Food Research International 198 (2024) 115418

Contents lists available at ScienceDirect

Food Research International

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

Investigation of Maillard reaction products in plant-based milk alternatives

Mariachiara Pucci [a,b], Halise Gül Akıllıoğlu [b], Marta Bevilacqua [b], Giulia Abate [a], Marianne Nissen Lund [b,c,*]

[a] Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
[b] Department of Food Science, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark
[c] Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

ARTICLE INFO

Keywords:
Plant-based foods
Advanced glycation end products
α-dicarbonyl compounds
Acrylamide
Protein modifications
Food processing
Maillard reaction

ABSTRACT

Over the past decade, plant-based milk alternatives (PBMAs) have gained increasing popularity. Several processing technologies, including heat treatment, are usually employed during their production in order to replicate the properties of cow's milk. These processes can trigger the Maillard reaction, producing Maillard reaction products (MRPs) and amino acid cross-links, which may alter the nutritional profile and digestibility of PBMAs. This study investigates PBMAs available in the Scandinavian market to assess their MRP and amino acid cross-link concentrations, aiming to understand the relationship between the formation of these heat-induced compounds and the specific chemical composition of individual PBMAs. Two types of UHT-treated cow's milk and ten UHT-processed PBMAs from different brands were analyzed. Quantitative analyses included early-stage MRPs (Amadori products detected as furosine), intermediate MRPs (α-dicarbonyl compounds and furans), advanced glycation end products (AGEs), acrylamide, and amino acid cross-links (lanthionine and lysinoalanine). Protein, carbohydrate, and amino acid profiles were also assessed using LC–MS and HPLC methods. PBMAs were found to differ substantially in carbohydrate and protein content, with soy-based drinks containing higher protein and rice and oat drinks having more carbohydrates. Essential amino acid (EAA) levels were found lower in all PBMAs, impacting their nutritional quality. MRP levels, such as furosine and AGEs, varied across PBMAs, indicating different heat-processing intensities. Specific α-dicarbonyl compounds, like 3-deoxyglucosone, were more concentrated in PBMAs than in UHT-treated cow's milk, and compounds like HMF, furfural, and acrylamide were also found in some PBMAs. Finally, correlations were observed between sugar content, α-dicarbonyls, and AGEs, which offer insights into possible chemical transformations in PBMAs during processing.

1. Introduction

Milk is widely recognized for its exceptional and balanced nutrient composition, which includes proteins, amino acids, lipids, vitamins, and minerals, making it an important component of human nutrition (Haug, Høstmark, & Harstad, 2007; Smith, Fletcher, Hill, & McNabb, 2022). Beyond its role as a complete food source, bovine milk consumption has been associated with various physiological and beneficial effects, such as anti-inflammatory (Da Silva & Rudkowska, 2014), anti-oxidant (Khan et al., 2019), anti-adipogenic (Rai, Nandini, & Priyadarshini, 2021) and anti-osteoporosis (Ratajczak, Zawada, Rychter, Dobrowolska, & Krela-Kaźmierczak, 2021) effects. However, in recent years, the dairy market has experienced a significant shift toward plant-based milk alternatives (PBMAs), water-based extracts derived from a variety of plant sources, including grains, pseudo-cereals, legumes, nuts and seeds (Sethi, Tyagi, & Anurag, 2016). The rising consumption of PBMAs is mainly driven by environmental sustainability, ethical concerns regarding animal welfare, and emerging scientific evidence suggesting potential health benefits from various functional components in plant-based beverages (Mäkinen et al., 2016; Sethi, Tyagi, & Anurag, 2016; Silva, Silva, &

Abbreviations: 1-DP, 1-deoxypentosone; 3-DG, 3-deoxyglucosone; AGEs, advanced glycation end products; CEL, N-Ɛ-(carboxyethyl)lysine; CML, N-Ɛ-(carboxymethyl)lysine; DETAPAC, diethylenetriaminepentaacetic acid; EAA, essential amino acids; GO-Hs, glyoxal-derived hydroimidazolone isomers; GO, glyoxal; GOLD, glyoxal lysine dimer; HMF, 5-hydroxymethylfurfural; LAL, lysinoalanine; LAN, lanthionine; MG-Hs, methylglyoxal-derived hydroimidazolone isomers; MGO, methylglyoxal; MOLD, methylglyoxal lysine dimer; MPA, 3-mercapto-propionic acid; MRPs, Maillard reaction products; OPA, O-phthaldialdehyde; OPD, orthophenilene diamine; PBMAs, plant-based milk alternatives.

* Corresponding author at: Department of Food Science, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark.
E-mail address: mnl@food.ku.dk (M.N. Lund).

https://doi.org/10.1016/j.foodres.2024.115418
Received 12 June 2024; Received in revised form 9 November 2024; Accepted 19 November 2024
Available online 20 November 2024

Ribeiro, 2020). For example, isoflavones in soy drinks have been shown to elicit protective effects against cardiovascular disease and osteoporosis (Omoni & Aluko, 2005). β-Glucans found in oat drinks were reported to reduce blood glucose and cholesterol levels (Truswell, 2002; Welch, 1989). A similar hypocholesterolemic effect was reported for β-sitosterol and γ-oryzanol in rice drinks, to which anti-diabetic, anti-inflammatory and anti-oxidant effects were also associated (Biswas, Sircar, Mitra, & de, 2011). Similarly, α-tocopherol found in almond drinks was reported to have antioxidant properties (Kodad, Socias i Company, & Alonso, 2018).

Besides the potential beneficial effects, the increased consumption of PBMAs calls for a thorough evaluation of their overall composition, nutritional value, and process-induced protein modifications. (Bocker & Silva, 2022).

In order to meet consumer demands and to replicate the sensory and nutritional qualities of cow's milk, PBMAs are subjected to numerous steps during their industrial production. Typically, PBMAs are obtained by soaking and grinding plant materials to release oils and proteins, resulting in a colloidal suspension that is stabilized with emulsifiers and thickening agents. Many PBMAs are fortified with essential nutrients like calcium, vitamin B12, and riboflavin, in order to compensate for their absence or low content in the raw materials used for PBMAs compared to cow's milk (Sethi et al., 2016; Tangyu, Muller, Bolten, & Wittmann, 2019; Vanga & Raghavan, 2018). PBMAs also rely on processing techniques, such as high-pressure homogenization, and sometimes advanced methods like ultrasound and pulsed electric fields, to improve product stability, extend shelf life, and reduce off-flavors that can result from plant ingredients and processing conditions (Aydar, Tutuncu, & Ozcelik, 2020; McClements, 2020; Tangyu et al., 2019; Trindler, Annika Kopf-Bolanz, & Denkel, 2022; Xing et al., 2020).

In addition, heat treatment is often essential to ensure product safety by preventing microbial growth and extending shelf-life. However, heat treatment can impact product quality, by inducing chemical changes and modifications of proteins, with potential short- and long-term effects on nutritional value (Čurlej et al., 2022; Liu et al., 2022; Roland, Aguilera-Toro, Nielsen, Poulsen, & Larsen, 2023). An important heat-induced reaction pathway leading to chemical modifications of proteins is the Maillard reaction, which takes place between the carbonyl group of reducing sugars and the amino group of amino acids, peptides, or proteins, resulting in the formation of Maillard reaction products (MRPs). The Maillard reaction is a cascade of reactions comprising three principal stages: early, advanced and late. The initial reaction between sugars and amino acids, peptides or proteins leads to the formation of a Schiff base. Rearrangements of this intermediate lead to early MRPs known as Amadori (or Heyns, depending on the sugar) products. During acid hydrolysis, which is a required step for quantitative analysis, Amadori products are converted into furosine, which represents an important marker of the early-stage Maillard reaction. Further rearrangements of Amadori products that include dehydration, elimination and condensations reactions typically occur during advanced Maillard reaction steps and result in the formation of α-dicarbonyl compounds, furanic compounds (e.g. 5-hydroxymethyl 2-furfural (HMF), 2-furaldehyde (furfural)), Strecker aldehydes and advanced glycation end products (AGEs), such as N-Ɛ-(carboxymethyl)lysine (CML), N-Ɛ-(carboxyethyl)lysine (CEL), methylglyoxal-derived hydroimidazolone isomers (MG-Hs), glyoxal-derived hydroimidazolone isomers (GO-Hs), methylglyoxal lysine dimer (MOLD) and glyoxal lysine dimer (GOLD). Acrylamide, which is a carcinogenic compound, is formed in the later stages of the Maillard reaction by the reaction of reducing sugars with free asparagine and typically in food products with low moisture content. Finally, additional polymerization reactions cause the formation of complex compounds that in turn can form melanoidins, which are brown pigments formed in the final stage of the Maillard reaction (Gökmen, Serpen, Açar, & Morales, 2008; Lund & Ray, 2017; Poojary & Lund, 2022).

Another chemical modification that occurs in food matrices during heat treatment is amino acid cross-linking. Unlike glycation, this type of protein modification is not dependent of the presence of sugars (Nielsen, Knudsen, Bækgaard, Rauh, & Larsen, 2022), but can manifest through various mechanisms, including oxidation, thiol-disulfide exchange reactions, and β-elimination. β-Elimination of serine or cysteine results in a dehydroalanine derivative, which then cross-links with lysine or cysteine residues, forming lysinoalanine (LAL) or lanthionine (LAN), respectively (Akıllıoğlu & Lund, 2022).

Formation of MRPs and protein cross-links in food have been drawing increasing attention, because they may be responsible for a reduction in the digestibility of proteins and the overall nutritional value of food (ALjahdali & Carbonero, 2019). Several studies have already been undertaken over the years to assess the safety of animal-derived foods and milk to assess their MRPs content (Nowotny, Schröter, Schreiner, & Grune, 2018; Stadler, 2005; Tamanna & Mahmood, 2015; Uribarri et al., 2010). On the contrary, very little is known about the formation of MRPs and amino acid cross-links in plant-based food and PBMAs (Nielsen et al., 2022). To address this knowledge gap, we have investigated several PBMAs currently available on the Scandinavian market to assess their concentrations of MRPs (Amadori compounds determined as furosine, AGEs, α-dicarbonyl compounds, furfurals and acrylamide) as well as amino acid cross-links. Particularly, our aim is to provide an insight into the relationship between these heat-induced compounds and the chemical composition of individual PBMAs. Plant proteins have different amino acid profiles from animal-based proteins, and PBMAs generally have a higher content of carbohydrates compared to cow's milk, therefore the susceptibility to formation of MRPs may differ from animal-based foods (Day, Cakebread, & Loveday, 2022; Walther et al., 2022). Thus, we hypothesized that PBMAs would have higher concentrations of MRPs than UHT-treated cow's milk due to the higher carbohydrate content of PBMAs combined with the heat treatment, and no presence of acrylamide due to the high moisture content in these products.

2. Materials and methods

2.1. Chemicals and consumables

The following standards and internal standards were purchased from Iris Biotech GmbH (Marktredwitz, Germany), with net weight values given in brackets; CEL (89.6 %), CML (95.5 %), GOLD TFA salt (94.1 %), MG-H3 TFA salt, GO-H3 TFA salt (48.6 %), furosine HCl (72.7 %), LAL HCl salt (mixture of two diastereoisomers, 62.7 %), MG-H1-d_3 (90.5 %), CEL-d_4 (78.3 %), MOLD-$^{15}N_2$ acetate salt (88.9 %), furosine-d_4 HCl (52.8 %), and CML-d_4 (94.4 %). In addition, MOLD acetate salt (≥96 %), GO-H1-$^{13}C_2$ (≥97 %) and GOLD-$^{15}N_2$ acetate salt (≥96 %) were also obtained from Iris Biotech GmbH; no net weight values were available from the supplier for these standards, so chromatographic purities are given in brackets. Glyoxal solution (40 % in water), methylglyoxal solution (40 % in water), dimethylglyoxal (≥97 %), 2-keto-D-glucose (D-glucosone, ≥98 %), 6-aminocaproic acid (≥99 %) and amino acid standard for protein hydrolysate were purchased from Sigma Aldrich (Copenhagen, Denmark). 2-(2′,3′,4′-trihydroxybutyl) quinoxaline (quinoxaline form of 3-deoxyglucosone) was obtained from Biosynth Carbosynth (UK). Orthophenilene diamine (OPD, 98 %), diethylenetriaminepentaacetic acid (DETAPAC, ≥99 %), 5-(hydroxymethyl) furfural (HMF) (99.5 %), furfural (≥99 %), sodium dihydrogen phosphate monohydrate (≥99 %), sodium phosphate dibasic anhydrous (≥99 %), sodium tetraborate decahydrate (99.5 %), sodium azide (≥99.99 %), O-phthaldialdehyde (OPA) (≥99 %), 3-mercapto-propionic acid (MPA) (≥99 %), acrylamide (≥99 %), acrylamide-d_3 standard solution (500 mg/L) were purchased from Sigma-Aldrich Chemie GmBh (Steinheim, Germany). Disodium hydrogen phosphate anhydrous (>99 %) and formic acid (≥99 %) were obtained from VWR Chemicals (Denmark). Carrez I (potassium hexacyanoferrate (II) trihydrate) and dextrin-15 from maize starch were purchased from Fluka Biochemika

(Switzerland) whereas Carrez II (zinc sulphate heptahydrate), 1-octanol anhydrous (≥99 %), maltose monohydrate, β-lactose, D-(+)-galactose, D-(+)glucose, D-(−)-fructose, sucrose, maltotriose were obtained from Sigma-Aldrich (Darmstadt, Germany). LC–MS grade acetonitrile, ammonium formate, methanol (99.995 %) and HPLC grade acetonitrile, methanol, sulfuric acid were purchased from Sigma Aldrich (Copenhagen, Denmark). Nylon filter membranes (0.20 μm pore size, 47 mm diameter) were obtained from Phenomenex (Aschaffenburg, Germany). Oasis MCX 1 cc Vac Cartridges were purchased from Waters (Taastrup, Denmark). Milli-Q water was produced from a Millipore purification

Table 1
Description of UHT milk and plant-based milk alternatives (PBMAs) under study in terms of ingredient list, nutrition facts, and nutrition claim.

