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Impact of Microfiltration on Particle Size Distribution, Volatile Compounds and Protein Quality of Pasteurized Milk during Shelf Life

Danfeng Wang[1,2], Yuanrong. Zheng[1], Zhenmin Liu[1], Guanlan Hu[2], Yun. Deng[2,*]

[1]State Key Laboratory of Dairy Biotechnology, Dairy Research Institute, Bright Dairy & Food Co., Ltd, Shanghai, China
[2]Department of Food Science & Technology, Shanghai Jiao Tong University, Shanghai, China
*Corresponding author: foodsjtu@gmail.com

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Abstract The effects of microfiltration on particle size, volatiles, protein quality and proximate compositions in pasteurized milk were studied over 7 days at 4°C. Changes in proximate compositions, pH, particle size, amino acids and volatile compounds of microfiltered and pasteurized milk (MPM) and pasteurized milk (PM) were evaluated. The MPM had lower values of proteins and total solids, and possessed higher particle size compared with PM. The D10 and D50 in MPM were individually reduced by 8.3% and 3.1% from day 0 to 7, and there were no differences for the D90. Sixty-one and 65 compounds were identified in the MPM and PM, respectively. The total contents of aliphatic hydrocarbons and alcohols in MPM decreased with storage length, while those of hydrocarbons, ketones, phenols, nitrogenous compounds and sulfide in PM increased with storage time. Other compounds clearly started to increase on day 4 and were reduced markedly by day 7. After 7 days, aliphatic hydrocarbons and alcohols decreased by 21.8% and 47.3% in MPM, while hydrocarbons, ketones and sulfide increased by 57%, 5.4% and 35.4% in PM, respectively. At the same storage time, the hydrocarbons, alcohols, aldehydes, acid and ketones were less in MPM than in PM. MPM had higher EAAI, BV and ePER values. These highlighted that microfiltration changes the compositions of volatiles and improves protein quality and stability during MPM shelf life.

Keywords: *microfiltration, particle size distribution, flavour, protein quality, milk*

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1. Introduction

Shelf-life extension and safety improvement are the major concerns of the dairy industry. Microfiltration (MF) has emerged as a new process that enables the removal of bacteria, defatting of whey, separation of micellar casein and selective fractionation of globular milk fat [1,2,3]. The combinations of MF and different heat treatments in dairy processing are more effective in the destruction of microbes and the extension of shelf life [2,3,4,5].

From the perspective of sensorial quality characteristics of milk, rheological properties, odor, taste and stability are important for consumers [6,7]. In milk, fat droplets are surrounded by a membrane named the milk fat globule membrane, which mainly comprises proteins and phospholipids [8]. However, when milk is subjected to processing treatments, the fat globules are disrupted and their average diameter decreases dramatically [6,9]. Changes in particle size and particle size distribution (PSD) are important contributors to surface area, opacity, reactivity, rheological properties, aggregation or dissociation phenomena of dispersed systems [8,10], which offer valuable indicators to predict the sensorial quality properties and stability of milk systems [6,11].

Milk represents a major source of dietary protein in humans because of its high nutritional quality. High milk protein quality is influenced by several factors, particularly amino acid composition and the bioavailability of the protein [12]. Heating could modify the structural, digestible and functional properties of proteins in the milk, depending on the applied thermal treatment and processing conditions [12,13,14]. An abundance of amino acids and peptides in milk contribute directly to its flavour; however, processing treatment could cause the formation of ammonia compounds, which result in unpleasant odors [12]. Lipid oxidation is also a major cause of the development of rancid flavour and odor, and a concomitant reduction in the acceptability and nutritional quality milk [15]. Milk flavour is delicate and subtle, and can be overshadowed by off-flavours, which reduce the sensory quality and economic value of dairy products [12]. Some distinctive volatiles have been used to identify freshness and deterioration, and to predict shelf life of milk [15]. Unfortunately, information about the changes of the particle size distribution, protein quality and volatiles of the microfiltered and pasteurized milk during shelf life is lacking.

To date, studies have investigated the effect of microfiltration on microorganisms, proximate compositions and main quality attributes of milk [1,2,3,4,16,17]. However, no detailed information is available on the influence of this combined processing on the particle size, protein quality and volatile components of milk during shelf life. Therefore, the objectives of this study were to identify and quantify the major classes of volatile compounds in the microfiltered and pasteurized milk (MPM) using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectroscopy (GC-MS), and to investigate the effect of the combination of MF with pasteurization on particle size distribution, amino acids and physiochemical quality during storage. Conventionally pasteurized milk (PM) was used as a comparator.

2. Materials and Methods

2.1. Raw Milk and Milk Processing

Fresh raw milk (3.4% fat), collected from the Jinshan dairy farm (Shanghai, China), was centrifuged to at 4000 g for 30 min to remove the fat and leucocytes. After centrifugation, the skim milk (less than 0.1% fat) was microfiltrated through ceramic membranes (Tami, France) (nominal pore size: 1.4 μm; total membrane surface area: 13.3 m²; flow rate : 5 $t \cdot h^{-1}$; inlet pressure: 2.5 bar) at 50°C. The separated cream stream (35% fat) of the fresh milk was heated at 120 °C for 4s and then mixed with the permeate of microfiltration to the fat-adjusted whole milk (3.4% fat). Then the mixture was homogenized (150/50 bar) at 65°C and pasteurized at 72°C for 15 s, and cooled to 4°C and then filled to give a stable product for volatiles analysis. After processing, all containers of all milk were stored in the dark at 4°C for the duration of the study. Analysis and testing were conducted at 0, 4, and 7 d

2.2. Chemical Analysis

Crude fat content was determined by ether extraction [18], the total nitrogen (N) by the Kjeldahl method [18], and total solids by forced air oven drying [18]. Protein was calculated using the factor N × 6.25.

