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Effect of green tea catechins on physical stability and sensory quality of lactose-reduced UHT milk during storage for one year

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ABSTRACT

Ultra-high temperature (UHT) processed lactose-reduced milk containing added green tea extract (GTE) at two concentrations (0.1% and 0.25%) was stored at 22 ± 2 °C for one year. The effect of GTE addition on physical stability, protein binding, and sensory quality was evaluated. Sedimentation in skim milk and creaming of full fat milk were inhibited by addition of GTE. The formation of Maillard-related flavour compounds was inhibited during storage as determined by dynamic headspace GC–MS. Using Western blot analysis, milk proteins were found to be highly conjugated to polyphenols. Addition of GTE before UHT treatment resulted in increased bitterness and astringency in UHT milk and this remained during storage. Even though GTE addition improved the physical stability and inhibited Maillard reactions in the milk, the taste and flavour contribution from GTE was dominating throughout storage, and alternative sources of polyphenols should be explored for increasing shelf-life stability of long-life milk.

1. Introduction

The shelf-life of long-life liquid dairy products, such as ultra-high temperature (UHT) milk, is limited by sensory deterioration due to physical and chemical changes, rather than microbial spoilage. Changes in sensory quality may cause consumers rejection of the product. Chemical changes include Maillard reactions and oxidation of lipids and proteins, resulting in off flavour generation and browning (Colahan-Sederstom & Peterson, 2005; Lund & Ray, 2017), which has been observed to be more challenging in lactose-reduced UHT milk compared with conventional UHT milk (Jansson et al., 2014). These chemical changes may additionally result in lowered nutritional value due to loss of key amino acids and formation of compounds that are potentially deleterious to human health (Hellwig, Gensberger-Reigl, Henle, & Pischetsrieder, 2018).

The most important physical changes are irreversible age gelation and sedimentation and creaming in fat-containing products. Gelation and sedimentation change the sensory perception and appearance of products in terms of clumps, and increased or decreased viscosity, resulting in an overall inhomogeneous product. Creaming leads to an unwanted fat layer or fat clumps in the product due to the density difference between the fat globules and the aqueous phase. Age gelation, sedimentation and creaming are a huge problem for shelf-life stability of UHT milk, and it is difficult to inhibit these conditions without compromising other quality parameters (Anema, 2019; Datta & Deeth, 2001).

Age gelation and sedimentation have been used in a broad sense to describe physical instability in sterilised milk and other heat-treated dairy products. Different causes for gelation and gel characteristics have been described and can roughly be divided into age gelation (non-enzymatic and proteolysis-induced) and sedimentation (Anema, 2019; Datta & Deeth, 2001; McMahon, 1996; Nieuwenhuijse & van Boekel, 2003). Age gelation refers to the formation of an irreversible three-dimensional protein network throughout the milk; it is often observed at later stages of storage, but the onset of gelation varies greatly with storage temperature, type of UHT processing, proteolysis, and the milk composition

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(Datta & Deeth, 2001). Sedimentation is a compact layer of protein-enriched material forming rapidly in the bottom of the pack after heat processing and is affected by similar factors as described for age gelation (Anema, 2019).

Protease-induced age gelation has mainly been ascribed to heat resistant indigenous and microbial enzymes. The major indigenous protease, plasmin, can withstand certain UHT treatments and hydrolyses mainly β- and α-caseins. The released peptides and polypeptides can dissociate from the casein micelle and aggregate resulting in a fine stranded gel (Manji & Kakuda, 1988). Limited proteolysis by plasmin can also lead to a destabilisation of casein micelles resulting in a gelled sediment. Heat-resistant microbial proteases often originate from psychrotrophic bacteria and vary greatly in specificity. In some cases, gels similar to those induced by plasmin can be formed, while other proteases predominantly hydrolyse κ-casein, resulting in a rennet-like gel (Datta & Deeth, 2001; McMahon, 1996).

Non-enzymatic age gelation is commonly described by two phases. The primary phase involves heat-induced denaturation of β-lactoglobulin and exposure of its free thiol group, which becomes involved in thiol-disulphide exchange reactions with κ-casein and whey proteins. The covalently bound β-lactoglobulin-κ-casein complex weakens the ionic bonds that anchor κ-casein to the casein micelles (Datta & Deeth, 2001; McMahon, 1996). In the secondary phase, the β-lactoglobulin-κ-casein complex accumulates in the serum phase as large protein aggregates that are partly or completely released from the casein micelles. A gel network is formed when a critical concentration of the β-lactoglobulin-κ-casein complex is obtained with any attached casein micelles being incorporated into the gel (McMahon, 1996; Nieuwenhuijse & van Boekel, 2003).

Sedimentation has been described to be caused by a release of κ-casein from the casein micelle, which may take place during heat treatment of milk. During storage, the κ-casein depleted casein micelles will be less stable and more prone to aggregation, which may be observed as sedimentation (Datta & Deeth, 2001; Gaur, Schalk, & Anema, 2018). Grewal et al. (2017) investigated sedimentation in stored UHT milk using Fourier transform infrared (FT-IR) spectroscopy showing an increased sedimentation in both skim milk and full-fat milk during storage, which was dependent on changes in structure and interactions between lipids and proteins, and increased intermolecular β-sheets formation, as well as a minor effect from the protein-carbohydrate interaction through the Maillard reaction.

Polyphenols from plants, such as green tea or olives, have shown to inhibit Maillard-related off-flavours in freshly produced UHT milk after 3 weeks of storage at 1 °C (Colahan-Sedersrom & Peterson, 2005; Troise et al., 2014), during short term storage at 30 °C for 30 days and at 40 °C for up to 42 days (Jansson et al., 2017; Kokkinidou & Peterson, 2014). The typical shelf-life of UHT milk is 3–9 months (dependent on the type of UHT treatment), and it is unknown how the sensory quality and physical characteristics of the milk with added polyphenols develops over these extended storage periods. We have recently shown that addition of polyphenols from green tea reduced heat- and storage-induced protein aggregation in UHT milk after storage at 40 °C for 42 days as evaluated by SDS-PAGE (Jansson et al., 2017).