Products			*Ingredients*	Nutritional Facts Label							*Nutrition Claims*
				Energy (kcal)	**Fat (g)**	**Carbohydrates (Sugar) (g)**	**Fiber (g)**	**Protein (g)**	**Salt (g)**	**Minerals and Vitamins**	
COW's MILK	**UHT milk**	**UHT 1.5%**	Semi-skimmed Milk (1,5% fat)	47	1.5	4.9 (4.9)	–	3.4	0.1	Ca: 125 mg	–
		UHT 3.5%	Whole Milk (3,5% fat)	64	3.5	4.9 (4.9)	–	3.4	0.1	Ca: 125 mg	–
PBMAs	**Brand A**	**Mix**	Water, SOY, rice, OATS, calcium carbonate, sea salt, ALMOND, vitamin D, vitamin B2 (riboflavin), vitamin B12, natural flavouring.	46	2	3.4 (2.3)	0.6	3.4	0.19	Ca: 120 mg Vit. D: 1.5 μg Vit. B12: 0.38 μg Riboflavin: 0.21 mg	–
		Soy	Water, SOY *(10 %) *organic ingredients.	37	2.1	0.6 (0.6)	0.6	3.7	0.09	–	–
		Almond	Water, cane sugar*, roasted ALMOND* (2 %), sea salt, stabilizer (guar gum*, gellan gum). *organic. Can contain traces of nuts	21	0.9	2.7 (2.4)	0.3	0.4	0.09	–	–
		Rice	Water, rice* (15 %), sunflower oil*, sea salt. *organic.	54	1.1	10 (6.5)	–	0.1	0.09	–	• Naturally lactose-free. • No added sugar-the sediment comes from the rice itself.
		Oat 1.9%	Water, OATS* (16 %), sunflower oil*, inulin*, sea salt. *organic ingredients. May contain traces of gluten.	52	1.9	8(3.2)	1	0.4	0.08	–	• Without added sugar-sweet comes from the oats themselves.
	Brand B	**Oat 0.5%**	Oat base (water, oats 10 %), sea salt. *organic Free from milk and soy.	37	0.5	6.7 (4.1)	0.8	1	0.11	–	• Natural sugars from oats
		Oat 1.5%	Water, oats 10 %, rapeseed oil, minerals (calcium carbonate, calcium phosphates), salt, vitamins (D2, riboflavin, B12). Free from milk and soy.	46	1.5	6.7 (4.1)	0.8	1	0.1	Ca: 120 mg Vit. D: 0.5 μg Vit. B12: 0.38 μg Riboflavin: 0.21 mg	• Natural sugars from oats
		Oat 3.0%	Water, oats 10 %, rapeseed oil, acidity regulator (dipotassium phosphate), minerals (calcium carbonate), salt, vitamins (D2, riboflavin, B12). Free from milk and soy.	61	3	7.1 (3.4)	0.8	1.1	0.1	Ca: 120 mg Vit. D: 0.5 μg Vit. B12: 0.38 μg Riboflavin: 0.21 mg	• Natural sugars from oats
	Brand C	**Oat 1.8%**	Oat base (water, oats (8.7 %)), sunflower oil, chicory root fiber, pea protein, calcium carbonate, acidity regulator (potassium phosphates), natural flavourings, sea salt, emulsifier (lecithins (sunflower)), stabiliser (gellan gum), vitamins (B12, D2). Free from milk. Naturally lactose-free	44	1.8	5.7 (0)	1	0.7	0.12	Ca: 120 mg Vit. D: 0.75 μg Vit. B12: 0.38 μg	• Source of calcium – no sugar – source of vitamins B12 – natural low content of saturated fat, • Naturally lactose free
		Oat 3.5%	Oat base (water, oats (8.7 %)), sunflower oil, chicory root fiber, pea protein, calcium carbonate, acidity regulator (potassium phosphates), natural flavourings, sea salt, emulsifier (lecithins (sunflower)), stabiliser (gellan gum), vitamins (B12, D2). Free from milk. Naturally lactose-free	59	3.5	5.7 (0)	1	0.7	0.12	Ca: 120 mg Vit. D: 0.75 μg Vit. B12: 0.38 μg	• Source of calcium – no sugar – source of vitamins B12 – natural low content of saturated fat, • Naturally lactose free

system (Millipore Corporation, Billerica, MA).

2.2. Food products

For this study, we utilized two types of UHT-treated cow's milk: one whole milk (UHT 3.5 %) and one semi-skimmed milk (UHT 1.5 %). Additionally, we included ten UHT-processed PBMAs derived from 3 different brands, comprising six oat drinks, one soy, one rice, one almond, and one mixed variant (consisting of soy, rice, almond, and oat). The oat drinks will be distinguished in the text based on their fat content: 0.5 %, 1.5 %, 1.8 %, 1.9 %, 3.0 % and 3.5 %. Furthermore, PBMAs from Brand A included soy, rice, almond, mixed, and oat with 1.9 % fat content. Brand B provided oat alternatives with fat content of 0.5 %, 1.5 %, and 3.0 %, while Brand C supplied oat options with fat content of 1.8 % and 3.5 %. Table 1 provides an overview of all milk and PBMAs used for the study, along with related information, such as the list of ingredients, nutrition facts label, and nutrition claim. All the milk and PBMAs used for the study were purchased from a local supermarket in Copenhagen (Denmark).

2.3. Freeze-drying of samples and dry matter determination

Aliquots of cow's milk and PBMAs were frozen overnight at −80 °C and then freeze-dried at −50 °C for 72 h under vacuum conditions (Modulyo, Edwards High Vacuum International, UK). After drying, dried drinks in the tubes were weighed and dry matter content was calculated gravimetrically as the percentage of weight loss. All cow's milk and PBMAs samples were stored in the freezer (−20 °C) until further analysis.

2.4. Determination of protein content

Protein contents of the samples were determined by analyzing the freeze-dried samples (approximately 0.4 g sample) by a standard Dumas method, ISO 14891:2008 (IDF 185:2008).

2.5. Hydrolysis of samples

For the determination of furosine, CEL, MG-Hs, GO-Hs, GOLD, MOLD, LAL, LAN and total amino acid content, samples were subjected to acidic hydrolysis with a previously validated and published method (Akıllıoğlu & Lund, 2022). Briefly, dried samples containing 3–5 mg protein were added to 3 mL of 6 M HCl in microwave glass tubes. Tubes were consequently flushed with nitrogen and tightly sealed. Afterwards, samples were hydrolyzed by microwave heating at 150 °C for 1 min followed by 10 min at 165 °C using a Biotage Initiator + microwave synthesizer. Aliquots of each hydrolysate were centrifuged at 22,000 *g* for 10 min at room temperature and the resulting supernatant (500 µL) were evaporated to dryness by using a centrifugal vacuum concentrator. The residue was dissolved in an equal amount of Milli-Q water, mixed vigorously and filtered through 0.22 µm nylon filters. For the determination of CML content, samples were reduced by sodium borohydride as previously described (Delatour et al., 2009) with minor modifications. In microwave glass tubes, dried samples with 3–5 mg protein were added 250 µL of Milli-Q water, 750 µL of sodium borate buffer (0.2 M, pH 9.2) and 500 µL of 1 M sodium borohydride in 0.1 M NaOH. Samples were then incubated at room temperature for 4 h. Afterwards, 50 µL of the antifoaming reagent 1-octanol was added to reduce foaming that will occur during the subsequent addition of HCl. Samples were finally hydrolyzed by 6 M HCl (1.45 mL, 12 M HCl) using the same microwave protocol and conditions described above.

2.6. Quantification of furosine, AGEs and amino acid cross-links

After proper dilutions (50:50 acetonitrile:water, v/v), the filtered hydrolysates were used for the analysis of furosine, AGEs (CML, CEL, MGO-Hs, GO-Hs, GOLD, MOLD) and amino acid cross-links (LAL and LAL) according to Akıllıoğlu and Lund (2022). Five µL of the sample was injected into the Dionex UltiMate 3000 LC system (Thermo Fisher Scientific Inc., Waltham, USA) equipped with a Syncronis HILIC column (100 mm length × 2.1 mm internal diameter × 1.7 µm particle size, Thermo Fisher Scientific Inc., USA) maintained at 40 °C. The UHPLC system was directly interfaced to an OrbiTrap Q Exactive mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) operated in positive ionization mode. The chromatographic conditions and mass spectrometry parameters are described elsewhere (Akıllıoğlu & Lund, 2022). The quantification of each compound was based on internal standard calibration by using a stable isotopically labeled internal standard.

2.7. Analysis of total amino acids

The analysis of total amino acids was performed according to Hildebrand et al. (2020). Briefly, working standard solutions were prepared by proper dilutions of the amino acid standard stock solution (500 µM) with 0.1 M HCl. Concentration levels of amino acids in the calibration solutions were in the range of 0–200 µM. Both diluted hydrolysates samples and working standard solutions were spiked with an internal standard (aminocaproic acid solution, 50 µM) in a ratio of 1:1. Samples and working standard solutions were filtered through a 0.22 µm syringe membrane filter before HPLC analysis. For the analysis, the primary amino acids were derivatized by OPA in the presence of MPA as described previously (Hildebrand et al., 2020; Vinther Schmidt, Olsen, & Mouritsen, 2021). Amino acid composition was analyzed by an ultra-high-performance liquid chromatography–fluorescence detection (UHPLC-FLD) system (Thermo Ultimate 3000 RS, Thermo Scientific, MA, USA) equipped with an Agilent AdvanceBio AAA column (100 mm length × 3.0 mm internal diameter × 2.7 µm particle size; Agilent Technologies, CA, USA) fitted to a guard cartridge. Mobile phase A consisted of 10 mM disodium hydrogen phosphate in 10 mM sodium tetraborate decahydrate (pH 8.2) whereas mobile phase B consisted of a mixture of acetonitrile, methanol and water at a ratio of 45:45:10 (v/v/v). A flow rate of 0.620 mL/min was used with the following gradient program: 0–0.35 min, 2 % B; 0.35–13.4 min, 57 % B; 13.4–13.5 min, 100 % B; 13.5–15.7 min, 100 % B; 15.7–15.8 min, 2 % B; 15.8–18.0 min, 2 % B. Fluorescence detection was carried out by using an excitation wavelength of 340 nm and an emission wavelength of 450 nm. The amino acids in the samples were quantified based on the internal standard calibration method using authentic amino acid calibration standards.

2.8. Preparation of water extract of the samples

Dried samples (0.5 g) were added to 10 mL of Milli-Q water and homogenized using an Ultra-turrax homogenizer (T 25 digital ULTRA-TURRAX®) for 2 min at 22,000 *g*. The homogenates were cooled in the freezer (−20 °C) for 15 min and then centrifuged for 15 min at 22,000 *g* at 0 °C to collect any major matrix components in the bottom pellet and if present, the top fat layer was removed. Aliquots of the clear supernatant were retrieved and stored in the fridge (5 °C) until further use. This water extract was used for the analysis of α-dicarbonyl compounds, HMF, furfural, acrylamide and carbohydrates.

2.9. Quantification of HMF and furfural

HMF and furfural analysis was performed as described in Akıllıoğlu, Chatterton, and Lund (2022). Briefly, aliquots of the water extract (1.5 mL) were added 50 µL Carrez I (7.5 g potassium hexacyanoferrate (II) trihydrate in 50 mL Milli-Q water) and 50 µL Carrez II (15 g zinc sulphate in 50 mL Milli-Q water) solutions. The tubes were vortexed and centrifuged for 10 min at 22,000 *g* at room temperature. The clear supernatant was collected and filtered directly into a UHPLC vial through a

0.22 μm nylon filter. Ten μL of the sample was injected into a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific Inc., Waltham, USA).

The chromatographic separation was performed using a Zorbax Eclipse XDB-C18 column (150 mm length × 4.6 mm internal diameter × 5 μm particle size; Agilent Technologies, CA, USA) maintained at 40 °C with isocratic conditions of 20% acetonitrile and 80% Milli-Q water at a flow rate of 0.500 mL/min. HMF (retention time (RT): 4.197) and furfural (RT: 6.783) were detected at 285 nm and 277 nm, respectively. External standard calibration curves were constructed in the range of 1 and 1000 μg/mL by using standards of HMF and furfural.

2.10. Quantification of α-dicarbonyl compounds

Analysis of α-dicarbonyl compounds was performed according to previous reports (Degen, Hellwig, & Henle, 2012; Kocadağli & Gökmen, 2014) with some modifications described in Akıllıoğlu et al. (2022). Aliquots of the water extract (1 mL) were mixed with ice-cold methanol (1:6) using a vortex mixer and then incubated at −80 °C for 2 h to allow protein precipitation. Afterwards, samples were centrifuged at 22,000 *g* for 15 min at 0 °C. Supernatants (500 μL) were then mixed with 150 μL of phosphate buffer (0.5 M, pH 7.0) and 150 μL of OPD (0.2 % w/v in 18.5 mM DETAPAC). They were then filtered through 0.22 μm nylon filters into HPLC vials and incubated at 37 °C for 2 h in the dark to allow the derivatization of α-dicarbonyl compounds. The soy drink required an alternative treatment compared to the other samples due to the naturally high concentration of calcium. The addition of phosphate buffer caused the formation of opalescence due to calcium phosphate precipitation. To prevent interference with the analysis, after the buffer was added, the soy samples were centrifuged at 22,000 *g* for 15 min at 0 °C to remove the precipitates. The OPD was then added to the clear supernatant, after which the mixture was filtered and incubated under the same conditions as the other samples. The quinoxaline derivatives of glucosone, 3-deoxyglucosone (3-DG), 1-deoxypentosone (1-DP), glyoxal (GO), methylglyoxal (MGO), and diacetyl were determined by LC–MS. Ten μL was injected into the same LC–MS system as described in section 2.6, equipped with an Acquity UPLC BEH Phenyl column (100 mm length × 2.1 internal diameter ×, 1.7 μm particle size; Waters, Taastrup, Denmark) at 55 °C. The chromatographic conditions and mass spectrometry parameters are reported elsewhere (Akıllıoğlu et al., 2022).

