made on freeze-distilled milk both HQ FD and UHT FD has a distinctly sweeter flavour, creamier texture and more intense aroma. It was also noticed that cappuccino samples prepared on regular milk were described as *bitter* that could be possibly associated with the ability of concentrated freeze-distilled milk to better mask the natural bitterness of the espresso coffee. As for the analysis of personal preferences of panellists, the majority of them indicated that they preferred the freeze-distilled milk sample in the pair of regular and freeze-distilled pasteurised milk and UHT milk sample.

To summarise, since espresso-based milk drinks, e.g. cappuccino, are expected to have stable milk foam during the average consumption time of 10 – 15 minutes, *microfoam* made from pasteurised milk in the temperature range of 50°C to 60°C with maximum stability at 50°C is more acceptable than UHT milk *microfoam* according to the parameters studied. Industrial heat treatment affects the milk protein content and structure. Both pasteurised and UHT (regular and lactose-free) milk can be used to make *microfoam*. However, the viscosity and

stability of pasteurised milk are higher in relation to the FDR, so it could be an advantage in achieving the *microfoam* properties. It is important not to exceed the temperature of 70°C, as our studies have shown that at higher temperatures (> 70°C) the amount of liquid draining off increases decreasing the stability of *microfoam*. Milk with a reduced fat content can be successfully used to prepare cappuccino-like beverages as it results in stable foaming; however, it is important to consider the effects of skim milk on coffee taste. It was foreseeable that the type of industrial heat treatment affected not only the components of milk, but also its aroma and taste. Our studies showed that consumers could distinguish UHT milk from pasteurised milk. In addition, panellists preferred UHT milk to pasteurised milk, describing it as sweeter and more traditional, less flat compared to pasteurised milk. So, when choosing milk for a bar or café, it is important to consider the difference in stability of milk froths from different types of milk and the differences in taste and aroma of milk.

Freeze-distillation increases the concentration of lactose and total protein in the milk samples as the water content decreases. As for the stability of the *microfoam*, it was found that microfoams with similar viscosity and stability could be obtained with regular and freeze-distilled milk samples, although the viscosity of the milk samples themselves was increased. The results of the sensory discrimination tests confirmed the alternative hypothesis, as the testers were able to distinguish between cappuccinos made from regular and freeze-distilled milk samples for both UHT and pasteurised milk. Most testers also preferred samples from freeze-distilled milk, describing them as more intense, sweeter and richer than samples from normal UHT or pasteurised milk.

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10. LIST OF PUBLICATIONS

Publications in peer-reviewed journals and book chapters

1. Klimanova, Y., Polzonetti, V., Pucciarelli, S., Perinelli, D. R., Bonacucina, G., Cespi, M., Gabrielli M. G., Santini, G., Fioretti, L., Cognigni, L., & Vincenzetti, S. (2022). Effect of Steam Frothing on Milk Microfoam: Chemical Composition, Texture, Stability and Organoleptic Properties. *International Dairy Journal*, 135, 105476.
2. Santini, G., Klimanova, Y., Pucciarelli, S., Polzonetti, V., Cespi, M., Perinelli, D. R., Polidori, P., Cognigni, L., Fioretti, L., Renzi, S., & Vincenzetti, S. (2023). Effects of different steam injection conditions on cappuccinos quality parameters.
   The paper was submitted to the *Food Chemistry* Journal and is currently going through the peer-review process.
3. Silvia Vincenzetti1.Effects of different steam injection conditions on cappuccinos quality parameters
4. Polidori, P., Rapaccetti, R., Klimanova, Y., Zhang, J.-J., Santini, G., & Vincenzetti, S. (2022). Nutritional Parameters in Colostrum of Different Mammalian Species. *Beverages*, 8(3), 54.
5. Polidori, P., Santini, G., Klimanova, Y., Zhang, J. J., & Vincenzetti, S. (2022). Effects of Ageing on Donkey Meat Chemical Composition, Fatty Acid Profile and Volatile Compounds. *Foods (Basel, Switzerland)*, 11(6), 821.
6. Vincenzetti, S., Cammertoni, N., Rapaccetti, R., Santini, G., Klimanova, Y., Zhang, J-J., & Polidori, P. (2022). Nutraceutical and Functional Properties of Camelids' Milk. *Beverages*, 8(1), 12.
7. Polidori, P., Cammertoni, N., Santini, G., Klimanova, Y., Zhang, J. J., & Vincenzetti, S. (2021). Effects of Donkeys Rearing System on Performance Indices, Carcass, and Meat Quality. *Foods (Basel, Switzerland)*, 10(12), 3119.

8. Polidori, P., Cammertoni, N., Santini, G., Klimanova, Y., Zhang, J.-J., & Vincenzetti, S. (2021). Nutritional Properties of Camelids and Equids Fresh and Fermented Milk. *Dairy*, 2, 288-302.
9. Vincenzetti, S., Santini, G., Polzonetti, V., Pucciarelli, S., Klimanova, Y., & Polidori, P. (2021). Vitamins in Human and Donkey Milk: Functional and Nutritional Role. *Nutrients,* 13(5), 1509.
10. Vincenzetti, S., Pucciarelli, S., Santini, G., Klimanova, Y., Polzonetti, V., & Polidori, P. (2020). B-Vitamins Determination in Donkey Milk. *Beverages,* 6(3), 46.
11. Santini, G., Bonazza, F., Pucciarelli, S., Polidori, P., Ricciutelli, M., Klimanova, Y., Silvi, S., Polzonetti, V., Vincenzetti, S. (2020). Proteomic characterization of kefir milk by two-dimensional electrophoresis followed by mass spectrometry. *Journal of Mass Spectrometry,* e4635.

10. ACCOMPLISHMENTS

UNICAM course of Italian language for foreigners, 60 hours, level B1 (final exam 78/81 points)
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ASSINT
Area Servizi agli Studenti e Internazionalizzazione
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Si attesta che la dottoranda

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Camerino, 7 maggio 2021

L'insegnante
Dott.ssa Rita Ortenzi

Il Delegato del Rettore
Sviluppo competenze linguistiche
Professor Renato De Leone