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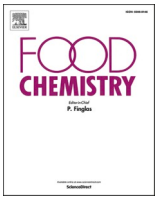

Effects of different steam injection conditions on cappuccino's nutritional profile

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ABSTRACT

The quality parameters of cappuccinos prepared with pasteurized milk or ultra-high-temperature milk steam-injected at different temperatures by a professional coffee machine have been assessed. In particular, the protein profile, the content of vitamins and lactose, the lipid peroxidation process, and the involvement of milk proteins in the foam formation were evaluated. The nutritional quality of milk seems not affected by the steam injection treatment carried out at a temperature of 60–65 °C, but at higher temperatures a decrement of lactoperoxidase, vitamin B6 and folic acid was observed. The milk used in cappuccino preparation is very important: pasteurized milk can form a more consistent and lasting foam with respect to ultra-high-temperature milk because of the presence of β-lactoglobulin and lactoferrin, both playing an important role in the foam formation and stability. This work would provide additional information to the coffee industry for the preparation of high nutritional and organoleptic quality cappuccinos.

1. Introduction

Among milk-based coffee drinks cappuccino is one of the most popular (Czarniecka-Skubina, Pielak, Sałek, Korzeniowska-Ginter, & Owczarek, 2021). In this beverage, an important quality characteristic for consumers is the foam layer at the top which must be as creamy as possible and leave a pleasant sensation in the mouth. Moreover, the global and European coffee markets, from 2022 to 2025, are forecast to grow at an annual rate of 7.64 % and 6.79 %, respectively. Considering the consumer's interest in cappuccino-like beverages, a more in-depth study of the nutritional aspects of the milk foaming process with steam-injection equipment, i.e. traditional coffee machines, is needed. A traditional cappuccino is made of 25 mL espresso coffee and 100 mL steam-foamed milk growing to a volume of about 125 mL. To get a high-quality final product, the selection of the raw material is very important; the cow milk used should have 3.2 % minimum protein content and 3.5 % minimum fat content in order to get the perfect balance of fat and protein fractions that ensures good taste and a creamy foam with a good texture. In general, a foam of excellent quality must meet specific parameters and it should be smooth, glossy, and moist, with no visible bubbles (Klimanova et al., 2022).

Milk foam is an air–liquid colloidal system in which milk proteins, due to the presence of hydrophilic and hydrophobic groups, act as surfactant agents. Milk proteins with good surfactant properties are both present in whey proteins (β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoferrin) and in caseins. Foam stability is enhanced when the protein content in the milk is high because of the formation of high viscoelastic films at the interface (Ho, Bhandari, & Bansal, 2021).

During steam injection, as soon as the gas is introduced into the milk, a gas–liquid interfacial film is formed that is stabilized by the surface tension between the gas and the liquid (milk) promoting the foam formation. After steam injection, denatured milk proteins are oriented at the air–liquid interface with the polar group rearranged towards the aqueous phase and the nonpolar group towards the non-aqueous one. The subsequent interactions among unfolded proteins give rise to a strong, viscous, and elastic interfacial film that stabilizes the air bubbles (Huppertz, 2010; Ho et al., 2021).

The behaviour of milk proteins at the air–water interface has been

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the object of several research studies that took into consideration the relationship between the chemical properties of milk proteins with the film formation, surface tension, and foam stability (Zhang, Dalgleish, & Goff, 2004). These studies evidenced that because of their flexible structure β-caseins can spread and absorb to the interface air–liquid very fast, contrarily to the globular whey proteins. Martin and co-workers (Martin, Grolle, Bos, Stuart, & van Vliet, 2002) have demonstrated that at a protein concentration of 0.1–3.0 % (w/v) and pH 6.7, β-lactoglobulin showed a lower foamability with respect to β-casein, because of its self-association that produces a dimer with a higher molecular size and its structural rigidity. However, β-lactoglobulin produced more stable foam than β-casein because of the strong internal structure, low adsorption probability, and the presence of free disulphide groups, which form a rigid network at the interface.

Besides the consistency of the foam, the nutritional quality of a good cappuccino deserves to be considered to make sure that the milk frothing process does not alter the characteristics of the milk. Normally cappuccino is prepared at a temperature of about 60 °C, but in some cases, the use of temperatures higher than 60 °C can be required which, however, can modify the nutritional quality, the organoleptic characteristics, and the foam structure of the beverage itself. It is for this reason that our attention focused on the nutritional characterization of cappuccinos prepared with commercial pasteurised high-quality milk (HQ milk) and ultra-high temperature milk (UHT milk) heated by a coffee machine steamer at different temperatures. In particular, the protein profile, the vitamin content (water- and fat-soluble), the lactose content, the lactoperoxidase activity and the lipid peroxidation process were evaluated. Vitamins are organic compounds required by the body in small amounts, very important for human nutrition. Water-soluble vitamins are the B vitamins and vitamin C, found in several vegetable and animal foods, including milk and its derivatives. In milk, the fat-soluble vitamins present in greater quantities are vitamins A, E, and K and their content is influenced by the food ration composition of the dairy cow, the season, and the lipid content (Mandrioli, Boselli, Fiori, & Rodriguez-Estrada, 2020).

Lactoperoxidase (LPO) is a member of the peroxidase-cyclooxygenase superfamily and one of the most abundant enzymes in bovine milk, making up about 1 % of the whey proteins in milk. This enzyme, together with its substrates, hydrogen peroxide, and oxidized products forms the “lactoperoxidase system” and has the function of protecting the mammary glands and the milk against pathogens. Lipid peroxidation is the degradation of lipids that occurs because of oxidative damage and therefore its evaluation is a useful marker for oxidative stress. Polyunsaturated lipids are in fact susceptible to an oxidative attack, typically by reactive oxygen species, resulting in the production of end products such as malondialdehyde (MDA). Lipid peroxidation products seem to be directly involved in the development of several pathologies such as atherosclerosis, cancer, and aging processes (Ajmal, Nadeem, Imran, & Junaid, 2018). In addition, they have a detrimental effect on milk flavour, texture and nutritive value.

Finally, since a high-quality cappuccino requires a consistent and lasting foam, the involvement of milk proteins in foam formation and stability has been investigated. The final objective of this study was to provide additional and useful information to the coffee industry for the preparation of milk and coffee-based beverages characterized by high nutritional and organoleptic quality.

2. Materials and methods

2.1. Chemicals

Tris (hydroxymethyl) aminomethane (trizma base), dithiotreitol (DTT), sodium dodecyl sulphate (SDS), lysozyme (from egg white), α-lactalbumin, β-lactoglobulin, β-casein, αs1-casein (from bovine milk), chlorogenic acid, trigonelline, caffeine, 2,2′-Azinobis (3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS), water- and fat-soluble vitamins standards, peroxidase (from horseradish), and other reagents were from Merck-Sigma Aldrich, Darmstadt, Germany.

Mini Protean III, and SDS-PAGE molecular weight standard low range were from Bio-Rad Laboratories (Hercules, CA). The HPLC system was an Agilent 1260 Infinity LC System (Agilent Technologies), consisting of a 1260 Infinity Quaternary Pump, 1260 Infinity Multisampler, 1260 Infinity Multicolumn Thermostat, and 1260 Infinity Diode Array Detector. Data were acquired by the OpenLab Software (Agilent technologies). The chromatographic columns were: 300 Å C4 Prosphere (5 μm, 4.6 mm I.D., 150 mm, Alltech), for protein profile evaluation and C18 HiQSil HS, 5 μm, 4.6 mm i.d, 250 mm (Kya Tech Corporation, Tokyo, Japan), for vitamins determination.

