Food Research International 130 (2020) 108923

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Evaluation of the effect of berry extracts on carboxymethyllysine and lysine in ultra-high temperature treated milk

Sarah Prestel[a], Bruna de Falco[a], Slim Blidi[a], Alberto Fiore[a], Keith Sturrock[b,*]

[a] School of Applied Sciences, Division of Engineering and Food Science, University of Abertay, Bell Street, DD1 1HG Dundee, Scotland, United Kingdom
[b] School of Applied Sciences, Division of Psychology and Forensic Science, University of Abertay, Bell Street, DD1 1HG Dundee, Scotland, United Kingdom

ARTICLE INFO

Keywords:
Silylation
Maillard reaction
Polyphenols
GC–MS
Metabolomics
FancyTiles

Chemical compounds:
Homovanillic acid (PubChem CID: 1738)
Lysine (PubChem CID: 5962)
Cycloleucine (PubChem CID: 2901)
N ε-(carboxymethyl)-lysine (PubChem CID: 123800)
L-Lysine (PubChem CID: 5962)
N-Methyl-N-(trimethylsilyl) trifluoroacetamide (PubChem CID: 32510)
N,O-Bis(trimethylsilyl)trifluoroacetamide (PubChem CID: 94358)
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (PubChem CID: 40634)
Gallic acid (PubChem CID: 370)

ABSTRACT

Both the Maillard reaction (MR) and thermal treatment influence the nutritional value of milk. In this paper, the capability of polyphenolic berry extract (PBE) to inhibit MR in an ultra-high temperature (UHT) treated milk was investigated. Total polyphenol content and antioxidant capacity of blueberry (BE) and raspberry extracts (RE) were also tested. A gas chromatography-mass spectrometry (GC–MS) method was developed to monitor the MR product N ε-(carboxymethyl)-L-lysine (CML) and L-lysine (LYS). PBE was added to milk at 0.05 and 0.1% w/v prior to UHT processing. Data revealed that formation of CML was significantly reduced (23.4 ± 5.1%) by addition of 0.1% w/v BE. The final concentrations of LYS measured following the addition of PBE prior to thermal treatment were statistically similar to the control milk which was not subjected to thermal processing. Additionally, the metabolic profile of milk samples was investigated by GC–MS and visualised using 'FancyTiles'.

1. Introduction

The composition of milk creates an ideal medium for the growth of many microorganisms and can harbour foodborne pathogens. This implies the need for thermal treatment of milk to ensure microbiological safety and also to extend shelf life (Oliver, Jayarao, & Almeida, 2005). The milk is exposed to high heat during UHT treatment with the temperature of heating in excess of 135 °C for a few seconds. However, thermal treatment leads to significant changes in the nutritional and sensory qualities of milk caused in part by the Maillard reaction (MR). This is due to the high heat used during processing and the milks natural high content of L-lysine (LYS) and reducing sugars (Pischetsrieder & Henle, 2012).

The MR is a combination of reactions starting with the condensation of amino groups with reducing sugars leading to the formation of a Schiff base and rearrangement to Amadori products (AP) or Heyns products (HP). These molecules are modified to reactive carbonyl species (RCS) such as glyoxal (GO) and methylglyoxal (MGO). These species undergo reactions with nucleophiles such as free amino acids to produce intermediate molecules that are in turn fragmented into Strecker aldehydes. Further subsequent reactions occur forming advanced glycation end-products (AGEs) (Lund & Ray, 2017).

The formation of MR products (MRPs) has been associated with negative consequences such as the loss of nutritional value due to protein degradation leading to impaired protein digestibility and bioavailability of the essential amino acid LYS (Rérat, Calmes, Vaissade, & Finot, 2002). Furthermore, formation of volatile products leads to undesirable off-flavours (Van Boekel, 1998).

Among the most studied AGEs in thermally treated milk is N ε-(carboxymethyl)-lysine (CML) (Meltretter, Seeber, Humeny, Becker, &

* Corresponding author.
E-mail address: k.sturrock@abertay.ac.uk (K. Sturrock).

https://doi.org/10.1016/j.foodres.2019.108923
Received 13 September 2019; Received in revised form 3 December 2019; Accepted 15 December 2019
Available online 18 December 2019
0963-9969/

Pischetsrieder, 2007) which can be formed either from oxidative degradation of the AP fructosyl-lysine (Ahmed, Thorpe, & Baynes, 1986), from MGO (Fu et al., 1996), from GO (Wells-Knecht, Thorpe, & Baynes, 1995) or via autoxidation in the presence of ascorbic acid and proteins (Dunn et al., 1990). There is growing evidence that food-derived AGEs contribute to the formation of increased oxidative stress in human tissue. This, in conjunction with inflammation processes originating from in vivo AGEs, is involved in the development of chronic diseases such as diabetes, atherosclerosis, Alzheimer's disease, and chronic renal failure (Uribarri et al., 2015). To monitor food quality, CML is acknowledged as a suitable marker compound in the analysis of foods which have been subjected to severe thermal treatment (Troise, Fiore, et al., 2014).

.As a result, the importance of being able to control MR and reduce the formation of dietary AGEs has emerged and different strategies have been proposed. For example, reactant encapsulation (Troise, Vitiello, Tsang, & Fiore, 2016), application of natural antioxidative compounds (Peng, Ma, Chen, & Wang, 2011), enzymatic approaches (Troise, Dathan, et al., 2014) or sugars substitution (Zhang et al., 2018) have already shown promising results in reducing MR derived compounds.

Polyphenols are secondary metabolites that exhibit an appreciable antioxidant activity and are found in vegetables, fruits, soybeans, grains, and other plant-derived food. They have also been found to inhibit MR (Lund & Ray, 2017) by trapping key intermediates such as GO and MGO. It has been proposed this takes place by an electrophilic aromatic substitution reaction (Totlani and Peterson, 2005, 2007). Moreover, phenols in their oxidised form can produce adducts, iminoquinones and iminophenols, via Schiff bases with amino groups of proteins and amino acids such as LYS, reducing this amino acid availability as a precursor for MR (Guerra & Yaylayan, 2014). Most recently, many studies have confirmed that polyphenols are effective in trapping carbonyls and mitigating the formation of CML using corn silk (Zhang, Wang, & Liu, 2020), Baijiu vinasse (Wang et al., 2019), sugarcane molasses (Yu, Xu, & Yu, 2017) and grape by-products extracts (Mildner-Szkudlarz, Siger, Szwengiel, & Bajerska, 2015). Nevertheless, these experiments were studying the effect of the extracts on simple model systems. On the other hand, green tea polyphenols decreased Strecker aldehydes in lactose-hydrolysed UHT milk (Jansson et al., 2017) but their effect on CML was not assessed.

Berries are well known sources of polyphenolic compounds. Blueberries and raspberries are particularly rich in anthocyanins, flavanols, and phenolic acids like hydroxycinnamic and hydroxybenzoic acids (Mattila, Hellström, & Törrönen, 2006). Additionally, epidemiological studies indicate that berry fruits have an emerging impact on cardiovascular health with respect to their polyphenolics (Rodriguez-Mateos, Heiss, Borges, & Crozier, 2013). Wang, Yagiz, Buran, do Nascimento Nunes, and Gu (2011) used berry products as a source of phenolic compounds to decrease AGE development in physiological model systems. Results showed that the phytochemical extracts were able to inhibit protein glycation due to the capacity of the phenolic compounds to scavenge RCSs.

In this paper, a novel GC–MS method has been developed for the analysis of CML and metabolites using an improved silylation protocol involving an excess of trifluoroacetamide (MSTFA) to ensure complete derivatisation of insufficiently volatile analytes.

Furthermore, the capacity of sugar-free polyphenolic berry extract (BE) and raspberry extract (RE) to prevent MR development in UHT infant milk was assessed and the polyphenolic composition of extracts was elucidated. The effectiveness of produced polyphenolic berry extract (PBE) on inhibition of formation of CML and reducing LYS blockage was examined by GC–MS in UHT milk at the laboratory-scale. Berries were sourced from local producers and were uncontaminated but deemed unfit for sale through normal outlets. These berries would normally go to waste and their use in applications such as those described here could significantly reduce said waste. Additionally, a readily accessible visual representation of key discovered nutrients was developed using FancyTiles by Troise, Ferracane, Palermo, and Fogliano (2014) and is presented.

