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## Clean-label smoothies with apple and carrot pomaces: rheology and antioxidant stability

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Smoothies are thick blended beverages made from fruits, vegetables, and additional ingredients such as yogurt, rice beverages, or honey. In this study, smoothies with different formulations were developed using whey, a by-product of the cheese industry, together with a rice beverage as a plant-based alternative for the growing vegan and lactose-free market. In this study, clean-label smoothies were formulated using a commercial rice beverage, liquid whey, and a 50:50 blend of rice beverage and whey. Each of these liquid bases was further enriched with 5% (w/w) apple or 5% (w/w) carrot pomace powder. The impact of the formulations on viscosity was evaluated, along with their effect on total phenolic compounds and antioxidant properties before and after *in vitro* gastrointestinal digestion. The viscosity of carrot pomace-based smoothies was approximately five times higher than that of apple pomace formulations. Smoothies made with whey exhibited higher initial total phenolic content and antioxidant activity (through DPPH and FRAP assays). However, their post-digestion bioaccessibility was significantly reduced, likely due to protein–polyphenol interactions and lower pH. In contrast, rice beverage-based smoothies demonstrated greater retention of antioxidant capacity after digestion, especially those containing carrot pomace. These findings emphasize the importance of matrix effects on the bioaccessibility of functional compounds and suggest that rice beverage-based formulations with fruit/vegetable pomaces may be preferable for developing functional beverages with improved post-digestive bioactivity.

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### Sustainability spotlight

This study focuses on the reutilization of side streams generated by fruit juice and cheese processing industries—specifically, apple and carrot pomaces and whey. The valorization of these by-products aligns with the United Nations Sustainable Development Goal (UN SDG) 2, Zero Hunger, by promoting the use of abundant and cost-effective raw materials. Furthermore, by reducing food loss and waste, the study supports UN SDG 12, Responsible Consumption and Production. Finally, the development of functional smoothies enriched with nutrients, antioxidants, and phenolic compounds contributes to UN SDG 3, Good Health and Well-being.

## 1 Introduction

The global challenge of food waste in the agri-food sector needs to be seriously addressed, driving efforts toward more sustainable food systems.<sup>1,2</sup> One promising approach is the upcycling of food industry by-products into high-value functional ingredients,

contributing to zero-waste strategies and circular economy principles.<sup>3</sup> Although recent reports indicate a slight reduction in global food loss – 13.8% in 2016 to 13.3% in 2022 – significant volumes of nutritious waste still demand innovative reuse.<sup>4,5</sup>

Among the most abundant food by-products are whey, a major residue from cheese production, and fruit and vegetable pomaces, generated primarily by the juice industry. In Europe alone, cheese manufacturing produced over 47.6 million tons of liquid whey in 2023, which accounts for nearly 81.8% of the milk used.<sup>6</sup> Due to its high organic load, whey disposal poses serious environmental issues.<sup>7</sup> However, it also retains valuable nutrients, including lactose, proteins, minerals, and bioactive peptides such as lactoferrin,  $\beta$ -lactalbumin, serum albumin, and immunoglobulin. These peptides can have health benefits by reducing blood pressure and cholesterol and lowering the risk of certain cancers.<sup>8,9</sup>

Commercial rice beverages can be produced from broken kernels, a by-product of rice milling, and have a mild, naturally

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sweet taste, a high whiteness index, and neutral pH. As a result, they integrate well with various fruits in smoothies, making them an appealing ingredient. These beverages are rich in carbohydrates which can serve as a rapid source of energy, while remaining low in fat and free of lactose, which is beneficial for individuals with dietary restrictions or lactose intolerance. Moreover, rice beverages exhibit noteworthy antioxidant activity due to the presence of tocopherol, tocotrienols,  $\gamma$ -oryzanol, and phenolic compounds.<sup>10</sup> Concerning sustainability aspects, the carbon footprint of rice beverages and their contribution to greenhouse gas emissions are relatively lower than those of milk.<sup>11</sup>

Similarly, apple and carrot pomaces represent underutilized sources of dietary fiber and phytochemicals, especially polyphenols and carotenoids. Apple pomace (AP) alone generates an estimated 5 to 7 million tons of waste annually, and global carrot production in 2019 reached 44.8 million tons with the production of high quantities of carrot pomace (CP).<sup>2,12</sup> Therefore, the potential for sustainable valorization is substantial and recent studies support their incorporation into different food matrices to enhance nutritional value and bioactivity.<sup>13–16</sup>

In parallel, the consumer's demand for clean-label and plant-based products is reshaping the beverage industry. In this study, the term clean-label is used in a formulation-oriented sense, referring to products developed without synthetic preservatives, stabilizers, or added thickeners, rather than implying the absence of technological processing. Drying of pomaces and mild pasteurization were applied as processing steps to ensure ingredient stabilization and product safety, but no synthetic texturizing or preservative additives were used. Smoothies are increasingly recognized for their convenience, pleasant sensory profile, and health-promoting attributes. Compared to fruit juices, smoothies offer higher dietary fiber, antioxidant, and vitamin C content,<sup>17</sup> aligning well with the functional food trend. Also, their market is projected to grow at a compound annual growth rate (CAGR) of 10.1% between 2025 and 2033.<sup>18</sup>

Limited information is available regarding how different beverage matrices modulate the physicochemical properties and post-digestion bioaccessibility of bioactive compounds in smoothies enriched with upcycled plant side streams. While previous studies have separately explored the incorporation of fruit and vegetable pomaces into beverages, the use of whey in functional formulations, and antioxidant behavior after *in vitro* digestion, the combined effect of pomace type and liquid matrix on rheology, composition, and antioxidant retention after digestion remains insufficiently understood. In this context, the novelty of the present work lies in the integrated evaluation of apple and carrot pomaces incorporated into three different liquid bases (rice beverage, whey, and their 50 : 50 blend), with emphasis on matrix-dependent effects on viscosity and antioxidant stability after *in vitro* gastrointestinal digestion.