Working solutions of glyoxal, methylglyoxal, diacetyl, glucosone and 1-DP in the range of 1–500 ng/mL were derivatized and analyzed as described above to build the external calibration curve of quinoxaline, 2-methylquinoxaline, 2,3-dimethylquinoxaline, glucosone and 1-DP quinoxaline forms, respectively. Quantification of 3-DG was based on an external calibration curve prepared with 2-(2′, 3′, 4′ −trihydroxybutyl) quinoxaline in Milli-Q water at a concentration range of 1–500 ng/mL.

2.11. Quantification of acrylamide

Analysis of acrylamide was performed according to Akillioglu and Gökmen (2014) with modifications. In details, 750 μL of water extract, 750 μL of formic acid (20 mM) containing acrylamide-d_3 (53.3 ng/mL), 50 μL Carrez I and 50 μL of Carrez II were mixed vigorously using a vortex mixer for at least 1 min. Thereafter samples were centrifuged for 15 min at 22,000 *g* at 10 °C. After centrifugation, 1 mL supernatant was filtered through a 0.22 μm nylon syringe filter. The filtrate was then passed through preconditioned MCX cartridges, where the first 8 drops were discarded, and the rest of the extract was collected into HPLC vials. Working concentrations of acrylamide were prepared in the range of 0.5–250 ng/mL. Standard solution (75 μL) were mixed with 75 μL of formic acid (20 mM) containing acrylamide-d_3 (53.3 ng/mL) and 10 μL of Milli-Q water directly in the HPLC vials. Five μL of samples were injected in a Vanquish LC system (Thermo Fisher Scientific Inc., Waltham, USA) equipped with an Acquity UPLC® HSS T3 column (100 mm length × 2.1 mm internal diameter × 1.8 μm particle size; Waters, Taastrup, Denmark) at 40 °C. Chromatographic separation was performed using isocratic conditions (98:2) of mobile phase A consisting of 10 mM formic acid and mobile phase B consisting of 10 mM formic acid in acetonitrile for a total run of 10 min and at a flow rate of 0.250 mL/min. The LC system was directly interfaced with a TSQ Quantis triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Waltham, USA) operated in positive heated electrospray ionization mode with a voltage of 3500 V and using the following interface parameters: ion transfer tube as well as the vaporizer temperature was 275 °C, sheath gas (60 arbitrary units), auxiliary gas (5 arbitrary units), sweep gas (1 arbitrary unit).

Data acquisition was performed by selected reaction monitoring (SRM) mode where acrylamide was monitored by the transitions of *m/z* values 72.08 → 55.04, 44.04 and 27.18 and acrylamide-d3 by *m/z* values of 75.01 → 58.05, 44.04 and 30.20. For the quantification, ions *m/z* 55.04 and *m/z* 58.05 were used for acrylamide and acrylamide-d_3, respectively.

2.12. Quantification of carbohydrates

Carbohydrates were analyzed by mixing 0.5 mL of the water extract with 100 μL of Carrez I and 100 μL Carrez II solutions. The tubes were vortexed vigorously and then centrifuged at 22,000*g* for 15 min at 22 °C. Clear supernatants (100 μL) were collected, added to 900 μL of Milli-Q water and then filtered through 0.22 μm nylon filters directly into HPLC vials. Twenty μL aliquots were injected into an HPLC system coupled with a refractive index detector (RID) equipped with an Aminex HPX-87H Ion Exclusion Column (300 mm length × 7.8 mm internal diameter × 9 μm particle size) at 30 °C. The chromatographic separation was performed under isocratic conditions (100 % of 5 mM sulfuric acid) at a flow rate of 0.500 mL/min for a total run time of 30 min. External standard calibration curves were constructed in the range of 0.1 and 10 mg/mL by using standards of dextrin-15, maltose, sucrose, lactose, glucose, maltotriose, galactose, fructose and xylose for quantification.

2.13. Data analysis

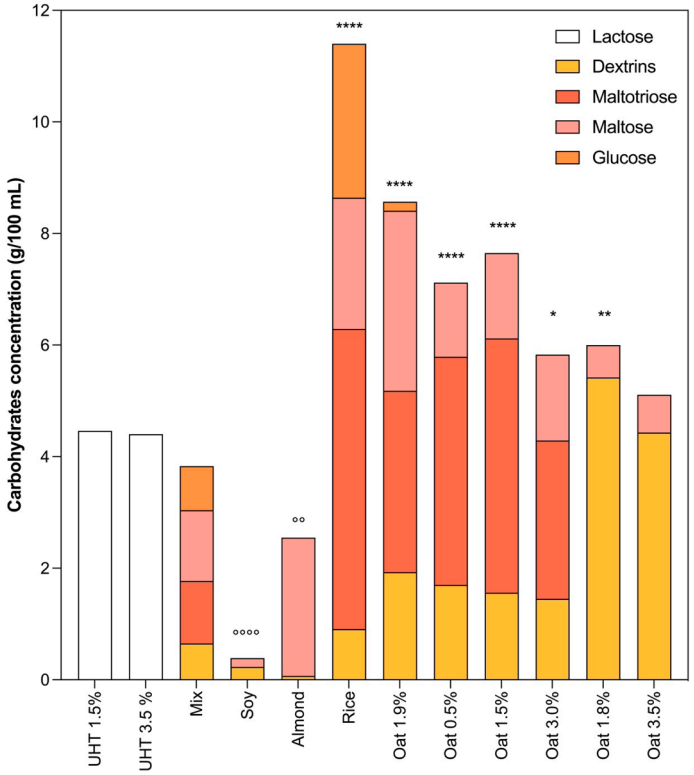
All measurements were performed at least in duplicates. One–way ANOVA was used to determine significant differences between means using Tukey's posthoc test (p values < 0.05). All statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego CA, USA), and data are presented as means ± standard deviation. Multivariate data analysis was performed by using MatLab R2023a (The MathWorks, Inc., Natick, MA, USA) with both in-house written routines and the use of PLS_Toolbox (Eigenvector Research, Inc., Manson, WA, USA).

3. Results and discussion

3.1. Carbohydrates, protein and amino acid composition

When considering PBMAs versus cow's milk, it is important to note the differences in nutritional profiles, which may lead to reduced intake of certain macro- and micronutrients (Zhang, Hughes, & Grafenauer, 2020). In the present study, we have conducted a thorough analysis of the content of carbohydrates and protein among various PBMAs, comparing them to traditional cow's milk. This dual examination is crucial for providing a comprehensive understanding of the nutritional profiles of these milk alternatives. The focus on carbohydrates and in particular on sugar content is principally relevant as excessive sugar intake has been linked to various health concerns (Shkembi & Huppertz, 2023). All oat drinks, except oat 3.5 %, and the rice drink showed a significantly higher concentration of carbohydrates than UHT milk (Fig. 1). Soy and almond drinks contained significantly lower levels of carbohydrates than UHT milk, whereas the mix drink had a similar content of carbohydrates. These data are in accordance with the ones

A



B

	Type of Carbohydrates	g/100 mL	Carbohydrates (Sugar) g/100 mL
UHT 1.5%	Lactose	4.46	4.46 (4.46)
UHT 3.5%	Lactose	4.40	4.40 (4.40)
Mix	Dextrins Maltotriose Maltose Glucose	0.65 1.12 1.27 0.79	3.83 (3.18)
Soy	Dextrins Maltose	0.23 0.16	0.39 (0.16)
Almond	Dextrins Maltose	0.07 2.48	2.55 (2.48)
Rice	Dextrins Maltose Maltotriose Glucose	0.91 2.35 5.38 2.76	11.40 (10.49)
Oat 1.9%	Dextrins Maltotriose Maltose Glucose	1.93 3.25 3.23 0.16	8.57 (6.64)
Oat 0.5%	Dextrins Maltotriose Maltose	1.70 4.09 1.33	7.12 (5.42)
Oat 1.5%	Dextrins Maltrotriose Maltose	1.56 4.56 1.53	7.65 (6.09)
Oat 3.0%	Dextrins Maltrotriose Maltose	1.45 2.84 1,54	5.83 (4.38)
Oat 1.8%	Dextrins Maltose	5.42 0.58	6.00 (0.58)
Oat 3.5%	Dextrins Maltose	4.43 0.68	5.11 (0.68)

Fig. 1. A) Carbohydrates content in milk and plant-based milk alternatives (PBMAs). Data are shown as the mean in g/100 mL. Statistical analysis of the data was performed by Tukey's multiple comparisons test and presented as means ± standard deviation. */°: $p < 0.05$. **/°°: $p < 0.01$. ***/°°°: $p < 0.001$. ****/°°°°: $p < 0.0001$ vs. control (UHT 1.5 %). * is used if values are higher vs. control while °if they are lower. B) Concentrations of identified carbohydrates in milk and PBMAs. Results are expressed as mean in g/100 mL.

observed previously (Angelino et al., 2021), where rice drinks and blended beverages were found to have the highest sugar content, while soy and almond drinks contained the lowest.

As for the type of carbohydrates identified, as expected, only lactose was detected in UHT milk samples, while PBMAs were characterized by the presence of dextrins, maltotriose, maltose, and in some cases, glucose. These differences can be attributed to the fact that starch is the storage polysaccharide in many plants and can be hydrolyzed into oligosaccharides and monosaccharides during production of PBMAs. One significant challenge in the preparation of a stable emulsion during the heat processing of PBMAs is the high concentration of starch. When heat is applied, starch begins to gelatinize, and the liquid attains a gel-like consistency with high viscosity and low consumer acceptability. To maintain fluidity and a beverage-like consistency, enzymatic hydrolysis is applied, which results in degradation of starch and thereby prevents gelatinization during thermal treatment (Deswal, Deora, & Mishra, 2014; Sethi et al., 2016). Another option besides enzymatic treatment is the natural germination of the plant raw material. This process activates enzymes such as proteases and amylases, which can significantly change the composition and functional characteristics of the plant material (Cichońska & Ziarno, 2022), resulting in an increase in free monosaccharides and amino acids that can be utilized to produce desirable aromas during fermentation (Yang & Li, 2010). It has been shown that in oat seeds, germination for 24–144 h reduced starch content from 60% to 21%, and increased the content of free monosaccharides from 5% to 28% (Tian et al., 2010).

Similar to the carbohydrate content, the different PBMAs also varied in protein content and in comparison to the UHT milk samples (Table 2A). The mix drink sample had protein levels comparable to those found in UHT milk, whereas soy drink exhibited significantly higher protein levels ($p < 0.01$) compared to UHT milk. Conversely, all other PBMAs displayed a significantly lower protein content ($p < 0.0001$) when compared to UHT milk. These lower protein levels could be attributed to the diverse nature of plant-based ingredients used in these alternatives, which may not inherently match the protein profile of cow's milk.

In addition, due to the limitation of essential amino acids (EAA), plant-based proteins often exhibit lower nutritional quality compared to animal-derived proteins. Amino acid composition of PBMAs are outlined in Table 2B. UHT cow's milk demonstrated the highest concentration of EAA (UHT 1.5%: 1480 ± 11 mg/100 mL; UHT 3.5%: 1375 ± 8 mg/100 mL). Among PBMAs, mix (1021 ± 8 mg/100 mL) and soy (1119 ± 5 mg/100 mL) drinks exhibited significantly lower EAA but at the same time, they showed the most comparable EAA levels to UHT milk (Table 2B). Apart from these two drinks, all the other analyzed PBMAs clearly contained significantly lower levels of EAA compared to the milk samples (Table 2B). These results are not surprising considering the protein content of the samples. In this context, we calculated the ratio between EAA and protein content. For most PBMAs, the lower EAA quantity in PBMAs is a consequence of overall lower protein content. Notably, in the case of rice and oat 1.9% drinks, the ratio of EAA to protein is even lower compared to cow's milk. This finding could have implications for the nutritional quality and amino acid balance in these two types of drinks.

Emphasizing the importance of specific EAAs on health, lysine supports the growth and repair of tissues, while leucine plays a key role in promoting muscle protein synthesis (Millward, 2012; Tomé & Bos, 2007). Another critical EAA is methionine, initiating the production of all eukaryotic proteins (Brosnan & ME, 2006). In this regard, in comparison with UHT cow's milk, comparable levels of lysine and leucine were found in soy (Lys: 217.1 ± 6.3 mg/100 mL; Leu: 285.2 ± 7.9 mg/100 mL) and mix (Lys: 181.6 ± 7.5 mg/100 mL; Leu: 249.3 ± 12.9 mg/100 mL) drinks, while in rice drink significantly lower contents were

Table 2
(A) Essential amino acid (EAA) content, protein content and the ratio between EAA and protein content in milk and plant-based milk alternatives (PBMAs). (B) Amino acid content (mg/100 mL) of milk and PBMAs. Asx and Glx represent the sum of Asp and Asn and Glu and Gln, respectively. The amount of Trp and Cys could not be determined as they were degraded during the hydrolysis step with acid. The asterisk in the table (*) refers to the statistical differences of $p < 0.0001$ between UHT milk 1.5 % and PBMAs.