2. Material and methods

2.1. Chemicals and reagents

Blueberries and raspberries were obtained from a local fruit grower. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin-Ciocalteu's phenol reagent (2 M), potassium persulfate, the ion pairing agent perfluoropentanoic acid (HFBA), D-(+)-glucose (≥99,5%), derivatisation agent N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), Amberlite XAD-7, the analytical standards LYS and cycloleucine (CYC), methylglyoxal (40% aqueous solution), glyoxal (40% aqueous solution), o-phenylenediamine dichloride (O-PD), polyphenols and organic acids used for identification and quantification were obtained from Sigma-Aldrich (Dorset, UK). *N,O*-Bis(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane (BSTFA with 1% TMCS) were from Supelco Analytical (Bellefonte, PA). Disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). Methanol, acetone and acetonitrile (all HPLC grade), hydrochloric acid (37%), formic acid (ACS), benzoic acid, pyridine and anhydrous sodium carbonate were purchased from Fisher Scientific (Loughborough, UK). Acetonitrile and pyridine were dried with sodium sulphate and stored in a desiccator before use.

2.2. Extraction and characterisation of berries

Extraction of raspberries and blueberries was carried out following the method of Wang et al. (2011) with some modification. Berries (500 g) were mixed with 300 mL acidified methanol (containing 1% formic acid) in a kitchen blender and then sonicated for 10 min in a water-bath sonicator (FB15063, Fisher Brand, UK), with cooling. The mixture was centrifuged (CR3.12, Jouan, France) at 10 °C for 5 min at 10,000 rpm and the supernatant was collected. This extraction process was repeated on the residue and the liquids obtained were combined and dried in a rotary evaporator (SciQuip Rotary-Pro, SciQuip, UK) at room temperature (19 ± 1 °C) under reduced pressure. The dried extract was dissolved in 50 mL distilled water acidified with formic acid (1%). To remove sugars, this solution was loaded onto a glass column packed with activated Amberlite XAD-7 resin. Using a peristaltic pump set at a flow rate of 3.5 mL/min, elution was obtained with 600 mL of distilled water containing 1% formic acid. Phytochemicals were desorbed from the resin with 300 mL of methanol (80%) containing 1% formic acid. The extract was reduced in volume on a rotary evaporator under reduced pressure at ambient temperature (19 ± 1 °C) to approximately 5 mL. Subsequently, this was frozen at −80 °C and then freeze dried (FT33-MKII vacuum freeze dryer, Armfield, UK). Extracts were diluted to final concentrations of 1000 μg/mL, 100 μg/mL and 10 μg/mL with distilled water immediately before further use to prevent degradation of polyphenols.

For characterisation of berry extracts, total polyphenol content (TPC), antioxidant capacity (AC) and their GC–MS profile were examined.

TPC was determined spectrophotometrically using Folin-Ciocalteu assay according to Singleton and Rossi (1965) method but with some modifications. A volume of 125 μL of diluted berry extract (100 μg/mL) was mixed with 500 μL distilled water in a UV-macro cuvette. After adding 125 μL of Folin & Ciocalteu's phenol reagent, the solution was allowed to stand for 6 min. Subsequently, 1.25 mL of a sodium carbonate solution (7.5% w/v) was added and the mixture was brought to a final volume of 3 mL with distilled water. The samples were then allowed to stand in the dark for 90 min at ambient temperature. Absorbance was measured at 760 nm (Thermo Scientific Genesys 10S UV–Vis Spectrophotometer, UK). Gallic acid was used to build an external

calibration curve in a concentration range between 5 and 200 μg/mL. TPC of the samples was expressed as gallic acid equivalents (GAE) in mg per mg extract (mg GAE/mg). Each sample was analysed in triplicate.

AC was measured with an ABTS radical cation ($ABTS^{\cdot+}$) decolourisation assay (Re et al., 1999). Therefore, a solution containing 2.5 mL of 7 mmol ABTS and 44 μL of 140 mmol potassium persulfate was prepared and stored in the dark for a minimum of 16 h before use. The $ABTS^{\cdot+}$ stock solution was then diluted with distilled water 1:90 to obtain an absorbance between 0.7 and 0.75 at 734 nm. Each sample (100 μL; 10 μg/mL PBE), was added to 1 mL of diluted $ABTS^{\cdot+}$ solution. Absorbance was read at 734 nm after 2.5 min. The percentage of inhibition of absorbance was calculated using the formula; $ABTS^{\cdot+}$ scavenging effect (%) = $((A_B - A_S)/A_B) \times 100$, where A_B is the absorbance of $ABTS^{\cdot+}$ in distilled water and A_S is the absorbance of $ABTS^{\cdot+}$ and sample (extract/standard). Results were expressed in Trolox equivalent antioxidant capacity (TEAC) in μM per mg extract (μM TE/mg) with a calibration curve ranging from 25 to 250 μM. All measurements were carried out in triplicate.

2.2.1. Metabolomic analysis of berry extracts

For GC–MS analysis, 500 μL of diluted PBE (1 mg/mL) was evaporated to dryness in a vacuum centrifuge (Eppendorf concentrator 5301) then resuspended in 50 μL anhydrous pyridine. To achieve volatile and stable products suitable for GC–MS analysis, dried samples were silylated according to the procedure of Zhang and Zuo (2004). Briefly, 100 μL BSTFA + 1% TMCS was added to the dried samples in pyridine and left in an air circulated oven (Memmert, Germany) for 4 h at 70 °C. Aliquots (1 μL) were injected in splitless mode into an Agilent Technologies 7820A GC system coupled to a 5977E MSD which was operated in EI mode at 70 eV. Helium was used as carrier gas, set at 40 psi, and column head pressure at 8 psi. Injector and MSD transfer line were operated at 280 °C. Separation was achieved on a HP-5MS fused-silica capillary column (30 m × 0.25 mm id, 0.25 μm) (Agilent technologies, UK). Column temperature profile and MSD settings were chosen according to the method of Zhang and Zuo (2004) with some modifications. Initially, column temperature was held at 80 °C for 1 min then raised to 220 °C at 10 °C/min, then raised to 310 °C at 20 °C/min and finally held for 16 min. Mass spectra were scanned from mass-to-charge ratio (m/z) 50–650 with a scan rate of 1.5 scans/s. Chromatograms and mass spectra were analysed using the MassHunter Qualitative Analysis B.07.00 software (Agilent Technologies, CA, USA). Data was then processed with AMDIS software (Agilent Technologies, CA, USA) to deconvolute coeluting peaks. Identification of phytochemicals was obtained by comparison of each extract sample with the retention time and mass spectra of authentic standards. Identification of other compounds was achieved by using National Institute of Standard and Technologies library NIST MS Search 2.2 (https://www.nist.gov). Relative quantification of compounds was carried out by measuring the areas of the total ion chromatogram (TIC) for each compound of interest and the area of the internal standard, homovanillic acid (HVA) (10 μL, 500 μg/mL), added to the extracts. Relative concentrations were established using the formula [(concentration HVA/peak area HVA) * peak area of compound of interest] and are listed in Table 1. HVA was chosen as internal standard due to its absence in any natural products. It was also fully resolved from other analytes and was stable under all experimental conditions.

2.3. Laboratory-scale UHT milk treatment

To each litre of milk, 0.5 g of RE or 0.5 g or 1 g of BE was added to obtain final concentrations of 0.5 mg/mL or 1 mg/mL, respectively. This was then mixed with a laboratory scale mixer (Silverston Machines Ltd, UK) for 5 min at room temperature. Each milk sample (1L) and one control sample, consisting of semi-skimmed, lactose free pasteurised milk, was then thermally processed in a laboratory-scale HTST/UHT system (FT74XTS-44-A, Armfield, UK). Processing pressure was set at 3.5 bar, preheating temperature was 75 °C then processed at 140 °C for two seconds. Flow rate was set at 10 L/h. These UHT milks were then immediately stored at −80 °C until analysis. It should be noted that reported values for naturally occurring CML in pasteurised milk range from 10.4 mg/kg (Assar, Moloney, Lima, Magee, & Ames, 2009) to 53.0 mg/kg (Ahmed et al., 2005). The amount discovered in our control sample was 11.9 mg/kg which falls within the reported values.