2.3. pH Analysis

The pH of the milk samples was monitored at 20°C using a standard pH meter (Model S400-K, METTLER TOLEDO, Switzerland).

2.4. Particle Size Distribution Determination

The particle size distribution of the milk was estimated by laser light scattering using a Malvern Mastersizer 3000 (Malvern Instruments, Malvern, UK). The samples were diluted in distilled water until an appropriate obscuration was obtained in the measurement cell. The stirred mixture was then continuously recycled through the sample cell of the Malvern with a laser wavelength of 633 nm. An optical model based on the Mie theory of light scattering by spherical particles was applied using the following conditions: real refractive index of 1.520; refractive index of fluid (water) of 1.330; and a pump speed of 21%. The size distribution was characterized by the diameter below which 50 or 90% of the volume of particles were found (D50 and D90, respectively).

2.5. Amino Acid Analysis

Amino acid compositions of milk samples were analyzed using an amino acid analyzer (L-8900, Hitachi, Japan) according to GB/T 5009.124-2003 [19]. On the basis of the amino acid composition, amino acid score (AAS), essential amino acid index (EAAI), estimated protein efficiency ratio (ePER), and biological value (BV) were calculated according to the reported method for evaluating protein quality [20].

2.6. Volatile Compound Analysis

Volatiles were collected by solid-phase micro-extraction (SPME) as described by Zhang *et al. [20]* with some modification. Briefly, a sample of 10 mL, 2 μL of 0.1 $mg.mL^{-1}$ 2,4,6-trimethylpyridine in methyl alcohol (internal standard solution) and 3.7 g of NaCl were placed in an annealed 20 mL brown glass vial. The SPME fiber used was 50/30 μm DVB/CAR on PDMS (Supelco, Bellefonte, PA, USA). The mixture was incubated at 50°C for 20 min with 500 rpm vibration and the SPME fiber was exposed to the headspace above the sample for 30 min at 50°C with 250 rpm vibration.

The headspace of the volatile compounds was analyzed using a 7890A gas chromatograph coupled with a 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). An HP-INNOWax column (30 m × 0.25 mm i.d., 0.25 μm df, Agilent) was used with helium as carrier gas at 1 $mL.min^{-1}$ in a splitless mode. The column temperature was held at 40°C for 5 min, increased to 220°C at 5°C.min^{-1}, then to 250°C at 20°C.min^{-1} and held for 7.5 minutes. MS conditions were as follows: source 230°C; electron impact ionization was recorded with an ionization energy of 70 eV and mass spectra were scanned from 20 to 350 amu to identify the compounds based on the Saturn spectra reported on NIST Library. Relative retention times of detected compounds were also determined by n-alkane standard solutions (ASTM D2887-01 Calibration Mix, Restek, Pennsylvania, USA).

2.7. Data Analysis

Statistical analysis of variance (ANOVA) was performed using SAS 8.0 statistical data analytical software (SAS Inst., Inc., Cary, NC, USA). Significant differences between means were determined by a least significant difference (LSD) test procedure at $P < 0.05$. All measurements were performed in triplicate.

3. Results and Discussion

3.1. Proximate Compositions and pH

The total protein, fat, solids contents and pH in PM and MPM are shown in Table 1. MPM had lower contents of proteins and total solids (TS) compared with PM (except for protein on day 7). However, the fat contents were not significantly different between PM and MPM. Thus, the reduction in the TS could be related to decreased protein contents. The result obtained for the loss of the total proteins on day 0, approximately 2.4%, is similar to that

of the study carried out by Debon *et al.* [17] in the MF process, where the retention of proteins was about 96%. The protein contents decreased slightly with storage time in PM, whereas the storage period did not influence (P > 0.05) the proteins in MPM, or the TS in all milk samples (Table 1). Debon *et al.* [17] also stated that the protein or lipid contents were not affected by the storage period. The fat contents of all milk samples showed no statistically significant change (P > 0.05) during storage, indicating that there was no significant lipolysis during the storage period. These results agree with a previous report on sheep milk yogurts [21]. Protein content in PM slightly decreased during storage, which was explained by the fact that Serra *et al.* [22] analyzed hydrolization of casein and observed an increase in soluble nitrogen at the end of storage of yogurt, which resulted in a reduction in total protein content.

No significant differences in the pH were detected between PM and MPM (except for day 0) (Table 1). During storage, small decreases in pH were observed in all samples, which were accompanied by an increase in acidity. These results are similar to the change trends in the pH of homogenized milks observed after 24 h of storage at 4°C [23]. Debon *et al.* [17] noted a similar behavior in milk, where the pH decreased on days 1 and 21. According to previous studies, the reductions in pH in milk could be caused by post-acidification [24] or the partial inactivation of milk lipoprotein lipase, an enzyme associated with the casein micelle [23].