## 2 Materials and methods

### 2.1. Materials

The rice beverage (carbohydrates 7.5 g/100 mL, proteins 0.6 g/100 mL, lipids 1 g/100 mL, fiber < 0.5 g/100 mL, salt 0.1 g/100

mL, and calcium 120 mg/100 mL) was purchased from the local market. The acidic, unsalted whey was provided by Queijos Santiago, Portugal. Apple and carrot pomace, obtained from a juice processing company in Portugal (ALITEC—Alimentos Tecnológicos, S.A., Nazaré, Portugal), were submitted to a process of tunnel-drying (Tecnofruta, Valencia, Spain) under specific conditions (80–85 °C, 110 min, 55 Hz of air flow) and grinding to a mesh size between 0.71 mm and 0.075 mm (Fernetto, Vagos, Portugal). The apple pomace powders were from Gala, Fuji, and Granny Smith cultivars, and the carrot pomace was from Berlicum, Nantes, and Kuroda ones.

### 2.2. Methods

**2.2.1 Smoothie preparation.** Smoothies were prepared using a Thermomix kitchen robot (Vorwerk, Wuppertal, Germany), operated at 20 °C and medium speed (position 3) for an emulsification time of 60 s. The preparation process began with the addition of the main aqueous phase (rice beverage, whey or their 50 : 50 blend), followed by the incorporation of a small bit of commercial honey. After an initial homogenization step, lemon juice was added, and finally, the AP and CP powder was introduced. All ingredients were blended until a uniform texture was obtained. The exact proportions of ingredients used in each formulation (100 g each) are presented in Table 1.

Liquid whey was filtered before blending and the smoothie was pasteurized at 63 °C for 30 min.

**2.2.2 Proximate composition analysis.** A proximate composition analysis of smoothies was carried out following different international standard methods. Each analysis was carried out in triplicate.

The water content of each sample was determined according to the standard method AACC 44-15.02:<sup>19</sup> 2 g of each sample was weighed (Denver Instrument Company, TC-403) and placed in an oven (BINDER, ED56, Germany) at 105 ± 1 °C until constant weight. The total ash content was measured *via* incineration according to the standard method AACC 08-01.01 (ref. 20) using a muffle furnace (SNOL, Lithuania) at 550 ± 1 °C overnight. The ash was cooled in a desiccator and weighed using a digital scale (Denver Instrument Company, TC-403). The mineral contents (Na, K, Ca, Mg, P, S, Fe, Cu, Zn, Mn, and B) were determined by inductively coupled plasma optical-emission spectrometry (iCap Series-7000 Plus Series ICP-OES, ThermoFisher Scientific, Waltham, MA, USA) following the method described by AACC 40-75.01.<sup>21</sup>

The protein content of the samples was determined using a combustion method following ISO 16634-2:2016 (ref. 22) using DUMAS equipment (VELP SCIENTIFICA, NDA 702, Italy) and a conversion factor of 6.25. The samples were combusted at a temperature ranging from 800–1000 °C, converting all nitrogen forms to nitrogen oxides. These nitrogen oxides were then reduced to nitrogen gas (N<sub>2</sub>) and measured using a thermal conductivity detector.

The total dietary fiber (TDF) content was determined following the enzymatic–gravimetric method based on AOAC 985.29 and AACC 32-05.01 standards<sup>23,24</sup> using a Megazyme TDF assay kit (Wicklow, Ireland). Carbohydrate content of the samples was calculated by subtracting the sum of water content,



**Table 1** Formulation for smoothies with apple or carrot pomace + rice beverage (AR : CR), apple or carrot pomace + 50 : 50 rice beverage and whey (ARW : CRW) and apple or carrot pomace + whey (AW : CW)

Ingredients, g per 100 g						
Sample	Apple pomace	Carrot pomace	Rice beverage	Whey	Honey	Lemon juice
AR	5	—	92	—	2	1
CR	—	5	92	—	2	1
ARW	5	—	46	46	2	1
CRW	—	5	46	46	2	1
AW	5	—	—	92	2	1
CW	—	5	—	92	2	1

protein, fat, fiber and ash from 100% wet weight basis following the proximate analysis.