Polyphenols with a catechol structure, such as those present in green tea, are readily oxidised to quinones, which react rapidly with nucleophilic sites on proteins such as thiol and amine groups by Michael addition (Li, Jongberg, Andersen, Davies, & Lund, 2016; Lund & Ray, 2017). When polyphenols were added to milk either before or after UHT treatment, proteins were found to be extensively bound to polyphenols (Jansson et al., 2017). O'Connell and Fox (1999) observed improved heat stability of milk in presence of polyphenols during heating at 140 °C for 10–20 min (O'Connell & Fox, 1999). They proposed that protein-quinone adduct formation could prevent the formation of β-lactoglobulin-κ-casein complexes (possibly through binding to the thiol group of β-lactoglobulin) and thereby inhibit the dissociation of κ-casein from the casein micelles, which is the primary phase of age gelation as described by McMahon (1996).

The present study evaluated the effect of adding two different concentrations of green tea extract (GTE) (0.1% and 0.25%) to lactose-reduced UHT milk (skim; 0.1% fat and full fat; 3.5% fat) on sensory quality and physicochemical characteristics during storage for 12 months at 22 ± 2 °C. Sensory descriptive analysis, dynamic headspace GC–MS analysis, and instrumental colour analysis were performed after 0, 4, 8, and 12 months of storage. Protein aggregation and polyphenol-binding to proteins were assessed by SDS-PAGE and Western blot analysis, respectively.

2. Materials and methods

2.1. Chemicals and reagents

Teavigo® green tea extract (GTE) was obtained from Taiyo International (Minneapolis, USA), and was reported to contain 95.3% epigallocatechin gallate (EGCG) based on chromatographic analysis. 2-Methylbutanal, 3-methylbutanal, 2-pentanone, 2-nonanone, benzaldehyde, dimethyl disulphide (DMDS), dimethyl trisulphide (DMTS), 2-pentylfuran, 2-ethylfuran, 4-methyl-1-pentanol and nitroblue tetrazolium (NBT) were obtained from Sigma Aldrich Inc. (Steinheim, Germany). Glacial acetic acid was purchased from VWR (Radnor, PA, USA). Ethanol was obtained from Kemetyl AB (Jordbro, Sweden). LDS sampling buffer, MOPS running buffer (20×), NuPAGE transfer buffer (20×), NuPAGE antioxidant and SYPRO® ruby protein gel stain were obtained from Invitrogen (Carlsbad, CA, USA). Pierce™ unstained protein MW marker was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). All other reagents were of analytical grade. Double-ionised water (Millipore, Bedford, MA) was used throughout.

2.2. Production and storage of lactose-reduced UHT milk drinks with added green tea extract

Preparation of lactose-reduced UHT milk with added green tea extract was performed at an Arla Foods dairy plant (Pronsfeld, Germany).

2.2.1. Lactose hydrolysis

Low-heat pasteurised (72 °C, 15 s) skim milk (0.1% fat) and full fat milk (3.5% fat) were used for the trials. Lactase (Opti-Lactase LX2, Optiferm, Germany) was added to the milk to a final concentration of 0.1%. Lactose was hydrolysed overnight at 8 °C.

2.2.2. Addition of GTE to lactose-reduced milk before UHT treatment

GTE was added to the milk before UHT treatment to a final concentration of either 0.1% (w/v) (GTE-low) or 0.25% (w/v) (GTE-high) using a high-shear mixer. Control milk without addition of GTE was included in the experimental design. Two independent batches of each milk sample (control, GTE-low, and GTE-high for both skim and full fat milk) were prepared, giving a total of six samples produced in two independent replicates.

2.2.3. UHT treatment and storage

The milk was heated to 75 °C, homogenised at 20 MPa and further preheated at 95 °C for 120 s, followed by UHT treatment using a tubular heat exchanger for 4 s at 141 °C, cooled to 20 °C, and aseptically filled in 320-mL bricks. Milk samples were stored at room temperature (22 ± 2 °C) in darkness for one year. All the milk

variants were evaluated using sensory analysis after 0, 4, 8, and 12 months and by instrumental colour analysis after 0, 4, 6, 8, 10, and 12 months. For GC–MS, SDS-PAGE and Western blot analyses, samples were frozen after 0, 4, 8 and 12 months of storage and kept at −80 °C until analysis.

Carbohydrate profile was analysed in the freshly produced UHT milk samples by the method described by Indyk, Edwards, and Woollard (1996) with modifications as described by Jansson et al. (2017). Lactose hydrolysis was found not to be completed as intended since only 65% of the lactose was hydrolysed in the milk samples, but the hydrolysis degree was the same in all samples.

2.3. Sensory evaluation

Descriptive sensory analysis of skim milk and full fat milk was conducted by a trained panel (n = 10) after 0, 4, 8, and 12 months, respectively. The panel was selected from the trained external sensory panel of the University of Copenhagen and kept together throughout the study. The preparation of the milk samples at the different storage intervals followed a similar protocol. Each milk variant was poured in 50-mL portions in 96-mL transparent, non-coloured, odourless polystyrene cups with a lid (Solo Cup Company, USA), and the samples were brought to room temperature (21 °C) to ensure that the panellists were able to detect the taste and flavour nuances in the milk. Each sample was blinded with three-digit random numbers. The descriptive sensory analyses consisted of 4 days of training (2 h per day) followed by 3 days of sensory profiling (2 h per day). A sensory vocabulary to describe the variation between the milk samples was generated by panel consensus during the first training sessions at month 0. Sensory descriptors describing well-known storage off-flavours in milk, such as cardboard flavour, were included in the vocabulary even though these were not relevant for describing the milk at month 0. This was performed to develop a vocabulary to be used throughout the entire storage period. The vocabulary development was supported by reference materials to increase the panellists' understanding of the descriptors. The final sensory vocabulary included 20 sensory descriptors and 'fresh milk' (Table 1). The 'fresh milk' descriptor referred to the sensory quality of fresh low-pasteurised milk of the respective milk variant (skim or full fat) being evaluated. The sensory descriptor 'gelation' referred to the degree of visually observable gelled sediment (clumps formed in the UHT milk) evaluated from the bottom of the transparent cup. The vocabulary was used to determine the sensory profile of the milk variants at 0, 4 and 8 months of storage. After 12 months of storage, the milk was not tasted by the trained panel due to too large sensory changes making the samples unrecognisable and too unpleasant to be tasted as milk. Thus, the sensory analysis of this milk included only the appearance descriptors.

The sensory profiling was conducted in test booths designed according to ISO (2007). The panel simultaneously evaluated the milk in triplicates over three sessions. For each session, the panel first evaluated the skim milk samples followed by full fat milk samples. The presentation order was monadic and randomised within milk type to reduce bias related to presentation order. The intensities of the descriptors were rated on a 15 cm-line scale anchored "none" to "a lot". The panel was provided with crackers, cold and lukewarm water to rinse their mouth after each milk evaluation. Data were collected using the Fizz Acquisition software, Version2.50B, Biosystemes, Couternon, France.

