



## Carbohydrate composition of cow milk and plant-based milk alternatives

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### ABSTRACT

The prevalence of lactose intolerance is one of the factors driving consumers toward plant-based milk alternatives (PBMA). This study aimed to analyze the carbohydrate profile of cows' milk (regular and lactose-free from both pasteurized and UHT milks;  $n = 80$ ) and PBMA ( $n = 60$ ) by HPLC. The study revealed that no significant difference was present in the energy content and total carbohydrate content between regular milk and lactose-free milk. Although milk and PBMA are entirely different food matrices, some PBMA types, as soya and coconut, may have energy contents and total carbohydrate contents comparable to those of milk. Furthermore, the variability observed in total carbohydrate content, as well as in carbohydrate profile, both between PBMA types and within samples of the same type, arises not only from variations in raw materials but also from the number of dilutions of the vegetable extract and the addition of different types and levels of carbohydrates, such as sucrose, fructose, or sorbitol, during PBMA manufacture. Although, milk presents a regular carbohydrate composition, differing solely between presentations (regular/lactose-free), the PBMA differs significantly between types and among the same type, not being for that reason regarded as a milk substitute.

**Key words:** pasteurized milk, ultra-high-temperature milk, plant-based milk alternatives, carbohydrates

### INTRODUCTION

Carbohydrates are a fundamental component of human nutrition, as a source of energy (Cummings and

Stephen, 2007), and in Western diet, they account for ~50% of total energy intake (Maughan, 2009). However, when intake exceeds this proportion, the energy surplus is converted into fatty acids and stored in adipose cells, leading to overweight and obesity (Cummings and Stephen, 2007; Maughan, 2009; WHO, 2021). In addition to energy provision, carbohydrates play vital roles in satiety, blood glucose regulation, insulin and lipid metabolism, colonic function, and the rheological properties of foods (Cummings and Stephen, 2007; Slavin and Carlson, 2014). According to the degree of polymerization, carbohydrates can be classified into monosaccharides (as galactose, glucose, and fructose), disaccharides (as maltose, sucrose, and lactose), oligosaccharides (as raffinose and maltodextrins), and polysaccharides (as starch and cellulose; Cummings and Stephen, 2007; Maughan, 2009).

Lactose is the natural sugar of milk, and this disaccharide is composed by glucose and galactose linked by a glycosidic bond, which is hydrolyzed by lactase (Heyman, 2006; EFSA NDA Panel, 2010). However, the inability to hydrolyze lactose, a consequence of lactase deficiency, results in lactose intolerance, which affects ~70% of the world's population (EFSA NDA Panel, 2010) and is one of the reasons why consumers have driven their choices to plant-based options, as plant-based milk alternatives (PBMA; Jeske et al., 2017).

PBMA are produced from a diversity of raw materials, including seeds, nuts, legumes, cereals, and pseudocereals (Sethi et al., 2016) and their formulation often encloses several additives (emulsifiers, stabilizers, flavors, sweeteners, and micronutrient supplementation; Mäkinen et al., 2016; Jeske et al., 2017). Such variability in ingredients and processing methodologies is translated into nutritional variability and some authors have expressed their concern regarding this subject (Bridges, 2018). The PBMA manufacturers add carbohydrates to their

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formulations to enhance the palatability and improve the rheological properties of the products (Slavin and Carlson, 2014). However, the inclusion of some sugars in the PBMA formulations increases their energy content, which may provide PBMA an energy load equivalent to whole milk (Mäkinen et al., 2016; Jeske et al., 2017). The WHO (2015) recommends reducing added sugar in the diet, particularly from sugar-sweetened beverages, to prevent weight gain and reduce the risk of obesity. Furthermore, such sugars may contribute to a high glycemic index (Chalupa-Krebsdak et al., 2018), which has been associated with increased risk of type 2 diabetes, obesity, and cardiovascular diseases (Ludwig, 2002; Bell and Sears, 2003; Brand-Miller, 2003). Contrariwise, the combination of lactose with the content and quality of milk protein has been shown to have a beneficial effect on glucose homeostasis, resulting in low glycemic indices (Anderson et al., 2011; Kung et al., 2018).

Considering the important role of carbohydrates in human health, as well as the negative consequences resulting from the ingestion of energy-dense foods, the work presented herein aimed to assess milk and PBMA total energy content, total carbohydrate content and characterize their carbohydrate profile, and it is sustained by the following scientific hypotheses: (1) regular milk and lactose-free milk share a similar carbohydrate profile; (2) milk and PBMA have different carbohydrate profile; and (3) PBMA types differ carbohydrate profile among themselves.