**2.2.3 Viscosity studies.** Viscosity measurements were performed using steady-state flow tests over a shear rate range of  $10^{-5}$  to  $500\text{ s}^{-1}$  at  $20 \pm 0.5\text{ }^{\circ}\text{C}$ . This range was within the measuring capability of the instrument, owing to its high low-torque sensitivity (minimum torque of  $0.01\text{ }\mu\text{N m}$  and a torque resolution of  $0.1\text{ nN m}$ ). Each measurement was carried out using 16 mL of each smoothie sample and a rheometer with a UTC-Peltier system (Haake Mars III, ThermoScientific, Dreieich, Germany) with a coaxial cylinder and rotor CC 25 DIN Ti (with a 6 mm gap). The flow behavior was analyzed using the Carreau model (eqn (1)), which was fitted to the experimental viscosity *versus* shear rate data using OriginPro 2019 software (OriginLab, Northampton, MA, USA).

$$\eta(\dot{\gamma}) = \eta_{\infty} + (\eta_0 - \eta_{\infty})[1 + (\lambda\dot{\gamma})^a]^{\frac{n-1}{a}} \quad (1)$$

In the Carreau model (eqn (1)), the parameters are  $\eta(\dot{\gamma})$  – the viscosity as a function of shear rate,  $\eta_0$  – the zero shear-rate viscosity (Pa s),  $\eta_{\infty}$  – the infinite shear-rate viscosity (Pa s),  $\lambda$  – the relaxation time – a time constant (s),  $\dot{\gamma}$  – the shear rate ( $\text{s}^{-1}$ ),  $a$  – a transition exponent, and  $n$  – the power-law index.

**2.2.4 Color evaluation and  $a_w$  and pH measurement.** Color measurements were performed using the CIE  $L^*a^*b^*$  color coordinate system with a Chroma Meter CR-400 colorimeter (Konica Minolta Business Technologies, Inc., Tokyo, Japan). The instrument was calibrated using the white standard plate ( $Y = 86.7$ ;  $x = 0.3160$ ;  $y = 0.3233$ ) prior to each measurement.

The pH was measured in triplicate using a PHM 92 Lab pH meter (Radiometer, Denmark) equipped with a glass electrode. Calibration was performed with standard buffer solutions at pH 4.0, 7.0, and 9.0, before analysis. Water activity ( $a_w$ ) of the samples was measured using a LabMaster-aw neo device (Novasina AG, Lachen, Switzerland) after calibrating with a SAL-T salt standard at 97% relative humidity.

**2.2.5 Sugar and organic acid composition.** The sample extracts (1 : 20 v/v) were diluted in 25 mM sulfuric acid. Organic acids and sugars were separated using a high-performance liquid chromatography (HPLC) system (Chromaster, Hitachi, Tokyo, Japan) equipped with a UV-Vis detector (model 5420) monitored at 210 nm for organic acids and a refractive index (RI) detector (model 5450) for sugars. Separation was carried out using an ion-exclusion column (Rezex<sup>TM</sup> ROA Organic Acid H<sup>+</sup> (8%),  $300 \times 7.8\text{ mm}$ , Phenomenex, Torrance, CA, USA)

maintained at  $65\text{ }^{\circ}\text{C}$ . The mobile phase consisted of 5 mM sulfuric acid at a flow rate of  $0.5\text{ mL min}^{-1}$ .

Organic acids were identified and quantified using standard solutions of oxalic, citric, lactic, and acetic acids over a concentration range of 0 to  $20\text{ g L}^{-1}$ . The results were expressed in  $\text{g L}^{-1}$  of each corresponding acid, based on the following calibration curves: oxalic acid ( $y = 3.87 \times 10^{-8}$ ,  $R^2 = 0.999$ ), citric acid ( $y = 4.12 \times 10^{-7}$ ,  $R^2 = 0.999$ ), lactic acid ( $y = 7.83 \times 10^{-7}$ ,  $R^2 = 0.999$ ), and acetic acid ( $y = 7.32 \times 10^{-7}$ ,  $R^2 = 0.999$ ). Sugar identification and quantification were performed using maltotriose, maltose, xylose, and fructose as standards in the range of 0 to  $40\text{ g L}^{-1}$ , with calibration curves of  $y = 1.36 \times 10^{-6}$  ( $R^2 = 0.997$ ) for maltotriose,  $y = 1.36 \times 10^{-6}$  ( $R^2 = 0.999$ ) for maltose,  $y = 1.37 \times 10^{-6}$  ( $R^2 = 0.999$ ) for xylose, and  $y = 1.43 \times 10^{-6}$  ( $R^2 = 0.999$ ) for fructose.

All experiments were conducted in triplicate, and data interpretation was carried out using ChromQuest software, version 4.2.

## 2.2.6 Total phenolic content and antioxidant activity

**2.2.6.1 Extract preparation.** The extraction was performed by the addition of 20 mL of EtOH to 2 g of the smoothie sample (1 : 10 ratio, w/v) followed by mixing for 6 h at room temperature under protection from light. Then, the supernatants were obtained through centrifugation at  $3340 \times g$  for 10 min at  $20\text{ }^{\circ}\text{C}$ . The supernatants were subsequently filtered and freeze-dried until further analysis.

**2.2.6.2 Total phenolic content.** Total Phenolic Content (TPC) was determined following the Folin-Ciocalteu (FC) method using a microplate reader. The analysis was performed according to the procedure previously described,<sup>25</sup> with modifications:<sup>26</sup> 20  $\mu\text{L}$  of sample was poured into a 96-well microplate (Nalge Nunc International, Rochester, NY, USA), followed by the addition of 100  $\mu\text{L}$  of FC reagent (1 : 4). The mixture was further left to settle for 5 min in the dark at room temperature. Then, 80  $\mu\text{L}$  of 7.5% sodium carbonate solution was added and the microplate was kept for 2 h in the dark at room temperature. After the incubation period, absorbance was measured at 760 nm using a Multiskan GO microplate reader (Thermo Scientific Waltham, MA, USA). The standard curve was prepared from a water solution of gallic acid at concentrations of 0 to  $120\text{ mg L}^{-1}$  ( $R^2 = 0.987$ ). The results were expressed as mg gallic acid equivalent per gram of sample (mg GAE per g).