3.2. Particle Size Distributions

The average diameter of milk fat globules has a remarkable contribution to the technological suitability and nutritional value of milk. Particle size parameters for the different milks are shown in Table 2. A small peak at 0.2 μm that corresponded to casein micelles and a main peak at 3.7 μm that corresponded to fat globules characterized the particle size distribution curve of raw milk [6]. Generally speaking, raw milk has only one peak after homogenization, and the mode depends on the pressure of the homogenization. The particle size distribution curve of PM was characterized by one peak at 0.719 μm, whereas MPM showed the presence of a second small at small peak at higher diameters reflecting the formation of large casein particles or fat aggregates, whose peaks were at 0.719 μm and 2.580 μm, respectively. These clusters can be formed through shared casein adsorbed onto the fat globules membrane or by coalescence of fat globules [8]. PM showed a reduction in fat globule size (P < 0.05) in all storage days studied compared with MPM, and the reduction in fat globule size achieved in the PMs for D90 values was highest, followed by D50 and D10. For PM, the fat globule size parameter (D1) on day 0 was higher than on day 4 and 7; however, no significant differences were detected for the D50 and D90 values over the 7-d shelf life. The values of D10 and D50 in MPM samples were greater on day 0 than day 4 and 7, and there were no significant difference for D90 values within 7 days, or for D10 and D50 values between day 4 and 7 (P > 0.05) (Table 2). Fat globule size parameters of the two treatments after 7 days of cold storage showed no significant changes, indicating that the shelf life was stable and fat and protein were not further aggregated.

Generally, aggregation is an energy consuming process: the higher the temperature, the faster the protein and fat aggregate. Therefore, stronger heating would theoretically cause faster and greater particle size aggregation. However, severe heating intensities enhance the denaturation of whey protein. In addition, the presence of denatured whey proteins on the surface of casein micelles or fat globules would also sterically restrain the approach of other casein particles or fat globules and reduce the formation of dense clusters, as has been observed in gels of unheated milks [11].

Table 1. Protein, crude fat, total solids and pH of milk during shelf life

	Protein (%)			Crude Fat (%)			Total solids (%)			pH		
	0d	4d	7d	0d	4d	7d	0d	4d	7d	0d	4d	7d
PM	3.33±0.03^{Aa}	3.31±0.04^{ABa}	3.26±0.01^{BCa}	3.41±0.01^{Aa}	3.39±0.03^{Aa}	3.42±0.02^{Aa}	12.46±0.04^{Aa}	12.50±0.05^{Aa}	12.46±0.03^{Aa}	6.63±0.01^{Aa}	6.54±0.01^{Ca}	6.58±0.01^{Ba}
MPM	3.25±0.05^{Ab}	3.22±0.03^{Ab}	3.21±0.03^{Aa}	3.42±0.03^{Aa}	3.42±0.04^{Aa}	3.32±0.05^{Aa}	12.32±0.03^{Ab}	12.31±0.03^{Ab}	12.34±0.06^{Ab}	6.56±0.02^{Ab}	6.53±0.02^{BCa}	6.55±0.04^{ABa}

PM: pasteurized milk; MPM: microfiltered and pasteurised milk; N: Total non-essential amino acids; E: Total essential amino acids; EAAI: essential amino acid index; BV: biological value; ePER: estimated protein efficiency ratio. Each value was expressed as mean ± standard deviation (n=3). Means in the same row with the same letters were not significantly different (P < 0.05).

Table 2. Particle size distributions of milk during shelf life

	D10 (μm)			D50 (μm)			D90 (μm)		
	0d	4 d	7 d	0d	4 d	7 d	0d	4 d	7 d
PM	0.27±0.00^{Ab}	0.25±0.01^{BCb}	0.25±0.0^{ABb}	0.62±0.00^{Ab}	0.60±0.01^{Ab}	0.61±0.00^{Ab}	1.26±0.00^{Ab}	1.27±0.00^{Ab}	1.25±0.02^{Ab}
MPM	0.48±0.02^{Aa}	0.44±0.01^{Ba}	0.44±0.02^{Ba}	1.94±0.03^{Aa}	1.88±0.04^{Ba}	1.90±0.03^{Ba}	4.84±0.23^{Aa}	4.71±0.24^{Aa}	4.82±0.33^{Aa}

PM: pasteurized milk; MPM: microfiltered and pasteurised milk.
Values were expressed as average ± standard error (SE) (n = 3). Means in same row with same online capital letters were not significantly different (P<0.05). Means in same column with same online lowercase letters were not significantly different (P<0.05).

3.3. Protein Quality Indices

The amino acid compositions of all samples are reported in Table 3. Among the amino acids in the milks, glutamic acid was the most abundant, followed by proline, lysine/leucine, and aspartic acid /lysine, while methionine was present in the least amount. Rutherfurd and Moughan [25] reported a similar result for several milk proteins. The contents of glutamic acid, proline, aspartic acid, glycine, isoleucine and leucine initially reduced on day 4 and then slightly increased on day 7 in all samples, while those of serine, tyrosine, histidine and methionine initially

increased on day 4 and then fell on day 7. No significant differences were found in the alanine contents (except for PM on day 4) (P >0.05). The levels of lysine increased on day 7 compared with other storage times. The results showed no homogeneous trend for other amino acids. Furthermore, the levels of essential amino acids (E), non-essential amino acids (N) or the ratio between E and N (E/N) did not show significant changes between PM and MPM for the same storage time. The contents of N were higher on day 4 than on day 0 or 7, and there were no differences between day 0 and 7. However, the values of E and E/N were lower on day 4 than on day 0 or 7, and there was no difference between day 0 and 7 for all samples (P > 0.05) (Table 3). Amino acids are usually susceptible to the processing conditions, which could decrease or maintain amino acid content depending on the material species, variety, age, amino acid type, process method and the part of the analyzed material [13,25]. Our experiments showed that microfiltration processing before pasteurization resulted in no visible changes in the concentration of E or N as well as the E/N ration in the milk at the same storage time compared with PM.