	COW'S MILK		PLANT-BASED MILK ALTERNATIVES (PBMAs)									
	UHT milk		*Brand A*					*Brand B*			*Brand C*	
	UHT 1.5%	UHT 3.5%	Mix	Soy	Almond	Rice	Oat 1.9%	Oat 0.5%	Oat 1.5%	Oat 3.0%	Oat 1.8%	Oat 3.5%
Total EAA (mg/100 mL)	1407.9	1374.5	1021.2*	1190.7*	114.3*	10.7*	48.8*	319.8*	298.5*	390.5*	208.6*	209.1*
Protein content (mg/100 mL)	3482.8	3426.7	3479.0	3718.0	565.9*	239.7*	566.5*	1326.3*	1294.7*	1572.1*	939.4*	871.6*
Ratio	0.404	0.401	0.294	0.320	0.202	0.045	0.086	0.241	0.231	0.248	0.222	0.240

		COW'S MILK		PLANT-BASED MILK ALTERNATIVES (PBMAs)									
		UHT milk		*Brand A*					*Brand B*			*Brand C*	
		UHT 1.5%	UHT 3.5%	Mix	Soy	Almond	Rice	Oat 1.9%	Oat 0.5%	Oat 1.5%	Oat 3.0%	Oat 1.8%	Oat 3.5%
Essential AA	**His**	91.9 ± 6.3	91.4 ± 4.2	75.8 ± 5.8	88.6 ± 4.8	10.6 ± 1.4	1.4 ± 0.9	4.0 ± 1.8	24.0 ± 2.3	22.7 ± 2.8	29.8 ± 4.6	14.6 ± 1.3	14.9 ± 2.1
	Ile	161.9 ± 10.4	163.4 ± 8.6	126.4 ± 7.0	148.1 ± 3.3	14.4 ± 1.2	0.9 ± 0.6	4.3 ± 0.3	36.4 ± 2.1	34.2 ± 3.2	44.9 ± 2.2	24.6 ± 1.8	24.5 ± 1.1
	Leu	351.6 ± 22.7	339.1 ± 14.2	249.3 ± 12.9	285.2 ± 7.9	32.9 ± 2.1	4.2 ± 1.0	14.1 ± 0.4	83.7 ± 3.6	78.2 ± 7.4	104.4 ± 2.5	53.3 ± 3.6	53.8 ± 3.5
	Lys	273.1 ± 20.2	252.8 ± 12.9	181.6 ± 7.5	217.1 ± 6.3	6.4 ± 0.4	1.3 ± 0.4	6.6 ± 0.5	37.6 ± 1.6	34.8 ± 3.3	42.2 ± 1.1	36.1 ± 1.8	36.2 ± 2.3
	Met	83.5 ± 4.9	81.8 ± 5.8	30.6 ± 2.3	35.9 ± 4.7	1.8 ± 0.3	<LOD	<LOD	13.0 ± 1.8	12.1 ± 1.6	16.3 ± 0.7	4.2 ± 0.6	4.3 ± 0.6
	Phe	155.8 ± 9.4	151.5 ± 7.9	148.6 ± 10.3	176.6 ± 5.9	21.8 ± 1.9	0.7 ± 0.6	5.7 ± 0.6	53.3 ± 2.4	49.5 ± 4.9	65.3 ± 2.6	31.2 ± 2.1	30.5 ± 1.2
	Thre	99.3 ± 5.4	98.5 ± 4.6	78.1 ± 5.1	94.0 ± 4.1	8.7 ± 0.7	0.9 ± 0.4	5.7 ± 0.6	24.0 ± 1.9	22.1 ± 2.3	27.9 ± 2.0	16.2 ± 1.1	16.1 ± 0.7
	Val	190.8 ± 12.1	195.9 ± 9.7	130.9 ± 11.2	145.2 ± 5.4	17.7 ± 2.3	2.9 ± 1.5	8.5 ± 0.5	47.8 ± 3.4	45.0 ± 5.8	59.8 ± 4.0	28.4 ± 2.4	28.8 ± 2.3
Non-essential AA	**Ala**	111.6 ± 7.7	107.2 ± 5.6	130.0 ± 5.4	145.2 ± 3.9	20.4 ± 1.3	4.2 ± 0.7	12.4 ± 0.2	46.5 ± 2.0	43.7 ± 3.6	56.7 ± 1.5	29.8 ± 2.1	29.5 ± 1.8
	Arg	112.2 ± 6.8	104.4 ± 8.0	237.9 ± 13.1	279.2 ± 12.3	45.4 ± 3.2	4.3 ± 0.9	12.3 ± 0.9	72.3 ± 2.1	66.1 ± 4.7	87.1 ± 1.7	48.9 ± 2.6	48.0 ± 2.5
	Asx	246.1 ± 15.9	263.7 ± 17.6	342.9 ± 24.3	403.3 ± 23.1	46.0 ± 3.1	6.5 ± 2.4	24.0 ± 2.3	83.7 ± 4.8	77.7 ± 6.2	101.3 ± 3.3	71.6 ± 6.4	71.0 ± 1.0
	Glx	781.4 ± 51.1	754.8 ± 43.5	623.3 ± 37.0	718.1 ± 44.0	125.3 ± 9.1	13.0 ± 2.6	62.3 ± 3.6	249.8 ± 10.4	234.8 ± 19.6	313.3 ± 8.6	131.9 ± 8.7	131.6 ± 6.3
	Gly	66.7 ± 4.0	62.4 ± 3.6	142.7 ± 6.9	152.2 ± 5.9	28.2 ± 1.6	4.1 ± 0.3	19.3 ± 0.1	53.9 ± 2.8	50.7 ± 3.7	63.8 ± 1.8	34.2 ± 1.9	33.2 ± 2.8
	Ser	151.4 ± 18.8	137.8 ± 15.2	125.3 ± 9.5	167.5 ± 21.5	14.4 ± 1.3	3.0 ± 0.6	15.2 ± 1.1	41.9 ± 1.1	39.6 ± 4.7	47.1 ± 2.1	30.0 ± 1.8	27.8 ± 2.2
	Tyr	165.3 ± 7.8	160.8 ± 7.1	104.0 ± 8.2	127.6 ± 4.1	11.5 ± 1.1	1.4 ± 0.6	7.3 ± 0.5	35.2 ± 1.4	33.2 ± 3.0	44.1 ± 1.7	21.5 ± 1.6	21.4 ± 1.6

found (Lys: 1.3 ± 0.4 mg/100 mL; Leu: 4.2 ± 1.0 mg/100 mL). Additionally, methionine contents were notably lower in all PBMAs when compared with UHT milk, showing considerable variation between PBMA drinks. These results align with those observed by Sousa and Bolanz (2017) highlighting some critical issues relating to the nutritional content of PBMAs.

3.2. Maillard reaction products and amino acid cross-links

Furosine is the acid derivative of Amadori compounds and a marker of early stages of the Maillard reaction. Our findings reveal notable concentrations of furosine in UHT milk (UHT 1.5%: 4270 μg/100 mL; UHT 3.5%: 4077 μg/100 mL). In contrast, the furosine content in all tested PBMA samples, except for the mix drink (3668 μg/100 mL), was significantly lower (Fig. 2A). However, expressing the concentrations of MRPs per gram of protein may be more beneficial for comparing the samples to accommodate variations in protein content among them. This conversion highlights how much the proteins in the samples were modified by glycation. Oat 1.9% was found to be the PBMA with the highest furosine concentration (3116 μg/g protein). UHT 1.5%, UHT 3.5%, mixed, and rice drinks exhibited comparable levels of furosine. Notably, all other PBMAs demonstrated significantly lower concentrations of furosine per gram of protein. As the Maillard reaction progresses, Amadori compounds can be further degraded into α-dicarbonyl compounds or oxidize to form AGEs. Therefore, the lower concentration of furosine in PBMAs may indicate a more advanced stage of glycation.

CML and CEL are the two most identified AGEs in different food items and are formed by the reaction between lysine and GO or MGO, respectively. UHT milk and the mix drinks were those with the highest CML content (UHT 1.5%: 324 μg/100 mL; UHT 3.5%: 299 μg/100 mL, mix: 298 μg/100 mL) and no statistical differences were observed among them. On the contrary, all other PBMAs showed significantly lower concentrations of CML in comparison with UHT milk (Fig. 2B). Besides the mix drink, oat beverages with concentrations of fat of 1.8% and 3.5% had a higher content of CML as compared to the other PBMAs. Notably, both these two oat drinks originate from Brand C. The similarity in their CML levels suggests that the elevated CML content may be attributed to the heat processing employed during their production. Surprisingly, a notable disparity in CML concentration was observed between oat 0.5% (47 μg/100 mL) and oat 3.0% (141 μg/100 mL) samples, both

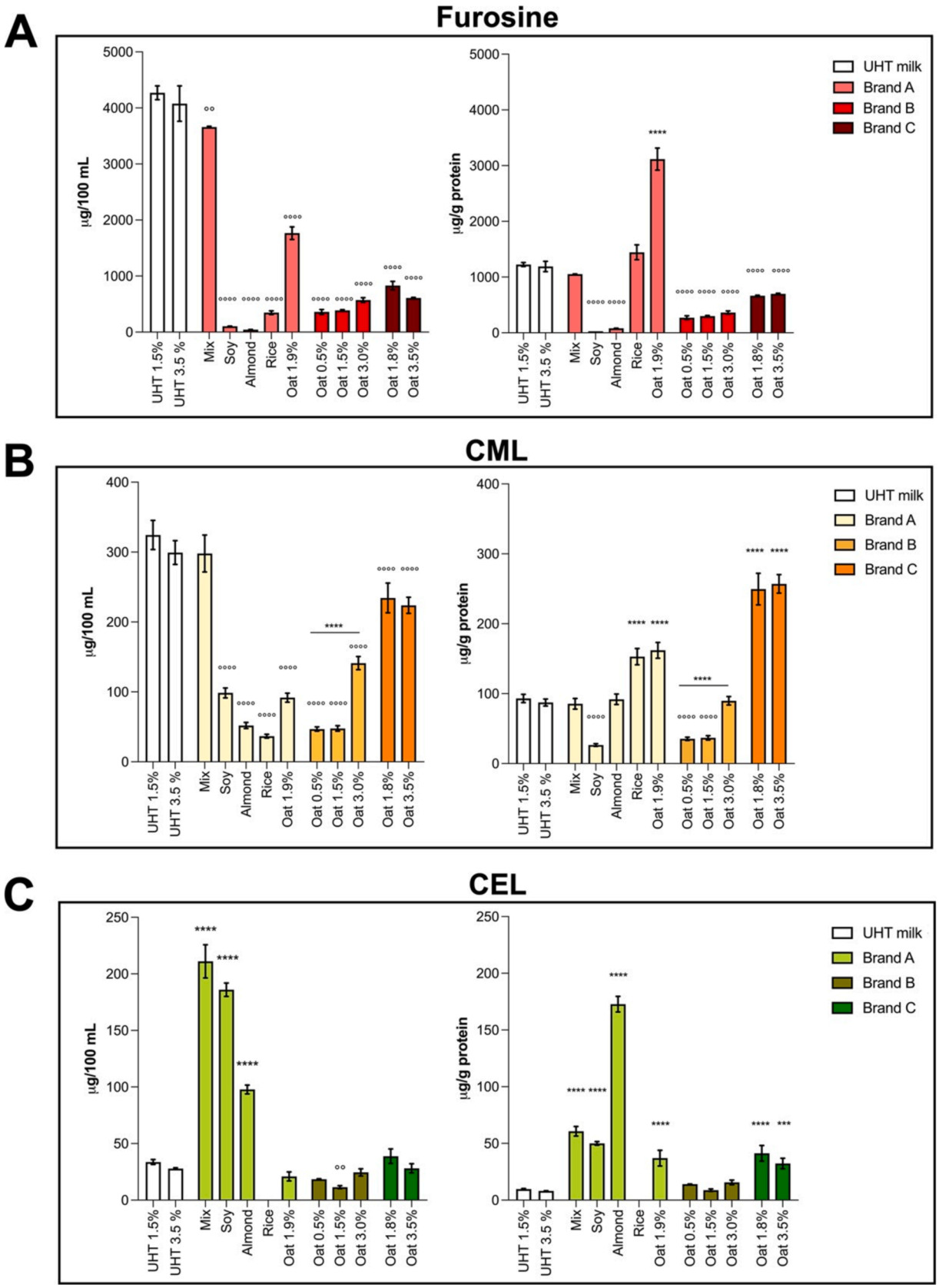


Fig. 2. Concentration (µg/100 mL on the right and µg/g protein on the left) of furosine (A), AGEs (CML (B), CEL (C). GO-Hs (D), MG-Hs (E)) and amino-acid crosslinks (LAN (F) and LAL (G)) in milk and plant-based milk alternatives (PBMAs). Statistical analysis of the data was performed by Tukey's multiple comparisons test and presented as means ± standard deviation. */°: $p < 0.05$. **/°°: $p < 0.01$. ***/°°°: $p < 0.001$. ****/°°°°: $p < 0.0001$ vs. control (UHT 1.5 %). * is used if values are higher vs. control while °if they are lower.