**2.2.6.3 DPPH assay.** The antioxidant activity was assessed using the DPPH radical scavenging assay adapted to a 96-well



microplate (Nalge Nunc International, Rochester, NY, USA). Briefly, 20  $\mu\text{L}$  of each sample extract was added to a well, followed by the addition of 180  $\mu\text{L}$  of a 60  $\mu\text{M}$  methanolic DPPH solution (prepared fresh). The plate was incubated in the dark at room temperature for 30 min. After incubation, absorbance was measured at 517 nm using a Multiskan GO microplate reader (Thermo Scientific Waltham, MA, USA). The results were expressed as  $\mu\text{mol}$  Trolox equivalent per gram of sample ( $\mu\text{mol}$  TEAC per g). The standard curve was prepared from a Trolox solution at concentrations of 0 to 120  $\mu\text{mol L}^{-1}$  ( $R^2 = 0.996$ ).

**2.2.6.4 FRAP assay.** The Ferric Reducing Antioxidant Power (FRAP) assay was performed using a 96-well microplate methodology. The FRAP reagent was prepared freshly by mixing 10 mL of 300 mM acetate buffer (pH 3.6), 1 mL of 20 mM ferric chloride hexahydrate (in distilled water) and 1 mL of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM HCl. For the assay, 25  $\mu\text{L}$  of sample extract was added to each well of the microplate (Nalge Nunc International, Rochester, NY, USA), followed by 175  $\mu\text{L}$  of the FRAP reagent. The plate was incubated in the dark at room temperature for 30 min. Absorbance was measured at 595 nm using a Multiskan GO microplate reader (Thermo Scientific Waltham, MA, USA). The results were expressed as  $\mu\text{mol}$  Trolox equivalent per gram of sample ( $\mu\text{mol}$  TEAC per g). The standard curve was prepared from a Trolox solution at concentrations of 0 to 120  $\mu\text{mol L}^{-1}$  ( $R^2 = 0.999$ ).

**2.2.7 In vitro digestion.** The static *in vitro* digestion of smoothie samples was carried out according to the standardized INFOGEST 2.0 protocol.<sup>27,28</sup> Each sample (2.5 g) was mixed with 5 mL of simulated salivary fluid (SSF) containing salivary amylase (75 U  $\text{mL}^{-1}$ ) and incubated for 2 min under gentle agitation. Subsequently, 10 mL of simulated gastric fluid (SGF) containing pepsin (2000 U  $\text{mL}^{-1}$ ) and gastric lipase (60 U  $\text{mL}^{-1}$ ) was added, and the mixture was adjusted to pH 3.0 and incubated for 120 min at 37 °C under continuous agitation. After the gastric phase, 20 mL of simulated intestinal fluid (SIF) containing pancreatin (100 U  $\text{mL}^{-1}$ ) and bile salts (10 mM) was added, the pH was adjusted to 7.0, and the mixture was incubated for another 120 min at 37 °C. The enzyme activities and the bile concentration were measured according to the assays described in the INFOGEST 2.0 protocol (Brodkorb *et al.*,<sup>27</sup> 2019; Duarte *et al.*,<sup>28</sup> 2022). The enzyme activity in the intestinal phase was stopped by the cold shock using the ice bath. The supernatants were collected after the centrifugation at 3340 $\times$ g for

10 min, followed by freeze-drying, and then kept in a freezer ( $-18$  °C) until the analysis.

**2.2.8 Data analysis.** All experiments were performed in triplicate. Data were analyzed using one-way analysis of variance (ANOVA), which was performed using SPSS version 29.0.1.0 (IBM, Armonk, NY, USA). *Post-hoc* comparisons were performed by using the Tukey test. The significance level was set at  $p < 0.05$ . Data are expressed as average  $\pm$  standard deviation.

## 3 Results and discussion

### 3.1. Proximate analysis

The nutritional composition of the smoothies (Table 2) underscores how the use of the rice beverage, whey, or their 50:50 blend and pomace type influences the water, protein, fat, carbohydrate, fiber, and ash contents of the formulations.

Water content varied considerably among smoothies' formulations. The highest values were observed in those prepared exclusively with liquid whey, particularly CW (92.2%) and AW (91.8%). This is consistent with the fact that liquid whey has a lower solid content, ranging from 90.1 to 96.5% water content.<sup>29</sup> In contrast, rice-based smoothies such as AR (83.6%) and CR (83.9%) have lower water content, as rice beverages generally contain around 84.7–94.2% water,<sup>30</sup> depending on added ingredients like oil, sugars, or thickeners. Intermediate values were found in the 50:50 blended formulations, CRW (88.4%) and ARW (87.7%), which combined both the rice beverage and whey. These findings show that liquid whey makes a major contribution to the total water content in the smoothies.