2.4. GC–MS analysis of milk and Maillard reaction products determination

In order to monitor CML, LYS and other MRPs, the UHT milk samples were treated according to the protocol of Troise, Fiore, Wiltafsky, and Fogliano (2015). Acidic hydrolysis was performed by adding 100 μL of milk sample and 4 mL of 6 M HCl to a screw capped flask with a PTFE septum. The mixture was saturated with nitrogen gas for 15 min at 2 bar and hydrolysed in an air forced circulating oven (Memmert, Germany) for 24 h at 110 °C before being centrifuged at ambient temperature for 15 min at 5000 rpm. This centrifugation step was repeated on the supernatant. 400 μL of the clear liquid phase were dried under nitrogen flow at 2 bar to stop oxidation of hydrolysed components. Samples were re-dissolved in 180 μL ultrapure water and 10 μL of each internal standard (CYC 1 mg/mL and d_2-CML 25 μg/mL) was added. As a clean-up step, samples were loaded onto equilibrated Oasis HLB 1 cc cartridges (Waters, Wexford, Ireland) and eluted with 1 mL of methanol: 20 mmol HFBA (1:1 *v/v*) into an Eppendorf tube. The eluate was evaporated to dryness in a vacuum centrifuge then derivatised following the method of de Falco, Fiore, Rossi, Amato, & Lanzotti (2018) with some modifications. Briefly, the dried sample was reconstituted in 50 μL anhydrous pyridine and 150 μL of MSTFA, mixed with a vortex mixer and transferred into a GC-vial. The mixture was allowed to react at 80 °C for 45 min before being analysis. Samples (1 μL) were injected into the GC in splitless mode operated at 220 °C utilising EI mode at 70 eV. Helium was used as carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed as following: 2 min of heating at 70 °C, then temperature was increased to 260 °C at 5 °C/min before being raised to 290 °C at 15 °C/min and held at 290 °C for 5 min. The mass spectrometer was operated both in scan mode at 10.8 scans/s and single ion monitoring (SIM) mode for the quantification of CML and LYS. Parameters for SIM mode were chosen according to the optimised protocol developed in this paper. For quantifying CYC, the first segment was set from 5 to 20 min and quadrupole was fixed to m/z 156.1 with 200 ms dwell time. Second segment was for the quantification of LYS 4TMS and set at m/z 174.1 from 0 to 30 min with a dwell time of 200 ms. Then from 30 to 47 min, the quadrupole was tuned to m/z 492.3 and 494.3 and 100 ms dwell time for the quantification of CML and d_2–CML, respectively. MSD transfer line was set at 280 °C, temperatures of the ion source and quadrupole were set at 200 °C.

Quantification of LYS, was carried out with a calibration curve of five points in the range 125 to 225 μg/mL using CYC as internal standard at 50 μg/mL; R^2 = 0.99; limit of detection (LOD) and limit of quantification (LOQ) were 0.15 and 0.45 μg/mL, respectively. The calibration curve for CML was prepared in the range 0.125 to 2.5 μg/mL with d_2-CML as internal standard at 1.25 μg/mL and five calibration points; R^2 = 0.99; LOD and LOQ were 0.03 and 0.09 μg/mL, respectively. All measurements were carried out in triplicate. Quantification was carried out in SIM mode using the following target ions: m/z 174.1 (LYS, 4TMS), m/z 156.1 (CYC, 2TMS), m/z 492.3 (CML, 4TMS), m/z 494.3 (d_2-CML, 4TMS). Peak area of compounds was compared to internal standard target ion areas. Other compounds, such as amino acids, were semi-quantified by measuring the area of target ions selected for quantification of each compound in relation to the area of the m/z 156.1 of CYC. An extracted ion chromatogram (EIC) at the mass of the selected target ion characteristic of the amino acid surrogate was used for quantification.

Table 1
Phytochemicals identified in blueberry (BE) and raspberry (RE) extract as TMS surrogates by GC–MS. In the table are reported retention time, *m/z* ratio and content related to each compound. Peak numbers correspond to Fig. 1.

Peak	Compound	RT [min]	Identified ions[1] [*m/z*]	Content in BE [μg/mg]	Content in RE [μg/mg]
1	Benzoic acid*	8.01	194, 179, 135, 105, 77	0.037 ± 0.002	0.218 ± 0.012
2	Salicylic acid*	11.69	267, 209, 193, 149, 135, 91, 73	0.120 ± 0.006	0.177 ± 0.010
3	4-Hydroxybenzoic acid	13.20	282, 267, 223, 193, 126, 73	n.d.	0.454 ± 0.018
4	Citric acid	15.36	375, 363, 273, 217, 147, 73	0.783 ± 0.050	1.128 ± 0.065
5	3,4-Dihydroxybenzoic acid	15.44	370, 355, 311, 281, 193, 147, 73	0.126 ± 0.012	0.477 ± 0.017
6	2,5-Dihydroxyphenylacetic acid	15.58	384, 341, 267, 252, 237, 73	0.147 ± 0.014	0.251 ± 0.013
7	Syringic acid	16.22	342, 327, 312, 197, 253, 223, 141, 73	1.090 ± 0.073	n.d.
8	Ascorbic acid*	16.61	374, 332, 259, 205, 147, 117, 73	n.d.	0.304 ± 0.024
9	p-Coumaric acid	16.65	308, 293, 249, 219, 179, 139, 73	0.022 ± 0.001	0.276 ± 0.013
10	Gallic acid*	16.71	399, 355, 311, 281, 179, 147, 73	1.694 ± 0.097	1.656 ± 0.089
11	Ferulic acid	17.85	338, 323, 308, 249, 219, 154, 117, 73	0.452 ± 0.019	0.644 ± 0.040
12	Caffeic acid*	18.13	396, 381, 307, 219, 191, 73	1.219 ± 0.068	0.827 ± 0.051
13	Sinapic acid*	18.83	368, 353, 338, 279, 249, 161, 73	0.463 ± 0.021	0.681 ± 0.033
16	Chlorogenic acid*	24.64	345, 307, 255, 219, 147, 73	50.882 ± 3.562	n.d.
	Total phenolic acids	–	–	**57.035 ± 3.565**	**7.093 ± 0.139**
14	Epicatechin*	22.33	649, 368, 355, 267, 179, 73	0.642 ± 0.026	9.195 ± 0.483
15	Catechin	22.37	650, 368, 355, 267, 179, 73	0.610 ± 0.034	0.600 ± 0.027
17	Quercetin*	25.20	647, 559, 487, 147, 73	n.d.	3.534 ± 0.244
	Total flavonols	–	–	**1.252 ± 0.043**	**13.329 ± 0.868**

n.d. = not detected.
* Identification obtained via authentic standard.

2.5. Quantification of glyoxal and methylglyoxal

Dicarbonyl quantification was determined as described by previous works (Hellwig, Degen, & Henle, 2010) with modifications. Briefly, 0.1 mL of acetonitrile (ACN) was added to 0.5 mL of sample before incubating the mixture in an Orbital shaker (Eppendorf, Germany) at room temperature for 15 min (500 rpm). The mixture was then centrifuged at 4 °C for 20 min (14,000*g*). 0.15 mL of 0.2% OPD in 9.6 mM EDTA solution and 0.15 mL of phosphate buffer solution (0.4 M, pH 7.0) were added to 0.5 mL of the supernatant in order to derivatise MGO and GO into 2 -methylquinoxaline (2-MQ) and 1-quinoxaline (1-Q), respectively. The samples were then incubated at 37 °C for 3 h.

The HPLC system consisted of a Thermo Scientific (Germany) Ultimate 3000 Pump, Dionex DDA-100 diode array detector, and a Dionex autosampler ASI-100 (San Jose, CA). Chromatographic separation was carried out onto an Eclipse Plus C18 column (250 mm × 4.6 mm, 5 μm) (Agilent, USA) with an integrated guard column (5 mm × 4 mm). The flow rate was 1.0 mL/min and the injection volume was 20 μL. A binary solvent system gradient of 0.075% acetic acid (A) and acetonitrile (B) was used; 5 min: 2% B, 22 min: 70% B, 25 min: 70% B, 28 min: 2% B, 33 min: 2% B. The total run was 33 min and chromatograms were recorded at 280 nm and 313 nm. 1-Q and 2-MQ were eluted at 15.20 and 16.86 min, respectively. Peaks were identified by comparison of the retention times with those of the standards. Quantification was carried out by an external calibration of six points in the range (0.1–2 μg/mL); R^2 = 0.99; LOD and LOQ were 0.010 and 0.030 μg/mL, respectively for both 1-Q and 2-MQ. All measurements were carried out in triplicate.