## MATERIALS AND METHODS

### Sampling

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board. Sampling was made in the Portuguese market, and we aimed to include as many brands as possible for each type of milk or beverage. However, the Portuguese market did not offer 10 different brands for each category. Therefore, to reach 10 samples per group, some brands were repeated, but with different manufacturing batches (Table 1). Each sample corresponds to one unit. A total of 80 packages (1 L) of milk (equally divided by pasteurized and UHT milk) and 60 packages (1 L) of PBMA ( $n = 10$  for each PBMA type) were purchased. Each type of milk enclosed samples from different technological treatments (regular milk, that is, milk without modification of the lactose content [ $n = 30$ ] and lactose-free [ $n = 10$ ]). The selection of PBMA excluded the blended options and was limited to distinct PBMA types made from a single raw material (soya, oat, rice, almond, coconut, and hazelnut). In Supplemental Table S1 (see Notes; Antunes, 2024), is

**Table 1.** Carbohydrates added on plant-based milk alternatives (PBMA) types during manufacturing

PBMA type	Brand	n	Carbohydrates added
Soya	A	2	Fructose, E460, E466
Soya	B	2	Sugar, gellan gum
Soya	C	1	Maltodextrin, gellan gum
Soya	D	1	Sugar, maltodextrin, gellan gum
Soya	E	1	Sugar, maltodextrin, gellan gum
Soya	F	1	Fructose, gellan gum
Soya	G	1	Sugar, maltodextrin, gellan gum
Soya	H	1	Glucose-fructose syrup, inulin, gellan gum
Oat	I	2	Gellan gum
Oat	J	2	Gellan gum
Oat	K	1	Inulin, maltodextrin, gellan gum
Oat	L	1	Inulin, gellan gum
Oat	M	1	—
Oat	N	1	Gellan gum
Oat	O	1	Gellan gum
Oat	P	1	Inulin, gellan gum
Rice	Q	2	Gellan gum
Rice	R	2	Gellan gum
Rice	S	1	Gellan gum
Rice	T	1	—
Rice	U	1	Inulin, gellan gum
Rice	V	1	Gellan gum
Rice	W	1	Inulin, gellan gum
Rice	Y	1	Gellan gum
Almond	X	3	Gellan gum, locust bean gum
Almond	Z	2	Gellan gum, locust bean gum
Almond	AA	1	Gellan gum
Almond	BB	1	Sugar, gellan gum, locust bean gum
Almond	CC	1	Sugar, gellan gum, locust bean gum
Almond	DD	1	Sugar, gellan gum
Almond	EE	1	Sugar, gellan gum
Coconut	FF	3	Guar gum, gellan gum, xanthan gum
Coconut	GG	7	Inulin, guar gum, gellan gum, xanthan gum
Hazelnut	HH	5	Sugar, gellan gum, locust bean gum
Hazelnut	II	5	Sugar, gellan gum, locust bean gum

presented the nutritional contents of each PBMA type according to package label. All PBMA samples were stored under the same conditions in the market where they were acquired, at room temperature in the supermarket shells, as they were all subject to UHT treatment. After acquisition, all samples remained in their original packaging and were transported in a refrigerated container to the laboratory. There, the contents of each package were distributed into small containers (100 mL) and stored in a freezer at  $-20^{\circ}\text{C}$  for subsequent analysis. The carbohydrates added on PBMA types during manufacturing is depicted in Table 1.

### Determination of Total Carbohydrate Content

The total carbohydrate content (TCC) was assessed by difference, using the equation (FAO, 2003) presented below:

$$\text{TCC (g/100 mL)} = 100 - \text{moisture content} - \text{total protein content} - \text{total fat content} - \text{ash content.}$$

**Table 2.** The carbohydrates standards with their retention time (RT), limit of detection (LOD) and the limit of quantification (LOQ), stock solutions, and the range of working solutions

Item	Category	RT (min)	LOD (mg/mL)	LOQ (mg/mL)	Stock solution (mg/mL)	Working solution (mg/mL)
Monosaccharides	Arabinose	12.01	0.17	0.51	34.83	0.1–35
	Fructose	11.64	0.09	0.28	36.54	0–36
	Fucose	11.81	0.02	0.07	15.29	0.1–15
	Galactose	10.82	0.06	0.17	30.09	0.1–30
	Glucose	9.86	0.08	0.25	33.02	0–34
	Mannose	7.93	0.05	0.14	28.85	0.1–30
	Rhamnose	10.74	0.09	0.27	51.21	0–50
	Ribose	17.10	0.16	0.50	32.95	0.5–30
	Xylose	10.70	0.03	0.08	15.14	0.1–15
	Disaccharides	Lactose	8.42	0.05	0.16	40.10
Lactulose		9.10	0.02	0.06	14.90	0.1–15
Maltose		8.17	0.03	0.08	14.36	0.1–15
Sucrose		8.04	0.05	0.16	40.14	0–40
Polyols	Mannitol	13.27	0.09	0.28	30.48	0–30
	Sorbitol	15.45	0.18	0.54	29.78	0–30
Oligosaccharides	Raffinose	7.15	0.02	0.06	30.18	0.1–30