2.2. Samples preparation

The commercial milk types used to prepare cappuccino samples belonged to the same batch, and were pasteurised high-quality (HQ milk, nutritional value per 100 mL: fats 3.6 g; proteins 3.2 g; sugars 5.0 g), and ultra-high temperature milk (UHT milk, nutritional value per 100 mL: fats 3.6 g; proteins 3.1 g; sugars 4.8 g). UHT milk has been taken into consideration in this study since this milk is sometimes used for the preparation of cappuccinos because it has a long shelf life and therefore may result in more practical than pasteurized milk. The espresso blend used for cappuccino preparation was a 100 % medium roasted Arabica coffee beans and consisted of beans from Brazil (Mundo Novo, natural), India (Plantation, S795 & Kent, fully washed), and Ethiopia (Yirgacheffe, Heirloom, fully washed). 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 with a built-in tamper. Each milk sample, with an initial volume of 150 mL and a temperature of 8 ± 1 °C, was heated with the steam nozzle (steamer) of the coffee machine in a metal jug with a capacity of 350 mL. Steam at a gauge pressure of 110 kPa was injected into milk to reach temperatures ranging from 40 °C to 80 °C. The quantity of water found in the sample after the steam injection treatment has been calculated and has been considered in the final calculation of the concentrations of the analytes under consideration. Furthermore, in all the analyses carried out in the cappuccino samples, the dilution made by the coffee (~1.18-fold) has been also considered.

2.3. Protein profile by reversed Phase-High Performance liquid chromatography (RP-HPLC)

Protein profile analysis was performed on HQ milk and on cappuccinos prepared with HQ and UHT milk (named HQ cappuccino and UHT cappuccino), and on the respective foams (named HQ foam and UHT foam). Samples were prepared as described in the “Sample preparation” section at a temperature range of 40–80 °C. Before being subjected to the Reversed Phase-High Performance Liquid Chromatography (RP-HPLC), the foams were left at 4 °C for about 2 h to liquefy, subsequently cappuccino and foam samples were skimmed by centrifugation at 5000 xg for 30 min at 15 °C. Each skimmed sample was then clarified by the addition of two volumes of CL buffer (0.1 M bis-tris, pH 8.0 containing 8 M urea, 1.3 % trisodium citrate, 0.3 % DTT), as described previously (Vincenzetti et al., 2008). One hundred microliters of clarified samples were loaded into the RP-HPLC column. The column was equilibrated in trifluoroacetic acid (TFA)/H_2O 1:1000 v/v (buffer A) and elution was achieved by the following step gradient with TFA/H_2O/acetonitrile 1:100:900 v/v (buffer B): %B = 0, time = 10 min; %B = 20, time = 10 min; %B = 40, time = 0.1 min; %B = 60, time = 40 min. The flow rate was 1 mL/min and fractions of 0.5 mL were collected. The proteins eluted from RP-HPLC columns were monitored at 280 nm. Each standard solution of bovine milk β-lactoglobulin (from 0.033 to 0.33 mg/mL), bovine milk α-lactalbumin (from 0.033 to 0.33 mg/mL), human lactoferrin (from 0.005 to 0.2 mg/mL), bovine β-casein (from 0.2 to 1.0

mg/mL) and bovine α_{s1}-casein (from 0.024 to 0.4 mg/mL), was prepared in CL buffer. A 100-μL solution of each standard was separately loaded on the RP-HPLC column. The area of each standard peak was measured using the valley-to-valley integration mode and quantification was achieved by a calibration curve obtained relating the concentration in micrograms of each standard loaded in the column to the peak area corresponding to each concentration. From each milk sample the quantity of lysozyme, β-lactoglobulin, α-lactalbumin, lactoferrin, β-casein, and α_{s1}-casein was determined by using the calibration curve. Furthermore, standard solutions of coffee compounds such as chlorogenic acid, trigonelline, and caffeine each prepared at the concentration of 0.1 mg/mL were separately loaded on RP-HPLC and used for the identification of the coffee compounds in the cappuccino preparations. All measurements were conducted in triplicate and the results were averaged and the standard deviations calculated.

2.4. SDS-PAGE, in-gel protein digestion and liquid chromatography-tandem mass spectrometry analysis

After RP-HPLC, the identification of the proteins eluted from the column was performed by loading each chromatographic to a 15 % SDS-PAGE. After electrophoresis the resulting protein bands were in-gel digested and subjected to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) for their identification. More in detail, 15 % SDS-PAGE was carried out as described by Laemmli (1970), under reducing conditions using a 15 % acrylamide-bis acrylamide solution. The electrophoretic gel was stained by Coomassie Blue staining (0.1 % Coomassie Brilliant Blue R250 in 50 % methanol and 10 % acetic acid). After electrophoresis, the protein bands were manually excised from the gel and subjected to the protein in-gel digestion procedure according to Shevchenko and co-workers (Shevchenko, Tomas, Havlis, Olsen, & Mann, 2006). The resulting tryptic peptides were resuspended in 100 μL of 0.1 % (v/v) trifluoroacetic acid and loaded into a reversed phase chromatography using the same chromatographic condition described in a previous work (Vincenzetti et al., 2016). The eluate was analysed by MS using an ESI/ion trap mass spectrometer (Agilent Technologies LC/MSD Trap SL) operating in positive ion mode over the mass range 300–2200 amu (atomic mass units). MS spray voltage was 3.5 kV and the capillary temperature was maintained at 300 °C. Obtained MS spectra were extracted and analysed by the MASCOT software (www.matrixscience.com) with the following search parameters: database, NCBInr taxonomy: *Bos taurus*; enzyme, trypsin; peptide tolerance, 1.2 Da; MS/MS tolerance, 0.6 Da and allowance of one missed cleavage.

2.5. Lactoperoxidase activity

Lactoperoxidase activity was evaluated by a continuous spectrophotometric rate determination using ABTS as substrate. In this method, reduced ABTS is oxidized by H_2O_2 to form oxidized ABTS and H_2O. The reaction mixture contained 75 mM potassium phosphate pH 5.5, 23 mM ABTS, and 0.0008 % (w/w) hydrogen peroxide and was started by the addition of 10 μL of each milk sample diluted 1:10 with the reaction buffer. The sample consisted of HQ milk heated for 20 s with the steam nozzle (steamer) of the coffee machine Appia II semi-automatic (Nuova Simonelli SpA, Belforte del Chienti, Italy), in the temperatures range of 50–80 °C. Absorbance measurement was made at $\lambda = 436$ nm in continuous after 5 min reaction. One unit of lactoperoxidase is defined as the amount of the enzyme that oxidizes 1.0 μmol of ABTS per minute at pH 5.5 and 25 °C.

2.6. Water- soluble and fat-soluble vitamins content determination

Water-soluble and fat-soluble vitamins content has been determined on HQ milk samples heated for 20–60 s with the steamer of the coffee machine Appia II semi-automatic (Nuova Simonelli SpA, Belforte del Chienti, Italy), in the temperatures range of 50–80 °C. During preparation, the samples were protected from light by wrapping tubes and flasks with aluminum foil. Before being analyzed for the water-soluble vitamin content by RP-HPLC, the milk samples were processed as described in a previous work (Vincenzetti, Pucciarelli, Santini, Klimanova, Polzonetti, Polidori, 2020). Standard solutions of vitamin B1 (from 0.1 to 18.7 μM), vitamin B2 (from 0.5 to 15 μM), vitamin B6 (from 0.75 to 20.0 μM) and folic acid (from 0.3 to 7.5 μM) were prepared in ultra-pure water.

Regarding the fat-soluble vitamin content determination, the method was based on the protocol of Albalá-Hurtado and co-workers with some modifications (1997). Before performing the analysis, milk was prepared by the following protocol: to 5 mL of milk in a graduated flask, 0.1 g of ascorbic acid was added followed by the addition of 10 mL of absolute ethanol and 2 mL of 60 % potassium hydroxide solution. The graduated flask was closed hermetically and kept in the dark, the mixture was incubated at 60 °C for 30 min under shaking, cooled for 5 min in cold water (water and ice) and 5 mL of *n*-hexane, were added followed by vigorous shaking for one minute. The content was transferred into a 50 mL separatory funnel and the phase was left to separate for 5 min; this step was repeated three times. All organic phases are combined. The collected solvent (approximately 15 mL) was evaporated by a SpeedVac vacuum system (Savant, Thermo Scientific).