Table 3. Amino acid compositions, essential amino acid index, estimated protein efficiency ratio and biological values of milk (%)

	PM			MPM		
Amino acids	0 d	4 d	7 d	0 d	4 d	7 d
Non-essential amino acids						
Aspartic acid	7.91±0.46b	7.59±0.18c	8.10±0.02ab	8.29±0.19a	7.99±0.05b	8.11±0.09ab
Arginine	3.34±0.01ab	3.38±0.02a	3.27±0.02c	3.37±0.04a	3.32±0.01b	3.29±0.04bc
Serine	4.87±0.13b	5.12±0.12a	4.77±0.14b	4.71±0.03b	5.08±0.05a	4.81±0.01b
Glutamic acid	19.92±0.07a	19.06±0.22b	20.14±0.18a	18.80±0.47b	18.31±0.14c	20.10±0.07a
Proline	10.01±0.07bc	9.80±0.03d	9.86±0.26cd	10.24±0.09a	9.94±0.04bcd	10.04±0.16b
Glycine	2.04±0.03bc	1.94±0.03d	2.01±0.02c	2.11±0.01a	1.92±0.02d	2.07±0.00ab
Alanine	3.29±0.01a	3.20±0.04b	3.35±0.01a	3.34±0.03a	3.31±0.08a	3.32±0.04a
Tyrosine	2.99±0.05bc	4.69±0.07a	2.70±0.01c	3.24±0.22b	4.61±0.38a	2.68±0.00c
Histidine	3.31±0.02d	3.53±0.03b	3.27±0.01d	3.44±0.08c	3.73±0.05a	3.25±0.01d
N	57.68±0.21b	58.31±0.26a	57.49±0.34b	57.55±0.12b	58.24±0.39a	57.68±0.10b
Essential amino acids						
Methionine	2.38±0.03c	2.53±0.08b	2.41±0.04c	2.49±0.03b	2.69±0.04a	2.41±0.04c
Isoleucine	5.57±0.24b	5.13±0.07d	5.78±0.05a	5.75±0.04a	5.29±0.12c	5.68±0.05ab
Leucine	9.94±0.02bc	9.79±0.02d	10.01±0.12b	10.21±0.06a	10.15±0.09a	9.88±0.05cd
Phenylalanine	5.46±0.09b	5.60±0.11ab	4.84±0.09c	5.69±0.04a	5.58±0.15ab	4.97±0.03c
Lysine	7.90±0.08b	8.05±0.14b	8.42±0.07a	7.24±0.23c	7.41±0.20c	8.42±0.07a
Threonine	4.27±0.02ab	4.26±0.05ab	4.25±0.02ab	4.26±0.04ab	4.38±0.17a	4.17±0.13b
Valine	6.78±0.03a	6.32±0.08b	6.80±0.00a	6.80±0.03a	6.27±0.11b	6.80±0.02a
E	42.31±0.30a	41.68±0.26b	42.51±0.34a	42.45±0.15a	42.39±0.08b	41.76±0.39a
E/N	73.38±0.65a	71.48±0.76b	73.96±1.02a	73.76±0.38a	71.72±1.14b	73.39±0.30a
Indices						
EAAI	95.98±0.44c	98.00±0.77b	95.84±0.61c	97.28±0.47b	99.35±0.15a	95.31±0.23c
BV	92.92±0.48c	95.12±0.84b	92.77±0.67c	94.34±0.51b	96.60±0.16a	92.18±0.25c
ePER	4.67±0.00b	4.42±0.00d	4.73±0.05a	4.76±0.03a	4.59±0.03c	4.67±0.02b

PM: pasteurized milk; MPM: microfiltered and pasteurised milk; N: Total non-essential amino acids; E: Total essential amino acids; EAAI: essential amino acid index; BV: biological value; ePER: estimated protein efficiency ratio. Each value was expressed as mean ± standard deviation (n=3). Means in the same row with the same letters were not significantly different (P < 0.05).

The EAAI and BV indexes demonstrated the same variation tendency during shelf life: the EAAI or BV values were higher in MPM on day 4 than in PM on day 4 and in MPM on day 0, they are lowest in other milks, moreover, they also showed no significant differences among MPM on day 7, PM on day 0 and 7. The ePER values of PM on day 7 and MPM on day 0 were the highest, followed by those of PM on day 0/MPM on day 7, and MPM on day 4, the smallest ePER values were found in PM on day 4. The EAAI, BV, and ePER indexes of MPM were higher than those of PM at the same storage time (except for on day 7). High EAAI, BV, and ePER values showed high protein quality of the milk. Overall, the MF combined with pasteurization could retain or improve the protein quality in the milk compared to the pasteurization alone.