2.4. Sediment analysis

After 12 months of storage, sensory assessed 'gelation' was clearly occurring in some of the milks. Wet sedimentation was determined by weight before and after the liquid was drained from the pack as described in detail by Gaur et al. (2018). Five individual packs of each milk were analysed.

2.5. Analysis of protein gross structure and protein-polyphenol binding

Protein composition in milk samples from 0 to 12 months of storage was evaluated by SDS-PAGE under reducing and non-reducing conditions as described by Jansson et al. (2017). Protein-polyphenol binding was evaluated by Western blot with NBT staining as described by Chen, Wang, Zhang, Ren, and Zeng (2011) with modifications described by Jansson et al. (2017).

2.6. Instrumental colour analysis

Colour of the milk was determined by Hunter L*, a*, b* values by a BYK-Gardner colour meter (Geretsried, Germany). Samples were poured into glass petri dishes and covered with plastic wrap. Air bubbles were removed and the measurements were performed in triplicates on three different positions in the petri dish. Brown colour (ΔC*) was determined according to equation (1) (0 is the a and b value at zero months, and x is the a and b value at 0, 4, 6, 8, 10, and 12 months) (Mokrzycki & Tatol, 2011).

$$\Delta C^* = \sqrt{\left(a_0^* - a_x^*\right)^2 + \left(b_0^* - b_x^*\right)^2} \quad [1]$$

2.7. Volatile profile by dynamic headspace gas chromatography

Dynamic headspace (DHS) GC–MS analysis was performed according to the method described by Jansson et al. (2017).

2.8. Data analysis

For the sensory profile data of each milk type data, the product effect was tested on each sensory descriptors by mixed model ANOVA, with product as fixed factor and batch as random factor using the lmerTest package (Kuznetsova, Brockhoff, & Christensen, 2015) in R v3.2.2. Models for appearance included all storage months, whereas models for the other sensory descriptors only included up to 8 months of storage. Post hoc multiple comparisons were made by Tukey HSD test ($P < 0.05$). Principal components analysis (PCA) with standardised variables was carried out to visualise relations between sensory attributes and milk samples. As the milk stored for 12 months was not tasted by the sensory panel, instrumental colour values, L*, a*, b*, were used to predict their changes at 12 months of storage using partial least squares (PLS) regression. For the GC–MS and the instrumental colour data, statistical differences between milk samples and storage time were determined by linear model ANOVA, with milk, storage and batch as factors. For pairwise comparison of the milk samples and storage time least squares means were applied on the data ($\alpha < 0.05$).

Correlation between GC–MS and sensory data was performed using Partial Least Squares (PLS) regression (LatentiX Aps version 2.12, Denmark) on auto scaled data.

3. Results and discussion

Sensory, physical and chemical analyses were performed on the lactose-reduced skim and full fat UHT milk with and without GTE.

The results showed that the effects from addition of GTE were similar in both skim and full fat milk but, since the effects were more pronounced in the skim milk, the results obtained for skim milk are presented in the following section. The distinguished results obtained for the full fat milk, including effects on creaming and lipid-derived flavour compounds, are presented in the Supplementary material.

3.1. Effect of green tea extract on physical stability of lactose-reduced UHT milk

The physical stability of the milk was evaluated throughout the 12 months storage period by the sensory descriptor 'gelation'. While age gelation describes the formation of a three-dimensional protein network formed throughout the milk at later storage times, sedimentation is usually observed within the first weeks of production (Anema, 2019).

In the present study, the changes in physical stability were only observed after 12 months of storage (Fig. 1), and as a sediment formed in the bottom of the pack of the UHT milk. This suggests that the 'gelation' descriptor assessed during the sensory analysis reflected an early stage of age gelation, and was defined as the degree of visually observable gelled sediment (Nieuwenhuijse & van Boekel, 2003). Evaluation of the physical stability of the milk after 2 years of storage showed that the milk had formed a gel throughout (data not shown), which also suggested that the 'gelation' observed in the current study was an early stage of age gelation. The amount of sedimentation, as determined by the weight of the gelled sediment after decantation, was only measured after 12 months of storage.

A significantly higher level of gelled sediment (i.e., sensory assessed 'gelation') was observed for batch 1 between 8 and 12 months of storage in the control skim milk (Fig. 1A, left y-axis and striped bars). A significant level of gelled sediment was also observed between 8 and 12 months for the skim milk with added GTE; however, this was significantly lower compared with the level of gelled sediment observed in the control milk. Therefore, sedimentation based on weight was also evaluated after 12 months of storage, as also presented in Fig. 1A (right y-axis and grey bars). Sedimentation analysed by weight showed similar effects as the sensory evaluation, i.e. while a pronounced sedimentation in control skim milk was found, no significant sedimentation was observed in skim milk containing added GTE at either dose used. A similar trend was observed for batch 2, but the sedimentation was much lower for this batch (Fig. 1B).

Sedimentation may have a negative effect on the shelf-life of the milk, which has also been observed in lactose-reduced UHT milk after 8 weeks of storage at 22 °C (Tossavainen, 2008). The two independent analyses supported that formation of the gelled sediment was highly batch dependent, and that addition of GTE, even at low dose, inhibited sedimentation in lactose-reduced UHT skim milk during storage.

SDS-PAGE analysis also showed inter-batch variation between the control samples stored for 12 months, where a decreased intensity of the band between 25 and 35 kDa (corresponding to the caseins) was observed for batch 1 compared with batch 2 (Fig. 2A). The observed variation could be caused by the fact that milk from a large area (and thus from cows of different breeds and age of the milk) was used for the two different batches. Analysis of the release of free amines according to the method described by Jansson et al. (2017) showed no difference in proteolytic activity between batch 1 and batch 2 after 0 and 12 months of storage (data not shown).