The standard stock solutions of the vitamins: retinol (vitamin A) (500 μg/mL), cholecalciferol (vitamin D3) (100 μg/mL) and α-tocopherol (vitamin E) (500 μg/mL) were prepared by weighing accurately 5, 10 and 50 mg of vitamins A, D3, and E in volumetric flasks of 10, 100 and 100 mL respectively. Sequentially, a volume of 2, 5, and 5 mL of absolute ethanol was added to each flask to aid solvation, and each flask was filled to the mark using methanol (HPLC PLUS Gradient grade, Carlo Erba reagents, Cornaredo, MI). 10 mg/mL of vitamin K1 stock solution was prepared by dissolving the vitamin in methanol. Standard solutions of vitamin A (from 0.05 to 10 μg/mL), vitamin D3 (from 0.05 to 10 μg/mL), vitamin E (from 0.05 to 50.0 μg/mL) and vitamin K1 (from 0.1 to 100 μg/mL) were prepared from the respective standard stock solution.

The chromatographic runs were performed using the Agilent 1260 Infinity LC System (Agilent Technologies) already described. The column was a C18 HiQSil HS. The mobile phase used for water-soluble vitamins separation was methanol (85:15), containing 0.5 % triethylamine, 2.4 % glacial acetic acid, and 5 mM octane sulfonic acid (pH 3.6) (Vincenzetti et al., 2020). The analyses were carried out isocratically at 25 °C, with a flow rate of 0.9 mL/min; the total run was 40 min. The eluent was detected at 254 and 270 nm. The optimized mobile phase for fat-soluble vitamins separation was an isocratic mixture of methanol and water (95:5, v/v), the flow rate was 1 mL/min, and the eluent was monitored at 280 nm (optimal absorbance for the detection of vitamins A, E and D3, simultaneously) (Albalá-Hurtado, Rodriguez, Veciana-Nogués, & Mariné-Font, 1997). The total analysis time was 15 min. In all cases, the injection volume was 100 μL. The area of each standard peak was measured using the valley-to-valley integration mode, and quantification was achieved by a calibration curve obtained relating the concentration (μM) of each standard loaded in the column to the respective peak area. The quantity of water- and fat-soluble vitamins in HQ milk was determined using the calibration curve. The vitamins identification was achieved by comparison of their retention times and UV spectra with those of the respective vitamin standards.

2.7. Lipid peroxidation assay (TBARS assay)

Lipid peroxidation has been determined by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a colorimetric product, proportional to the MDA present, that can be evaluated at $\lambda =$ 532 nm by a spectrophotometer. The assay has been performed by using the Lipid Peroxidation (MDA) Assay Kit (Sigma-Aldrich, St. Louis, MO). The sample was HQ milk treated by the steamer of the coffee machine, in the temperatures range of 50–80 °C. All samples and standards were run in triplicate. Ultrapure water has been used for the preparation of all

standards and samples. MDA standard concentration was 0.02, 0.04, 0.06, 0.08, 0.1 mM. Before performing the assay, milk samples were prepared as follows: to 150 μL of milk, 3 μL of butylhydroxytoluene (BHT) and 150 μL of 10 % trichloroacetic acid (TCA) were added and the mixture was centrifuged at 13000g for 5 min to remove the proteins. 200 μL of supernatant was directly used for the MDA assay strictly following the protocol described in the kit datasheet.

2.8. Hydrogen peroxide determination in freshly brewed espresso

The hydrogen peroxide (H_2O_2) concentration in freshly-brewed espresso has been determined by spectrophotometric method proposed by Cai and co-workers (Cai et al., 2018) that exploits the peroxidase-catalyzed oxidation of ABTS to form the stable green radical $ABTS^{+}$. The product formation can be evaluated at 415 nm, in presence of different H_2O_2 concentrations. The reaction mixture consisted of 75 mM potassium phosphate buffer (PPB) pH5.5, 23 mM ABTS and freshly-brewed espresso diluted 1:1 with ultrapure water. The reaction was started by the addition of 0.1 μg (25 mU) of peroxidase.

The change in absorbance at 436 nm (ΔA_{436}) was evaluated after 30 s and the corresponding blank value was subtracted (75 mM PPB pH5.5; 23 mM ABTS; the peroxidase but without the diluted espresso sample). A calibration line was also generated by relating the ΔA_{436} subtracted with the blank, versus increasing concentrations of standard H_2O_2 solutions. The concentration of H_2O_2 in the freshly-brewed espresso was calculated through the following equation (1):

$$[H_2O_2] = \frac{\Delta A^* V}{\gamma^* \varepsilon^* v} \quad (1)$$

where:$\Delta A = \Delta A_{436}$ subtracted with the blank;V = final volume of the reaction mixture;γ = *stoichiometric factor of ABTS+ generation which is calculated by dividing the slope of the calibration curve by the millimolar extinction coefficient of oxidized ABTS at 436 nm;*ε (millimolar extinction coefficient of oxidized ABTS at 436 nm) = 29.3;v = volume of the sample.

2.9. Other analytical procedures

The total protein concentration was determined according to the Bradford method Bradford (1976). The quantitative determination of lactose was carried out through the Lactose/D-Galactose kit (Boehringer Mannheim/R-Biopharm, Darmstadt) following the instruction provided in the datasheet. Before the lactose determination, milk samples were treated as follows: 2 g of milk was placed into a 100 mL volumetric flask and diluted with 20 mL of ultrapure water. Proteins were precipitated by adding 1.0 mL of 3 M TCA. After 10 min incubation the mixture was neutralized with 1 M NaOH and to the mixture was added ultrapure water up to 100 mL. This final solution was used for the lactose determination.

2.10. Statistical analysis

Samples comparison was performed using *t*-test, two-way ANOVA or factorial ANOVA according to the number of factors and levels involved. The level of significance was set at $p < 0.05$. When a factors having more than two levels resulted statistically significant the post-hoc Tukey's test was carried out setting a family-wise level of significance of 5 %. *T*-test, two-way ANOVA and respective Tukey's tests were carried out with the software Prism 6 (GraphPad Inc., USA), while factorial ANOVA and respective post-hoc tests was performed using the framework of the General Linear Model (GLM) with the software Minitab 18 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Protein profile analysis on cappuccino and foam samples

It is well-known that the optimum temperature used for the preparation of cappuccino is 60 °C. However, in some takeaway cappuccinos the use of temperatures higher than 60 °C for their preparation is required which, however, can modify the nutritional quality, the organoleptic characteristics, and the foam structure of the beverage itself. The objective of these experiments was to know the effects of the steam injection at different temperatures (from 40° to 80 °C) on milk protein concentration to better understand their involvement in the formation and stability of the foam. In particular, the concentration of β-casein, α_{s1}-casein, β-lactoglobulin, α-lactalbumin, and lactoferrin has been determined in HQ milk, HQ cappuccino, UHT cappuccino, HQ foam, and UHT foam. **Table S1** shows the equations of the calibration lines of the milk protein standard with the respective retention time (RT) and r^2 values. The RP-HPLC protein profile of the HQ milk sample, along with the 15 % SDS-PAGE of each eluted peaks is shown in Fig. 1**A**, whereas in Table 1 are listed the milk proteins identified after the LC-MS/MS analysis carried out as described in the Materials and Methods section.

β-lactoglobulin (peaks A and B, Fig. 1**A**) is present as two main isoforms with different retention times (RT): one at RT = 34.3 min (peak A) and the other one at RT = 34.8 min (peak B). These two isoforms showed a similar molecular weight (19.4 kDa), after 15 % SDS-PAGE analysis (Fig. 1**, D).** From the literature, is known that β-lactoglobulin exists in two main genetic variants namely A and B, and other β-lactoglobulin variants that have been identified by several authors. The difference between the two variants is the substitutions in variant A of Asp and Val at positions 80 and 134, respectively, by Gly and Ala in variant B (Ng-Kwai-Hang and Kim, 1996). Thus, it could be assumed that peak A, with an RT = 34.3 min, corresponds to the isoform A which possesses the Asp at 80 aminoacidic position, whereas peak B, with an RT = 34.8, may correspond to the isoform B since it possesses the glycine at 80 position of the aminoacidic sequence and therefore is more retained by the RP column. In the HQ milk, the concentration of each β-lactoglobulin isoform, calculated after RP-HPLC analysis as described in the Materials and Methods section, is 3.68 ± 0.79 mg/mL for the isoform A, and 3.94 ± 0.39 mg/mL for the isoform B.