Protein content increased with whey incorporation: smoothies containing whey (ARW, CRW, AW, and CW) showed higher protein content (0.68% to 0.83%) compared to those made only with the rice beverage (AR: 0.6% and CR: 0.6%). As expected, AW (0.8%) and CW (0.8%) showed the highest protein content, consistent with whey's well-known protein richness. As a by-product of cheese manufacturing, whey is particularly high in soluble, bioavailable proteins such as  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin,<sup>31,32</sup> which are complete proteins with all essential amino acids.<sup>33,34</sup> In contrast, the rice beverage is made from a cereal grain, rice, and is naturally low in protein.<sup>35</sup>

Regarding fat content, no significant differences were observed among smoothies, rice beverages, and whey, apart

**Table 2** Proximate composition of smoothies (g per 100 g w/w). Results are expressed in % of "as is" (wet weight) basis as average  $\pm$  standard deviation ( $n = 3$ )<sup>a</sup>

Sample	Water content	Protein	Fat	Carbohydrate	Fiber	Ash
Rice beverage	88.7 $\pm$ 0.07 <sup>d</sup>	0.1 $\pm$ 0.00 <sup>d</sup>	0.5 $\pm$ 0.05 <sup>b</sup>	10.3 $\pm$ 0.17 <sup>b</sup>	n/a	0.3 $\pm$ 0.03 <sup>cd</sup>
Whey	95.0 $\pm$ 0.02 <sup>a</sup>	0.6 $\pm$ 0.00 <sup>bc</sup>	0.5 $\pm$ 0.16 <sup>b</sup>	3.3 $\pm$ 0.01 <sup>e</sup>	n/a	0.4 $\pm$ 0.01 <sup>bc</sup>
AR	83.6 $\pm$ 0.03 <sup>h</sup>	0.6 $\pm$ 0.01 <sup>c</sup>	0.9 $\pm$ 0.14 <sup>b</sup>	11.9 $\pm$ 0.11 <sup>a</sup>	2.6 $\pm$ 0.02 <sup>a</sup>	0.4 $\pm$ 0.03 <sup>bc</sup>
CR	83.9 $\pm$ 0.10 <sup>g</sup>	0.7 $\pm$ 0.06 <sup>bc</sup>	1.5 $\pm$ 0.52 <sup>a</sup>	11.5 $\pm$ 0.26 <sup>a</sup>	2.2 $\pm$ 0.04 <sup>b</sup>	0.6 $\pm$ 0.03 <sup>a</sup>
ARW	87.7 $\pm$ 0.01 <sup>f</sup>	0.7 $\pm$ 0.02 <sup>bc</sup>	0.6 $\pm$ 0.09 <sup>b</sup>	9.1 $\pm$ 0.06 <sup>c</sup>	1.6 $\pm$ 0.01 <sup>c</sup>	0.4 $\pm$ 0.02 <sup>cd</sup>
CRW	88.4 $\pm$ 0.07 <sup>e</sup>	0.7 $\pm$ 0.03 <sup>ab</sup>	0.6 $\pm$ 0.09 <sup>b</sup>	8.6 $\pm$ 0.14 <sup>c</sup>	1.2 $\pm$ 0.01 <sup>d</sup>	0.5 $\pm$ 0.08 <sup>ab</sup>
AW	91.8 $\pm$ 0.10 <sup>c</sup>	0.8 $\pm$ 0.07 <sup>a</sup>	0.7 $\pm$ 0.08 <sup>b</sup>	5.7 $\pm$ 0.10 <sup>d</sup>	0.9 $\pm$ 0.02 <sup>e</sup>	0.3 $\pm$ 0.07 <sup>d</sup>
CW	92.2 $\pm$ 0.14 <sup>b</sup>	0.8 $\pm$ 0.02 <sup>a</sup>	0.4 $\pm$ 0.08 <sup>b</sup>	5.5 $\pm$ 0.08 <sup>d</sup>	0.7 $\pm$ 0.00 <sup>f</sup>	0.4 $\pm$ 0.07 <sup>cd</sup>

<sup>a</sup> Different letters mean different significant results (Tukey's HSD;  $p \leq 0.05$ ). n/a: Not available.



from CR, which exhibited the highest fat level. This result may be attributed to the heterogeneous composition of the pomace and the uneven distribution of carrot peel.<sup>45</sup>

The carbohydrate content of the smoothies was inversely affected by whey addition. As shown in Table 2, an increase in whey content resulted in a significant reduction in carbohydrate levels. Smoothies formulated solely with the rice beverage, such as AR (11.9 ± 0.11%) and CR (11.5 ± 0.26%), exhibited the highest carbohydrate content. In contrast, whey-based formulations, AW (5.7 ± 0.10%) and CW (5.5 ± 0.08%), demonstrated markedly lower values. These findings align with the existing literature on the composition of liquid whey and rice, the latter being well recognized as a rich source of carbohydrates.<sup>36,37</sup>

Maximum fiber content was present in the rice-based smoothies, particularly AR (2.6%) and CR (2.2%), reflecting the fiber-rich nature of the added pomaces. The higher fiber in apple-based smoothies when compared to carrot-based ones is in line with previously reported differences in dietary fiber content between apple and carrot pomace, where apple pomace showed considerably higher levels of total dietary fiber.<sup>16</sup> Since whey is a filtered dairy liquid lacking dietary fiber,<sup>38</sup> its inclusion diluted fiber levels in the mixed formulations: ARW (1.6%) and CRW (1.2%). The whey-only smoothies, AW (0.9%) and CW (0.7%), showed the lowest fiber content, and the absence of rice beverage contributed to this reduction.

Ash, as a parameter of overall mineral content, was also influenced by the smoothie composition (Table 2). As expected, carrot-based smoothies showed higher ash content, particularly CR (0.6%), CRW (0.5%), and CW (0.4%), because of the natural composition of carrot that, being a root, is highly enriched in minerals.<sup>16,39</sup> Apple smoothies (AR, ARW, and AW) yielded lower ash content, the lowest being observed in AW (0.3%).