3.4. Volatile Compounds Profiles

Table 4 shows that 61 and 65 volatile compounds could be identified in MPM and PM, respectively, which were organized into the following chemical groups: aliphatic hydrocarbons, alcohols, aldehydes, ketones, acids, esters, aromatic hydrocarbons, phenols, nitrogenous and sulfo compounds. About 55 volatiles were detected in both MPM and PM. In particular, six compounds (β-pinene, o-cymene, tetrahydro-6-propyl-2H-pyran-2-one, propanoic acid butyl ester, butanoic acid butyl ester, and 1,2-benzenedicarboxylic acid bis(2-methylpropyl) ester) were only characterized in MPM. The analysis of PM showed an additional detection of 2,2,4,6,6-pentamethyl-heptane, longifolene, benzene, ethanol, 1-octen-3-ol, Propanoic acid, n-hexadecanoic acid, acetoin, acetic acid 2-ethylhexyl ester, 2-Ethylhexyl acrylate, compared with MPM.

The hydrocarbons are numerically the most representative class, which can be formed by lipid autoxidation processes, or by the decomposition of carotenoids [12]. They have a very limited influence on foodstuffs because they have a high perception threshold [12,15,26]. Storage led to a decrease in heptane, dodecane, tridecane, o-xylene and styrene, but increased or retained the levels of decane, 6-ethyl-2-methyl- octane, tetradecane, pentadecane, toluene and ethylbenzene in both milks. The

levels of β-pinene (the smell of pine needles or resin) and o-cymene decreased or remained unchanged for MPM depending on storage time, while those of longifolenes increased over the storage period in PM. These terpenes originate from pasture plants and are then transferred to milk and milk products by animal grazing [27]. Styrene, which has a strong plastic odor, was found at the highest concentration (5.65 ng/mL) in PM on day 0 and at the lowest level (1.53 ng/mL) in MPM on day 7.

Acids or ketones, numerically the second class after the hydrocarbons, were present in all the analyzed samples. Acids are derived from lipolysis, proteolysis or the fermentation of lactose, which are responsible for the lipolyzed flavour in milk [12]. In milk, carboxylic acids are not only crucial aromas themselves, but also are important precursors of other compounds, including methyl ketones, alcohols, aldehydes and esters [12]. The concentrations of butanoic acid, hexanoic acid, n-decanoic acid, 9-decenoic acid and tetradecanoic acid increased from day 0 to 4 and decreased or slightly changed from day 4 to 7 for all samples. Compared with day 0, storage decreased the levels of acetic acid, octanoic acid and dodecanoic acid for MPM, but increased those of heptanoic acid and n-hexadecanoic acid for PM. methyl ketones are the principal flavour compounds in Blue cheese and are formed by enzymatic oxidation of FFA to *β*-keto acids and their consequent decarboxylation to methyl ketones, which contribute to the pungent aroma of such dairy products [27]. Approximately 11 ketones were identified in all samples. Compared with day 0, storage caused the loss in acetone, 2-pentanone, 2-nonanone and 2-undecanone compounds from MPM, and enhanced the levels of acetone, 2-heptanone and 2-nonanone in PM as well as those of 1-phenyl-2-butanone and tetrahydro-6-pentyl-2H-pyran-2-one in both MPM and PM. No differences were found for tetrahydro-6-propyl-2H-pyran-2-one between day 4 and 7 for MPM, and the level of acetone initially increased after 4 days and markedly declined after 7 days.

The next groups numerically were the esters and alcohols. Esters are formed through two enzymatic reactions: esterification and alcoholysis. Esterification is the formation of esters from alcohols and carboxylic acids, whereas alcoholysis is the production of esters from alcohols and acylglycerols, or from alcohols or acyl-coenzyme A [27]. The important contributions of ester compounds to food aromas are undisputed: esters with low numbers of carbon atoms are highly volatile at ambient temperatures and the perception thresholds are ten times lower than their alcohol precursors [12]. For MPM, storage treatments had positive effects on ethyl acetate, butyl ester acetic acid, and butyl ester propanoic acid (except at day 4), whereas the levels of methyl ester butanoic acid, butyl ester butanoic acid and butyrolactone decreased. For PM, the levels of ethyl acetate and butyrolactone decreased, while those of methyl ester butanoic acid and 2-ethylhexyl ester acetic acid increased compared with day 0.The content of 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester was higher on day 0 than on other days, and there were no changes between day 4 and 7 for MPM. Alcohols are derived from many metabolic pathways, i.e., metabolism of lactose and AA, degradation of methyl ketones, and the secondary hydroperoxides of fatty acids, and are considered to be important volatile compounds that may impart an alcoholic, winey, sweet, fruity, and harsh note in dairy products. Unsaturated alcohols, such as 1-octen-3-ol, play an important role in product odor, even if present in very small amounts. During the storage period, the contents of all alcohols (except for 2-ethoxy-ethanol) in MPM as well as 2-ethoxy-ethanol and 2-(2-ethoxyethoxy)-ethanol in PM decreased. For PM, the levels of 1-octen-3-ol and 2-ethyl-1-hexanol increased during storage, and those of ethanol and 1-butanol initially increased and then decreased over the storage period.