Age gelation is often initiated by denaturation of β-lactoglobulin during heating, which exposes its free thiol group that is involved in thiol-disulphide exchange reactions with κ-casein. Preferential

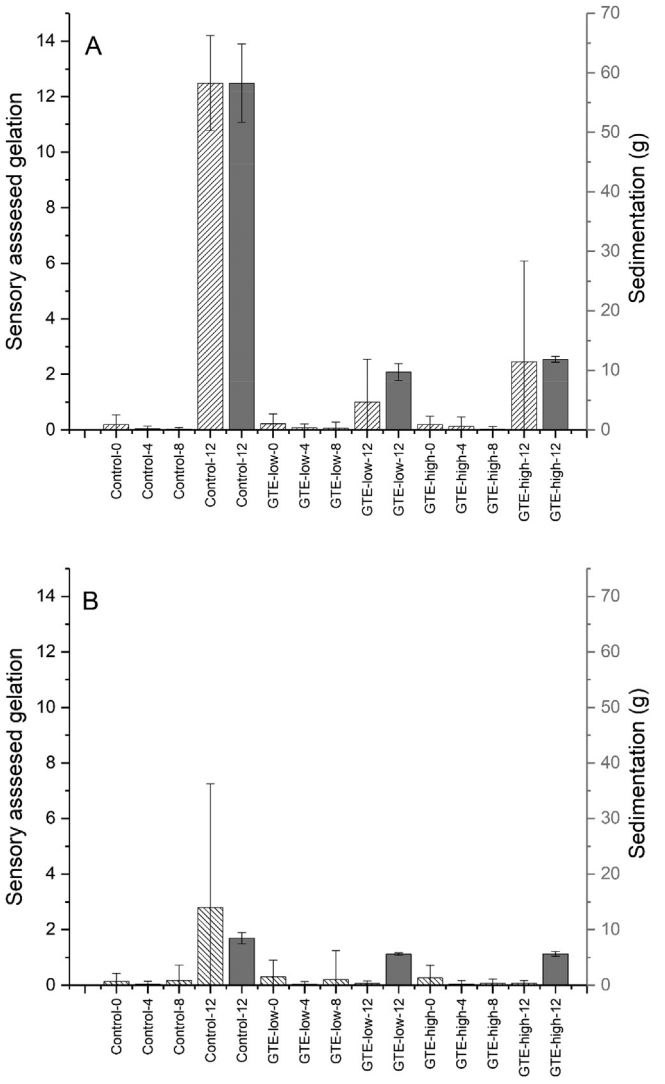

Fig. 1. Sensory assessed gelation (appearance descriptor) (left y-axis, striped bars) in lactose-reduced UHT skim milk without GTE added (control), 0.1% GTE (GTE-low) or 0.25% GTE (GTE-high) before UHT treatment and stored for up to 12 months (22 ± 2 °C), and sedimentation in weight (g) in the milk as determined after storage for 12 months (right y-axis, grey bars). Data are shown separately for batch 1 (A) and batch 2 (B) as high standard deviation and consequently significant differences between the two independent batches ($p < 0.001$) were observed for sedimentation in the control milk.

reaction between nucleophilic free thiol in β-lactoglobulin and the electrophilic α,β-unsaturated moiety in quinones, the oxidised form of epigallocatechin gallate, would therefore inhibit the formation of the β-lactoglobulin-κ-casein complex, which is formed in the primary phase of age gelation (McMahon, 1996). Fig. 2A shows that the milk proteins were more aggregated after 12 months of storage compared with 0 days of storage, as evaluated by SDS-page analysis, but it was not possible to observe any major differences between control samples and samples added GTE in their degree of aggregation. Similar results were obtained when samples were analysed under reduced and non-reduced conditions by SDS-PAGE, but only the results from the reduced samples are presented in Fig. 2A.

Western blot analysis with NBT staining is an established method to evaluate the extent of protein-polyphenol binding (Chen et al., 2011). The Western blot showed that milk proteins were already highly bound to polyphenols on day 0 in milk added GTE,

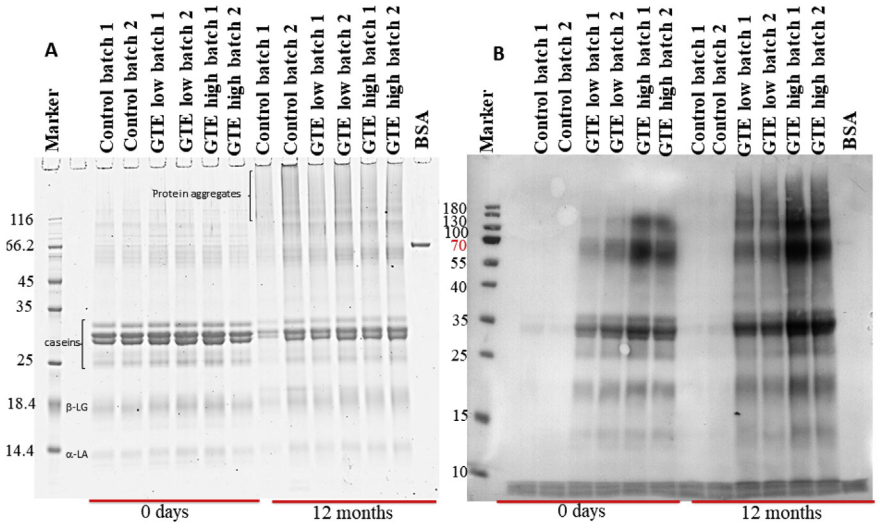

Fig. 2. (A) SDS-page (reduced) and (B) NBT stained Western Blots (assessment of polyphenol-protein binding) of the two batches of lactose-reduced UHT treated skim milk without GTE added (control), 0.1% GTE (GTE-low), or 0.25% GTE (GTE-high) before UHT treatment and stored for 0 days and 12 months at 22 °C. α-LA, α-lactalbumin; β-LG, β-lactoglobulin; BSA, bovine serum albumin. BSA was included as a negative control in the Western blots.

with the extent of binding increasing after 12 months of storage, while no binding was observed in the control milk without GTE (Fig. 2B). In addition, the protein-polyphenol binding appeared to be concentration-dependent as a more intense staining of polyphenol-bound proteins in the Western blot was observed in milk with the higher dose of GTE compared with the milk with a low dose of GTE. It is unknown whether or not the Western blot method discriminates fully between covalent and non-covalent protein-polyphenol binding (for a more detailed discussion on the method, see Jansson et al., 2017).