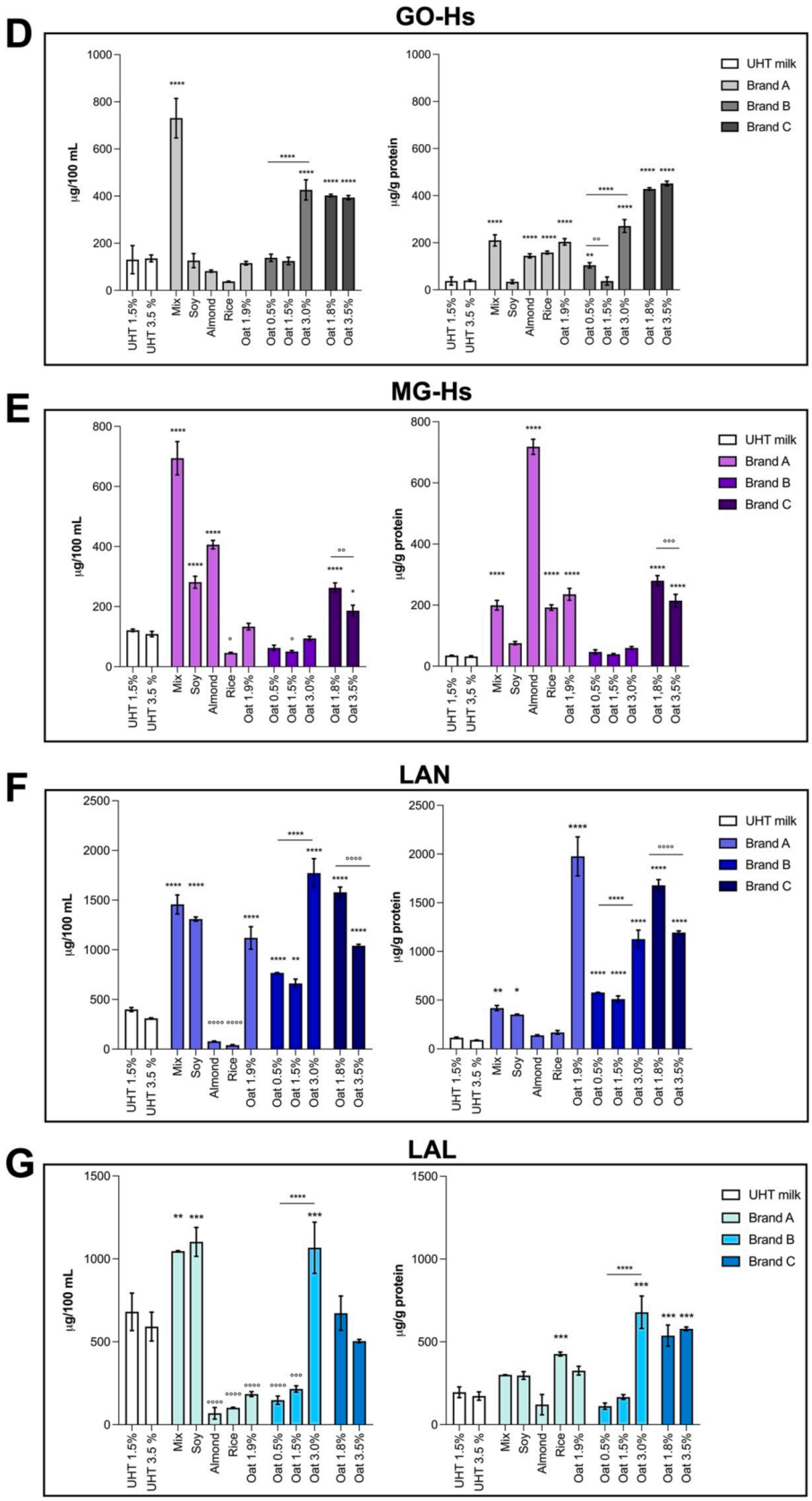


Fig. 2. (*continued*).

originating from Brand B. As these two beverages belong to the same brand, they should most probably share similar processing conditions and similar protein and carbohydrate levels, discrepancy in CML content could be due to variations in fat content. The non-enzymatic oxidation of polyunsaturated fatty acids can produce peroxide intermediates, which may further degrade into diverse oxidative products, including glyoxal,

thus contributing to CML formation (Vistoli et al., 2013). The role of lipid oxidation in comparison to carbohydrate chemistry in the formation of glyoxal and CML is unclear as there are contradictory findings in different food matrices (Eggen, Merboth, Neukirchner, & Glomb, 2022; Fu et al., 1996; Han et al., 2013). Further investigations, particularly in the context of plant-based foods, are necessary to provide clarity on this matter.

When data is analyzed in relation to their protein content (μg/g protein), the CML content in mix (86 μg/g protein), almond (92 μg/g protein), and oat 3.0% (90 μg/g protein) drinks was comparable to UHT milk (UHT 1.5%: 93 μg/g protein; UHT 3.5%: 87 μg/g protein). The highest concentrations of CML were observed in oat 1.8% (239 μg/g protein) and oat 3.5% (257 μg/g protein), followed by rice (153 μg/g protein) and oat 1.9% (162 μg/g protein). The lowest CML content was observed in soy (27 μg/g protein), oat 0.5% (35 μg/g protein), and oat 1.5% (37 μg/g protein). The formation of CML takes place through two pathways: i) the Hodge pathway, which includes the oxidation of the Amadori compound, or ii) the Namiki pathway, which involves the direct reaction of glyoxal (GO) with the ε-amino group of lysine. The higher variability in CML formation in PBMAs may be attributed to differences in the concentration of GO in the samples, which will be discussed in section 3.3.

Compared to UHT milk, CEL levels were higher in mix (211 μg/100 mL), soy (186 μg/100 mL), and almond (98 μg/100 mL) drinks, lower in oat 1.5% drink (11 μg/100 mL), and undetectable in rice drink (Fig. 2C). When expressed in μg/g protein, almond drinks had the highest CEL content (173 μg/g protein). The variation in CEL formation could be attributed to differences in the concentrations of methylglyoxal (MGO) formed in the samples.

Arginine-derived AGEs include MGO- and GO-derived hydroimidazolones (MG-Hs and GO-Hs), which exist as three different isomers (H1, H2, H3), and have been quantified as MG-H3 equivalents and GO-H3 equivalents corresponding to the sum of MGO- or GO-isomers as described previously (Akıllıoğlu & Lund, 2022). High concentrations of GO-Hs were found in mix (730 μg/100 mL), oat 3.0% (426 μg/100 mL), oat 1.8% (402 μg/100 mL) and oat 3.5% (393 μg/100 mL) (Fig. 2D), similar to what was observed for CML concentrations. In particular, mix, oat 1.8% and oat 3.5% drinks were found to contain significantly higher levels of CML as well as GO-Hs among the PBMAs.

MG-Hs have demonstrated to be another important AGE formed in food (Akıllıoğlu & Lund, 2022; Antonova et al., 2019). The content of MG-Hs was found to be higher in mix (694 μg/100 mL), soy (281 μg/mL), almond (406 μg/100 mL), oat 1.8% (263 μg/100 mL) and oat 3.5%, (187 μg/100 mL) drinks as compared to UHT milk, while oat 1.5% (50 μg/100 mL) and rice (46 μg/mL) contained significantly lower levels (Fig. 2E). Mix, soy and almond drinks contained a significant concentration of both MG-Hs and CEL.

MGO- and GO-Lys dimers (MOLD and GOLD), which are cross-links formed between two Lys residues, were below the limit of detection (data not shown). These results are in agreement with previous studies showing that MOLD and GOLD have only been detected in food at minor levels (Akıllıoğlu & Lund, 2022).

Finally, LAN and LAL are amino-acid cross-links formed during heat treatment by the reaction of cysteine and lysine with dehydroalanine, respectively. The content of LAN was found to be higher in all tested PBMAs in comparison to UHT milk, except for rice and almond where it was lower (rice: 40 μg/100 mL; almond: 78 μg/100 mL) (Fig. 2F). The content of LAL was higher for mix (1048 μg/100 mL), soy (1102 μg/100 mL) and oat 3.0% (1067 μg/100 mL) drinks than UHT milk but was found to be significantly lower in almond (68 μg/100 mL), rice (102 μg/100 mL), oat 1.9% (184 mg/100 mL), oat 0.5% (147 μg/100 mL), and oat 1.5% (215 μg/mL) (Fig. 2G). The disparities in LAN and LAL levels may be attributed to the manufacturing methods employed. In the production of soy protein, alkali extraction is a prevalent technique. Alkali processes elevate the formation of dehydroalanine by facilitating the beta-elimination of serine and cysteine residues, leading to an increase in LAL and LAN formation (Friedman, 1999). Thus, it is plausible that an alkaline extraction method during the production of protein isolate or protein concentrate contributed to elevated levels of LAL and LAN (Boschin, D'Agostina, Rinaldi, & Arnoldi, 2003; Friedman, 1999; Rombouts, Lambrecht, Carpentier, & Delcour, 2016). Proteins derived from oat can also be extracted under alkaline conditions, as indicated by Deleu, Lambrecht, Van de Vondel, and Delcour (2019). Similarly, it has been noted that the alkali extraction process can lead to the formation of cross-linked amino acids. These alterations result in a reduction in product quality due to lowered protein digestibility and loss of essential amino acids (ALjahdali & Carbonero, 2019).

3.3. Content of α-dicarbonyl compounds

In general, in comparison to UHT milk, higher concentrations of α-dicarbonyl compounds were detected in PBMAs (Fig. 3) as hypothesized. This is mainly due to the higher concentration of carbohydrates in the origin of plants (Table 1). α-Dicarbonyl compounds can be formed from the degradation of Amadori products through the Maillard reaction pathway or can also be formed directly from the degradation of carbohydrates (Zheng, Ou, & Ou, 2019). GO was found higher in mix (147 μg/100 mL), rice (246 μg/100 mL), oat 1.9% (125 μg/100 mL), oat 1.5% (135 μg/100 mL), oat 3.0% (439 μg/100 mL), oat 1.8% (236 μg/100 mL), and oat 3.5% (380 μg/100 mL) drinks (Fig. 3A), compared to UHT milk. This result is in line with the AGE results. Higher concentrations of GO in these samples correspond to the higher CML and GO-H levels observed (Fig. 2). Soy, oat0.5 % and oat 1.5% together with almond drinks were found to have lower concentrations of GO, corresponding to lower CML and GO-H concentrations in these samples.

A similar trend was observed for MGO (Fig. 3B), with mainly oat 1.8%, oat 3.5% and oat 1.9% having the highest concentrations of MGO amongst the samples analyzed. In these samples, higher concentrations of CEL and MG-H were also observed. Diacetyl was only detected in soy (9 μg/100 mL), oat 1.8% (73 μg/100 mL) and oat 3.5% (57 μg/100 mL) drinks (Fig. 3C). Interestingly, 3-deoxyglucosone (3-DG) was detected in significantly higher concentrations in mix (4252 μg/100 mL), rice (12169 μg/100 mL), oat 1.9% (27672 μg/100 mL), oat 1.8% (4964 μg/100 mL), and oat 3.5% (5647 μg/100 mL) drinks as compared to UHT milk (Fig. 3D). Notably, among the α-dicarbonyl compounds, 3-DG was identified with the highest concentrations in the PBMAs. The distinction between the C6 dicarbonyls (3-DG) and the smaller dicarbonyls (GO and MGO) becomes apparent in our study, with the former detected at higher concentrations. This divergence can be elucidated by the differing reactivity of these compounds. The more reactive nature of GO and MGO implies that they are prone to undergo more extensive reactions during the thermal treatment, leading to reduced concentrations (and thus increased formation of their derived AGEs as also observed in the present study). On the contrary, 3-DG may accumulate in the product before undergoing further reactions.

In this investigation, the exploration of α-dicarbonyl compounds in various beverages aligns with previous studies that have assessed concentrations in diverse consumables such as coffee, soft drinks, and fermented products like beer, wine, and soy sauce (Degen et al., 2012). A recent contribution (Maasen et al., 2021) developed a food composition database for α-dicarbonyl compounds highlighting that the lowest total amount of α-dicarbonyl compounds in terms of GO, MGO and 3-DG, were found in tea, dairy and light soft drinks (<10 mg/kg). The low concentrations of MGO, GO, and 3-DG in our milk and soy samples are consistent with findings in existing literature (Hellwig, Degen, & Henle, 2010; Maasen et al., 2021).

According to the literature, alcoholic beverages tend to exhibit higher α-dicarbonyl compound content due to the fermentation step in their manufacturing process. Within the plant-based food segment, fermentation is a frequently employed technique to enhance sensory characteristics, nutritional value, texture, and microbial safety of PBMAs (Tangyu et al., 2019). Consequently, an additional plausible explanation

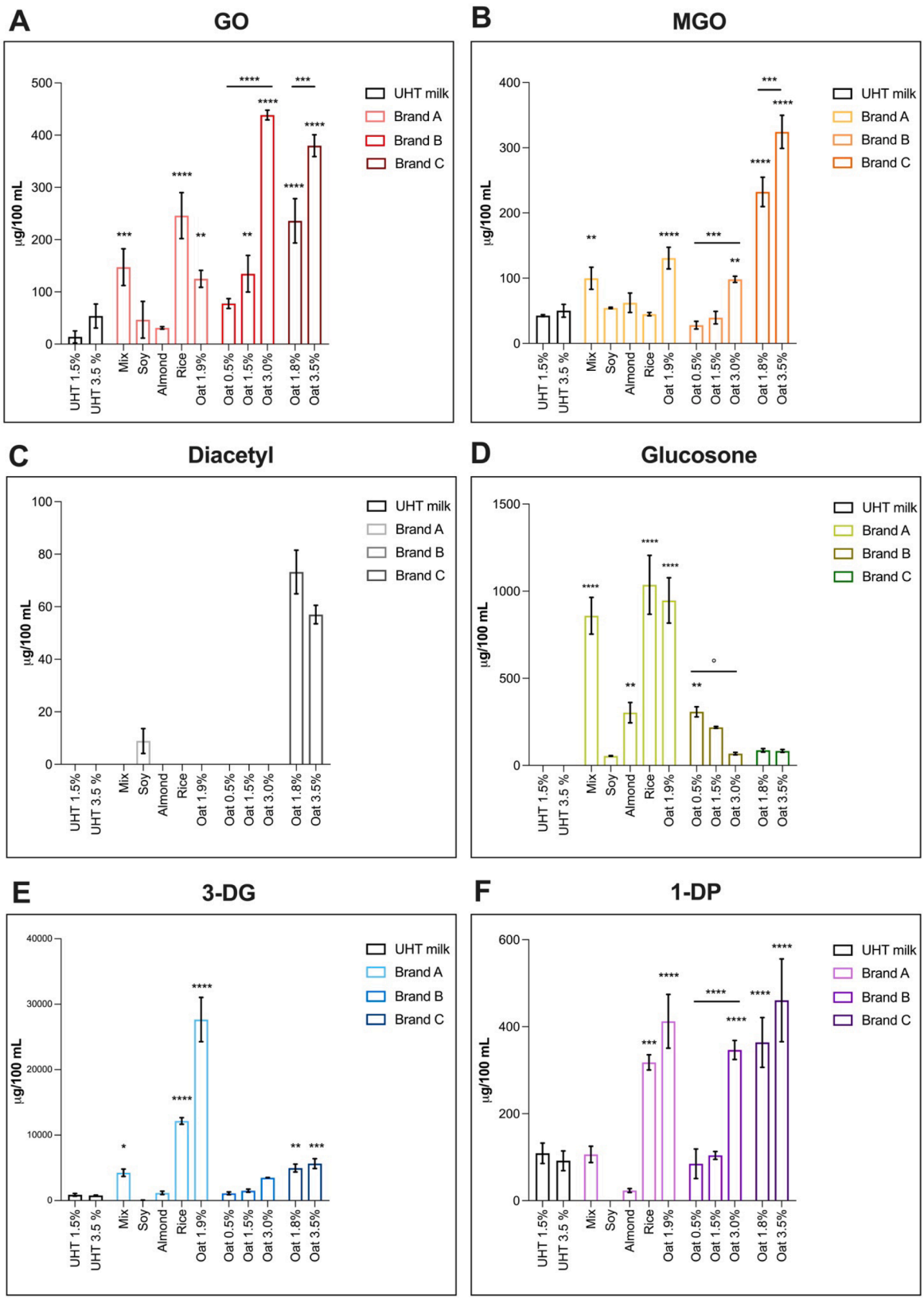


Fig. 3. Concentration (µg/100 mL) of α-dicarbonyl compounds (GO (A), MGO (B), diacetyl (C), 3-DG (D), glucosone (E), 1-DP (F)) in milk and plant-based milk alternatives (PBMAs). Statistical analysis of the data was performed by Tukey's multiple comparisons test and presented as means ± standard deviation. */°: $p < 0.05$. **/°°: $p < 0.01$. ***/°°°: $p < 0.001$. ****/°°°°: $p < 0.0001$ vs. control (UHT 1.5 %). * is used if values are higher vs. control while °if they are lower.

for elevated 3-DG levels could be due to the fermentation process, routinely applied to the raw materials utilized in the production of PBMAs. This speculative link underscores the potential impact of processing techniques on the composition of α-dicarbonyl compounds in PBMAs and warrants further exploration in future studies.