2.6. Statistical analysis

Each milk sample was analysed in triplicate and results were reported as μg/mL of milk. Data were analysed by ANOVA using XLStat statistical software (Addinsoft, New York, NY, USA). Results were reported as mean ± standard deviation (SD). Significant differences between samples were examined using a Duncan test, with a confidence interval of 95%.

FancyTiles were built according to Troise, Ferracane, et al. (2014) using XLStat.

3. Results and discussion

3.1. Method development

In this work, mass spectrometric parameters and derivatisation processes were optimised for the identification and quantification of CML and LYS. A SIM acquisition method was established and accordingly, appropriate ions were selected for the detection of each compound of interest.

Instrumentation was optimised with respect to temperature of quadrupole and dwell time. The first was chosen according to the highest intensity obtained after analysing a sample solution by multiple injection. Dwell time was optimised by analysing sample solutions with respect to optimum peak shape and amount of data points.

Derivatisation is a crucial step in the analysis and is not always easy to accomplish. It is dependent on many parameters such as choice of the appropriate derivatisation reagent, solvent, temperature and time (Knapp, 1979). For this work, silylation was chosen as the derivatisation strategy and acetonitrile (ACN) and pyridine were tested for their suitability as solvents. As it can be seen in Fig. S1 (please see Supplementary material), the choice of solvent affects the derivation process. When ACN was used, three derivatives were generated for CML (2TMS, 3TMS and 4TMS). When the dried sample was dissolved in pyridine, only two of these CML derivatives (3TMS and 4TMS) were detected in the chromatogram. This had an impact on quantification, in that the sum of both product (3TMS and 4TMS) peaks had to be measured. Since the derivatisation could not be driven to completion under the initial set of conditions, the amount of silylation reagent was optimised. Often, artefact peaks which are due to incomplete derivatisation can be eliminated when the volume of silylation reagent is increased (Knapp, 1979). Samples were then derivatised at 80 °C for 45 min before being analysed with GC–MS. This temperature was chosen as it was considered to be a compromise between accelerating the reaction and not increasing it to the point where the thermal stability of the product is threatened. Chromatograms were then evaluated in terms of number of derivatised product peaks, peak shape and intensity of signals observed. The introduction of more silylation reagent increased the total solution volume and hence dilution of CML, thus intensity of signal decreased. When the amount of MSTFA was increased to 150 μL from 50 μL, the intensity of signal of the 3TMS derivative was reduced, as can be seen in Fig. S2 (please see Supplementary material), and in the

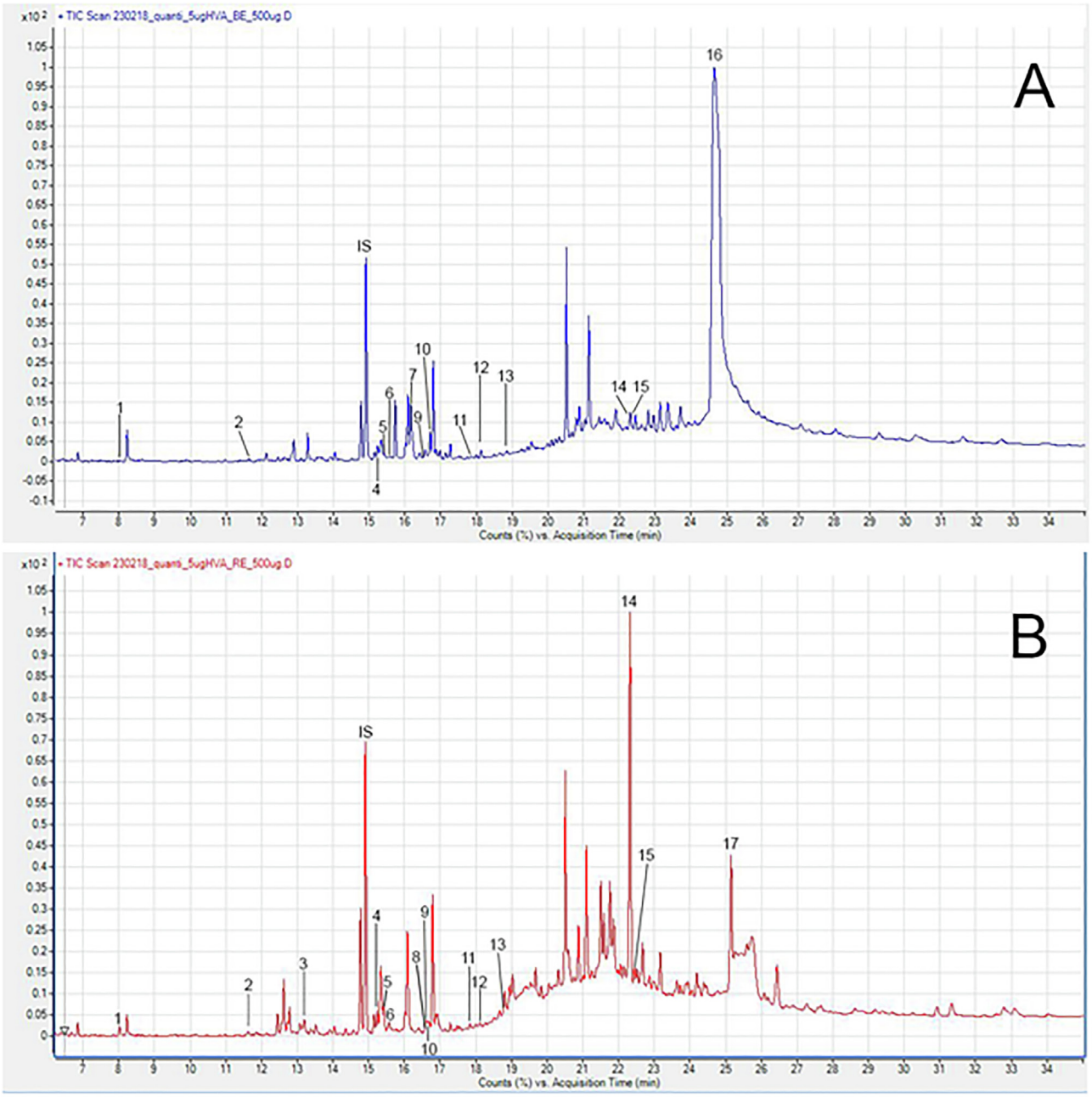

Fig. 1. Representative TIC of the BE (A) and RE (B). Peaks correspond to numbering of compounds in Table 1.

chromatogram obtained with 150 µL MSTFA, the signal of the 3TMS derivative was no longer detectable. Regarding peak shape, the symmetry of the signal of the 4TMS derivative was also enhanced when 150 µL MSTFA was used. Based on these findings, 150 µL of MSTFA was used in all subsequent derivatisations and these reactions were examined in detail. In comparison to earlier experiments, only one silylated derivative of CML was identified instead of three. This derivative was found to be stable over a considerable period of time. Hasenkopf, Rönner, Hiller, and Pischetsrieder (2002) obtained two silylation products of CML.

This problem is avoided when the optimised silylation procedure developed in this study is applied as only the 4TMS derivatives was observed meaning that the reaction had gone to completion. The derivatisation procedure for LYS was also successfully optimised. Two samples containing both 10 µg LYS dissolved in 50 µL solvent were analysed, one utilising the initial protocol and one the optimised protocol. Example chromatograms obtained are shown in Fig. S3 (please see Supplementary material). As can be seen, only the 4TMS derivative was detected in the sample which was analysed with the new derivatisation procedure.

After solvent optimisation, different time/temperature combinations, that is 60 °C/45 min, 90 °C/30 min, and 70 °C/4h, were carried out on identical sample solutions to determine their effect on speed and ease of derivatisation.

In summary, changing solvent to ACN, increasing the volume of silylation reagent to 150 µL and carrying out the derivatisation at 80 °C for 45 min produced the best results.

3.2. Characterisation of PBE

Absence of sugars in extracts was verified with a DMA 35 Portable Density Meter (Anton Paar, Austria).