β-casein has been recognized in a peak with RT = 31.6 min and a molecular weight of 30.4 kDa. α_{s1}-casein and α-lactalbumin co-elute in the same peak (RT = 29.6), and the respective molecular weight was 32.7 and 13.4 kDa. The concentration of HQ milk β-casein is 11.37 ± 2.49 mg/mL, whereas α_{s1}-casein and α-lactalbumin amount were 1.44 ± 0.21 mg/mL and 0.87 ± 0.1 mg/mL, respectively.

Lactoferrin has been identified in the chromatographic peak with an RT of 28.2 min, the PDQuest calculated molecular weight was 82.1 kDa, consistent with that found in the literature (Indyk, Filonzi, & Gapper, 2006). The concentration of HQ milk lactoferrin is 0.32 ± 0.024 mg/mL.

Fig. 1**B** shows the protein profile of a cappuccino prepared with HQ at the standard temperature of 60 °C. In addition to the above-identified caseins and whey proteins, there are three further peaks, coming from the coffee used for the cappuccino's preparation, with an RT of 15.9 min, 16.3 min, and 23.1 min. These peaks were identified by comparing their retention times and UV spectra with those of coffee compound standard solutions. The first two peaks were identified as chlorogenic acid and caffeine respectively while the third (RT = 23.1 min) could not be identified.

The protein profile of UHT cappuccino milk, obtained after the RP-HPLC analysis revealed that the ultra-high temperature (UHT) treatment strongly affected β-lactoglobulin content (Fig. 1**C**) that in this sample resulted completely absent.

A detailed analysis of the content of main milk proteins has been performed in HQ cappuccino, UHT cappuccino, HQ foam, and UHT foam samples obtained by steam injection with a professional coffee

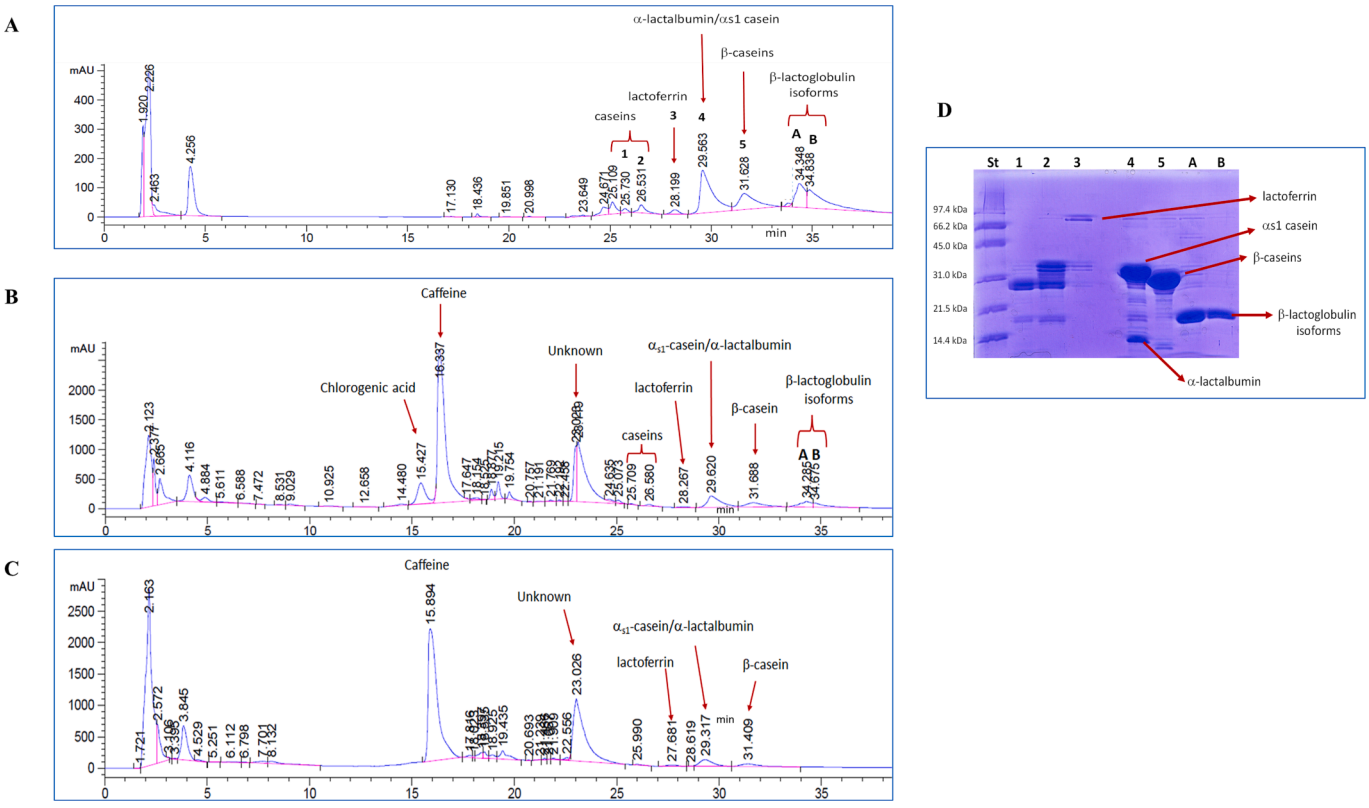

Fig. 1. Protein profile of: **A**) HQ milk, **B**) HQ cappuccino, **C**) UHT cappuccino after RP-HPLC performed as described in the Materials and Methods section. **D**) 15 % SDS-PAGE of the eluted chromatographic peaks from A.

Table 1
Identification milk proteins from HQ milk by LC-MS/MS followed by MASCOT software (http://www.matrixscience.com/search_form_select.html) analysis.

Sample ID[a]	Protein name[b]	Score[b]	Mr (kDa)[b]	Mr (kDa)[c]	Sequence
A	β-lactoglobulin (*Bos taurus*)	361	19.8	19.4	VAGTWYSLAMAASDISLLDA
B	β-lactoglobulin (*Bos taurus*)	361	19.8	19.4	VAGTWYSLAMAASDISLLDA
4	α_{s1}-casein (*Bos taurus*)	260	24.5	32.7	HQGLPQEVLNENLLRFFV
4	α-lactalbumin (*Bos taurus*)	61	16.2	13.4	KVGINYWLAHK
5	β-casein (*Bos taurus*)	160	25.1	30.4	PIQAFLLYQEPVLGPVRGPF

Abbreviations:
Mr, molecular mass;
[a] Assigned sample ID as indicated in Fig. 1.
[b] MASCOT results (SwissProt databases).
[c] Experimental values were calculated from the electrophoretic gel (15 % SDS-PAGE) by the PDQuest software.

machine at different temperatures (from 40 to 80 °C). Specifically, for each sample, the concentration of β-casein, α_{s1}-casein, β-lactoglobulin isoforms, α-lactalbumin, and lactoferrin has been determined by RP-HPLC and the results are shown in Figs. 2 and 3.

3.1.1. *α_{s1}- and β-casein behaviour in cappuccino and foam samples*

The ANOVA results indicate as the concentration of both caseins, β and α_{s1}, depends exclusively on the milk type used. More in detail, the concentration of β and α_{s1} caseins is higher in HQ milk with respect to the UHT milk and this is due to the different heat treatments (pasteurization or ultra-high temperature). This is evident also in the main effects plots (Fig. 2**E, F**). On the other hand, the processing temperature after steam injection treatment as well as the sampling zone (liquid body or foam) do not affect in a statistically significant manner their concentration (Fig. 2**A and B**). This result indicates that, in general, there are no phenomena of aggregation and precipitation of α_{s1}- and β-caseins induced by temperature and steam injection.