Polyphenols may bind to proteins by covalent binding (e.g., via quinone formation as described in the introductory section) and by non-covalent interactions (hydrophobic interactions, ionic binding, and hydrogen bonding) (Le Bourvellec & Renard, 2012), which change the secondary protein structure as observed for β-lactoglobulin and the major polyphenols present in tea: catechin, epicatechin, epicatechin gallate, and EGCG (Kanakis et al., 2011). In the study by Kanakis et al. (2011), both hydrophilic and hydrophobic binding between EGCG and β-lactoglobulin was observed. The hydrophilic binding may be due to both ionic and hydrogen bonding; the pKa values of EGCG have been reported to be 7.6–10.7 dependent on the hydroxyl group and to be lowered to 3.8–7.2 in the presence of metal ions (Fe, Cu, Al, Zn) (Kumamoto, Sonda, Nagayama, and Tabata (2001). It is therefore possible that EGCG is partly deprotonated and thereby negatively charged at pH of milk (pH 6.8). Hence, non-covalent binding between EGCG and milk proteins may include ionic and hydrogen binding as well as hydrophobic interactions. Both covalent and non-covalent binding between proteins and polyphenols may therefore affect sedimentation by inhibiting protein–protein binding and complexation.

3.2. Effect of green tea extract on colour

Freshly produced lactose-reduced UHT milk added GTE had a significantly higher a* value (red colour) compared with the lactose-reduced control UHT milk (Fig. 3A). This observation is in agreement with sensory assessed pink colour, Pink-A, as addition of GTE (both low and high doses) also significantly increased the pink colour of the milk (Fig. 3B). These results are in agreement with other studies where the a* value has increased in milk after addition of EGCG (Jansson et al., 2017; Schamberger & Labuza, 2007). During storage, the a* value increased in all milk variants (Fig. 3A); however, the sensory assessed pink colour decreased during storage in milk containing the high dose of GTE. This could be explained by an increase in brown colour during storage. The brown colour, Brown-A, formation was observed in all milk variants from the sensory profiling (Fig. 3C). Due to the intense browning effect, the data obtained from the instrumental colour analysis was converted to ΔC*, which corresponds to brown colour (Fig. 3D), and ΔC* showed a similar pattern as observed for the sensory assessed brown colour. The intense browning observed in all milk samples during storage may be attributed to formation of Maillard-derived pigments. However, the browning was significantly higher in milk with added GTE compared with control milk, so either this observation is caused by more intense Maillard reactions induced by GTE, incorporation of the GTE in the melanoidins, or polymerisation of EGCG, which occurs during oxidation and also creates brown-coloured pigments (Marco, Fischer, & Henle, 2011; Wang, Kim, & Lee, 2000).

3.3. Effect of addition of GTE and storage time on the taste and flavour profile

Significant product effects were found for all taste and flavour descriptors (Table 1). The freshly produced lactose-reduced UHT milk at room temperature, was described as having sweet taste, corn, mushroom, and hay/cereal flavours and 'fresh milk' taste. The addition of GTE had a greater impact on changing the sensory properties than storage time (up to 8 months) (Fig. 4A). This was evident from PCA of the descriptive sensory data, which showed mainly a separation according to the concentration of the GTE in PC1 (70%), while PC2 (15%) separated the milk samples mostly according to storage time.

Compared with the control milk without GTE, the freshly produced UHT skim milk with the high dose of GTE had a significantly higher perceived bitter taste and aftertaste, metallic flavour and aftertaste, strawberry, cardboard and rancid flavour and astringent aftertaste. Furthermore, the high dose of GTE significantly decreased the sweet, fresh milk, corn and mushroom flavours (Fig. 4 and Table 1). The increased bitterness, astringency, metallic, cardboard and rancid flavours are often related to flavour defects, which may decrease consumer acceptance of the milk added the

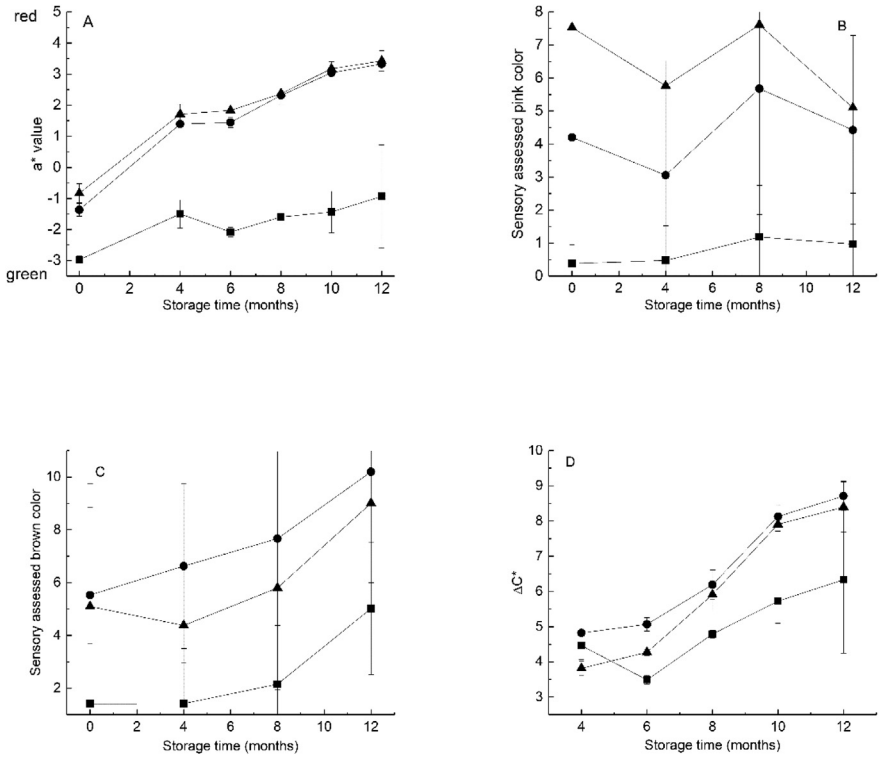

Fig. 3. Colour of lactose-reduced UHT skim milk during storage at 22 °C for 12 months evaluated by sensory analysis (B and C) and instrumentally by the CIELAB LAB system (A and D). (A) a* value (negative values indicate green, positive values indicate red); (B) sensory assessed pink colour; (C) sensory assessed brown colour; (D) ΔC* (brown colour); control milk without GTE (square symbols), milk with 0.1% GTE (GTE-low) (circular symbols), milk with 0.25% GTE (GTE-high) (triangular symbols). Means are based on two independent batches.