The moisture content (AOAC 931.04), total protein content (AOAC 970.22), and ash content (AOAC 13.003) were determined according to the Association of Official Analytical Chemists (1990) methods, whereas the total fat content was determined according to the methodology previously described by Folch et al. (1957).

#### Determination of Energy Content

The energy content was calculated according to the Atwater specific factor system (FAO, 2003), as follows:

$$\text{Energy (kcal/100 mL)} = (\text{protein content} \times 4) \\ + (\text{carbohydrate content} \times 4) + (\text{fat content} \times 9).$$

#### Carbohydrate Reagents and Standard Solutions

Proanalysis grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). The Carrez I (500 mM aqueous potassium ferrocyanide), Carrez II (500 mM zinc acetate) and EDTA Ca (1 mM) solutions were prepared with distilled water. The carbohydrate standards (sucrose, glucose, rhamnose, fructose, mannitol, sorbitol, raffinose, lactose, galactose, arabinose, ribose, maltose, lactulose, xylose, fucose, mannose) were acquired from Sigma Chemical Co. (St. Louis, MO). The standard stock solutions were freshly prepared with distilled water and the working standard solutions were prepared by several dilutions of stock solutions with distilled water (Table 2). The limit of detection and the limit of quantification (LOQ) for each carbohydrate standard were also calculated (Table 2). The carbohydrates arabi-

nose, fucose, mannose, rhamnose, xylose, and lactulose were not detected in our samples, therefore they are not presented in the results.

#### Carbohydrate Extraction

The extraction of carbohydrates was performed as previously described by Sharma et al. (2009). Briefly, 2 mL of sample was pipetted into a test tube, to which 1.5 mL of distilled water was added, and incubated in a water bath at 60°C for 10 min. Then 0.25 mL of Carrez I solution, 0.25 mL of Carrez II solution, and 1 mL of acetonitrile were added. The contents were mixed gently and kept for 1 h at room temperature. At the end of this time, the precipitate obtained was removed by centrifugation (8 min, 10,000 × g, 20°C). The resulting supernatant was filtered through a 0.45 µm nylon syringe filter (Filter laboratory, Barcelona, Spain) and injected on the HPLC system. For all calculations, a density of 1 g/mL was assumed for all beverages analyzed.

#### Carbohydrate High-Performance Liquid Chromatography Analysis

For the identification and quantification of carbohydrates an HPLC system (Waters Corporation, Milford, MA) was used. This included an autosampler (model 717Plus), 2 pumps (model 510), a column oven (model Col. HTR), and a refractive index detector set at 35°C (model 2414). The system was connected to the Waters Empower software for acquisition and control. The separation was performed using a Waters Sugar-Pak I column (6.5 mm × 300 mm, 10 µm; Waters Corporation, Milford,

MA) maintained at 85°C with a mobile phase of EDTA Ca 0.01 mM, a flow rate of 0.5 mL/min, an injection volume of 20 µL, and a runtime of 30 min.

### Statistical Analysis

The Mixed procedure of SAS version 9.4 (SAS Institution, Cary, NC) was used in the statistical analysis. A first statistical analysis, using a single fixed effect, was performed to test potential differences between regular and lactose-free milk samples from both pasteurized and UHT milk groups. Because no statistical ( $P > 0.05$ ) differences were observed, milk samples were grouped as regular milk and lactose-free milk, independently of heat-treatment. A second statistical analysis was performed to compare the carbohydrate profile of regular milk, lactose-free milk, and PBMA types using a single fixed effect statistical model considering the type of beverage as an independent variable and the different samples of each beverage as the experimental unit. In some cases, the variance heterogeneity was introduced in the model through the “group” option in the “repeated” statement (Milliken and Johnson, 2009). For the determination of multiple comparisons of the least squares means, the PDIFF with Tukey–Kramer adjustment options of SAS was used. Furthermore, a principal component analysis (PCA) was performed with all variables through PRINCOMP procedure of SAS. The PCA was applied to 140 samples and 11 variables with 2 principal components being retained to describe the variability. In some samples, some carbohydrate contents were not detected, or their content was below the LOQ, therefore, these values were replaced by  $LOQ/\sqrt{2}$  value, which was included in the PCA calculations. The principal components’ eigenvalues, loadings of the

variables, and principal components’ loadings of samples are presented as supplemental material (Supplemental Tables S1, S2, S3, and S4; see Notes; Antunes, 2024).