In milk, caseins are organized to form macromolecular aggregates named casein micelles, however, during cappuccino preparation, the steam injection causes the breakdown of the micelles and the release of the individual caseins. Our data suggests that the caseins released after the steam injection process do not undergo aggregation phenomena and this may indicate that the caseins located at the air–liquid interface are not aggregated but stretched out. This is consistent to the findings of Ipsen and Otte (2004) who found that caseins, less structured and more flexible than the globular whey proteins, can occupy a larger area at the interface which results to be soft and compressible. Other authors evidenced that at the interface air–liquid, there is the presence of non-micellar β-casein which can occupy a larger surface than intact casein micelles (Zhang et al., 2004).

In this work it was found that after steam injection β- and a_{s1}-caseins behaved similarly, however in previous studies, it was found that β-casein with a less ordered secondary structure with respect to α_{s1}-casein, diffuses more extensively at the interface due to the hydrophobic amino acid region anchored at the layer surface and the hydrophilic region directed towards the aqueous phase (Marinova et al., 2009). α_{s1}-casein, more structured with respect to β-casein, forms a more voluminous and stable foam with respect to β-casein but is not able to form a

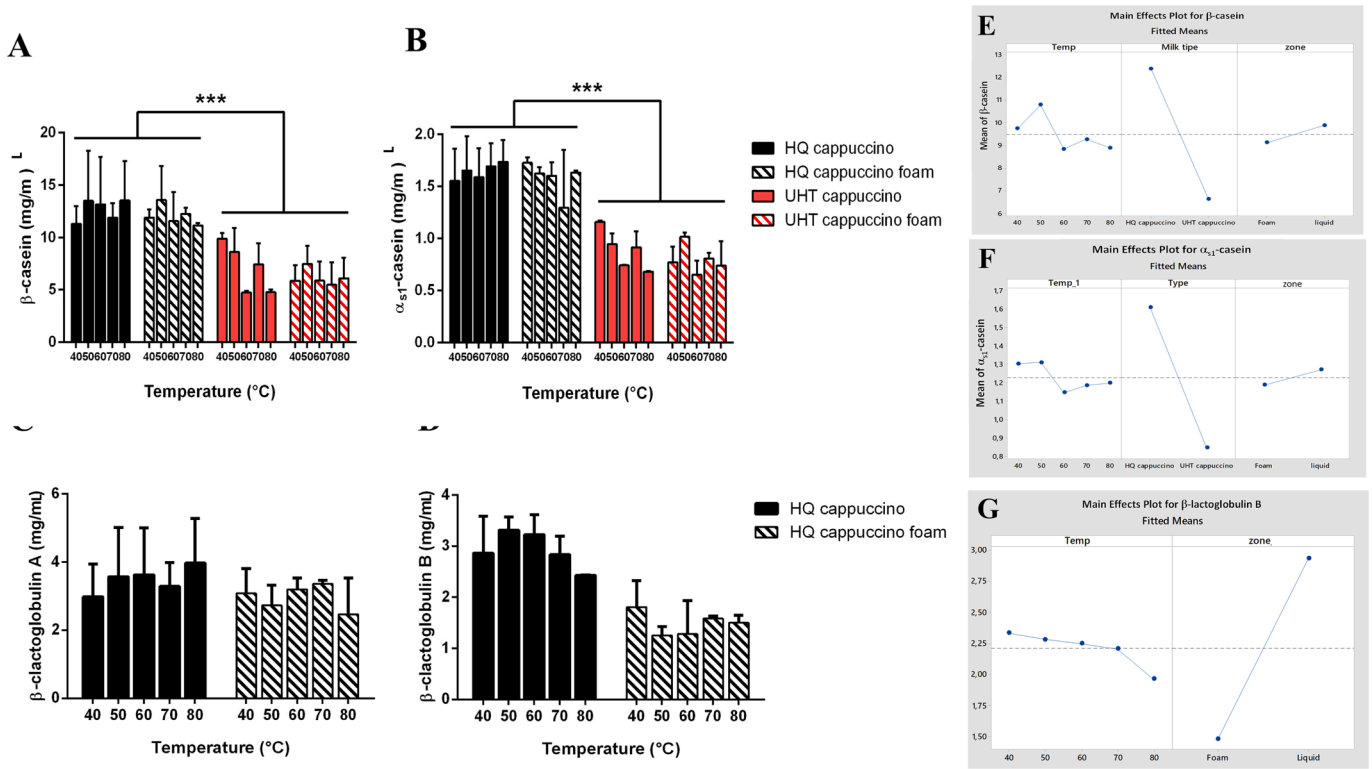

Fig. 2. **A**) β-casein, **B**) αs1-casein, **C**) β-lactoglobulin isoform A and **D**) β-lactoglobulin isoform B concentration in HQ cappuccino and HQ cappuccino foam, UHT cappuccino and UHT cappuccino foam at different temperatures obtained varying the steam injection conditions. Main effects Plot for β-casein (**E**), α_{s1}-casein (**F**), and β-lactoglobulin isoform B (**G**). Temp: effect of increasing temperature by steam injection; Milk type: HQ or UHT milk; Zone: liquid or foam phase of cappuccino.

strong interfacial layer (Ipsen & Otte, 2004).

3.1.2. *Whey proteins behaviour in cappuccino and foam samples*

Unlike caseins, whey proteins have a globular structure and are characterized by the presence of secondary, tertiary, and, in some cases, quaternary structures. β-lactoglobulin, α-lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins, and other minor proteins are part of the whey proteins. Considering the β-lactoglobulin concentration behaviour in HQ cappuccino, the results indicated that the isoforms A and B seem not to be greatly affected by the heat treatment carried out by the steamer throughout the temperature range considered (Fig. 2**C and D, respectively**). However, the concentration of isoform B was significantly lower in the HQ cappuccino foam with respect to the HQ cappuccino ($P < 0.0001$), this is also shown in the main effects plot for β-lactoglobulin (Fig. 2**G**). Both β-lactoglobulin variants A and B are responsible for foam stability because of their ability to form intermolecular disulphide bonds at the interface. Isoform B forms rapidly a stronger interfacial layer and therefore initially forms a more stable foam than the A variant (Ipsen & Otte, 2004).

The decreased concentration of β-lactoglobulin B found in this study, may be due to the formation of an unfolded protein that allows hydrophobic interactions and the compaction of the protein molecules at the interface. This could result in a strong interfacial film and good bubble coverage. In fact, from the literature, it is known that the greater foam stability can be due to an increase in the viscosity of the protein solution because of the presence of aggregates that slowed the rate of liquid drainage due to the interfacial film compacting (Sarkar & Singh, 2015; Ho et al., 2021).

β-lactoglobulin was not observed in our UHT cappuccino and UHT foam samples (Fig. 1**C**), indicating that the ultra-heat treatment negatively affected the content of this protein, this is consistent with the findings from Krishna and co-workers (Krishna et al., 2021). The absence of β-lactoglobulin in UHT milk could be one of the factors that determine the poor stability and consistency of the foams produced with this type of milk.

The concentration of α-lactalbumin is not affected by the increasing temperature after the steam injection treatment (Fig. 3**A**). This is consistent with the literature where it is reported that at the temperature of 63.7 °C (which is close to the optimal temperature for a cappuccino preparation) α-lactalbumin is denatured but does not aggregate. However, the concentration of α-lactalbumin is slightly lower ($P < 0.005$) in the foam with respect to the liquid phase, and this occurs in both HQ and UHT milk, as shown in the main effects plot (Fig. 3**C**). The plot shows also that the concentration of α-lactalbumin is significantly lower ($P < 0.0005$) in the UHT milk with respect to the HQ milk because of the different heat treatments. At the temperature and steam injection conditions used to prepare the cappuccino, α-lactalbumin may denature but not aggregate, therefore this protein can absorb at the air–liquid interface but, differently from β-lactoglobulin, is not able to form a strong interfacial film. This agrees with the observations of Ipsen and Otte (2004) who have proved that α-lactalbumin, more structured than β-lactoglobulin, is able to form a voluminous but unstable foam.