Table 1
Product mean values for taste, flavour, mouthfeel, aftertaste and fresh milk sensory descriptors during storage of skim milk with 0.10% or 0.25% GTE added.[a]

Sample	Storage time (months)									Product effect
	0			4			8			
	Control	GTE-low	GTE-high	Control	GTE-low	GTE-high	Control	GTE-low	GTE-high	
Sweet-T	10.6^{c}	9.5^{bc}	8.0^{ab}	11.3^{c}	10.4^{c}	9.9^{bc}	9.7^{bc}	8.4^{ab}	7.4^{a}	<0.001
Bitter-T	2.5^{a}	3.4^{a}	7.0^{b}	2.2^{a}	3.6^{a}	6.1^{b}	2.1^{a}	2.9^{a}	6.7^{b}	<0.001
Cream-F	3.7^{cd}	3.7^{cd}	2.2^{ac}	3.8^{d}	2.4^{ad}	1.8^{a}	3.4^{bcd}	2.4^{ad}	1.9^{ab}	<0.001
Corn-F	8.4^{e}	6.1^{d}	3.0^{abc}	4.9^{cd}	2.9^{abc}	2.0^{a}	4.3^{bd}	3.1^{abc}	2.5^{ab}	<0.001
Strawberry-F	1.7^{a}	4.0^{bc}	5.8^{cd}	1.3^{a}	5.1^{cd}	6.9^{d}	2.0^{ab}	5.2^{cd}	5.5^{cd}	<0.001
Boiled milk-F	4.8^{bd}	4.3^{abc}	2.4^{a}	6.1^{cd}	5.2^{bd}	4.0^{ab}	6.8^{d}	5.5^{bd}	4.0^{ab}	<0.001
Caramel-F	4.9^{b}	4.3^{ab}	2.4^{a}	6.3^{b}	6.2^{b}	4.8^{ab}	6.5^{b}	5.7^{b}	4.3^{ab}	<0.001
Fermented-F	0.7^{ab}	0.7^{ab}	1.6^{b}	0.4^{a}	0.7^{a}	0.8^{ab}	0.4^{a}	0.5^{a}	1.2^{ab}	<0.001
Mushroom-F	6.2^{e}	5.4^{de}	3.9^{bcd}	4.9^{de}	4.6^{ce}	2.8^{ac}	4.4^{ce}	2.3^{ab}	1.3^{a}	<0.001
Hay/cereal-F	6.4^{c}	4.9^{bc}	3.8^{ab}	2.5^{a}	2.8^{a}	2.5^{a}	3.4^{ab}	3.9^{ab}	3.6^{ab}	<0.001
Rancid-F	2.4^{a}	2.4^{a}	4.7^{b}	0.9^{a}	1.0^{a}	2.3^{a}	1.3^{a}	2.1^{a}	4.2^{b}	<0.001
Cardboard-F	3.0^{a}	3.5^{ab}	5.2^{b}	2.5^{a}	3.8^{ab}	5.1^{b}	4.3^{ab}	5.1^{b}	7.7^{c}	<0.001
Metallic-F	2.8^{a}	4.4^{a}	8.7^{b}	2.9^{a}	4.3^{a}	8.0^{b}	3.1^{a}	4.4^{a}	8.0^{b}	<0.001
Sulphur-F	3.8^{b}	3.7^{b}	4.2^{b}	0.3^{a}	0.3^{a}	0.4^{a}	1.0^{a}	0.8^{a}	1.3^{a}	<0.001
Viscous-MF	2.4^{ac}	2.5^{bc}	2.0^{ac}	1.9^{ac}	1.7^{ab}	1.3^{a}	3.1^{c}	2.7^{bc}	2.2^{ac}	<0.001
Coating-MF	3.4^{ac}	3.5^{ac}	2.8^{ab}	3.2^{ac}	2.8^{ab}	2.5^{a}	4.0^{bc}	4.7^{c}	4.0^{ac}	<0.001
Metallic-AT	4.1^{a}	5.4^{ab}	10.4^{c}	4.6^{a}	6.9^{b}	9.7^{c}	5.6^{ab}	5.9^{ab}	9.7^{c}	<0.001
Bitter-AT	2.5^{a}	3.6^{ab}	10.0^{c}	3.2^{a}	5.1^{b}	8.6^{c}	3.2^{a}	3.9^{ab}	9.0^{c}	<0.001
Astringent-AT	5.0^{a}	6.7^{ab}	10.4^{d}	6.6^{ab}	8.1^{bc}	10.0^{cd}	6.7^{ab}	7.5^{b}	10.1^{cd}	<0.001
Stable-AT	4.8^{c}	3.2^{bc}	1.9^{ab}	0.9^{a}	1.6^{a}	0.5^{a}	2.0^{ab}	2.0^{ab}	1.4^{a}	<0.001
Fresh milk	7.5^{d}	7.2^{cd}	5.3^{bd}	5.0^{bc}	4.1^{ab}	2.4^{a}	5.0^{bc}	4.1^{ab}	2.1^{a}	<0.001

[a] Abbreviations are: T, taste; F, flavour; MF, mouthfeel; AT, aftertaste; GTE-low, 0.10% GTE added; GTE-high, 0.25% GTE added. Data are based on a 15 cm unstructured intensity scale; values in rows with different superscript letters are significantly different ($p < 0.05$) in Tukey's HSD test. For the fresh milk descriptor, high values indicate a greater similarity to the sensory quality of fresh low-pasteurised milk.

high dose of GTE (Costell, Tárrega, & Bayarri, 2010). No significant difference was observed for these attributes between the milk containing the low dose of GTE and the control milk. This is consistent with the study of Colahan-Sederstom and Peterson (2005), who investigated the effect of adding 0.2%, 0.1% and 0.01% epicatechin to UHT milk, and found a significantly higher bitterness in the milk added 0.2%, while no difference was found for the lower concentrations (Colahan-Sederstom & Peterson, 2005).