## RESULTS AND DISCUSSION

### Energy Content

The total energy content (expressed as kcal/100 mL), TCC (expressed as g/100 mL) and carbohydrate profile (expressed as g/100 mL) of both milk (regular milk and lactose-free milk) and PBMA types are depicted in Table 3. The highest total energy contents were observed simultaneously in both regular and lactose-free milk, as well as in rice and oat PBMA (averaging 49 kcal/100 mL). In contrast, hazelnut and almond PBMA presented the lowest total energy content (averaging 29 kcal/100 mL). Significant differences ( $P < 0.05$ ) were observed between beverages presenting the highest and lowest total energy contents, whereas soya and coconut PBMA presented energy contents in-between (averaging 36 kcal/100 mL), not differing significantly ( $P > 0.05$ ) with all other beverages in analysis. Such energy contents are in the range of those previously reported by other authors (Singhal et al., 2017; Walther et al., 2022). In addition to differences observed between PBMA types, a considerable variability was also observed among samples of the same PBMA type. In this regard, coconut and soya PBMA types were the ones displaying the highest variability among samples. Such variability was also reported by other authors (Mäkinen et al., 2016; Bridges, 2018; Chalupa-Krebzdak et al., 2018; Walther et al., 2022), and may be a consequence of differences in PBMA formulation between brands, namely the number

**Table 3.** Energy content (expressed as kcal/100 mL), total carbohydrate content (expressed as g/100 mL), and carbohydrate profile (expressed as g/100 mL) of cow milk (regular milk and lactose-free milk) and plant-based milk alternative types (presented as mean ± SE)

Item	Cow milk		Plant-based milk alternative						P-value
	Regular	Lactose-free	Soya	Oat	Rice	Almond	Coconut	Hazelnut	
	60	20	10	10	10	10	10	10	
Energy	49.1 ± 1.52 <sup>a</sup>	49.0 ± 0.60 <sup>a</sup>	40.1 ± 4.15 <sup>ab</sup>	48.0 ± 0.89 <sup>a</sup>	49.4 ± 1.01 <sup>a</sup>	27.6 ± 0.89 <sup>b</sup>	32.1 ± 5.87 <sup>ab</sup>	30.2 ± 0.94 <sup>b</sup>	<0.001
Total carbohydrates	4.9 ± 0.07 <sup>b</sup>	5.0 ± 0.12 <sup>b</sup>	5.6 ± 0.67 <sup>bc</sup>	9.2 ± 0.16 <sup>a</sup>	11.3 ± 0.71 <sup>a</sup>	4.1 ± 0.17 <sup>c</sup>	5.6 ± 1.01 <sup>bc</sup>	3.7 ± 0.17 <sup>c</sup>	<0.001
Lactose	4.6 ± 0.13	<LOQ <sup>1</sup>	—	—	—	—	—	—	—
Glucose	—	2.3 ± 0.11 <sup>a</sup>	0.1 ± 0.19 <sup>b</sup>	2.6 ± 0.19 <sup>a</sup>	2.5 ± 0.18 <sup>a</sup>	—	1.9 ± 0.61 <sup>ab</sup>	—	<0.001
Galactose	—	2.5 ± 0.08	—	—	—	—	—	—	—
Raffinose	—	—	0.3 ± 0.08	0.6 ± 0.10	—	—	—	—	0.104
Sucrose	—	—	1.3 ± 0.17 <sup>b</sup>	—	—	1.8 ± 0.04 <sup>a</sup>	0.1 ± 0.06 <sup>c</sup>	1.6 ± 0.14 <sup>ab</sup>	<0.001
Maltose	—	—	—	2.6 ± 0.45	3.8 ± 0.47	—	2.9 ± 0.63	—	0.172
Fructose	—	—	0.02 ± 0.017 <sup>b</sup>	0.07 ± 0.012 <sup>ab</sup>	0.10 ± 0.014 <sup>a</sup>	—	0.03 ± 0.013 <sup>b</sup>	—	0.029
Sorbitol	—	—	3.0 ± 0.99	3.2 ± 0.93	3.2 ± 2.06	1.0 ± 0.24	0.6 ± 0.20	0.9 ± 0.15	0.050

<sup>a-c</sup>Different superscripts correspond to significant differences ( $P < 0.05$ ).