Moreover, the high concentration of 3-DG in the oat 1.9% drink is noteworthy and may be related to the addition of inulin to this drink (Table 1) as oligosaccharides have been demonstrated to break down during thermal treatment to generate 3-DG (Nomi et al., 2022; Zhang et al., 2019, Zhang, Poojary, et al., 2020). This hypothesis emphasizes the importance of considering additives and their thermal behaviors in understanding the composition and dynamics of α-dicarbonyl compounds in PBMAs.

Reports on glucosone in various foods is limited and have so far primarily focused on high-fructose corn syrups, carbonated soft drinks and honeys (Ruiz-Matute, Castro Vazquez, Hernández-Hernández, Sanz, & Martínez-Castro, 2015). Additionally, glucosone has been detected in infant formula (Akıllıoğlu et al., 2022). In our study, glucosone was detected in significantly higher concentrations in mix (859 μg/100 mL), almond (311 μg/100 mL), rice (1037 μg/100 mL), oat 1.9% (947 μg/100 mL), and oat 0.5% (308 μg/100 mL) drinks compared to the other drinks and was not detected in UHT milk (Fig. 3E). Finally, 1-DP was identified in significantly higher concentrations in oat 1.9% (412 μg/100 mL), oat 3.0% (346 μg/100 mL), oat 1.8% (364 μg/100 mL), oat 3.5% (461 μg/100 mL), and rice (318 μg/100 mL) drinks than in UHT milk (Fig. 3F). In summary, our study highlights the presence of α-dicarbonyl compounds in various PBMAs, with notable differences in concentrations among different products. Factors such as additives, processing techniques, and ingredient compositions likely influence the formation of these compounds. The findings underscore the importance of considering processing methods and additives in understanding the composition of α-dicarbonyl compounds in PBMAs. Further research is needed to elucidate the mechanisms driving their formation, their reactions and potential implications for consumer health.

3.4. HMF, furfural and acrylamide analyses

The mechanisms for the formation of HMF and furfural are either through the Maillard reaction or caramelization via sugar condensation (Capuano & Fogliano, 2011). HMF is generated from hexose degradation while furfural is primarily formed from pentose degradation or can also result from the thermal degradation of HMF (Kroh, 1994). HMF and furfural have been identified as indicators of the severity of heat treatment or length of storage in several beverages including fruit juices (Gökmen & Acar, 1999), UHT milk (Ferrer et al., 2000; Morales, Romero, & Jimenez-Pérez, 1992; Morales, Romero, & Jiménez-Pérez, 1997), wine and other alcoholic beverages, vinegars and coffee (Teixidó et al., 2006; 2011). In the present study, furfural was detected only in almond drinks (1.00 μg/100 mL), whereas HMF was detected in almond, mix, oat 1.9% and oat 3.5%, exhibiting notably elevated concentrations in oat 1.9% (28.9 μg/100 ml) and in almond (19.6 μg/100 mL) drinks. The high levels of HMF observed in oat 1.9% could be explained by the fact that HMF is formed from the dehydration of 3-deoxyosones (Hodge, 1953), as a notably high concentration of 3-DG was found in oat 1.9%. However, the concentration of HMF is relatively low compared to 3-DG, which is a consequence of low reaction rate of dehydration in high moisture environment. Low pH, low water activity and high temperatures are key factors for HMF and furfural generation (Ameur, Trystram, & Birlouez-Aragon, 2006; Gökmen, Açar, Köksel, & Acar, 2007; Purlis, 2010). The higher concentration of these two compounds in almond drink samples is likely due to the fact that this type of PBMA was made from roasted almonds. Furans, such as furfural and HMF have already been detected in toasted almonds (Agila & Barringer, 2012; Valdés et al., 2015; Xiao et al., 2014) as well as other roasted food such as coffee (Capuano & Fogliano, 2011). Besides HMF and furfural, acrylamide was also detected in almond drink (1.08 μg/100 mL), oat 1.8% (0.64 μg/100 mL), oat 1.9% (2.93 μg/100 mL), and oat 3.5% (1.08 μg/100 mL) drinks. Acrylamide is formed by the reaction between asparagine and reducing sugars (Yaylayan & Stadler, 2005; Keramat, LeBail, Prost, & Soltanizadeh, 2011; Granvogl & Schieberle, 2006) and can be found in a wide range of heat-processed foods, such as fried potatoes, baked goods, and roasted food (Michalak, Gujska, Czarnowska, & Nowak, 2014). Acrylamide formation is not usually observed at elevated levels in food containing high content of water such as PBMAs or milk. Hence, the presence of acrylamide in our samples was unexpected, as the formation of this compound primarily occurs in conditions of intense heat treatment and low moisture environment. Therefore, the presence of acrylamide, as well HMF and furfural, is more likely to be due to treatments, such as roasting, applied to the raw material used for the production of PBMAs.

The European Food Safety Agency (EFSA) identifies the presence of acrylamide in foods as a public health concern (EFSA, 2015). The Commission Regulation (EU) 2017/2158, established mitigation strategies and benchmark levels to reduce acrylamide in foods but mainly focused on potatoes, cereal products, coffee, and coffee substitutes (EU., 2017). In 2019, EC recommended expansion of monitoring of the presence of acrylamide in other food items, including roasted nuts (European Commission, 2019). (Mesías, Palenzuela, Olombrada, Holgado, & Morales, 2024)proposed a reference value of 470 μg/kg for almonds, to guide manufacturers in their control and mitigation efforts. However, there are no reports of acrylamide in almond drinks or other PBMAs up to date. Considering the reference acrylamide concentrations in various food, the levels obtained in the PBMA samples analyzed in the current study are very low.

3.5. Principal component analysis (PCA)

A PCA analysis (Wold, Esbensen, & Geladi, 1987) of all data was performed in order to further interpret differences among our samples. The score plot shows that UHT milk, soy, and mix drinks were notably different as compared to all the other PBMAs as these were separated along the first principal component (PC1), which explains more than 56 % of the sample variation (Fig. 4A, left). In the loadings plot, it can be seen, that this variation is linked to the higher protein or amino acid content observed in UHT milk, soy, and mixed drinks compared to the remaining samples. The majority of amino acids appear to exhibit a significant correlation among themselves, which is expected as they collectively contribute to the total protein content. On the other hand, sugar content, α-dicarbonyls, AGEs and cross-links are the main responsible factors for the differences among the samples along PC2 that explains 15 % of the variation. Some α-dicarbonyls, specifically MGO, GO, and diacetyl, demonstrate remarkable correlations among themselves. Additionally, these α-dicarbonyls exhibit correlations with AGEs such as GO-Hs and MG-Hs. This interdependence demonstrates the shared biochemical pathways or reactions involving these compounds. A discernible inverse correlation exists between sugar content and certain α-dicarbonyls (MGO, GO, diacetyl). In instances where these α-dicarbonyls are elevated, sugar content tends to be lower and vice versa. This is because α-dicarbonyls are formed from the sugars.

Further considerations regarding the samples entail assessing their degree of similarity (Fig. 4A, left). For example, the two UHT milk samples, despite disparities in fat content, exhibit notable resemblance across all measured variables, and are therefore placed close to each other in the plot. Contrastingly, the oat samples do not uniformly demonstrate a high degree of similarity. In order to better explain the variations among the oat samples, only oat drinks were analyzed in a second PCA plot (Fig. 4B). Specifically, while the two oat drinks from Brand C (oat 1.8 % and 3.5 %) exhibit considerable resemblance, such uniformity is not consistently observed among those from Brand B (Fig. 4B). Within the oat drink category, the primary determinant in distinguishing between samples are the sugar and protein content. Investigation into Brand B products reveals “non-standardized

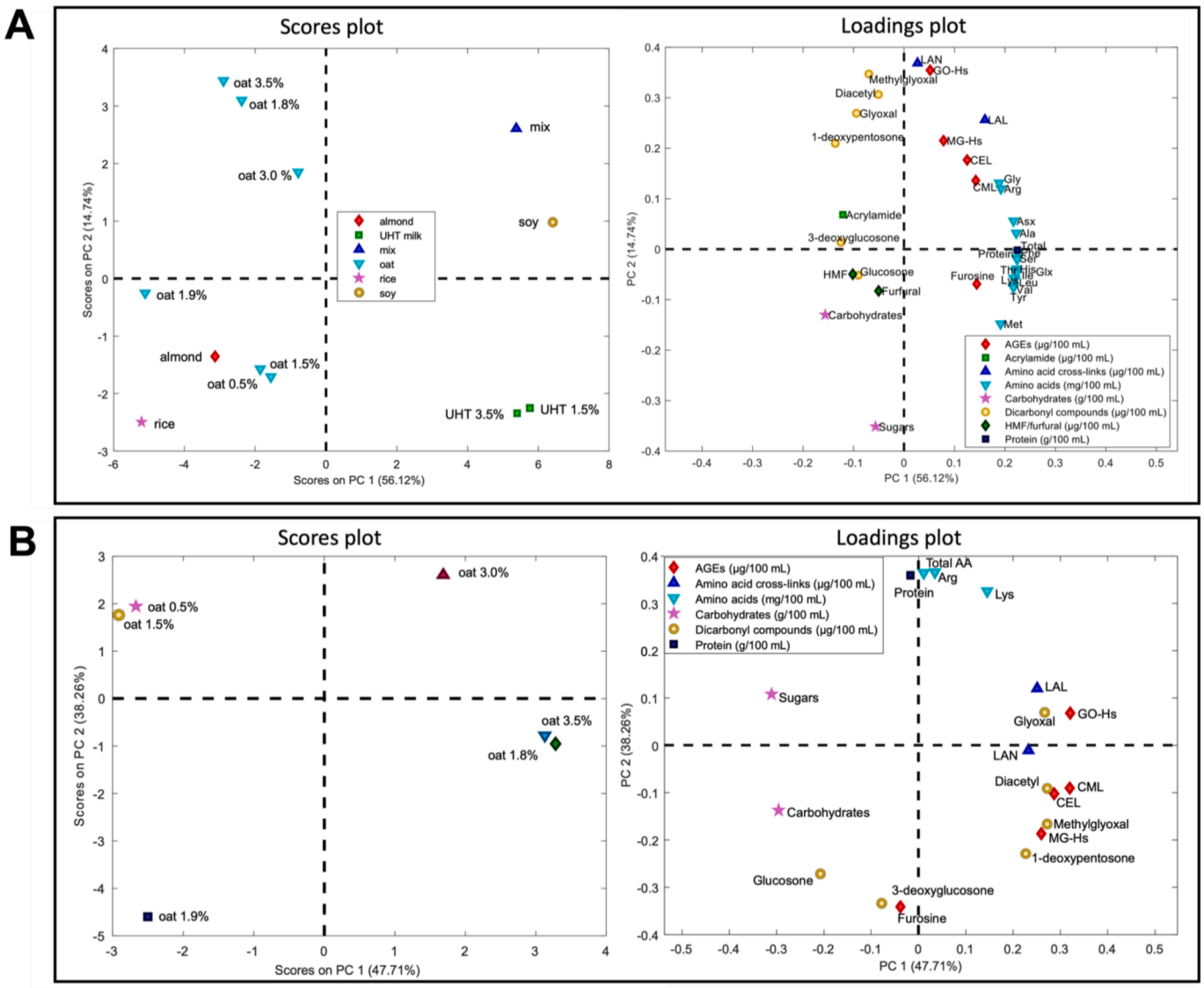


Fig. 4. Principal component analysis (PCA) performed with the milk and plant-based milk alternatives (PBMAs). Scores plots on the left and loadings plot on the right. A) Comprehensive PCA plots encompassing all beverages examined in the study; B) PCA plots focusing exclusively on oat.

compositions across markets" (as the website of Brand B declares), indicating potential variability in their manufacturing processes or raw ingredients. The observed inverse correlation between sugar/carbohydrates and small α-dicarbonyls persists, suggesting a potential transformation from the former to the latter. Particularly, a correlation between fat content and PC2 can be noted implicating a potential contribution to the formation of α-dicarbonyls and, consequently, AGEs (Fig. 4B). In the case of oat drinks, pronounced correlations are evident between MG-Hs and MGO, as well as between GO-Hs and GO.

4. Conclusions

In conclusion, our comprehensive analysis of PBMAs compared to traditional cow's milk not only unveiled substantial variations in nutritional profiles, particularly in terms of carbohydrate, protein and amino acid composition, but also highlighted distinct differences in the content of MRPs. Oat and rice drinks exhibited higher carbohydrate concentrations, while soy and almond drinks showed lower levels compared to UHT milk. Not surprisingly, the type of carbohydrates identified in PBMAs differed from lactose found in cow's milk, attributing variations to starch hydrolysis during the production of PBMAs. The protein content in PBMAs varied, with soy drinks demonstrating a significantly higher content, while the other alternatives showed a lower content compared to UHT milk. A lower content of total EAAs was found for most PBMAs, impacting their nutritional quality and amino acid balance with noteworthy variations in lysine, leucine, and methionine levels.