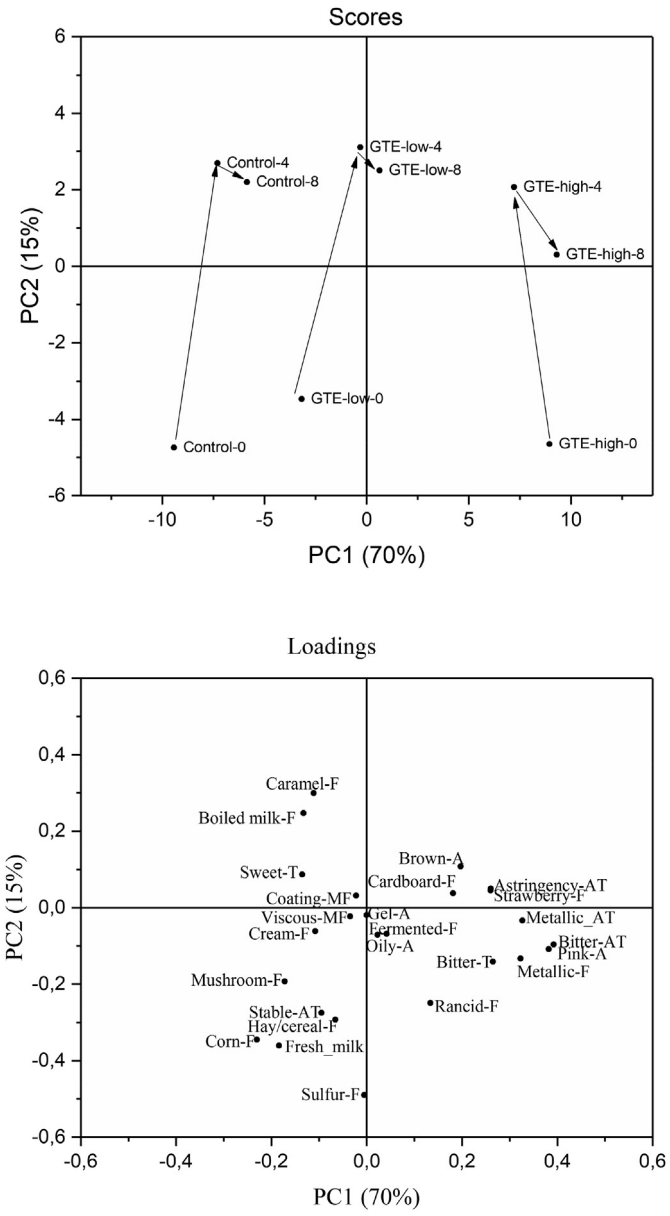

Fig. 4. (A) PCA score plot and (B) PCA loading plot: Sensory profile of lactose-reduced UHT skim milk containing no GTE (control), 0.1% GTE (GTE-low) or 0.25% GTE (GTE-high). The plots show the first two principal components.

Nevertheless, the high dose of GTE was deliberately included in the experimental design to study how a milk with an 'overdose' of GTE would affect sensory and physical characteristics of UHT milk during storage.

The high dose of GTE had a significantly decreased perception of boiled milk compared with the control milk, which is likely to be comparable with the significantly lower cooked flavour evaluated by Colahan-Sederstom and Peterson (2005) in milk with added epicatechin compared with control milk. Hay/cereal flavour was significantly higher in the fresh milk with the high dose of GTE compared with the fresh control milk; however, the difference was insignificant at 4 and 8 months. The sulphur flavour significantly decreased during storage, as reported by Al-Attabi (2009); however, no significant difference was observed between the control and milk with added GTE. The mushroom flavour significantly decreased during storage for both the low and high dose GTE milk, and, after 8 months of storage, the milk with added GTE had a significantly lower perceived mushroom flavour compared with the control milk.

Overall, addition of a low dose of GTE was not found to have any superior influence on the taste and flavour of the milk up to 8 months of storage compared with the control milk, apart from a significant decrease in mushroom flavour, which may improve the flavour of the GTE-added milk.

3.4. Prediction of taste and flavour at 12 months of storage

Since the milk stored for 12 months could not be tasted by the sensory panel, instrumental colour values were used in the flavour prediction by PLS regression. The colour measurements could predict the flavour development of the milk at 12 months (PLS regression correlation coefficients > 0.7, RMSE: 0.45–1.85). The model indicated that boiled milk and caramel flavour continued to increase during storage in all milk samples, and that the level was lower in the milk added GTE compared with the control milk. The 'fresh milk' characteristic decreased in all milk samples, and a further decrease in corn and mushroom flavour was predicted in the milk with added GTE at 12 months.

3.5. Effect of green tea extract on volatile profile

In total, 35 volatile compounds were detected in the skim milk samples during storage by DHS GC–MS, excluding alkanes since these are not very aroma active and were therefore not expected to affect the overall flavour profile. A complete list of all volatile compounds detected in skim milk and full fat milk during storage is included in Supplementary material, Tables S1 and S2, including statistical information.

The concentration of a number of Maillard and oxidation-derived volatile compounds increased during storage; 2-methylbutanal, benzaldehyde, 2-ketones (2-heptanone, 2-nonanone), pyrazine and 2-ethylfuran, while the concentration of furfural, DMS and dimethyl trisulfide (DMTS) decreased. This is in agreement with previous studies describing flavour development of UHT milk during storage (Jansson et al., 2014; Troise et al., 2016; Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001). Addition of GTE was found to significantly lower the concentrations of Maillard and oxidation-derived volatiles detected in the milk (Fig. 5). These compounds include 2-methylbutanal (a Strecker aldehyde) (Belitz, Grosch, & Schieberle, 2009), benzaldehyde (an oxidation product from the Strecker aldehyde phenylacetaldehyde) (Chu & Yaylayan, 2008), furfural (formed during Maillard reactions and sugar condensation reactions), pyrazine (formed either by condensation between α-amino carbonyl compounds followed by dehydration or via α-dicarbonyl compounds that react with amino acids) (Guerra & Yaylayan, 2012; Shibamoto & Bernhard, 1977), 2-ethylfuran (formed by sugar and Strecker degradation and lipid oxidation) (Märk, Pollien, Lindinger, Blank, & Märk, 2006), and DMTS (formed by degradation of methionine) (Al-Attabi, 2009; Ballance, 1961; Belitz et al., 2009).

The effects observed for Strecker aldehydes and benzaldehyde in the present study are in agreement with our previous study where we found a significant decrease in Strecker aldehydes and benzaldehyde when 0.275% GTE was added to UHT milk and stored at 40 °C for 42 days (Jansson et al., 2017), but in our previous study we did not observe an effect of adding GTE on pyrazine and furfural. In addition, the levels of 2-methylbutanal and pyrazine were found to be dependent on the concentration of GTE added; the highest GTE concentration added to the milk resulted in the lowest concentration of these volatile compounds.