<sup>1</sup><LOQ = below limit of quantification.

of dilutions of the vegetable extract and the addition of some ingredients, such as carbohydrates (Bridges, 2018; Scholz-Ahrens et al., 2020).

One of the main reasons supporting the growing popularity of PBMA is their lower energy content compared with milk (Vanga and Raghavan, 2018). Nevertheless, such concept is not truly correct when considering total energy content, because PBMA supplementation with sugars, to enhance palatability and improve the rheological properties of the beverages (Slavin and Carlson, 2014), may increase their energy content, making them comparable to that of milk, as demonstrated herein and previously observed by others (Mäkinen et al., 2016; Jeske et al., 2017).

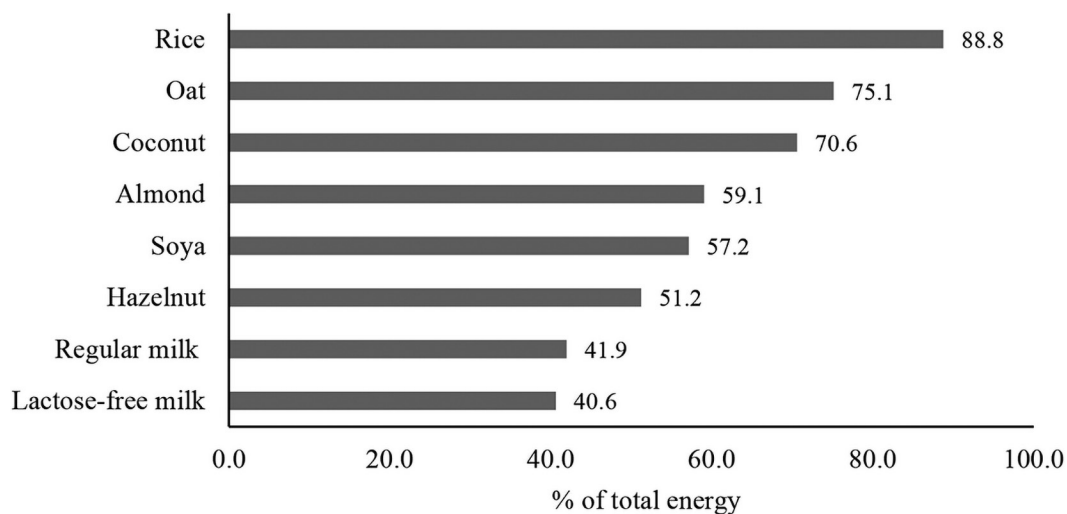
The consumption of beverages with high energy content is worrisome because prolonged consumption of energy-rich foods can increase adipose reserves in the human body, leading to weight gain and, in more severe cases, to obesity. This condition affects a significant portion of the global population, with estimates indicating that in 2016, 13% of adults were obese, and over 340 million children and adolescents were either overweight or obese (WHO, 2021). The prevalence of obesity and its detrimental effects on health are major concerns for medical professionals, as it poses a risk factor for cardiovascular diseases, diabetes, musculoskeletal disorders, and even to some cancer types (WHO, 2021).

### Total Carbohydrate Content

Significant differences ( $P < 0.001$ ) on TCC were observed between PBMA with the highest (rice and oat) and lowest (almond and hazelnut) TCCs (averaging 10.3 and 3.9 g/100 mL, respectively; Table 3). In contrast,

soya and coconut PBMA presented intermediate TCC (averaging 5.6 g/100 mL), differing significantly ( $P < 0.05$ ) from those PBMA with the highest TCC, but not from those PBMA with the lowest TCC. Milk presented a midway value of TCC (regular and lactose-free averaged 5.0 g/100 mL), differing significantly ( $P < 0.05$ ) from the PBMA with the highest and lowest TCCs, but not differing from the PBMA with intermediate TCCs, namely soya and coconut PBMA (averaged 5.6 g/100 mL). The highest TCCs in rice and oat may be attributed to their high levels on starch (Qamar et al., 2020). The TCC of milk aligns with previous findings, but the same researchers reported lower TCCs for soya, almond, coconut and hazelnut PBMA (Singhal et al., 2017; Angelino et al., 2020; Pointke et al., 2022; Walther et al., 2022). Differences between studies in PBMA composition may be attributed to differences in the raw materials used in their preparation and differences in the processing methodologies between brands (Bridges, 2018; Scholz-Ahrens et al., 2020). During product formulation, the addition of carbohydrates to enhance the beverage's rheological properties, as well as the number of dilutions of the vegetable extract, will affect the TCC of the beverage (Slavin and Carlson, 2014; Bridges, 2018; Scholz-Ahrens et al., 2020).