In addition, four aldehydes (hexanal, octanal, benzaldehyde and nonanal), four nitrogenous and sulfo compounds (dimethyl sulfoxide, benzonitrile, methoxy-phenyl-oxime and dimethyl sulfone), and two phenols (phenol, 2,5-bis(1,1-dimethylethyl)-Phenol) were detected. Aldehydes are produced by the catabolism of fatty acids or amino acids via decarboxylation or deamination, which can make quite a marked contribution to the overall flavour of the product because of their low olfactory perception level [27]. Hexanal is a volatile compound produced by the oxidation of linoleic acid, which contributes to the smells of oxidate and cod-liver oil, as well as the typical smell of grass and plants, whilst nonanal can be traced to the degradation of the fatty acids of the n-9 series, and Benzaldehyde is generally responsible for the almond, fruit and hazelnut flavours [12]. The amount of hexanal and nonanal firstly increased and then decreased during the storage period. The amounts octanal and benzaldehyde decreased by varying degrees depending on the storage time. The phenols were more or less increased in all samples during the storage period. Bendall [28] suggested that phenol compounds impart woody, smoky and burnt aromas to milk and have been considered as the agents responsible for the smell of cow urine. The sulfur-containing volatile compounds are potent odor-active compounds, even at low concentrations; therefore, they can rapidly cause a strong odor in food.

As shown in Table 4, among the various groups, ketones were present at the highest concentration (42.8%–51.5%), followed by acids, aromatic hydrocarbons, esters, nitrogenous compounds and sulfo compounds; the levels of phenols were the lowest (0.1%–0.7%) (except for MPM on day 4) in both the MPM and PM. Among all identified volatile components, the dominant components were acetone, octanoic acid, methoxy-phenyl-oxime, hexanoic acid and n-decanoic acid in the milk on day 0 and day 4. The major components were acetone, octanoic acid, methoxy-phenyl-oxime, hexanoic acid and 2,5-bis(1,1-dimethylethyl)-phenol on day 7.

Table 4. Comparative volatile compositions of milk during storage. Average of three brands (ng mL^{-1})

RT (min)	Identifcation Compounds	CAS number	MPM			PM		
			0 d	4 d	7 d	0 d	4 d	7 d
Aliphatic Hydrocarbons								
1.91	Heptane	142-82-5	2.39 ± 0.39	1.50	0.92 ± 0.09	2.44 ± 0.43	1.94	1.94 ± 0.48
4.30	Heptane, 2,2,4,6,6-pentamethyl-	13475-	-	-	-	1.96 ± 0.22	2.40	2.40 ± 0.29