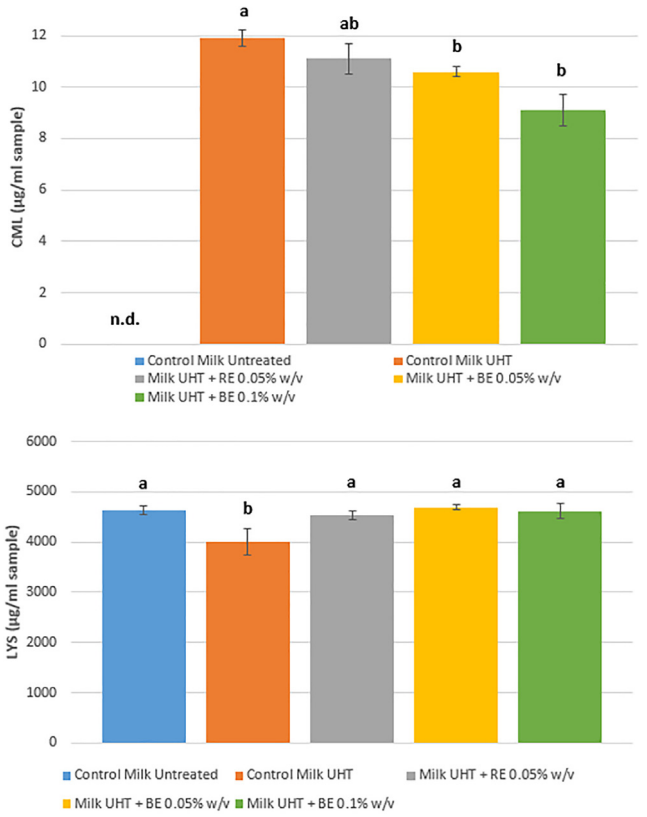

Fig. 2. CML (top) and LYS (bottom) content in UHT treated infant formula milk samples and addition of different amounts of PBE. Samples were analysed by GC–MS after derivatisation. Significant differences were determined by ANOVA analysis and Duncan test ($p \leq 0.05$). Different letters indicate significant differences. n.d.: not detected.

An ABTS decolourisation assay was used to evaluate the AC of the berry extracts. The RE showed a higher AC (6.30 ± 0.11 µmol TEAC/mg) than the BE (5.45 ± 0.07 µmol TEAC/mg). These results are perceptibly higher than the ones obtained by Wang et al. (2011). This may be due to several factors, which have to be taken into account, such as the ripeness of the berries at harvest, genotype, growing season, geographical origin, and farming practise (Josuttis et al., 2012; Zhao, Yuan, Fang, Yin, & Feng, 2013).

BE showed a higher TPC (0.619 ± 0.001 mg GAE/mg dry extract) than that of RE (0.479 ± 0.001 mg GAE/mg dry extract). These results are in accordance with those of Wang et al. (2011). Although, values obtained in this study are slightly higher for both extracts. This can be due to several reasons as the TPC in fruits and berries, similar to the AC, is affected by a similar host of factors (de Falco, Incerti, Pepe, Amato, & Lanzotti, 2016; Josuttis et al., 2012; Zhao et al., 2013). When comparing the TPC values from this study with the values presented in the literature (Skrede, Martinsen, Wold, Birkeland, & Aaby, 2012; Wang, Chen, Camp, & Ehlenfeldt, 2012), it can be seen that in our study, the RE has an approximately 400-fold increase in TPC. While for the BE, the increase ranges from roughly 200 – 800-fold. These increases of TPC in both extracts can be mainly attributed to the extraction process, which included phytochemical enrichment steps (that is drying of aqueous extracts with a rotary evaporator or purification of sugars with Amberlite XAD-7 resin).

The metabolomic profile was obtained by GC–MS analysis. Compounds with their corresponding retention times and *m/z* values are listed in Table 1, and Fig. 1 shows the respective TIC for both BE and RE. The metabolomics approach with GC–MS allowed the identification of 17 phenolic compounds including aromatic acids, benzoic and hydroxycinnamic, and the flavonoids quercetin, catechin and epicatechin. These findings are in line with those of Zhang and Zuo (2004). The overall profile of these compounds in terms of those found are broadly consistent with those previously reported. However the concentrations (when reported as mg/mg extract and as mg/100 g of fresh fruit) were found to be higher than reported elsewhere (Mattila et al., 2006; Rodriguez-Mateos, Cifuentes-Gomez, Tabatabaee, Lecras, & Spencer, 2012).

3.3. Milk samples characterisation and semi-quantification of metabolites

Prior to being subjected to thermal processing, PBE was added to the milk. Subsequently, concentrations of LYS and CML were determined and a metabolomic profile, utilising the so-called FancyTiles, was created for each sample. From GC–MS analysis, common mass fragments of TMS derivatives of amino acids in EI mass spectra are as following: $[M-15]^+$ -CH_3, $[M-72]^+$ -TMS, $[M-85]^+$ –$C(CH_3)$–CO, $[M-89]^+$ - OTMS, $[M-117]^+$ - COOTMS, $[M-89-117]^+$ -OTMS -COOTMS.

Results for the quantification of CML and LYS are depicted in Fig. 2. When PBE was added at 0.5 mg/mL of milk, levels of CML decreased from 11.9 ± 0.3 µg/mL in the control UHT milk to 11.1 ± 0.6 µg/mL for RE, and to 10.6 ± 0.2 µg/mL for BE, respectively (both as 0.05% solutions). However, the difference compared to the UHT treated control milk sample was not significant when RE was used. Concerning the BE, some statistical changes could be observed. In order to determine whether this trend could be increased, milk was spiked with a higher dosage of BE, 1 mg/mL, resulting in a reduction of 23.4% (9.1 ± 0.6 µg/mL) of CML. The result was statistically different to the control milk sample but not significantly so. Nevertheless, the addition of the BE prior to thermal processing does inhibit the formation of the CML. This is in line with work reported on the inhibition of CML in heat-treated milk. For example, Troise, Fiore, et al. (2014) reported a reduction in CML on treatment of milk with olive mill wastewater (OMWW) prior to heat treatment in the range 11.2–16.2%. On the other hand, these inhibitory rates are lower compared to those of Baijiu vinasse extracts (43.2%) in a casein and D-glucose model system (Wang et al., 2019), corn silk extracts (76.6%) in a casein glucose-fatty acid model system (Zhang et al., 2020) or sugarcane molasses extracts (34.8%) in glucose- LYS model reaction systems (Yu et al., 2017). This can be explained by the food matrix effect that might implicate antagonistic interactions to CML inhibition.

Regarding the results of dicarbonyls quantification, MGO levels did not significantly drop with decreases of 9.6% (1.09 ± 0.02 µg/mL) and 7.8% (1.12 ± 0.03 µg/mL) in 0.05% BE and RE UHT milk samples of intermediate concentration, respectively, comparing to the control sample (1.21 ± 0.02 µg/mL). MGO concentration significantly decreased by 25.1% (0.90 ± 0.01 µg/mL) in the UHT milk spiked with 1 mg/ml of BE comparing to the control sample. On the other hand, no significant change was observed in the levels of GO in all samples. These results imply that the antiglycative effect of the BE is exerted through trapping MGO via the aromatic rings of the polyphenols present in the extract, resulting in the inhibition of CML. This is in agreement with the findings of Yang et al. (2018) that stipulate that low concentration of (+)-catechin or certain stoichiometry of catechin: MGO can remarkably mitigate the formation of CML.

LYS levels found in the UHT milk (4004.2 ± 267.2 µg/ml) were statistically lower than those in the untreated milk (4640.0 ± 94.1 µg/ml) by 13.70% (Fig. 2). This result is not surprising, since it is well-known that thermal treatment can lead to LYS loss due to blockage of the amino acid and supplementation of glucose prior to thermal processing which was done to facilitate an increase in the yield of MRPs. The works of Schmitz-Schug, Kulozik, and Foerst (2014), and Schmitz, Gianfrancesco, Kulozik, and Foerst (2011) have shown that heating at 90 °C for 30 min has caused around 70% to 81% LYS loss in powdered dairy formulations, respectively. Aalaei, Rayner, and Sjöholm (2018) reported a decrease of approximately 30% of available LYS in