As shown in Table 3, smoothies formulated with carrot pomace contained higher levels of potassium, calcium, sulfur and manganese, whereas apple-based smoothies were richer in sodium, magnesium, phosphorus, iron and zinc. The addition of liquid whey notably increased the concentrations of potassium, calcium, magnesium, sulfur, and manganese, particularly in the case of carrot-based smoothies, thereby enhancing the overall mineral density of the beverages. These trends reflect the strategic role of ingredient selection in modulating the nutritional profile of functional smoothies. For instance, mineral enrichment can support specific health outcomes, including bone health (*via* calcium, magnesium, and phosphorus), electrolyte balance (sodium and potassium), and antioxidant defense mechanisms (magnesium and zinc).<sup>40</sup> The relatively high standard deviations observed for some minerals, especially in ARW and CRW, likely reflect the natural heterogeneity of the pomaces and the intrinsic variability associated with mineral distribution in upcycled plant matrices. Further research is needed to evaluate the bioaccessibility of these minerals after the consumption of smoothies.

### 3.2. Physicochemical parameters

**3.2.1 Viscosity.** The rheological behavior of the smoothies was evaluated according to the Carreau model, commonly

**Table 3** Mineral contents of the smoothies. The results are the average of triplicates, shown as the average ± standard deviation, and are reported as mg of mineral per 100 g (mg per 100 g)<sup>a</sup>

Smoothies	Na	K	Ca	Mg	P	S	Fe	Cu	Zn	Mn	B
AR	125.6 ± 2.10 <sup>a</sup>	232.8 ± 3.14 <sup>c</sup>	22.1 ± 0.43 <sup>c</sup>	56.8 ± 1.07 <sup>ab</sup>	88.7 ± 1.72 <sup>a</sup>	54.1 ± 7.61 <sup>b</sup>	1.6 ± 0.38 <sup>c</sup>	0.2 ± 0.01 <sup>abc</sup>	0.9 ± 0.03 <sup>a</sup>	0.4 ± 0.01 <sup>c</sup>	0.5 ± 0.03 <sup>a</sup>
CR	103.1 ± 7.60 <sup>b</sup>	273.9 ± 20.86 <sup>c</sup>	35.2 ± 3.78 <sup>ab</sup>	47.2 ± 3.44 <sup>ab</sup>	96.5 ± 6.69 <sup>a</sup>	51.2 ± 3.67 <sup>b</sup>	1.7 ± 0.10 <sup>c</sup>	0.3 ± 0.06 <sup>ab</sup>	0.8 ± 0.10 <sup>a</sup>	0.4 ± 0.03 <sup>c</sup>	0.4 ± 0.02 <sup>ab</sup>
ARW	112.2 ± 16.35 <sup>b</sup>	298.9 ± 45.10 <sup>c</sup>	38.1 ± 6.54 <sup>ab</sup>	50.5 ± 7.67 <sup>ab</sup>	107.7 ± 17.15 <sup>a</sup>	54.0 ± 8.19 <sup>b</sup>	5.0 ± 0.73 <sup>ab</sup>	0.3 ± 0.04 <sup>a</sup>	0.7 ± 0.11 <sup>a</sup>	0.4 ± 0.07 <sup>c</sup>	0.4 ± 0.04 <sup>ab</sup>
CRW	84.7 ± 6.41 <sup>c</sup>	422.5 ± 31.80 <sup>b</sup>	36.8 ± 1.87 <sup>ab</sup>	48.6 ± 3.60 <sup>ab</sup>	51.2 ± 4.11 <sup>b</sup>	62.6 ± 5.20 <sup>a</sup>	2.2 ± 0.15 <sup>bc</sup>	0.1 ± 0.02 <sup>d</sup>	0.3 ± 0.08 <sup>c</sup>	2.3 ± 0.15 <sup>b</sup>	0.6 ± 0.04 <sup>ab</sup>
AW	97.6 ± 2.56 <sup>b</sup>	263.3 ± 6.69 <sup>c</sup>	33.3 ± 1.61 <sup>bc</sup>	45.2 ± 1.17 <sup>b</sup>	90.9 ± 2.56 <sup>a</sup>	48.0 ± 1.51 <sup>b</sup>	2.1 ± 0.18 <sup>bc</sup>	0.2 ± 0.01 <sup>bcd</sup>	0.6 ± 0.02 <sup>ab</sup>	0.4 ± 0.01 <sup>c</sup>	0.3 ± 0.01 <sup>ab</sup>
CW	102.5 ± 9.36 <sup>b</sup>	511.4 ± 48.00 <sup>a</sup>	46.7 ± 7.73 <sup>a</sup>	59.1 ± 5.47 <sup>a</sup>	63.1 ± 6.38 <sup>b</sup>	77.3 ± 6.60 <sup>a</sup>	2.8 ± 0.22 <sup>b</sup>	0.2 ± 0.03 <sup>cd</sup>	0.5 ± 0.15 <sup>bc</sup>	2.8 ± 0.24 <sup>a</sup>	0.8 ± 0.07 <sup>b</sup>

<sup>a</sup> Different letters mean different significant results (Tukey's HSD;  $p \leq 0.05$ ).