5.29	Decane	124-18-5	0.67 ± 0.12	1.04	0.88 ± 0.14	0.99 ± 0.15	1.61	1.61 ± 0.3
5.61	Octane, 6-ethyl-2-methyl-	62016-	2.01 ± 0.37	3.72	3.34 ± 0.73	2.98 ± 0.23	3.30	3.30 ± 0.41
8.09	Undecane	1120-21-	1.91 ± 0.26	0.83	1.27 ± 0.20	1.01 ± 0.26	1.10	1.10 ± 0.11
8.21	β-Pinene	127-91-3	1.77 ± 0.03	0.83	0.91 ± 0.01	-	-	-
11.52	Dodecane	112-40-3	2.13 ± 0.29	1.44	1.34 ± 0.30	1.94 ± 0.15	1.33	1.33 ± 0.05
14.70	Tridecane	629-50-5	0.82 ± 0.05	0.61	0.47 ± 0.05	0.76 ± 0.17	0.67	0.67 ± 0.12
17.57	Tetradecane	629-59-4	1.64 ± 0.45	1.40	1.22 ± 0.27	1.17 ± 0.16	1.66	1.66 ± 0.44
20.24	Pentadecane	629-62-9	0.13 ± 0.04	0.22	0.18 ± 0.06	0.30 ± 0.12	0.96	0.96 ± 0.01
21.87	Longifolene	475-20-7	-	-	-	1.81 ± 0.36	4.01	4.01 ± 0.46
Aromatic Hydrocarbons								
4.02	Benzene	71-43-2	-	-	-	1.62 ± 0.41	1.41	1.41 ± 0.14
6.55	Toluene	108-88-3	5.54 ± 0.88	10.54	7.06 ± 0.87	6.36 ± 0.28	15.73	15.73 ± 3.69
9.14	Ethylbenzene	100-41-4	2.98 ± 0.17	5.16	4.17 ± 0.65	3.41 ± 0.40	6.66	6.66 ± 1.05
9.40	p-Xylene	106-42-3	1.12 ± 0.19	1.48	0.84 ± 0.12	1.80 ± 0.35	2.41	2.41 ± 0.77
9.62	o-Xylene	95-47-6	4.20 ± 0.36	3.87	2.72 ± 0.47	2.79 ± 0.44	5.67	5.67 ± 0.21
13.52	Styrene	100-42-5	4.84 ± 0.37	2.82	1.53 ± 0.26	5.65 ± 0.22	4.41	4.41 ± 1.01
13.83	o-Cymene	527-84-4	2.10 ± 0.32	1.5	1.55 ± 0.15	-	-	-
14.16	Benzene, 1,2,3-trimethyl-	526-73-8	1.00 ± 0.23	0.78	0.53 ± 0.09	1.05 ± 0.30	2.18	2.18 ± 0.53
18.58	Benzene, 1,2,4,5-tetramethyl-	95-93-2	0.53 ± 0.02	0.36	0.39 ± 0.11	0.57 ± 0.07	0.69	0.69 ± 0.14
26.09	Naphthalene	91-20-3	1.17± 0.20	0.94	0.71 ± 0.12	1.45 ± 0.28	2.34	2.34 ± 0.88
Alcohols								
3.88	Ethanol	64-17-5	-	-	-	2.27 ± 0.38	5.07	5.07 ± 1.43
10.12	1-Butanol	71-36-3	2.56 ± 0.38	2.04	0.72 ± 0.10	1.85 ± 0.04	2.46	2.46 ± 0.48
12.47	Ethanol, 2-ethoxy-	110-80-5	1.24 ± 0.19	1.65	1.25 ± 0.36	2.53 ± 0.17	1.90	1.90 ± 0.55
19.08	1-Octen-3-ol	3391-86-	-	-	-	0.49 ± 0.08	0.61	0.61 ± 0.06
20.08	1-Hexanol, 2-ethyl-	104-76-7	0.74 ± 0.12	0.73	0.67 ± 0.09	3.32 ± 0.23	4.39	4.39 ± 0.41
21.77	1-Octanol	111-87-5	1.85 ± 0.29	1.08	0.87 ± 0.03	0.50 ± 0.08	0.52	0.52 ± 0.08
23.26	Ethanol, 2-(2-ethoxyethoxy)-	111-90-0	1.69 ± 0.23	0.98	0.75 ± 0.03	2.14 ± 0.41	1.38	1.38 ± 0.22
Aldehydes								
7.88	Hexanal	66-25-1	1.77 ± 0.22	4.41	2.55 ± 0.42	10.53 ± 1.65	14.64	14.64 ± 3.68
14.49	Octanal	124-13-0	1.94 ± 0.33	0.88	0.43 ± 0.13	1.88 ± 0.30	1.06	1.06 ± 0.27
17.50	Nonanal	124-19-6	0.59 ± 0.10	0.91	0.50 ± 0.08	0.81 ± 0.08	1.25	1.25 ± 0.16
20.98	Benzaldehyde	100-52-7	3.31 ± 0.65	2.38	2.07 ± 0.11	3.02 ± 0.42	2.49	2.49 ± 0.52
Acids								
19.47	Acetic acid	64-19-7	0.93 ± 0.23	0.51	0.45 ± 0.02	0.83 ± 0.06	1.95	1.95 ± 0.33
21.60	Propanoic acid	79-09-4	-	-	-	0.46 ± 0.03	1.3 ±	1.3 ± 0.62
23.73	Butanoic acid	107-92-6	9.30 ± 1.11	11.17	6.38 ± 0.56	15.88 ± 2.02	24.01	24.01 ± 3.60
28.54	Hexanoic acid	142-62-1	44.42 ±	47.67	37.19 ± 1.76	69.46 ± 7.35	82.27	82.27 ± 8.22
30.75	Heptanoic acid	111-14-8	0.33 ± 0.08	0.64	0.34 ± 0.07	0.52 ± 0.13	0.96	0.96 ± 0.13
32.83	Octanoic acid	124-07-2	73.10 ±	66.94	45.43 ± 3.85	104.79 ±	101.0	101.05 ±
34.84	Nonanoic acid	112-05-0	0.71 ± 0.02	0.70	0.45 ± 0.03	1.29 ± 0.25	0.97	0.97 ± 0.59
36.74	n-Decanoic acid	334-48-5	30.39 ±	42.56	18.07 ± 2.83	56.11 ± 4.02	71.98	71.98 ± 2.32
37.84	9-Decenoic acid	14436-	1.80 ± 0.47	2.22	0.76 ± 0.10	2.72 ± 0.43	4.18	4.18 ± 0.45
40.32	Dodecanoic acid	143-07-7	3.88 ± 0.74	1.02	1.86 ± 0.41	5.16 ± 0.75	9.28	9.28 ± 1.06
42.93	Tetradecanoic acid	544-63-8	1.32 ± 0.38	1.72	0.65 ± 0.04	1.4 ± 0.20	2.81	2.81 ± 0.41
45.25	n-Hexadecanoic acid	57-10-3	-	-	-	0.87 ± 0.01	3.28	3.28 ± 0.87
Ketones								
2.48	Acetone	67-64-1	182.27 ±	174.8	152.17 ±	307.58 ±	318.5	318.51 ±
3.34	2-Butanone	78-93-3	11.20 ±	32.55	15.24 ± 1.17	33.86 ± 0.87	67.43	67.43 ±
4.79	2-Pentanone	107-87-9	5.21 ± 0.99	4.33	3.63 ± 0.66	4.00 ± 0.59	6.24	6.24 ± 0.66
11.15	2-Heptanone	110-43-0	20.02 ±	22.19	17.85 ± 0.19	24.71 ± 3.79	29.08	29.08 ± 1.33
14.37	2-Octanone	111-13-7	0.26 ± 0.05	0.27	0.22 ± 0.03	0.74 ± 0.14	0.57	0.57 ± 0.15
14.44	Acetoin	513-86-0	-	-	-	0.93 ± 0.19	1.02	1.02 ± 0.11
17.36	2-Nonanone	821-55-6	7.44 ± 0.88	7.25	6.99 ± 0.69	8.47 ± 0.21	10.95	10.95 ± 0.80
20.49	2,5-Hexanedione	110-13-4	0.45 ± 0.05	0.41	0.31 ± 0.02	0.55 ± 0.13	0.52	0.52 ± 0.18
22.76	2-Undecanone	112-12-9	5.60 ± 0.78	3.55	3.80 ± 0.10	6.73 ± 1.00	6.32	6.32 ± 1.58
27.51	1-Phenyl-2-butanone	1007-32-	2.01 ± 0.51	2.30	2.61 ± 0.44	0.91 ± 0.22	1.29	1.29 ± 0.77
30.90	2H-Pyran-2-one, tetrahydro-6-propyl-	698-76-0	0.42 ± 0.12	0.30	0.30 ± 0.05	-	-	-
35.23	2H-Pyran-2-one, tetrahydro-6-pentyl-	705-86-2	1.41 ± 0.26	1.67	1.73 ± 0.26	1.71 ± 0.11	2.39	2.39 ± 0.24
Esters								
3.17	Ethyl Acetate	141-78-6	7.05 ± 0.76	16.60	17.11 ± 2.27	4.35 ± 0.52	4.03	4.03 ± 0.71
5.05	Butanoic acid, methyl ester	623-42-7	2.73 ± 0.41	1.33	2.49 ± 0.50	2.34 ± 0.63	2.75	2.75 ± 0.14
7.64	Acetic acid, butyl ester	123-86-4	1.41 ± 0.23	1.65	2.69 ± 0.07	1.90 ± 0.32	3.01	3.01 ± 0.62
9.82	Propanoic acid, butyl ester	590-01-2	1.78 ± 0.25	1.73	2.06 ± 0.20	-	-	-
12.36	Butanoic acid, butyl ester	109-21-7	1.26 ± 0.24	0.36	1.16 ± 0.26	-	-	-