The concentration of lactoferrin is significantly affected by the milk type since it is lower in the UHT milk with respect to HQ milk ($P < 0.0005$), as shown in Fig. 3**B** and the main effects plot (Fig. 3**D**). Furthermore, in the foam samples (both in the UHT and HQ cappuccinos), the concentration of this protein is significantly lower than that found in the liquid phase ($P < 0.0005$). The concentration of lactoferrin in the HQ cappuccino foam sample is also affected by the increased temperature by steam injection, especially at 80 °C ($P < 0.0005$), as shown by the main effects plot.

From the literature, it is known that lactoferrin exhibits good foam formation but poor foam stability properties (Dai et al., 2021). Other studies showed that lactoferrin can form complexes with some polyphenols such as proanthocyanidins, epigallocatechin gallate, and chlorogenic acid and some of them led to better foamability and foam

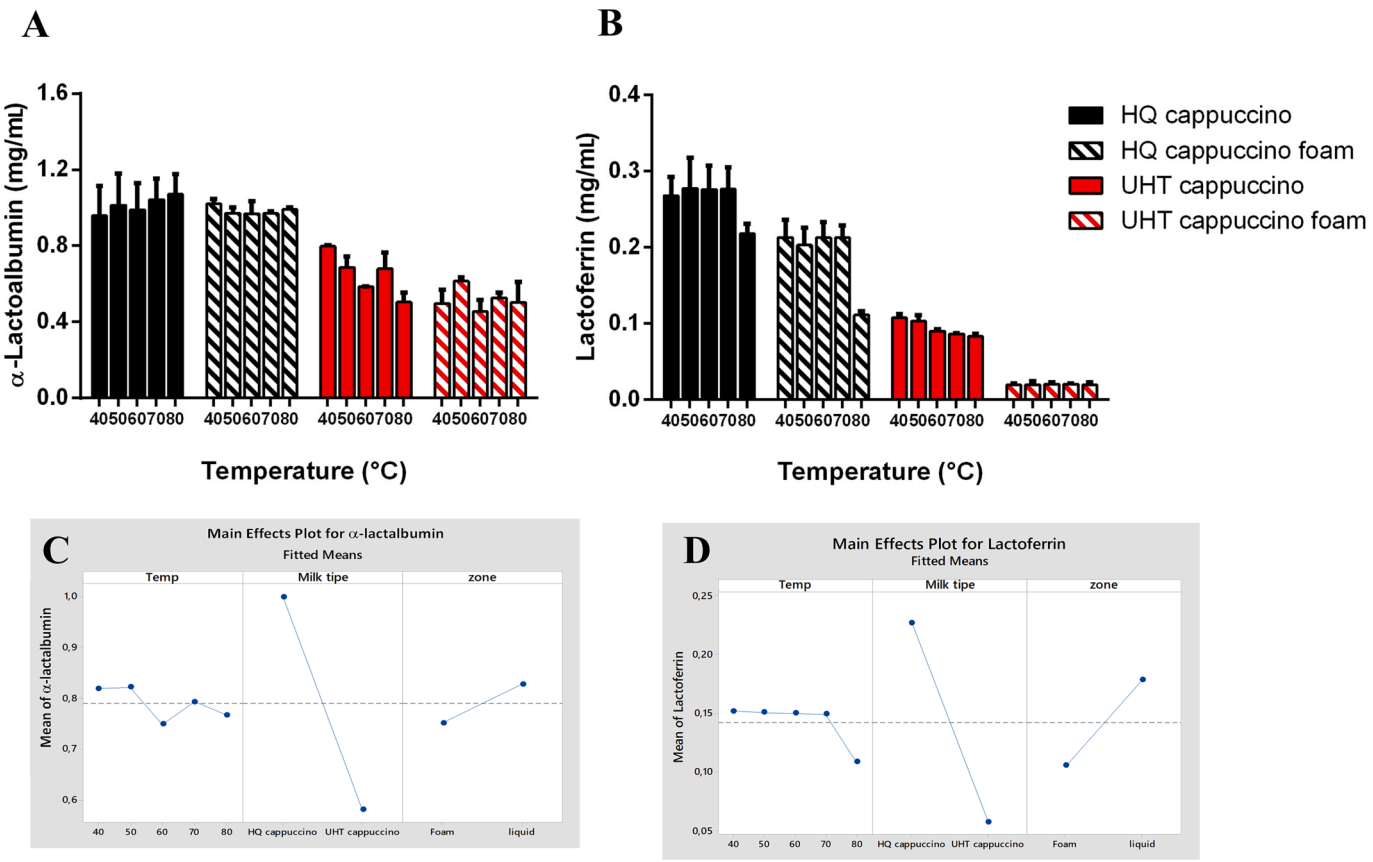

Fig. 3. **A**) α-lactalbumin and **B**) Lactoferrin concentration in HQ cappuccino and HQ cappuccino foam, UHT cappuccino and UHT cappuccino foam, at different temperature and steam injection conditions. Main effects Plot for α-lactalbumin (**C**) and Lactoferrin (**D**). Temp: effect of increasing temperature by steam injection; Milk type: HQ milk or UHT milk; Zone: liquid or foam phase of cappuccino.

stability than the lactoferrin alone (Zhang et al., 2019; Li et al., 2021). In another work, the complex between lactoferrin and tannic acid has been studied and it was shown that the complexes were more hydrophilic and larger than the lactoferrin alone and this made them absorb more slowly to the air–water interface leading to a reduced foamability. However, the lactoferrin-tannic acid complexes can form a thick viscoelastic layer around the bubble that increases the foam stability (Dai et al., 2021).

The concentration of lactoferrin decrement in the foam samples could be due to the possible formation of aggregated denatured protein or to the formation of complexes with compounds derived from the coffee that may be involved in the foam formation and stability. Lactoferrin is strongly affected by the UHT treatment and its concentration is significantly lower in the UHT cappuccino with respect to the HQ counterpart. This is not surprising since work by Brick and co-workers (Brick et al., 2017) described the effects of the milk processing methods, including the ultra-high temperature treatment, on the immunologically active bovine milk serum proteins, indicating that heating led to a reduced amount of lactoferrin, lactoperoxidase, and lactadherin.

The decreased amount of lactoferrin in the UHT cappuccino samples, together with the lack of β-lactoglobulin, may be responsible for the different foamability and foam stability that occurs when a cappuccino is prepared with UHT milk. These data agree with our previous work where microfoam produced from commercial HQ milk and UHT milk were analyzed using the steaming function of the espresso machine (Klimanova et al., 2022). It was shown that the foam stability, viscosity and the bubble size distribution behaved differently depending on the type of milk used and steam heating temperature. Foams made from HQ milk were more stable with respect to UHT milk, also bubbles were better distributed in HQ milk especially at the temperature of 60 °C.

Taking all these findings together, it could be stated that whey proteins have an important role in foam formation and stability, especially β-lactoglobulin, and the use of UHT milk to produce cappuccino could lead to a final product characterized by poor-quality foam. This was also demonstrated by Kamath and co-workers (2008).

It is important to take into account, when interpreting these results, that the decreased concentration of β-lactoglobulin and lactoferrin observed in the cappuccino foam samples is not due to the different system that occurs in the foam phase (air bubble) with respect to the liquid phase of cappuccino since the concentration of other milk proteins such αs1-casein, and β-casein is the same in both phases (see Fig. 2**E, F**). An explanation of the decreased concentration of β-lactoglobulin and lactoferrin in the foam samples observed in this study could be due to protein aggregation at the air-milk interface in the cappuccino foam. When foam samples are subjected to RP-HPLC, these aggregates of protein can precipitate after the centrifugation to which samples are subjected before the chromatographic run. In ligh.t of this, it could be supposed that the decreased concentration of β-lactoglobulin and lactoferrin that has been found in the cappuccino foams through these experiments could be considered indirect evidence that the aggregation process of denatured proteins at the air–liquid interface has occurred.