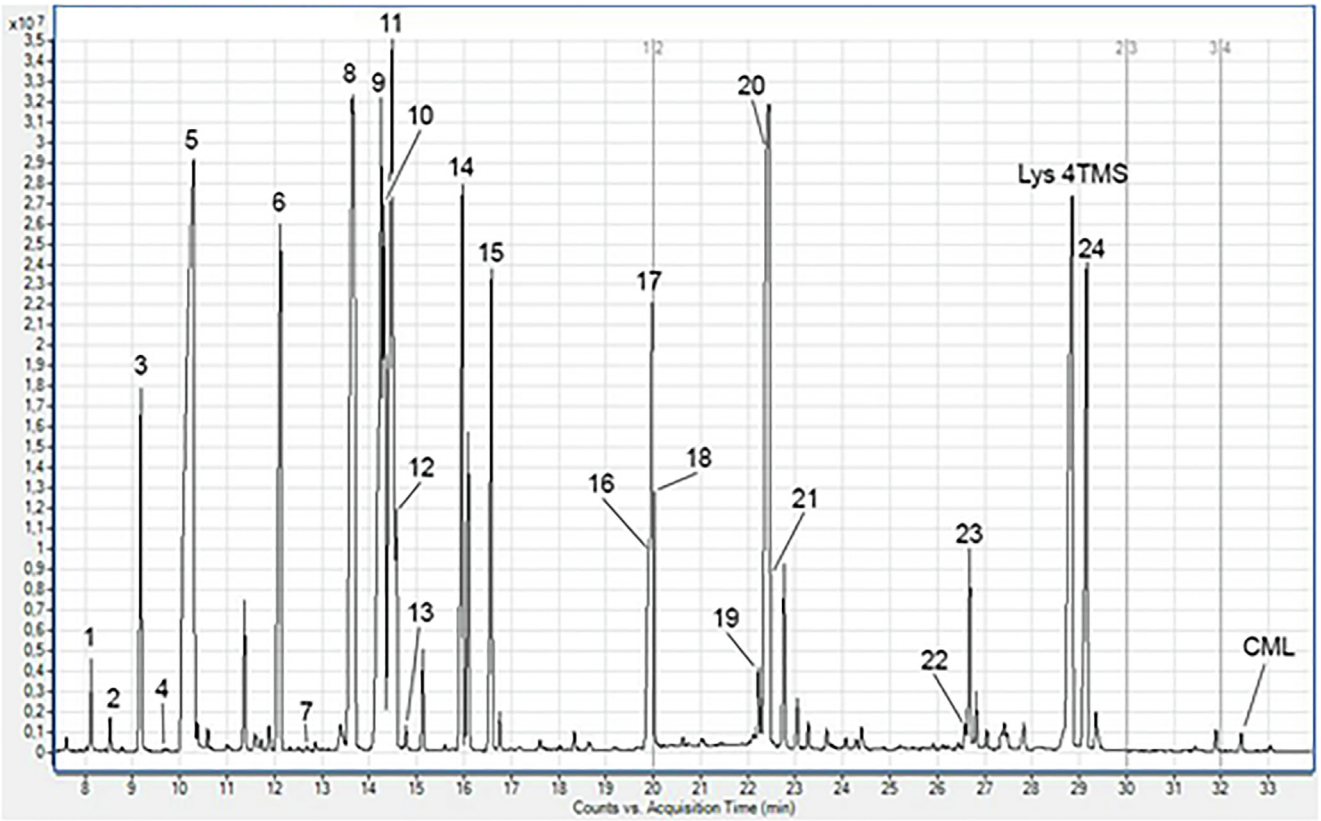

Fig. 3. Representative TIC acquired by injecting a milk sample after preparation. Peaks correspond to numbering of compounds in Table 2.

Table 2
List of compounds detected as TMS surrogates by GC–MS analysis with corresponding target ions and expected retention times. Milk samples were analysed according to the optimised protocol with respect to sample preparation and silylation.

Peak	Compound	Target ion [*m/z*]	RT [min]
1	Lactic acid 2 TMS	147	8.1
2	Glycolic acid TMS	147	8.51
3	Alanine 2 TMS	116.1	9.18
4	Glycine 2 TMS	102	9.67
5	Levulinic acid TMS	75	10.24
6	Valine 2 TMS	144.1	12.12
7	4-Hydroxybutanoic acid 2 TMS	147	12.66
8	Leucine 2 TMS	158.1	13.62
9	Isoleucine 2 TMS	158.1	14.23
10	Proline 2 TMS	142.1	14.3
11	Pentenoic acid 2 TMS	143.1	14.45
12	Glycine 3 TMS	174.1	14.5
13	Butanedioic acid 2 TMS	147	14.76
14	Serine 3 TMS	204.1	15.96
15	Threonine 3 TMS	218.2	16.58
16	Metionine 2 TMS	176.2	19.89
17	PyrGlu 2 TMS	156.1	19.96
18	Aspartic acid 3 TMS	232.1	20
19	Ornithine 3 TMS	142.1	22.23
20	Glutamic acid 3 TMS	246.1	22.43
21	Phenylananine 2 TMS	192.1	22.45
22	Ornithine 4 TMS	142.1	26.58
23	Citric acid 4 TMS	273.1	26.68
24	Tyrosine 3 TMS	218.1	29.15

commercial skim milk powder after storage at 35 °C for 30 days. When polyphenolic extracts were added prior to the thermal treatment, samples showed higher LYS values which were significantly different from the UHT treated control milk. They were statistically similar to the control milk which was not subjected to further thermal processing. These findings indicate that PBE can be used as a functional ingredient to inhibit blockage of LYS caused by thermal treatment of milk. Besides the formation of CML via oxidative cleavage of the AP Klicken oder tippen Sie hier, um Text einzugeben., CML can be also formed from RCS such as methylglyoxal and glyoxal, towards which polyphenols show a trapping activity (Nguyen, Van der Fels-Klerx, & Van Boekel, 2014). This can explain the observed reduction of CML and simultaneous decrease of LYS loss.

A representative GC–MS chromatogram for milk samples is given in Fig. 3 and identified compounds as well as their target ions are listed in Table 2 From these an EIC at the mass-to-charge ratio of each target ion was created, allowing greater accuracy in quantification as, through this, interference with coeluting substances and overlapping peaks, which could lead to inaccuracies, was avoided (e.g. peak number 9, 10, 11 and 12 in Fig. 3). It is challenging to analyse all amino acids with a single derivatisation procedure due to various chemical properties of amino acids, resulting in different degrees of derivatisation. Compared to the study conducted by Guida, Salvatore, and Salvatore (2015), fewer artefact peaks for some amino acids were detected in the TIC. For example, only one TMS derivative of tyrosine and phenylalanine, the 3TMS and 2TMS silyl derivatives respectively, were produced when the silylation technique developed in this study was applied. Also, for LYS the 4TMS derivative was the only one observed. Guida et al. (2015) obtained three silylation products from LYS and the methyl ester TMS was the principle one discovered. However, utilising our optimised silylation procedure, only the 4TMS derivative was generated.

For each milk sample, a FancyTile diagram (Fig. 4) was created by plotting the semi-quantitative amount of each metabolite into a square based on a colorimetric scale from blue (minimum value) to red (maximum value). Each set of these squares can be thought of as a fingerprint, representing the metabolite profile.

In Fig. 4, the effect of the PBE on the amino acid profile is reported. Scaling is set in a range from 29.8 to 4691.4 μg PBE/mL milk which