This is the first study where a comprehensive evaluation was performed on the MRPs in PBMAs. Quantification of MRPs and amino acid cross-links uncovered large differences among UHT milk and PBMAs. Furosine concentrations per mL of drink were higher in UHT milk, indicating early Maillard reaction stages. However, when considered on protein level, rice and oat 1.9 % showed higher concentrations of furosine. CML levels were generally lower in PBMAs (per mL of drink), with notable variability among oat drinks. However, when evaluated as per gram of protein, all AGEs were found to be higher in PBMAs compared to UHT milk. LAL and LAN concentrations were also higher in PBMAs. Overall, these results indicate that the proteins in PBMAs are modified to a higher extent in comparison to UHT milk. These variations could be connected to processing methods and additives. α-Dicarbonyl compounds, indicative of Maillard and carbohydrate degradation pathways, displayed distinct patterns across PBMAs. Notably, 3-DG

exhibited higher concentrations in oat drinks, possibly linked to the fermentation process and/or the addition of inulin. Analysis of HMF, furfural, and acrylamide indicated varying levels in PBMAs, potentially influenced by the treatments of the raw materials. Unexpectedly, acrylamide was detected in almond and some of the oat drink samples, which is likely due to the roasting of the raw materials in the production of these drinks.

In summary, this study sheds light on the intricate composition and processing dynamics of PBMAs, emphasizing the need for further research to enhance understanding and address nutritional implications and product quality in the burgeoning PBMAs industry. The European Food Safety Authority plays a vital role in evaluating food contaminants, diligently studying their impact and establishing benchmark values through various research methods. Current EFSA regulations target specific MRPs and cover only certain categories of foods (Benford, Bignami, Chipman, & Ramos Bordajandi, 2022; European Commission, 2021; Zheng, Ou, & Ou, 2019; European Commission, 2003; EFSA, 2015). Regulatory bodies like EFSA have set guidelines primarily for specific compounds when a clear, food-contact-related health risk is present, but gaps exist in the application of these regulations to emerging food technologies and novel ingredients. However, the complexity of food processing necessitates more extensive studies in this domain. To comprehensively understand the potential risks and establish clearer guidelines, exploratory studies like ours as well as multidisciplinary studies integrating both pharmacological and chemical analytical approaches become paramount. In addition, given the exploratory nature of this study, we focused on a specific, clearly defined sample size of commercially available PBMAs to assess variability within typical market products; however, future studies with larger sample sizes will be essential for broader generalization of these findings.

CRediT authorship contribution statement

Mariachiara Pucci: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Halise Gül Akıllıoglu:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marta Bevilacqua:** Writing – review & editing, Formal analysis. **Giulia Abate:** Writing – review & editing, Supervision. **Marianne Nissen Lund:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Funding

This work was supported by funding from the Novo Nordisk Foundation (grant no. NNF21OC0066330) to MNL, and from "SOCIETÀ ITALIANA DI FARMACOLOGIA (SIF)" to MP. The funding agency had no involvement in the experimental design or interpretation of the results.

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.

Acknowledgements

The authors thank Bente Pia Danielsen and Mahesha Manjunatha Poojary for their assistance throughout the amino acid analysis and Emilie Hornum Hansen for assistance with sample preparation.

Data availability

Data will be made available on request.

References

Agila, A., & Barringer, S. (2012). Effect of roasting conditions on color and volatile profile including HMF level in sweet almonds (Prunus dulcis). *Journal of Food Science, 77*(4), 1–8. https://doi.org/10.1111/j.1750-3841.2012.02629.x

Akillioglu, H. G., & Gökmen, V. (2014). Mitigation of acrylamide and hydroxymethyl furfural in instant coffee by yeast fermentation. *Food Research International, 61*, 252–256. https://doi.org/10.1016/j.foodres.2013.07.057

Akıllıoğlu, H. G., Chatterton, D. E. W., & Lund, M. N. (2022). Maillard reaction products and amino acid cross-links in liquid infant formula: Effects of UHT treatment and storage. *Food Chemistry, 396*(January). https://doi.org/10.1016/j.foodchem.2022.133687

Akıllıoğlu, H. G., & Lund, M. N. (2022). Quantification of advanced glycation end products and amino acid cross-links in foods by high-resolution mass spectrometry: Applicability of acid hydrolysis. *Food Chemistry, 366*(December 2020). https://doi.org/10.1016/j.foodchem.2021.130601

ALjahdali, N., & Carbonero, F. (2019). Impact of Maillard reaction products on nutrition and health: Current knowledge and need to understand their fate in the human digestive system. *Critical Reviews in Food Science and Nutrition, 59*(3), 474–487. https://doi.org/10.1080/10408398.2017.1378865

Ameur, L. A., Trystram, G., & Birlouez-Aragon, I. (2006). Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method. *Food Chemistry, 98*(4), 790–796. https://doi.org/10.1016/j.foodchem.2005.07.038

Angelino, D., Rosi, A., Vici, G., Dello Russo, M., Pellegrini, N., Martini, D., & Group, and on behalf of the S. Y. W. (2021). Nutritional quality of plant-based drinks sold in Italy: The food labelling of Italian products (FLIP) study. *Nutrients, 13*(1), 1–12. https://doi.org/10.3390/nu13010171

Antonova, K., Vikhnina, M., Soboleva, A., Mehmood, T., Heymich, M. L., Leonova, T., … Frolov, A. (2019). Analysis of chemically labile glycation adducts in seed proteins: Case study of methylglyoxal-derived hydroimidazolone 1 (MG-H1). *International Journal of Molecular Sciences, 20*(15). https://doi.org/10.3390/ijms20153659

Aydar, E. F., Tutuncu, S., & Ozcelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *Journal of Functional Foods, 70*(April), Article 103975. https://doi.org/10.1016/j.jff.2020.103975

Benford, D., Bignami, M., Chipman, J. K., & Ramos Bordajandi, L. (2022). Assessment of the genotoxicity of acrylamide. *EFSA Journal, 20*(5). https://doi.org/10.2903/j.efsa.2022.7293

Biswas, S., Sircar, D., Mitra, A., & de, B. (2011). Phenolic constituents and antioxidant properties of some varieties of Indian rice. *Nutrition and Food Science, 41*(2), 123–135. https://doi.org/10.1108/00346651111117391

Bocker, R., & Silva, E. K. (2022). Innovative technologies for manufacturing plant-based non-dairy alternative milk and their impact on nutritional, sensory and safety aspects. *Future Foods, 5*(July 2021), 100098. https://doi.org/10.1016/j.fufo.2021.100098

Boschin, G., D'Agostina, A., Rinaldi, A., & Arnoldi, A. (2003). Lysinoalanine content of formulas for enteral nutrition. *Journal of Dairy Science, 86*(7), 2283–2287. https://doi.org/10.3168/jds.S0022-0302(03)73820-X

Brosnan, J., & Me, B. (2006). The sulfur-containing amino acids: an overview. Retrieved from *The Journal of Nutrition, 136*(6), 16365–16405 https://www.pkdiet.com/pdf/eaa/aminoacidwrksp.pdf.

Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *LWT - Food Science and Technology Journal, 44*(4), 793–810. https://doi.org/10.1016/j.lwt.2010.11.002

Cichońska, P., & Ziarno, M. (2022). Legumes and legume-based beverages fermented with lactic acid bacteria as a potential carrier of probiotics and prebiotics. *Microorganisms, 10*(1). https://doi.org/10.3390/microorganisms10010091

Čurlej, J., Zajác, P., Čapla, J., Golian, J., Benešová, L., Partika, A., … Jakabová, S. (2022). The effect of heat treatment on cow's milk protein profiles. *Foods, 11*(7), 1–15. https://doi.org/10.3390/foods11071023

Da Silva, M. S., & Rudkowska, I. (2014). Dairy nutrients and their effect on inflammatory profile in molecular studies Marine. *Molecular Nutrition & Food Research, 1–44*. https://doi.org/10.1002/mnfr.201400569.This

Day, L., Cakebread, J. A., & Loveday, S. M. (2022). Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends in Food Science & Technology, 119*, 428–442. https://doi.org/10.1016/j.tifs.2021.12.020

Degen, J., Hellwig, M., & Henle, T. (2012). 1,2-Dicarbonyl compounds in commonly consumed foods. *Journal of Agricultural and Food Chemistry, 60*(28), 7071–7079. https://doi.org/10.1021/jf301306g

Delatour, T., Hegele, J., Parisod, V., Richoz, J., Maurer, S., Steven, M., & Buetler, T. (2009). Analysis of advanced glycation endproducts in dairy products by isotope dilution liquid chromatography-electrospray tandem mass spectrometry. The particular case of carboxymethyllysine. *Journal of Chromatography A, 1216*(12), 2371–2381. https://doi.org/10.1016/j.chroma.2009.01.011

Deleu, L. J., Lambrecht, M. A., Van de Vondel, J., & Delcour, J. A. (2019). The impact of alkaline conditions on storage proteins of cereals and pseudo-cereals. *Current Opinion in Food Science, 25*, 98–103. https://doi.org/10.1016/j.cofs.2019.02.017

Deswal, A., Deora, N. S., & Mishra, H. N. (2014). Optimization of enzymatic production process of oat milk using response surface methodology. *Food and Bioprocess Technology, 7*(2), 610–618. https://doi.org/10.1007/s11947-013-1144-2

EFSA. (2015). Scientific Opinion on acrylamide in food. *EFSA Journal, 13*(6), 4104.

Eggen, M. D., Merboth, P., Neukirchner, H., & Glomb, M. A. (2022). Lipid peroxidation has major impact on malondialdehyde-derived but only minor influence on glyoxal and methylglyoxal-derived protein modifications in carbohydrate-rich foods. *Journal*