3.2. *Lactoperoxidase activity*

In general, the presence of active LPO ensures a longer shelf-life of the milk, and it is well known from the literature that the pasteurization process does not inactivate LPO contrarily to the UHT treatment (Østdal, Bjerrum, Pedersen, & Andersen, 2000). In the unheated HQ milk, LPO activity was 7.96 ± 0.6 mU/mg. If the HQ milk is subjected to the steam injection treatment at increasing temperatures, the enzymatic activity

remains constant up to a temperature of about 55 °C, and then it begins slowly to decrease until showing residual activity of 11 % to 80 °C (Fig. 4**A**). The fact that at the cappuccino preparation temperature (60–65 °C) LPO is still active is very important to ensure the good quality of the beverage itself.

3.3. Water-soluble and fat-soluble vitamins determination on pasteurized milk subjected to steam injection treatment.

The content of water-soluble and fat-soluble vitamins was determined on pasteurized HQ milk used for cappuccinos preparation subjected to different steam injection conditions, to verify if the thermal treatment affected the nutritional properties of this milk and coffee-based beverage. Linearity was assessed by building external calibration curves for each water- and fat-soluble vitamin using the respective standard solutions. **Table S2** shows the retention times, and the equation of the calibration lines for each vitamin standard, with the respective correlation coefficient (r^2) values. Correlation coefficients ($r2$) > 0.99 were obtained for all compounds studied. The vitamins in the milk samples were identified by comparison of their retention times and UV-spectra with those of the respective vitamin standards.

The vitamins determined in the HQ milk samples before steam injection treatment, were vitamin B6 (1,58 ± 0.38 μM), vitamin B2 (3.40 ± 0.14 μM), folic acid (0.45 ± 0.1 μM), vitamin A, (1.07 ± 0.23 μM); vitamin E, (1.24 ± 0.44 μM); and vitamin D, (0.1 ± 0.009 μM).

These values agreed with those already reported in literature for cow's milk where it was shown that vitamin B6 and vitamin B2 (Riboflavin) ranged from 0 1.0–11.23 and 2.12 to 6.91 μM, respectively (INRAN, 2006; Graulet & Girard, 2017; Schmidt, Pratsch, Schreiner, & Mayer, 2017). In pasteurized whole bovine milk, the vitamin B2 content has been reported to be around 4.8 μM (INRAN, 2006; Poulsen et al., 2015).

The concentration of folic acid in raw cow's milk is about 0.18 μM. However, the amount of this vitamin is influenced by several factors such as cattle nutrition, the presence of antioxidant vitamins, and the presence of oxygen. From the literature, it is known that milk pasteurization causes a loss of some vitamins sensitive to the heat treatment, mainly ascorbic acid (18,7 %), folates (12 %), vitamin B1 (3 %), and vitamin B12 (10 %) (Andersson & Öste, 1994).

In cow's raw milk the content of vitamin A is in the range of 0.35–3.14 μM (INRAN, 2006; Haug, Høstmark, & Harstad, 2007), but following the UHT treatment of the milk, cis isomers of the retinol esters are formed which are due precisely to the heat treatment and a decreased amount of vitamin A has been observed.

On the contrary, the concentration of vitamin A in pasteurized milk (HQ milk) is stable, showing the same concentration found in raw milk, as observed by Le Maguer and Jackson (1983). In bovine milk, the most abundant form of vitamin E is α-tocopherol. The average content of α-tocopherol in milk is in the range of 0.46 to 1.62 μM (INRAN, 2006). Niero and co-workers (Niero et al., 2018) found that after heat treatments of milk, vitamin E, calculated as the sum of α-tocopherol and γ-tocopherol, was similar in whole pasteurized and raw milk, as a mean concentration of averaging 3.64 and 3.62 μM, respectively, whereas in the whole UHT was 3.08 μM. Like all fat-soluble vitamins, the concentration of vitamin E is influenced by the amount of fat present in milk and by the conditions of livestock farming. The concentration of vitamin D in raw cow's milk ranges between 0.26 nM and 0.73 μM. Some authors determined the content of vitamin D3 in pasteurized milk (HQ milk) and found that its content varied from 2.6 nM to 0.044 μM (Mandrioli et al., 2020).

When the HQ-milk is treated with the steam injection, the concentration of vitamin B6 decreases with increasing temperature, and at 75 °C, it is completely absent. The same behaviour has been observed in the case of the folic acid concentration. On the contrary, vitamin B2 was not affected by the steam injection treatment (Table 2). The fat-soluble vitamins A, D, and E concentration remained constant even after a steam injection treatment for 20–60 s (data not shown).

These results seem to indicate that, considering the overall vitamin

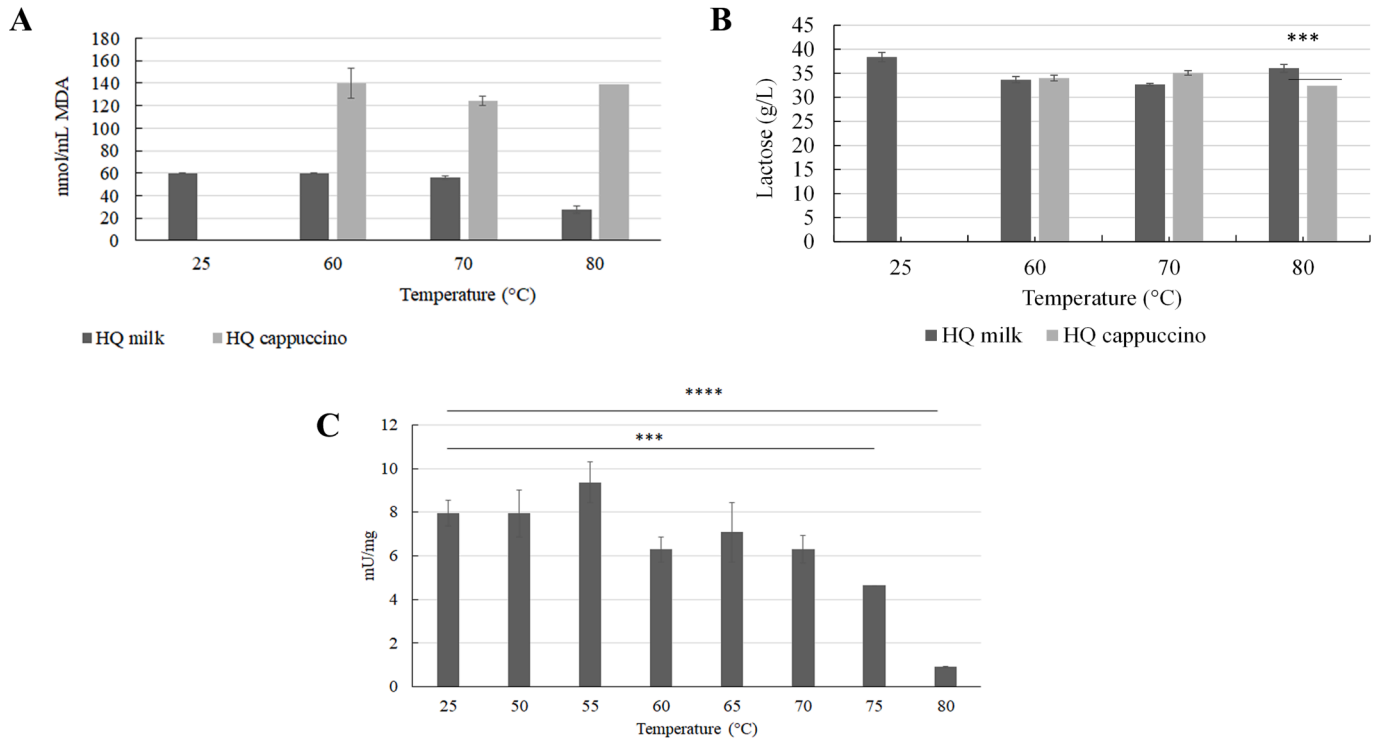

Fig. 4. A) Lactoperoxidase activity of HQ milk sample at 25 °C and after stem injection treatment in the temperature range of 50–80 °C. **B)** Behaviour of MDA in HQ milk and HQ cappuccino after steam injection at different temperatures. **C**) Lactose content (g/l) in HQ milk and in cappuccinos prepared with HQ milk, both treated at increasing temperature (up to 80 °C) with the steam injection. ***P < 0.005; ****P < 0.001.