Despite similar TCC on milk (regular and lactose-free) and some PBMA types (soya and coconut PBMA), the contribution of total carbohydrate to the final energy content of milk was much lower (41.3% vs. 63.9% of total energy; Figure 1) than that in these PBMA types. For the remaining PBMA types, the TCC accounts for the majority of the total energy content in rice PBMA (89%), oat PBMA (75%), and coconut PBMA (71%). In contrast, the contribution is much lower in almond PBMA (59%) and hazelnut PBMA (51%), but still above half of total



**Figure 1.** The contribution of total carbohydrate content of regular milk, lactose-free milk, and plant-based milk alternative types to their total energy content.

energy content. Such differences between PBMA types may be attributed to variations in raw materials, as well as the addition or not of carbohydrates and oils to enhance the beverages' rheological properties (Slavin and Carlson, 2014; Bridges, 2018).

### Carbohydrate Profile

Milk and PBMA are distinct food matrices, thus a direct comparison of the carbohydrate profile is unfeasible due to their considerable differences, with glucose being the sole common sugar. Despite these limitations, important observations can still be made, which are vital for making informed dietary choices.

Regular milk averaged a lactose content of 4.6 g/100 mL, and glucose and galactose were not detected in regular milk samples (Table 3). These findings contradict the results of other authors, who reported the presence of glucose and galactose in regular milk (Walstra et al., 2006; Jeske et al., 2017; Walther et al., 2022). The lactose contents observed herein are in the range reported by other authors (Jeske et al., 2017; Bridges, 2018; Walther et al., 2022). In contrast, lactose-free milk presented 2.3 g/100 mL of glucose and 2.5 g/100 mL of galactose, whereas the lactose content was <LOQ (0.16 mg/mL), agreeing with Mäkinen et al. (2015). Furthermore, it has been reported that heat-treatment can affect the lactose content through its isomerization into lactulose, when milk is intensely heated (Walstra et al., 2006; Muehlhoff et al., 2013). Such effect was not detected in our milk samples, as lactulose was neither identified nor quantified, which contradicts the findings of other authors (Walstra et al., 2006; Manzi et al., 2013).

Regarding the carbohydrate profile of PBMA, it encloses 6 different carbohydrates, namely 2 monosaccharides (glucose and fructose), 2 disaccharides (maltose and sucrose), one trisaccharide (raffinose) and one sugar alcohol (sorbitol), distributed by different PBMA (Table 3). Sorbitol was the only carbohydrate quantified in all 6 PBMA types in evaluation, its content ranging between 0.6 and 3.2 g/100 mL, and it was associated with a strong statistical tendency ( $P = 0.05$ ). Sorbitol was the prime carbohydrate in both soya and oat PBMA, accounting for 54 and 35% of the total carbohydrate content. Sorbitol is a sugar alcohol commonly used as a sweetener due to its low caloric load, high sweetening capacity, and low glycemic response, making it a good alternative to sucrose in the diets of diabetics (Zhang et al., 2020). Thus, we hypothesize that the presence of sorbitol in the PBMA composition may be due to its addition during PBMA processing, even though it was not listed among the ingredients. However, it would be important to understand, when applicable, the percentage and origin of sorbitol that is added to these beverages, not only because it is a

food additive with laxative effect (due to its water holding ability; Peters and Lock, 1958; Zaccheria et al., 2017), but also, because it can be synthesized through lactose's hydrolysis and hydrogenation (Zaccheria et al., 2017; Zhang et al., 2020), which may raise questions regarding its source and use as a component for beverages that may be consumed by vegetarian and vegan consumers.

Glucose and fructose were simultaneously quantified in coconut, oat, rice, and soya PBMA, and their contents varied significantly ( $P < 0.05$ ) between them. Oat and rice PBMA displayed the highest contents of both glucose and fructose (averaging 2.6 and 0.09 g/100 mL, respectively), whereas soya presented the lowest content of both glucose and fructose (0.1 and 0.02 g/100 mL, respectively). Glucose accounted for 22%, 28%, and 34% of total carbohydrate content in rice, oat, and coconut PBMA, respectively, and fructose was responsible for 1% of total carbohydrate content in these 3 PBMA types. In soya PBMA, glucose and fructose accounted for 2% and 0.4% of total carbohydrate content, respectively. The presence of glucose in certain PBMA types might be attributed to the addition of gellan gum or maltodextrin, and the detected fructose could be linked to the inclusion of inulin. (Table 1). Finally, lactose-free milk presented a glucose content (2.3 g/100 mL) not significantly ( $P > 0.05$ ) different from the content presented by oat and rice PBMA.