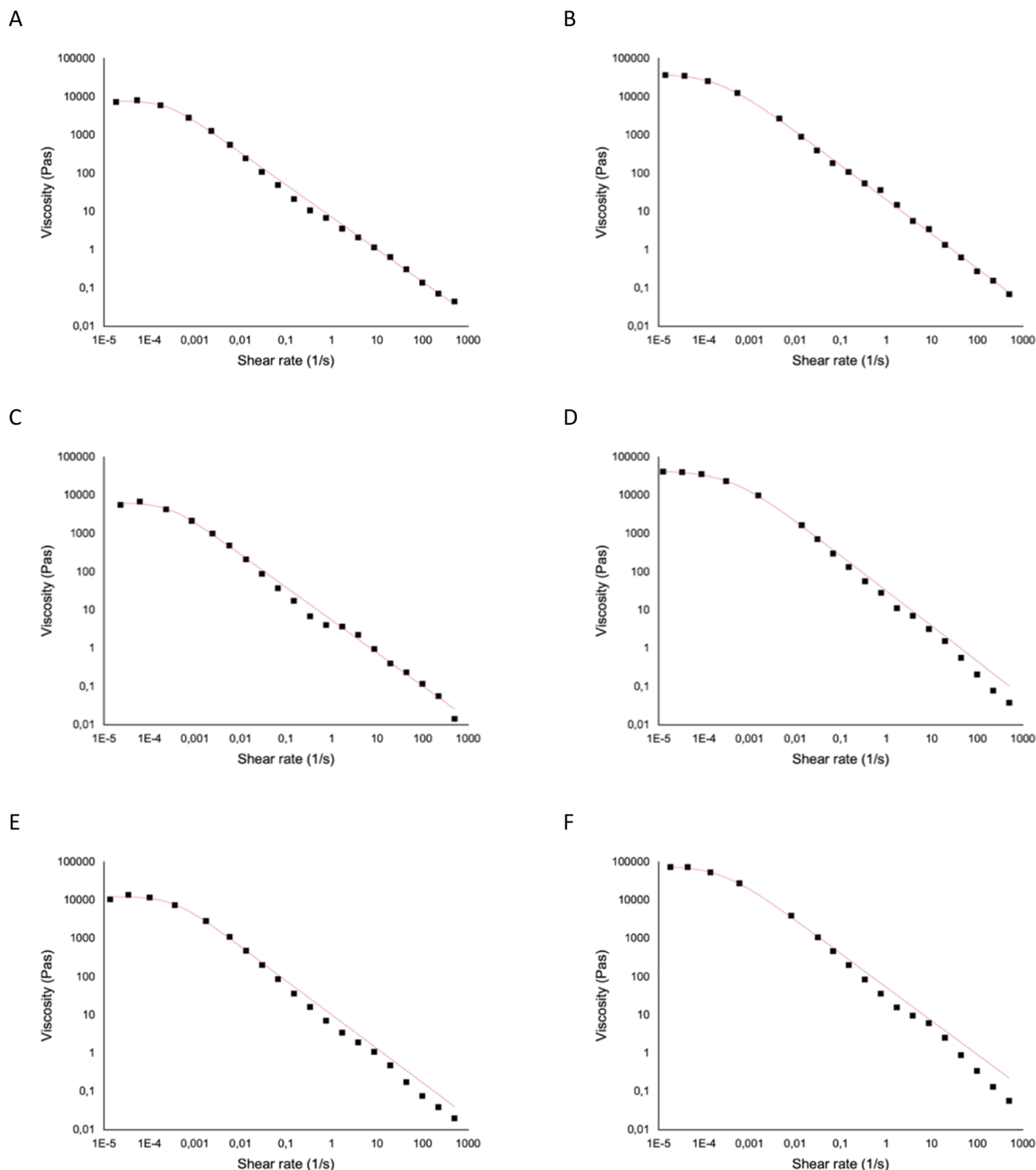


Fig. 1 Viscosity of smoothies: (A) apple pomace and rice beverage AR; (B) carrot pomace and rice beverage CR; (C) apple pomace with rice beverage and whey ARW; (D) carrot pomace with rice beverage and whey CRW; (E) apple pomace and whey AW; and (F) carrot pomace and whey CW.

applied to describe the flow characteristic of non-Newtonian fluids, in this case, the most common, shear-thinning systems. From the model (eqn (1)) application, significant differences in the flow properties of smoothies with variable composition were obtained as a function of the pomace type

(apple or carrot) and level of liquid whey incorporation (Fig. 1 and Table 4).

The zero-shear rate viscosity ( $\eta_0$ ), which describes fluid behavior at rest or very low shear rates, is presented in Table 4. Carrot pomace-based smoothies exhibited markedly higher  $\eta_0$



**Table 4** Rheological parameters from the Carreau model for the smoothies. The results are the average of triplicates, shown as the mean  $\pm$  standard deviation<sup>a</sup>