Table 2
Concentration (μM) of folic acid, vitamin B2 and vitamin B6 in HQ milk subjected to steam injection (from 50 to 80 °C for 20, 40, 60 s).

Temperature (°C)	FOLIC ACID			VITAMIN B2			VITAMIN B6		
	Time (sec)			*Time (sec)*			*Time (sec)*		
	20	40	60	20	40	60	20	40	60
25	0.46	0.46	0.46	3.41	3.41	3.41	1.58	1.58	1.58
50	0.29	0.25	0.29	3.62	3.00	3.26	1.57	0.96	1.34
60	0,27	0.32	0.32	3.27	3.24	3.31	1.47	1.51	1.71
65	0.27	0.26	0.28	3.28	3.30	2.76	1.15	1.06	1.53
70	0.21	0.26	0.23	3.31	3.22	3.11	1.5	1.43	0.00
75	0.26	0.00	0.00	3.41	2.60	3.10	0.00	0.00	0.00
80	0.00	0.00	0.00	3.27	3.28	3.27	0.00	0.00	0.00

concentration, the nutritional quality of HQ milk, is not influenced by the steam injection treatment at the temperature normally used for the cappuccino preparation (about 60 °C).

3.4. Evaluation of lipid peroxidation and hydrogen peroxide in freshly-brewed espresso

As discussed in the introduction section, MDA is one of the end products of lipid peroxidation, therefore the measurement of its concentration in milk is directly proportional to the extent of lipid peroxidation. In these experiments, the concentration of MDA has been evaluated in the HQ milk, in the coffee used for cappuccino preparation, and in HQ cappuccinos produced at increased temperature by the steam injection treatment. As discussed above, the greater the amount of MDA, the greater the rate of lipid peroxidation in the milk sample tested. The equation of the calibration obtained plotting the concentration of MDA versus the absorbance at $\lambda = 532$ nm resulted to be $y = 0.0959x + 0.0744$ ($r^2 = 0.99$), whereas the MDA content in HQ milk and HQ cappuccino samples after treatment with steam injection at different temperature is shown in Fig. 4**B.**

MDA content in HQ milk not heated (25 °C) was 60.17 ± 0.2 nmol/mL, very close to 48–86 nmol/mL, found by Kapusta and co-workers (Kapusta, Kuczyńska, & Puppel, 2018).

If HQ milk is steam injected to the temperature of 60 °C, which is the one normally used in the production of cappuccino, the concentration of MDA was the same as the untreated milk (60.1 ± 0.2 nmol/mL), and only at 80 °C there was a significative decrement of MDA concentration (27.5 ± 2.9 nmol/mL). The content of MDA is significantly higher in the HQ cappuccino (139.9 ± 9.9 nmol/mL) with respect to the HQ milk (without coffee) in the temperature range 60–80 °C (Fig. 4**B**), indicating that the lipid peroxidation process is faster in cappuccino if compared to the same milk sample but without coffee.

Lipid peroxidation has been also evaluated in the coffee sample used for the cappuccinos preparation and the content of MDA resulted to be 99.4 ± 6.1 nmol/mL, and this could explain the highest MDA value found in the cappuccino samples with respect to the milk alone.

The high amount of MDA observed in coffee, may be due to the presence of hydrogen peroxide (H_2O_2) as recently described by Uppu and co-workers (Uppu & London, 2020). These authors measured H_2O_2 in freshly brewed coffee from different coffee companies and found that its content ranged between 0.29 and 0.82 mM, a value considered 5- to 20-fold higher than the concentration of H_2O_2 that is known to induce cytotoxic effects in most mammalian cell types in culture.

Considering this evidence, in this work the concentration of H_2O_2 has been determined in the espresso blend used for cappuccino preparation (100 % medium roasted Arabica coffee), using the enzymatic method described in the Materials and Methods section. The concentration of H_2O_2 in freshly brewed espresso resulted to be 32.5 ± 1.5 μM,

The amount of H_2O_2 found in the coffee blend used in this work is much lower than that found by Uppu and co-workers (Uppu & London, 2020) but is very similar to the value 30–50 μM found by other authors (Hiramoto, Kida, & Kikugawa, 2002). At this concentration level, the H_2O_2 in coffee should not have cytotoxic effects but could enhance the process of lipid peroxidation in coffee and consequently in a cappuccino preparation (Siddique, Ara, & Afzal, 2012).

3.5. Lactose content evaluation

Lactose, a disaccharide consisting of glucose and galactose, is one of the main constituents of milk and acts as an energy-carrier in milk. Cow's milk typically contains about 4.8 % lactose, and this value decreases with advancing lactation and mastitis infection. There is little effect of breed or individuality or nutrition of the animal on the lactose content of its milk.

In this work, lactose content has been determined on HQ milk (without coffee) and on cappuccino samples prepared with the HQ milk, both treated with steam injection at increasing temperatures, as described in the Materials and Methods section. From Fig. 4**C** it is evident that there is a decrement in the concentration of lactose among the HQ milk samples induced by the temperature ($P < 0.005$ between HQ milk not treated and HQ milk at 60 °C). There are no differences in the lactose concentration between HQ milk and HQ cappuccino, only when these two samples are steam injected at 80 °C a slight decrement in lactose content can be observed ($P < 0.005$ between HQ milk and HQ cappuccino at 80 °C).

4. Conclusions

This work has shown that the nutritional quality of milk is almost unchanged after the steam injection treatment carried out with the coffee machine at a temperature of 60–65 °C. However, at higher temperatures (>70 °C), sometimes used for the preparation of milk and coffee-based beverages, a decrease in the activity of lactoperoxidase, and in the content of vitamin B6 and folic acid has been observed. The type of milk used in cappuccino preparation is very important: pasteurized milk (HQ milk) contains a higher amount of whey proteins with respect to UHT milk and therefore can form a more consistent and lasting foam. In particular, the main difference between cappuccino foams prepared with UHT milk and HQ milk is the presence in the latter of β-lactoglobulin, and lactoferrin, both playing an important role in the foam formation and stability.

It is known that the predominant class of casein responsible for the surface properties is β-casein which, as discussed before, is less structured than α-caseins. Since caseins are resistant to temperature, the increasing temperature induced by the steamer during cappuccino preparation does not affect casein's concentration even at 80° for 60 s, therefore no change in the distribution of caseins in the adsorption layer is expected, which remains dense and thick ensuring the foam stabilization. On the other hand, globular whey protein adsorbs almost intact at the interface, however at increasing temperature induced by the stem injection β-lactoglobulin and lactoferrin concentration decreases in the foam samples indicating that these proteins could form aggregates that are trapped in the films. This phenomenon can affect their digestibility in cappuccino. The concentration of α-lactalbumin is not affected by the

increasing temperature and probably could not compact well to ensure the necessary film and foams stabilization.

The outcomes of this work may be exploited by the coffee industry for the realization of milk and coffee-based beverages characterized by high nutritional and organoleptic values.

CRediT authorship contribution statement

Giuseppe Santini: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Yulia Klimanova:** Conceptualization, Validation, Writing – review & editing. **Stefania Pucciarelli:** Conceptualization, Validation, Writing – review & editing. **Valeria Polzonetti:** Conceptualization, Validation, Writing – review & editing. **Marco Cespi:** Data curation, Formal analysis, Software. **Diego Romano Perinelli:** Data curation, Formal analysis, Software. **Paolo Polidori:** Conceptualization, Validation, Writing – review & editing. **Luca Cognigni:** Conceptualization, Supervision, Writing – review & editing. **Lauro Fioretti:** Conceptualization, Supervision, Writing – review & editing. **Sofia Renzi:** Conceptualization, Formal analysis, Investigation, Writing-original draft. **Silvia Vincenzetti:** Conceptualization, Data curation, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136757.

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