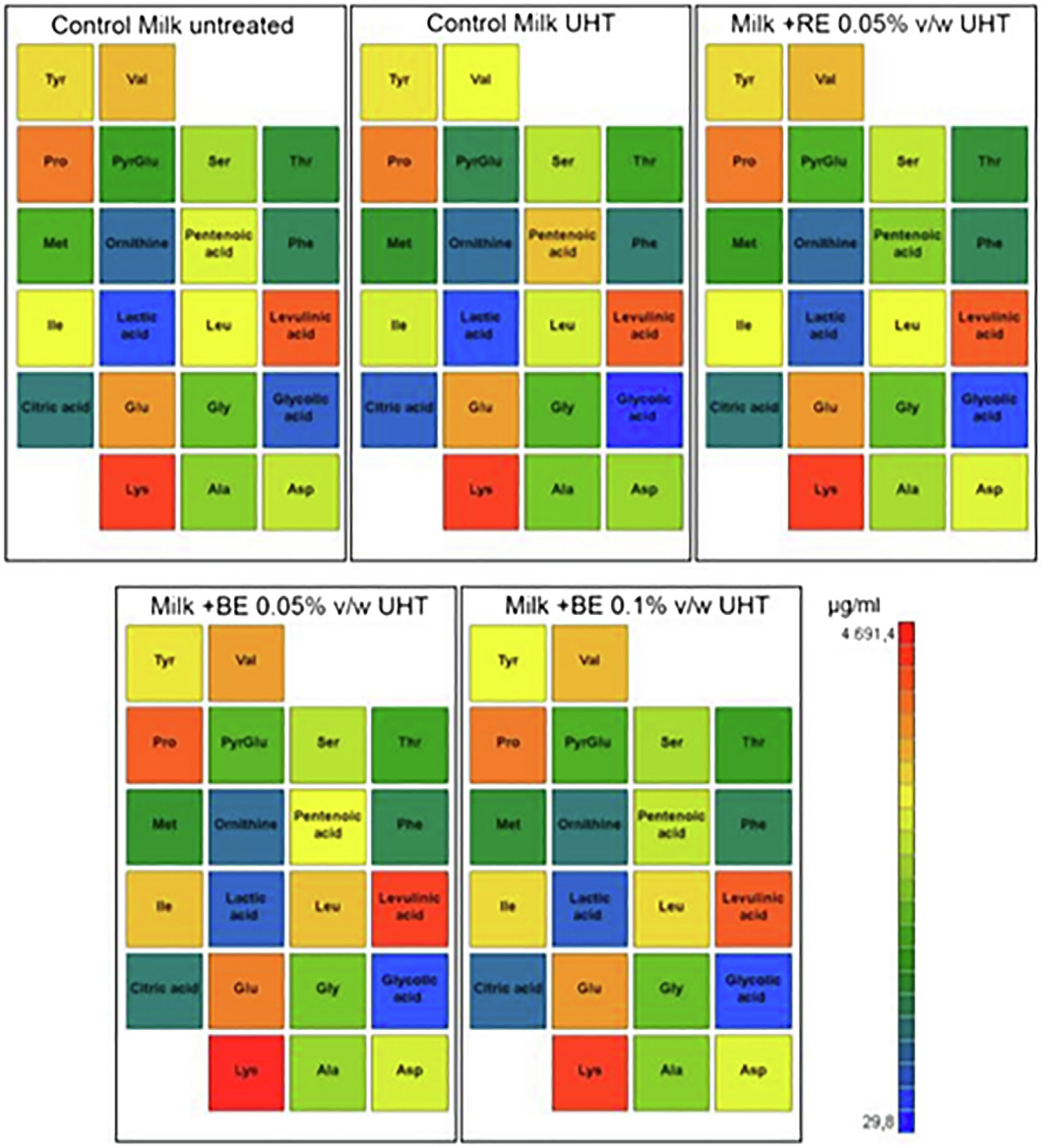

Fig. 4. FancyTiles of amino acids and other organic acids in milk samples before and after thermal treatment, depicted on a spectrum inverted chromatic scale. In order to highlight the differences between compounds, CML was excluded from this analysis to allow better visualisation of smaller differences in the amino acids content between samples. The range was built by using minimum and maximum values.

captures the overall minimum and maximum values found over all samples. In order to highlight differences in the relative abundance of compounds, CML was excluded. Selection of FancyTiles for data presentation proved an effective method to illustrate the effectiveness of PBE in controlling aspects of the MR during UHT treatment. The results for thermally treated milk samples supplemented with PBE's were more similar to those of the untreated control milk than processed control milk. As an example of this, the squares representing the amino acid valine in the FancyTile charts are of similar shade and hence of similar concentrations for the PBE treated milk and the untreated control milk, when compared to the processed control milk, where a lighter colour represents a lower concentration. This trend can also be seen for pyroglutamic acid, phenylalanine, leucine, isoleucine, and asparagine, indicating that not only the LYS, but also other amino acids were less prone to becoming modified by the addition of berry extracts.

4. Conclusions

In this study a novel use of poor condition raspberries and blueberries is presented. We report the ability of sugar-free extracts from these to reduce the formation of AGEs i.e. the reduction in the formation of CML, and protect amino acids from degradation was successfully demonstrated in infant formula milk. An optimised derivatisation and GC–MS method was developed for compounds of interest particularly CML. The derivatisation process developed improved the quantification of the selected analytes.

For each treated and untreated milk sample analysed in this study, a FancyTiles chart was created. These FancyTiles provide an illustration of the data obtained to compare differences in metabolic profiles in a quick and easy way. A significant decrease of CML was observed in milk samples in the presence of 1 mg/mL BE. This can be attributed to the trapping ability of MGO by the aromatic rings of the polyphenols present in the PBE.

In conclusion this procedure allows to plot semi-quantitative data depicting the effectiveness of PBE as a functional ingredient for controlling MR during UHT treatment in milk.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Sarah Prestel: Investigation, Formal analysis, Data curation, Writing - original draft. **Bruna de Falco:** Investigation, Formal analysis, Data curation, Writing - original draft. **Slim Blidi:** Investigation, Formal analysis, Data curation, Writing - review & editing. **Alberto Fiore:** Conceptualization, Methodology, Supervision, Project administration, Visualization, Data curation, Writing - review & editing. **Keith Sturrock:** Conceptualization, Methodology, 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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108923.

References

Aalaei, K., Rayner, M., & Sjöholm, I. (2018). Kinetics of available lysine in stored commercial skim milk powder at moderate temperatures. *International Journal of Food Science & Technology, 53*(9), 2159–2165.

Ahmed, N., Mirshekar-Syahkal, B., Kennish, L., Karachalias, N., Babaei-Jadidi, R., & Thornalley, P. J. (2005). Assay of advanced glycation endproducts in selected beverages and food by liquid chromatography with tandem mass spectrometric detection. *Molecular Nutrition & Food Research, 49*(7), 691–699.

Ahmed, M. U., Thorpe, S. R., & Baynes, J. W. (1986). Identification of N epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *The Journal of Biological Chemistry, 261*(11), 4889–4894.

Assar, S. H., Moloney, C., Lima, M., Magee, R., & Ames, J. M. (2009). Determination of N ε-(carboxymethyl) lysine in food systems by ultra performance liquid chromatography-mass spectrometry. *Amino Acids, 36*(2), 317–326.

de Falco, B., Fiore, A., Rossi, R., Amato, M., & Lanzotti, V. (2018). Metabolomics driven analysis by UAEGC-MS and antioxidant activity of Chia (Salvia hispanica L.) commercial and mutant seeds. *Food Chemistry, 254*, 137–143.

de Falco, B., Incerti, G., Pepe, R., Amato, M., & Lanzotti, V. (2016). Metabolomic fingerprinting of Romaneschi Globe Artichokes by NMR spectroscopy and multivariate data analysis. *Phytochemical Analysis, 27*(5), 304–314.

Dunn, J. A., Ahmed, M. U., Murtiashaw, M. H., Richardson, J. M., Walla, M. D., Thorpe, S. R., & Baynes, J. W. (1990). Reaction of ascorbate with lysine and protein under autoxidizing conditions: Formation of N. epsilon.-(carboxymethyl) lysine by reaction between lysine and products of autoxidation of ascorbate. *Biochemistry, 29*(49), 10964–10970.

Fu, M. X., Requena, J. R., Jenkins, A. J., Lyons, T. J., Baynes, J. W., & Thorpe, S. R. (1996). The advanced glycation end product, Nepsilon-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *The Journal of biological chemistry, 271*(17), 9982–9986.

Guerra, P. V., & Yaylayan, V. A. (2014). Interaction of flavanols with amino acids: Postoxidative reactivity of the B- ring of catechin with glycine. *Journal of Agricultural and Food Chemistry, 62*(17), 3831.

Guida, M., Salvatore, M. M., & Salvatore, F. (2015). A strategy for GC/MS quantification of polar compounds via their silylated surrogates: silylation and quantification of biological amino acids. *Journal of Analytical & Bioanalytical Techniques, 6*(263).

Hasenkopf, K., Rönner, B., Hiller, H., & Pischetsrieder, M. (2002). Analysis of glycated and ascorbylated proteins by gas chromatography − mass spectrometry. *Journal of Agricultural and Food Chemistry, 50*(20), 5697–5703.

Hellwig, M., Degen, J., & Henle, T. (2010). 3-Deoxygalactosone, a "new" 1, 2-dicarbonyl compound in milk products. *Journal of Agricultural and Food Chemistry, 58*(19), 10752–10760.

Jansson, T., Rauh, V., Danielsen, B. P., Poojary, M. M., Waehrens, S. S., Bredie, W. L., ... Lund, M. N. (2017). Green tea polyphenols decrease strecker aldehydes and bind to proteins in lactose-hydrolyzed UHT milk. *Journal of Agricultural and Food Chemistry, 65*(48), 10550–10561.