The disaccharide sucrose was quantified in 4 PBMA types (soya, almond, coconut, and hazelnut) and its content differed significantly between PBMA types ( $P < 0.001$ ), being highest in almond and hazelnut PBMA types (averaging 1.7 g/100 mL, and accounting for 44% of total carbohydrate content), lowest in coconut PBMA (0.1 g/mL) and intermediate in soya (1.3 g/100 mL). The presence of sucrose in these PBMA types is due to its addition during the PBMA manufacturing process, as it is listed among the ingredients (Table 1).

Maltose was quantified in oat, rice, and coconut PBMA types, its content showed no significant differences ( $P > 0.05$ ) between PBMA types, averaging 3.1 g/mL. Mäkinen et al. (2015) have also reported maltose as a prime carbohydrate in rice PBMA and oat PBMA. Such higher maltose content, along with higher glucose contents, may be a consequence of starch hydrolysis throughout processing (Jeske et al., 2017). Conversely, coconut's PBMA high maltose content may indicate that, contrary to our initial stipulation of not including blended beverages, this group erroneously may have enclosed some beverages that are blended but do not report that information in packaging.

Raffinose was only quantified in soya PBMA and oat PBMA but without significant ( $P > 0.05$ ) differences in its contents (averaging 0.5 g/100 mL). We may hypothesize that such PBMA types presented raffinose in

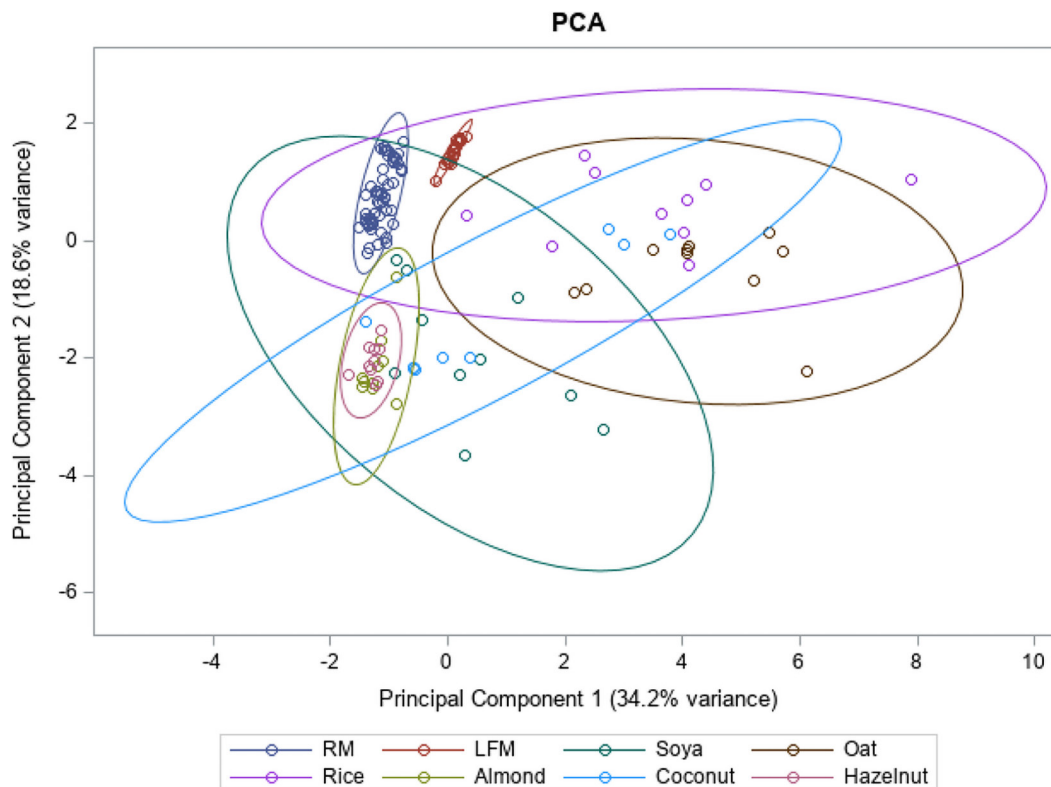
their carbohydrate profile because it was extracted from their raw materials during processing (Kuo et al., 1988; Elango et al., 2022). However, the consumption of raffinose by humans should be moderate, once humans lack  $\alpha$ -galactosidase, the enzyme required for raffinose digestion, leading to its fermentation by bacteria in the colon with consequent flatulence (Naczki et al., 1997; Han and Baik, 2006; Elango et al., 2022).