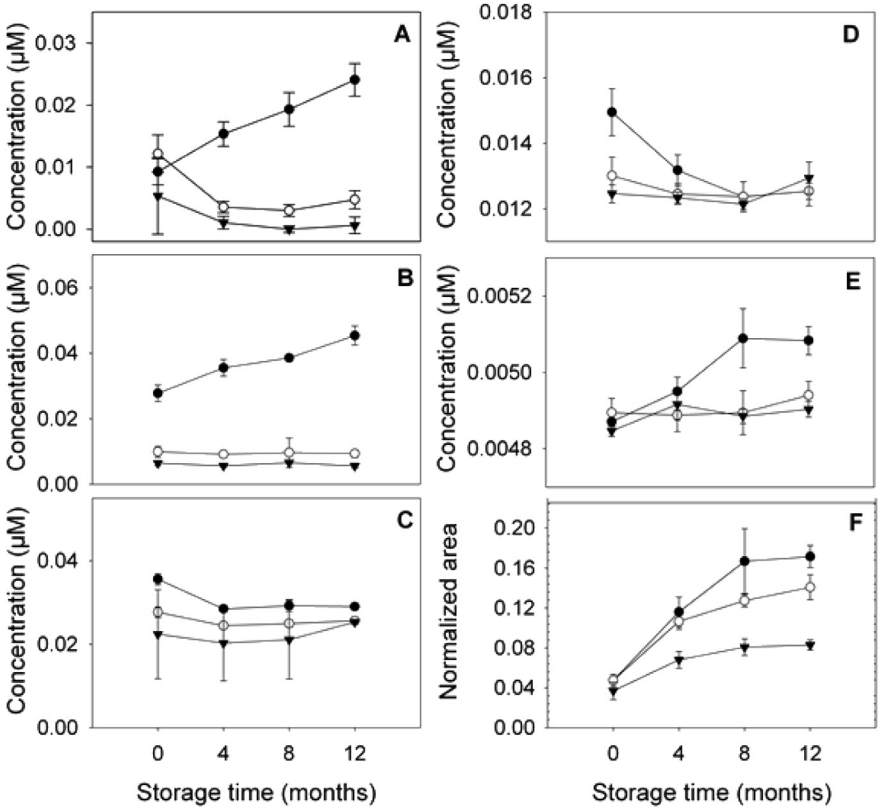

Fig. 5. Concentration of 2-methylbutanal (A), benzaldehyde (B), furfural (C), dimethyl trisulphide (DMTS, D), 2-ethylfuran (E) and normalised area of pyrazine (F) in lactose-reduced UHT skim milk without addition of GTE (●), with added low (0.1%) dose (◯) and high (0.25%) dose (▼) of green tea extract (GTE) stored for 12 months at 22 °C.

3.6. Correlation between sensory descriptors and volatile compounds determined by GC–MS

To examine if certain volatile compounds were associated with the sensory descriptors that were affected by addition of GTE and storage, correlation analyses were performed between the most pronounced sensory descriptors and the volatile compounds known to be aroma-active in milk. The volatile compounds that were most affected by addition of GTE were also the volatile compounds that provided the best correlation to sensory descriptors.

The sensory descriptors that provided correlations with a PLS regression coefficient >0.5 were: cream, mushroom, corn, cardboard and sulphur flavours. Mushroom flavour was positively correlated with the contents of 2-methylbutanal, 3-methylbutanal, dimethyl disulphide (DMDS), DMTS, furfural, benzaldehyde, and 2-methylfuran, 2-pentylfuran and to a minor degree also hexanal, octanal, nonanal and 2-methyl-1-propanol, and negatively correlated to some alcohols, alkenes and 2-ketones (Fig. 6). These compounds are all known to arise from heat-induced reactions such as Maillard reactions and lipid oxidation in milk. Mushroom, often related to the formation of 1-octen-3-one, has also been discussed as an oxidation product (Karagül-Yüceer, Cadwallader, & Drake, 2002) and, in a review by Moliszewska (2014), several aroma compounds, in addition to 1-octen-3-one, were reported in different fungi, such as octanal, hexanal, 3-methylbutanal, benzaldehyde, DMDS and DMTS.

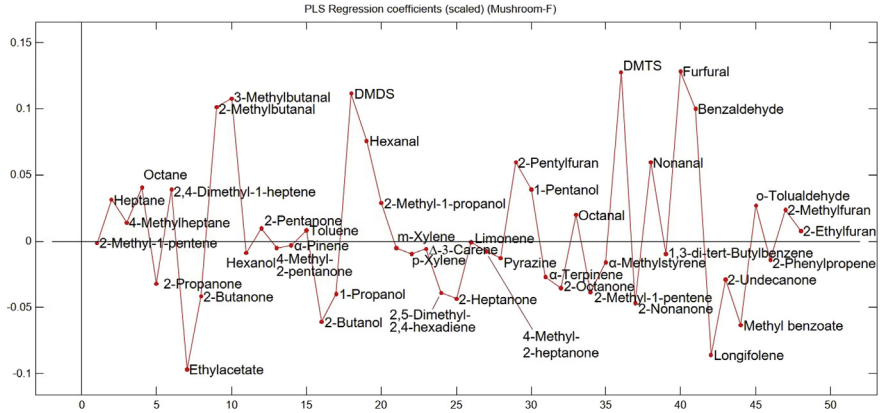

Fig. 6. Partial least squares regression between the sensory descriptors "mushroom flavour" and volatile compounds detected in lactose-reduced UHT skim milk containing no GTE, 0.1% GTE, or 0.25% GTE and stored for 0, 4, and 8 months.

It was not possible to identify 1-octen-3-one in the milk with the present method; however, from the correlation analyses, it does not appear to be possible to associate any of the sensory descriptors by only one or a few volatile compounds. This suggests that the changes observed in the sensory profile may be attributed to multiple changes in the composition of flavour compounds in the milk variants. This is in agreement with a previous study showing that a specific flavour (cardboard) was typically derived from a mixture of different volatile compounds at very specific concentrations (Whitson, Miracle, & Drake, 2010). The lower perceived mushroom flavour could indicate a lower level of oxidation in the milk with added GTE compared with the control.

4. Conclusion

Addition of both the low (0.1%) and high (0.25%) dose of GTE showed a very pronounced inhibitory effect on sedimentation in lactose-reduced skim milk and creaming in full fat milk. This observation could be explained by covalent or non-covalent binding of polyphenols from GTE to milk proteins, but further studies are required to elucidate the exact mechanism. Our study showed that addition of a high dose of GTE clearly had negative effects on some sensory descriptors by providing bitter, metallic and astringent taste, while this effect was less pronounced when GTE was added at the lower concentration. Addition of GTE at both concentrations also created a pink colour in the fresh milk and a more brown colour in the stored milk compared with the control milk. Reduction in the concentration of unwanted flavour compounds, such as Strecker aldehydes and sulphur-derived compounds, was apparent at the low dose of GTE, and sensory analysis showed a lower degree of mushroom flavour following addition of the low dose of GTE. Nevertheless, the negative effects observed on colour and sensory bitterness and astringency shows that it is necessary to find alternative sources of plant polyphenols for application in UHT milk than GTE.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2019.03.007.

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