Josuttis, M., Carlen, C., Crespo, P., Nestby, R., Toldam-Andersen, T., Dietrich, H., & Krüger, E. (2012). A comparison of bioactive compounds of strawberry fruit from Europe affected by genotype and latitude. *Journal of Berry Research, 2*(2), 73–95.

Knapp, D. R. (1979). *Handbook of analytical derivatization reactions.* John Wiley & Sons.

Lund, M. N., & Ray, C. A. (2017). Control of maillard reactions in foods: Strategies and chemical mechanisms. *Journal of Agricultural and Food Chemistry, 65*(23), 4537–4552.

Mattila, P., Hellström, J., & Törrönen, R. (2006). Phenolic acids in berries, fruits, and beverages. *Journal of Agricultural and Food Chemistry, 54*(19), 7193–7199.

Meltretter, J., Seeber, S., Humeny, A., Becker, C., & Pischetsrieder, M. (2007). Site-specific formation of Maillard, oxidation, and condensation products from whey proteins during reaction with lactose. *Journal of Agricultural and Food Chemistry, 55*(15), 6096–6103.

Mildner-Szkudlarz, S., Siger, A., Szwengiel, A., & Bajerska, J. (2015). Natural compounds from grape by-products enhance nutritive value and reduce formation of CML in model muffins. *Food Chemistry, 172*, 78–85.

Nguyen, H. T., Van der Fels-Klerx, H., & Van Boekel, M. (2014). N ∈-(carboxymethyl) lysine: A review on analytical methods, formation, and occurrence in processed food, and health impact. *Food Reviews International, 30*(1), 36–52.

Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. *Foodbourne Pathogens & Disease, 2*(2), 115–129.

Peng, X., Ma, J., Chen, F., & Wang, M. (2011). Naturally occurring inhibitors against the formation of advanced glycation end-products. *Food & function, 2*(6), 289–301.

Pischetsrieder, M., & Henle, T. (2012). Glycation products in infant formulas: Chemical, analytical and physiological aspects. *Amino acids, 42*(4), 1111–1118.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9), 1231–1237.

Rérat, A., Calmes, R., Vaissade, P., & Finot, P. (2002). Nutritional and metabolic consequences of the early Maillard reaction of heat treated milk in the pig. *European journal of nutrition, 41*(1), 1–11.

Rodriguez-Mateos, A., Cifuentes-Gomez, T., Tabatabaee, S., Lecras, C., & Spencer, J. P. (2012). Procyanidin, anthocyanin, and chlorogenic acid contents of highbush and lowbush blueberries. *Journal of Agricultural and Food Chemistry, 60*(23), 5772–5778.

Rodriguez-Mateos, A., Heiss, C., Borges, G., & Crozier, A. (2013). Berry (poly) phenols and cardiovascular health. *Journal of Agricultural and Food Chemistry, 62*(18), 3842–3851.

Schmitz-Schug, I., Kulozik, U., & Foerst, P. (2014). Reaction kinetics of lysine loss in a model dairy formulation as related to the physical state. *Food and Bioprocess Technology, 7*(3), 877–886.

Schmitz, I., Gianfrancesco, A., Kulozik, U., & Foerst, P. (2011). Kinetics of lysine loss in an infant formula model system at conditions applicable to spray drying. *Drying Technology, 29*(16), 1876–1883.

Singleton, V., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Skrede, G., Martinsen, B. K., Wold, A., Birkeland, S., & Aaby, K. (2012). Variation in quality parameters between and within 14 Nordic tree fruit and berry species. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 62*(3), 193–208.

Totlani, V., & Peterson, D. (2007). Influence of epicatechin reactions on the mechanisms of maillard product formation in low moisture model systems. *Journal of Agricultural and Food Chemistry, 55*(2), 414–420.

Totlani, V. M., & Peterson, D. G. (2005). Reactivity of epicatechin in aqueous glycine and glucose maillard reaction models: Quenching of C2, C3, and C4 sugar fragments. *Journal of Agricultural and Food Chemistry, 53*(10), 4130.

Troise, A. D., Ferracane, R., Palermo, M., & Fogliano, V. (2014a). Targeted metabolite profile of food bioactive compounds by Orbitrap high resolution mass spectrometry: The "FancyTiles" approach. *Food Research International, 63*, 139–146.

Troise, A. D., Vitiello, D., Tsang, C., & Fiore, A. (2016). Encapsulation of ascorbic acid promotes the reduction of Maillard reaction products in UHT milk. *Food & Function, 7*(6), 2591–2602.

Troise, A. D., Dathan, N. A., Fiore, A., Roviello, G., Di Fiore, A., Caira, S., ... Monti, S. M. (2014b). Faox enzymes inhibited Maillard reaction development during storage both in protein glucose model system and low lactose UHT milk. *Amino acids, 46*(2), 279–288.

Troise, A. D., Fiore, A., Colantuono, A., Kokkinidou, S., Peterson, D. G., & Fogliano, V. (2014c). Effect of olive mill wastewater phenol compounds on reactive carbonyl species and Maillard reaction end-products in ultrahigh-temperature-treated milk. *Journal of Agricultural and Food Chemistry, 62*(41), 10092–10100.

Troise, A. D., Fiore, A., Wiltafsky, M., & Fogliano, V. (2015). Quantification of Nepsilon-(2-Furoylmethyl)-L-lysine (furosine), Nepsilon-(Carboxymethyl)-L-lysine (CML), Nepsilon-(Carboxyethyl)-L-lysine (CEL) and total lysine through stable isotope dilution assay and tandem mass spectrometry. *Food Chemistry, 188*, 357–364.

Uribarri, J., del Castillo, M. D., de la Maza, M. P., Filip, R., Gugliucci, A., Luevano-Contreras, C., ... Menini, T. (2015). Dietary advanced glycation end products and their role in health and disease. *Advances in Nutrition, 6*(4), 461–473.

Van Boekel, M. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry, 62*(4), 403–414.

Wang, S. Y., Chen, H., Camp, M. J., & Ehlenfeldt, M. K. (2012). Genotype and growing season influence blueberry antioxidant capacity and other quality attributes. *International Journal of Food Science & Technology, 47*(7), 1540–1549.

Wang, W., Yagiz, Y., Buran, T. J., do Nascimento Nunes, C., & Gu, L. (2011). Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. *Food Research International, 44*(9), 2666–2673.

Wang, Y., Liu, H., Zhang, D., Liu, J., Wang, J., Wang, S., & Sun, B. (2019). Baijiu vinasse extract scavenges glyoxal and inhibits the formation of Nε-carboxymethyllysine in dairy food. *Molecules, 24*(8), 1526.

Wells-Knecht, M. C., Thorpe, S. R., & Baynes, J. W. (1995). Pathways of formation of glycoxidation products during glycation of collagen. *Biochemistry, 34*(46),

15134–15141.
Yang, L., Li, X., Wu, Z., Feng, C., Zhang, T., Dai, S., & Dong, Q. (2018). Inhibition of methylglyoxal-induced histone H1 Nε-carboxymethyllysine formation by (+)-catechin. *Journal of Agricultural and Food Chemistry, 66*(23), 5812–5820.
Yu, P., Xu, X.-B., & Yu, S.-J. (2017). Inhibitory effect of sugarcane molasses extract on the formation of N ε -(carboxymethyl)lysine and N ε -(carboxyethyl)lysine. *Food Chemistry, 221*, 1145–1150.
Zhang, D., Wang, Y., & Liu, H. (2020). Corn silk extract inhibit the formation of Nε-carboxymethyllysine by scavenging glyoxal/methyl glyoxal in a casein glucose-fatty acid model system. *Food Chemistry, 309*.
Zhang, W., Ray, C., Poojary, M. M., Jansson, T., Olsen, K., & Lund, M. N. (2018). Inhibition of Maillard reactions by replacing galactose with galacto-oligosaccharides in casein model systems. *Journal of Agricultural and Food Chemistry, 67*(3), 875–886.
Zhang, K., & Zuo, Y. (2004). GC-MS determination of flavonoids and phenolic and benzoic acids in human plasma after consumption of cranberry juice. *Journal of Agricultural and Food Chemistry, 52*(2), 222–227.
Zhao, X., Yuan, Z., Fang, Y., Yin, Y., & Feng, L. (2013). Characterization and evaluation of major anthocyanins in pomegranate (Punica granatum L.) peel of different cultivars and their development phases. *European Food Research and Technology, 236*(1), 109–117.