The carbohydrate content of PBMA varies widely, as seen in the results presented herein, influenced by the composition of the raw materials, and the type and amount of added carbohydrates (Bridges, 2018; Chalupa-Krebzdak et al., 2018; Scholz-Ahrens et al., 2020). It has been reported that apple concentrate, sucrose, or maple syrup, which are high in fructose or sucrose are used as sweeteners in PBMA production (Mäkinen et al., 2015; Jeske et al., 2017). Given that some carbohydrates are a crucial energy source in human nutrition, their excessive intake can lead to being overweight and having obesity (Cummings and Stephen, 2007; Maughan, 2009; WHO, 2021). The addition of carbohydrates to PBMA formulations is made to enhance their sensorial and rheological properties (Slavin and Carlson, 2014), but it might raise health concerns because this addition could lead to an increase in the glycemic index (GI), and potentially, raise

the risk of obesity, type 2 diabetes, and cardiovascular diseases (Ludwig, 2002; Bell and Sears, 2003; Brand-Miller, 2003; Chalupa-Krebzdak et al., 2018). Herein, we were unable to estimate the GI, but we have previously addressed this issue in a review article (Antunes et al., 2023). The GI values of PBMA and their potential effects on human health require additional research to fully assess the metabolic effects of PBMA and to refine their formulations for improved health outcomes. Additionally, it is noteworthy that milk, despite its high carbohydrate content compared with some PBMA, exhibits a moderate GI value. This is likely attributable to its high protein content, as the combination of lactose and high-quality milk protein seem to positively affect glucose homeostasis, resulting in lower GI values compared with many PBMA (Anderson et al., 2011; Kung et al., 2018). This suggests that lactose might play a role in managing blood glucose levels and decrease the risk of metabolic diseases.

### Principal Component Analysis

A PCA was accomplished using all the variables analyzed in this study to describe the data's variability in 2 dimensions. The score plot indicates that the first



**Figure 2.** Principal component plot for carbohydrate profile of cow milk (RM = regular milk; LFM = lactose-free milk) and plant-based milk alternatives (soya, oat, rice, almond, coconut, and hazelnut).

2 principal components (PC) account for 52.8% of the variability, with 34.2% attributed to PC1 and 18.6% to PC2. Figure 2 illustrates the positioning of each group of beverages within the multivariate space of these first 2 PC. There was a distinct separation between the regular and lactose-free milk samples, with the latter exhibiting much lower variability than the former, as indicated by the size of their ellipses. Soya PBMA and hazelnut PBMA were the only PBMA types whose ellipses overlapped with those of milk. Almond PBMA's ellipse was positioned close to that of regular milk, whereas the ellipses of the other PBMA types (oat, rice, and coconut) were distant from those of both regular and lactose-free milk. Based on the size of their ellipses, PBMA types exhibited significant variability both between different types and within samples of the same PBMA type. This variability might result from differences in PBMA formulation between brands, such as the number of dilutions of the vegetable extract and the use of additives as carbohydrates (Bridges, 2018; Scholz-Ahrens et al., 2020). Soya PBMA, rice PBMA, oat PBMA, and coconut PBMA displayed great variability, whereas almond PBMA and hazelnut PBMA showed much less variability. Furthermore, the overlap of the 2 ellipses indicates that the hazelnut PBMA samples are very similar to the almond PBMA samples.

## CONCLUSIONS

The carbohydrate content and profile of regular milk and lactose-free milk, already known, is limited to lactose or glucose plus galactose, and their contents remain constant between different brands. In contrast, each PBMA type presents a single carbohydrate profile and different TCC. The PBMA carbohydrate profile encloses different combinations of glucose, fructose, sucrose, maltose, raffinose, and sorbitol, which are presented in different amounts in the various PBMA types and even within the same type. Such variability between PBMA types and even within a single type is regarded as a negative attribute, because the lack of a standardized composition, may make an informed decision very difficult to take. This observed variability in PBMA is a significant limitation for their nutritional assessment. Based on the results presented here, PBMA can be an alternative to milk, especially in the diet of people with lactose intolerance. However, due to the differences between these products, PBMA cannot be considered as a milk substitute.

## NOTES

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**Nonstandard abbreviations used:** GI = glycemic index; LOD = the limit of detection; LOQ = the limit of quantification; PBMA = plant-based milk alternatives; PCA = principal component analysis; RT = retention time; TCC = total carbohydrate content.

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