of Agricultural and Food Chemistry, 70(33), 10271–10283. https://doi.org/10.1021/acs.jafc.2c04052
EU. (2017). Commission Regulation (EU) 2017/2158: establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. *Official Journal of the European Union, L 304*(315), 24–44. Retrieved from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2158&from=EN.
European Commission. (2003). Opinion of the Scientific Committee on Food on furfural and furfural diethylacetal, (December 2002), 16.
European Commission. (2019). Commission Recommendation (UE) 2019/1888 on the monitoring of the presence of acrylamide in certain foods. *Official Journal of the European Union, 882*, 31–33.
European Commission. (2021). Scientific Opinion on Flavouring Group Evaluation 13 Revision 3 (FGE.13Rev3): furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14. *EFSA Journal, 19*(2). https://doi.org/10.2903/j.efsa.2021.6386
Ferrer, E., Alegría, A., Courtois, G., & Farré, R. (2000). High-performance liquid chromatographic determination of Maillard compounds in store-brand and name-brand ultra-high-temperature-treated cows' milk. *Journal of Chromatography A, 881*(1–2), 599–606. https://doi.org/10.1016/S0021-9673(00)00218-1
Friedman, M. (1999). Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *Journal of Agricultural and Food Chemistry, 47*(4), 1295–1319. https://doi.org/10.1021/jf981000+
Fu, M. X., Requena, J. R., Jenkins, A. J., Lyons, T. J., Baynes, J. W., & Thorpe, S. R. (1996). The advanced glycation end product, N∈-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *Journal of Biological Chemistry, 271*(17), 9982–9986. https://doi.org/10.1074/jbc.271.17.9982
Gökmen, V., & Acar, J. (1999). Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography, *847*, 69–74.
Gökmen, V., Açar, Ö.Ç., Köksel, H., & Acar, J. (2007). Effects of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies. *Food Chemistry, 104*(3), 1136–1142. https://doi.org/10.1016/j.foodchem.2007.01.008
Gökmen, V., Serpen, A., Açar, Ö.Ç., & Morales, F. J. (2008). Significance of furosine as heat-induced marker in cookies. *Journal of Cereal Science, 48*(3), 843–847. https://doi.org/10.1016/j.jcs.2008.06.007
Granvogl, M., & Schieberle, P. (2006). Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *Journal of Agricultural and Food Chemistry, 54*(16), 5933–5938. https://doi.org/10.1021/jf061150h
Han, L., Li, L., Li, B., Zhao, D., Li, Y., Xu, Z., & Liu, G. (2013). Review of the characteristics of food-derived and endogenous Nε-carboxymethyllysine. *Journal of Food Protection, 76*(5), 912–918. https://doi.org/10.4315/0362-028X.JFP-12-472
Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition - A review. *Lipids in Health and Disease, 6*, 1–16. https://doi.org/10.1186/1476-511X-6-25
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. https://doi.org/10.1021/jf102388v
Hildebrand, G., Poojary, M. M., O'Donnell, C., Lund, M. N., Garcia-Vaquero, M., & Tiwari, B. K. (2020). Ultrasound-assisted processing of Chlorella vulgaris for enhanced protein extraction. *Journal of Applied Phycology, 32*(3), 1709–1718. https://doi.org/10.1007/s10811-020-02105-4
Hodge, J. E. (1953). Browning reactions in model systems. *Journal of Agricultural and Food Chemistry, 1*, 928–943.
Keramat, J., LeBail, A., Prost, C., & Soltanizadeh, N. (2011). Acrylamide in foods: chemistry and analysis. A review. *Food and Bioprocess Technology, 4*(3), 340–363. https://doi.org/10.1007/s11947-010-0470-x
Khan, I. T., Bule, M., Ullah, R., Nadeem, M., Asif, S., & Niaz, K. (2019). The antioxidant components of milk and their role in processing, ripening, and storage: Functional food. *Veterinary World, 12*(1), 12–33. https://doi.org/10.14202/vetworld.2019.12-33
Kocadağli, T., & Gökmen, V. (2014). Investigation of α-dicarbonyl compounds in baby foods by high-performance liquid chromatography coupled with electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 62*(31), 7714–7720. https://doi.org/10.1021/jf502418n
Kodad, O., Socias i Company, R., & Alonso, J. M. (2018). Genotypic and environmental effects on tocopherol content in almond. *Antioxidants, 7*(1), 1–9. https://doi.org/10.3390/antiox7010006
Kroh, L. W. (1994). Caramelisation in food and beverages. *Food Chemistry, 51*(4), 373–379. https://doi.org/10.1016/0308-8146(94)90188-0
Liu, S., Sun, H., Ma, G., Zhang, T., Wang, L., Pei, H., … Gao, L. (2022). Insights into flavor and key influencing factors of Maillard reaction products: A recent update. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.973677
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. https://doi.org/10.1021/acs.jafc.7b00882
Maasen, K., Scheijen, J. L. J. M., Opperhuizen, A., Stehouwer, C. D. A., Van Greevenbroek, M. M., & Schalkwijk, C. G. (2021). Quantification of dicarbonyl compounds in commonly consumed foods and drinks; presentation of a food composition database for dicarbonyls. *Food Chemistry, 339*(September 2020), 128063. https://doi.org/10.1016/j.foodchem.2020.128063
Mäkinen, O. E., Wanhalinna, V., Zannini, E., & Arendt, E. K. (2016). Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical Reviews in Food Science and Nutrition, 56*(3), 339–349. https://doi.org/10.1080/10408398.2012.761950
McClements, D. J. (2020). Development of next-generation nutritionally fortified plant-based milk substitutes. *Structural Design Principles*, 271–314. https://doi.org/10.1142/9781848161672_0006
Mesías, M., Palenzuela, C., Olombrada, E., Holgado, F., & Morales, F. J. (2024). Acrylamide and hydroxymethylfurfural formation in roasted almonds (Prunus dulcis). *Food Control, 156*(July 2023), 110140. https://doi.org/10.1016/j.foodcont.2023.110140.
Michalak, J., Gujska, E., Czarnowska, M., & Nowak, F. (2014). Study on the acrylamide content in selected groups of food products. *Polish Journal of Commodity Science, 4 (41)* (December 2016), 60–66.
Millward, D. J. (2012). Knowledge gained from studies of leucine consumption in animals and humans. *Journal of Nutrition, 142*(12). https://doi.org/10.3945/jn.111.157370
Morales, F. J., Romero, C., & Jimenez-Pérez, S. (1992). An enhanced liquid chromatographic method for 5-hydroxymethylfurfural determination in UHT milk. *Chromatographia, 33*(1–2), 45–48. https://doi.org/10.1007/BF02276850
Morales, F. J., Romero, C., & Jiménez-Pérez, S. (1997). Chromatographic determination of bound hydroxymethylfurfural as an index of milk protein glycosylation. *Journal of Agricultural and Food Chemistry, 45*(5), 1570–1573. https://doi.org/10.1021/jf960930v
Nielsen, S. D., Knudsen, L. J., Bækgaard, L. T., Rauh, V., & Larsen, L. B. (2022). Influence of lactose on the Maillard reaction and dehydroalanine-mediated protein cross-linking in casein and whey. *Foods, 11*(7). https://doi.org/10.3390/foods11070897
Nomi, Y., Sato, T., Mori, Y., & Matsumoto, H. (2022). Evaluation of fructo-, inulin-, and galacto-oligosaccharides on the Maillard reaction products in model systems with whey protein. *Journal of Agricultural and Food Chemistry, 70*(29), 9154–9165. https://doi.org/10.1021/acs.jafc.2c03197
Nowotny, K., Schröter, D., Schreiner, M., & Grune, T. (2018). Dietary advanced glycation end products and their relevance for human health. *Ageing Research Reviews, 47*, 55–66. https://doi.org/10.1016/j.arr.2018.06.005
Omoni, A. O., & Aluko, R. E. (2005). Soybean foods and their benefits: Potential mechanisms of action. *Nutrition Reviews, 63*(8), 272–283. https://doi.org/10.1301/nr.2005.aug.272-283
Poojary, M. M., & Lund, M. N. (2022). Chemical stability of proteins in foods: oxidation and the maillard reaction. *Annual Review of Food Science and Technology, 13*(1), 35–58. https://doi.org/10.1146/annurev-food-052720-104513
Purlis, E. (2010). Browning development in bakery products - A review. *Journal of Food Engineering, 99*(3), 239–249. https://doi.org/10.1016/j.jfoodeng.2010.03.008
Rai, C., Nandini, C. D., & Priyadarshini, P. (2021). Composition and structure elucidation of bovine milk glycosaminoglycans and their anti-adipogenic activity via modulation PPAR-γ and C/EBP-α. *International Journal of Biological Macromolecules, 193*(PA), 137–144. https://doi.org/10.1016/j.ijbiomac.2021.10.076
Ratajczak, A. E., Zawada, A., Rychter, A. M., Dobrowolska, A., & Krela-Kaźmierczak, I. (2021). Milk and dairy products: Good or bad for human bone? practical dietary recommendations for the prevention and management of osteoporosis. *Nutrients, 13*(4). https://doi.org/10.3390/nu13041329
Roland, I. S., Aguilera-Toro, M., Nielsen, S. D. H., Poulsen, N. A., & Larsen, L. B. (2023). Processing-induced markers in proteins of commercial plant-based drinks in relation to compositional aspects. *Foods, 12*(17). https://doi.org/10.3390/foods12173282
Rombouts, I., Lambrecht, M. A., Carpentier, S. C., & Delcour, J. A. (2016). Identification of lanthionine and lysinoalanine in heat-treated wheat gliadin and bovine serum albumin using tandem mass spectrometry with higher-energy collisional dissociation. *Amino Acids, 48*(4), 959–971. https://doi.org/10.1007/s00726-015-2139-2
Ruiz-Matute, A. I., Castro Vazquez, L., Hernández-Hernández, O., Sanz, M. L., & Martínez-Castro, I. (2015). Identification and determination of 3-deoxyglucosone and glucosone in carbohydrate-rich foods. *Journal of the Science of Food and Agriculture, 95*(12), 2424–2430. https://doi.org/10.1002/jsfa.6965
Sethi, S., Tyagi, S. K., & Anurag, R. K. (2016). Plant-based milk alternatives an emerging segment of functional beverages: A review. *Journal of Food Science and Technology, 53*(9), 3408–3423. https://doi.org/10.1007/s13197-016-2328-3
Shkembi, B., & Huppertz, T. (2023). Glycemic responses of milk and plant-based drinks: food matrix effects, 1–18.
Silva, A. R. A., Silva, M. M. N., & Ribeiro, B. D. (2020). Health issues and technological aspects of plant-based alternative milk. *Food Research International, 131*(December 2019), 108972. https://doi.org/10.1016/j.foodres.2019.108972
Smith, N. W., Fletcher, A. J., Hill, J. P., & McNabb, W. C. (2022). Modeling the contribution of milk to global nutrition. *Frontiers in Nutrition, 8*(January), 1–7. https://doi.org/10.3389/fnut.2021.716100
Sousa, A., & Bolanz, K. A. K. (2017). Nutritional implications of an increasing consumption of non-dairy plant-based beverages instead of cow's milk in Switzerland. *Advances in Dairy Research, 05*(04). https://doi.org/10.4172/2329-888x.1000197
Stadler, R. H. (2005). Acrylamide formation in different foods and potential strategies for reduction. *Advances in Experimental Medicine and Biology, 561*(April 2002), 157–169. https://doi.org/10.1007/0-387-24980-X_13
Tamanna, N., & Mahmood, N. (2015). Food processing and maillard reaction products: Effect on human health and nutrition. *International Journal of Food Science, 2015*. https://doi.org/10.1155/2015/526762
Tangyu, M., Muller, J., Bolten, C. J., & Wittmann, C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. *Applied Microbiology and Biotechnology, 103*(23–24), 9263–9275. https://doi.org/10.1007/s00253-019-10175-9
Teixidó, E., Santos, F. J., Puignou, L., & Galceran, M. T. (2006). Analysis of 5-hydroxymethylfurfural in foods by gas chromatography-mass spectrometry. *Journal of Chromatography A, 1135*(1), 85–90. https://doi.org/10.1016/j.chroma.2006.09.023

Teixidó, E., Núñez, O., Santos, F. J., & Galceran, M. T. (2011). 5-Hydroxymethylfurfural content in foodstuffs determined by micellar electrokinetic chromatography. *Food Chemistry, 126*(4), 1902–1908. https://doi.org/10.1016/j.foodchem.2010.12.016

Tian, B., Xie, B., Shi, J., Wu, J., Cai, Y., Xu, T., … Deng, Q. (2010). Physicochemical changes of oat seeds during germination. *Food Chemistry, 119*(3), 1195–1200. https://doi.org/10.1016/j.foodchem.2009.08.035

Tomé, D., & Bos, C. (2007). Lysine requirement through the human life cycle. *Journal of Nutrition, 137*(6). https://doi.org/10.1093/jn/137.6.1642s

Trindler, C., Annika Kopf-Bolanz, K., & Denkel, C. (2022). Aroma of peas, its constituents and reduction strategies – Effects from breeding to processing. *Food Chemistry, 376* (August 2021), 131892. https://doi.org/10.1016/j.foodchem.2021.131892

Truswell, A. S. (2002). Cereal grains and coronary heart disease. *European Journal of Clinical Nutrition, 56*(1), 1–14. https://doi.org/10.1038/sj.ejcn.1601283

Uribarri, J., Woodruff, S., Goodman, S., Cai, W., Chen, X. U. E., Pyzik, R., … Striker, G. E. (2010). AGE's in Foods and practical ways to reduce them. *Journal of the American Dietetic Association, 110*(6), 911–916. https://doi.org/10.1016/j.jada.2010.03.018. Advanced

Valdés, A., Beltrán, A., Karabagias, I., Badeka, A., Kontominas, M. G., & Garrigós, M. C. (2015). Monitoring the oxidative stability and volatiles in blanched, roasted and fried almonds under normal and accelerated storage conditions by DSC, thermogravimetric analysis and ATR-FTIR. *European Journal of Lipid Science and Technology, 117*(8), 1199–1213. https://doi.org/10.1002/ejlt.201400384

Vanga, S. K., & Raghavan, V. (2018). How well do plant based alternatives fare nutritionally compared to cow's milk? *Journal of Food Science and Technology, 55*(1), 10–20. https://doi.org/10.1007/s13197-017-2915-y

Vinther Schmidt, C., Olsen, K., & Mouritsen, O. G. (2021). Umami potential of fermented beverages: Sake, wine, champagne, and beer. *Food Chemistry, 360*(January), Article 128971. https://doi.org/10.1016/j.foodchem.2020.128971

Vistoli, G., De Maddis, D., Cipak, A., Zarkovic, N., Carini, M., & Aldini, G. (2013). Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): An overview of their mechanisms of formation. *Free Radical Research, 47*(S1), 3–27. https://doi.org/10.3109/10715762.2013.815348

Walther B, Guggisberg D, Badertscher R, Egger L, Portmann R, Dubois S, Haldimann M, Kopf-Bolanz K, Rhyn P, Zoller O, Veraguth R, Rezzi S. Comparison of nutritional composition between plant-based drinks and cow's milk. Front Nutr. 2022 Oct 28;9: 988707. doi: 10.3389/fnut.2022.988707. PMID: 36386959; PMCID: PMC9650290.

Welch, R. W. (1989). Oats in human nutrition. *Archiv Für Tierernährung, 39*(7), 603–610. https://doi.org/10.1080/17450398909428330

Wold, S., Esbensen, K., & Geladi, P. (1987). Decret_Du_7_Mai_1993_Fixant_Les_Modalites_D_Application_De_La_Loi_Relative_Aux_Recensements_Et_Enquetes_Statistiques.Pdf. *Chemometrics and Intelligent Laboratory Systems, 2*(1–3), 37–52. Retrieved from http://files.isec.pt/DOCUMENTOS/SERVICOS/BIBLIO/Documentos de acesso remoto/Principal components analysis.pdf.

Xiao, L., Lee, J., Zhang, G., Ebeler, S. E., Wickramasinghe, N., Seiber, J., & Mitchell, A. E. (2014). HS-SPME GC/MS characterization of volatiles in raw and dry-roasted almonds (Prunus dulcis). *Food Chemistry, 151*, 31–39. https://doi.org/10.1016/j.foodchem.2013.11.052

Xing, Q., Ma, Y., Fu, X., Cao, Q., Zhang, Y., & You, C. (2020). Effects of heat treatment, homogenization pressure, and overprocessing on the content of furfural compounds in liquid milk. *Journal of the Science of Food and Agriculture, 100*(14), 5276–5282. https://doi.org/10.1002/jsfa.10578

Yang, M., & Li, L. (2010). Physicochemical, textural and sensory characteristics of probiotic soy yogurt prepared from germinated soybean. *Food Technology and Biotechnology, 48*(4), 490–496.

Yaylayan, V. A., & Stadler, R. H. (2005). Acrylamide formation in food: A mechanistic perspective. *Journal of AOAC International, 88*(1), 262–267. https://doi.org/10.1093/jaoac/88.1.262

Zhang, W., Poojary, M. M., Olsen, K., Ray, C. A., & Lund, M. N. (2019). Formation of α-dicarbonyls from dairy related carbohydrates with and without Nα-Acetyl- l -Lysine during Incubation at 40 and 50 °c. research-article *Journal of Agricultural and Food Chemistry, 67*(22), 6350–6358. https://doi.org/10.1021/acs.jafc.9b01532.

Zhang, W., Poojary, M. M., Rauh, V., Ray, C. A., Olsen, K., & Lund, M. N. (2020). Limitation of maillard reactions in lactose-reduced UHT milk via enzymatic conversion of lactose into galactooligosaccharides during production. *Journal of Agricultural and Food Chemistry, 68*(11), 3568–3575. https://doi.org/10.1021/acs.jafc.9b07824

Zhang, Y. Y., Hughes, J., & Grafenauer, S. (2020). Got mylk? The emerging role of australian plant-based milk alternatives as a cow's milk substitute. *Nutrients, 12*(5). https://doi.org/10.3390/nu12051254

Zheng, J., Ou, J., & Ou, S. (2019). *Chemical hazards in thermally-processed foods*. *Chemical Hazards in Thermally-Processed Foods*. https://doi.org/10.1007/978-981-13-8118-8