17.24	Acetic acid, 2-ethylhexyl ester	103-09-3	-	-	-	0.81 ± 0.13	1.11	1.11 ± 0.45
19.94	2-Ethylhexyl acrylate	103-11-7	-	-	-	1.67 ± 0.17	2.82	2.82 ± 0.63
23.57	Butyrolactone	96-48-0	6.92 ± 0.17	1.98	1.24 ± 0.19	10.38 ± 1.29	1.17	1.17 ± 0.49
41.16	1,2-Benzenedicarboxylic acid, bis(2-	84-69-5	1.59 ± 0.23	1.35	1.35 ± 0.24	-	-	-
Phenols								
31.79	Phenol	108-95-2	0.88 ± 0.14	9.34	2.04 ± 0.48	1.05 ± 0.14	7.46	7.46 ± 2.71
37.41	Phenol, 2,5-bis	96-76-4	1.20 ± 0.28	1.23	54.7 ± 5.85	1.12 ± 0.22	1.72	1.72 ± 0.22
Nitrogenous Compounds & sulfide								
21.95	Dimethyl Sulfoxide	67-68-5	0.74 ± 0.11	0.81	0.74 ± 0.02	0.73 ± 0.13	-	-
23.01	Benzonitrile	100-47-0	0.68 ± 0.10	0.40	0.39 ± 0.10	1.05 ± 0.14	0.69	0.69 ± 0.20
26.68	Oxime-, methoxy-phenyl-_	-	67.51 ±	51.58	35.51 ± 5.03	71.47 ± 8.85	133.9	133.92 ±
29.65	Dimethyl sulfone	67-71-0	0.88 ± 0.09	1.57	1.43 ± 0.29	1.05 ± 0.05	2.6 ±	2.6 ± 0.89

RT: Retention time (min); MPM: microfiltered and pasteurised milk; PM: pasteurized milk.
Values were expressed as average ± standard error (SE) (n = 3).

The evolution of volatiles was different in MPM and PM, and several qualitative and quantitative changes were observed during storage (Table 4). The total contents of aliphatic hydrocarbons and alcohols in MPM or those of esters in PM decreased after 7 days of storage. The total levels of hydrocarbons, ketones, phenols, nitrogenous and sulfide compounds in PM or those of esters, phenols in MPM increased with storage time. Other compounds, such as aromatic hydrocarbons, aldehydes, acids, ketones and phenols in MPM as well as alcohols, aldehydes and acids in the PM, clearly started to increase on day 4 but were reduced markedly on day 7. At the same storage time, the total concentrations of hydrocarbons, alcohols, aldehydes and acid ketones were lower in MPM than in PM. However, esters, nitrogenous compounds and sulfo compounds showed the reverse trend. These differences between MPM and PM might reflect MF's ability to remove microbes and selectively fractionate the components in the milk, resulting in different reactions and compositions during processing and storage. Reports on the changing behavior of volatiles during storage did not show the same clear trend, or are contradictory, which may be influenced by milk source, compositions, processing and storage conditions [12,15,26,29].

4. Conclusions

The combination of MF and a subsequent pasteurization treatment (72°C for 15s) has proved to be an adequate tool for the extension of milk's shelf life. The changes in particle size, volatiles and quality of the proteins in MPM during storage were studied for the first time. Compared with PM, MF treatment enhanced protein quality, and decreased the levels of proteins and total solids. The essential amino acids (E), non-essential amino acids (N), the E/N ration, EAAI and BV indexes did not vary widely at the same storage time among all samples. MF also can cause varying degrees of alteration of the number, concentrations and groups in MPM, which results in the differences in flavour between MPM and MP during storage. The separation, identification and quantification of the changes in the key aroma compounds during storage of MPM will be addressed in the future.

Abbreviations

PM: pasteurized milk; MPM: microfiltered and pasteurised milk; N: Total non-essential amino acids; E: Total essential amino acids; EAAI: essential amino acid index; BV: biological value; ePER: estimated protein efficiency ratio.

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