Smoothies	$\eta_0$ (Pa s)	$\lambda$ (s)	$a$	$n$
AR	6648.6 $\pm$ 1126 <sup>c</sup>	3868.3 $\pm$ 432.08 <sup>a</sup>	1.7 $\pm$ 0.18 <sup>a</sup>	0.2 $\pm$ 0.02 <sup>a</sup>
CR	35 460.7 $\pm$ 10 018.39 <sup>b</sup>	3910.6 $\pm$ 800.98 <sup>a</sup>	1.1 $\pm$ 0.23 <sup>bc</sup>	0.1 $\pm$ 0.02 <sup>a</sup>
ARW	6192.9 $\pm$ 148.78 <sup>c</sup>	2662.6 $\pm$ 930.25 <sup>a</sup>	1.3 $\pm$ 0.13 <sup>b</sup>	0.1 $\pm$ 0.07 <sup>a</sup>
CRW	41 844.4 $\pm$ 4922.17 <sup>ab</sup>	2339.2 $\pm$ 492.50 <sup>a</sup>	1.0 $\pm$ 0.09 <sup>c</sup>	0.1 $\pm$ 0.05 <sup>a</sup>
AW	11 465.3 $\pm$ 1080 <sup>c</sup>	3223.8 $\pm$ 388.07 <sup>a</sup>	1.5 $\pm$ 0.13 <sup>ab</sup>	0.1 $\pm$ 0.03 <sup>a</sup>
CW	60 282.5 $\pm$ 250.46 <sup>a</sup>	3855.3 $\pm$ 250.46 <sup>a</sup>	1.0 $\pm$ 0.07 <sup>c</sup>	0.1 $\pm$ 0.01 <sup>a</sup>

<sup>a</sup> Different letters mean different significant results (Tukey's HSD;  $p \leq 0.05$ ).

values – CR (35 460.7 Pa s), CRW (41 844.4 Pa s) and CW (60 282.5 Pa s) – reflecting a more viscous matrix.<sup>41</sup> Also the highest viscosity was observed when carrot pomace powder was incorporated into the gluten-free batter compared to apple and orange pomace powders. In contrast, apple pomace formulations, AR (6648.6 Pa s), ARW (6192.9 Pa s) and AW (11 465.3 Pa s), displayed much lower  $\eta_0$  values. As many studies state, for example,<sup>42</sup> the viscosity is mainly influenced by soluble dietary fibers (SDFs), in this case, mainly due to the composition and structural differences of SDF in apple and carrot pomace.<sup>43,44</sup> Additionally, the smaller particles of carrot pomace<sup>45</sup> create a larger surface area and have higher water holding and swelling capacity<sup>46</sup> and this facilitates the formation of more extensive, complex networks within the fluid matrix, increasing the resistance against the flow, therefore viscosity. The high  $\eta_0$  values obtained for carrot-based smoothies indicate the presence of a semi-solid structure under near-rest conditions. However, these samples showed marked shear-thinning behavior, with viscosity decreasing substantially as the shear rate increased, which is consistent with their pourable character despite high  $\eta_0$  values.

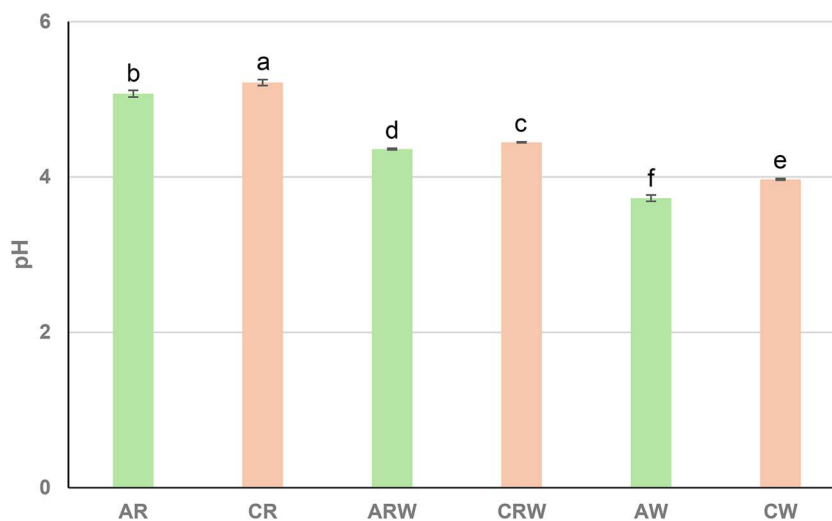
The relaxation time ( $\lambda$ ), as seen in eqn (1), representing the onset of shear-thinning behavior, did not differ significantly

between formulations. However, differences emerged in the transition index ( $a$ ), which describes how rapidly viscosity decreases with increasing shear rate;<sup>47</sup> for the apple pomace-based smoothies, AR, ARW and AW, slightly higher  $a$ -values ranging from 1.3 to 1.7 were observed, suggesting a smoother, more gradual transition from Newtonian to shear-thinning behavior. Carrot pomace formulations, with lower  $a$ -values (1.0–1.1), underwent a sharper transition, indicating more pronounced shear-thinning.

All formulations had flow behavior indices ( $n$ )  $< 1$ , confirming their shear-thinning flow, typical of fiber- and pulp-rich beverages.

Overall, rheology properties were strongly influenced by pomace type and, to a lesser extent, by whey addition. Carrot pomace, particularly when combined with whey, produced the highest viscosities and stronger structural networks, consistent with a gel-like texture. Apple pomace yielded more fluid smoothies with weaker structures.

**3.2.2 Water activity.** Water activity ( $a_w$ ) is an important parameter to determine the microbial stability, enzymatic activity, and chemical reactivity of food systems.<sup>48</sup> In the current research, as expected, all smoothie formulations exhibited high



**Fig. 2** pH of the smoothies. Results are expressed as average  $\pm$  standard deviation ( $n = 3$ ), followed by a letter. Different letters mean significantly different results (Tukey's HSD;  $p \leq 0.05$ ). AR is apple pomace and rice beverage; CR is carrot pomace and rice beverage; ARW is apple pomace with rice beverage and whey; CRW is carrot pomace with rice beverage and whey; AW is apple pomace and whey; and CW is carrot pomace and whey.



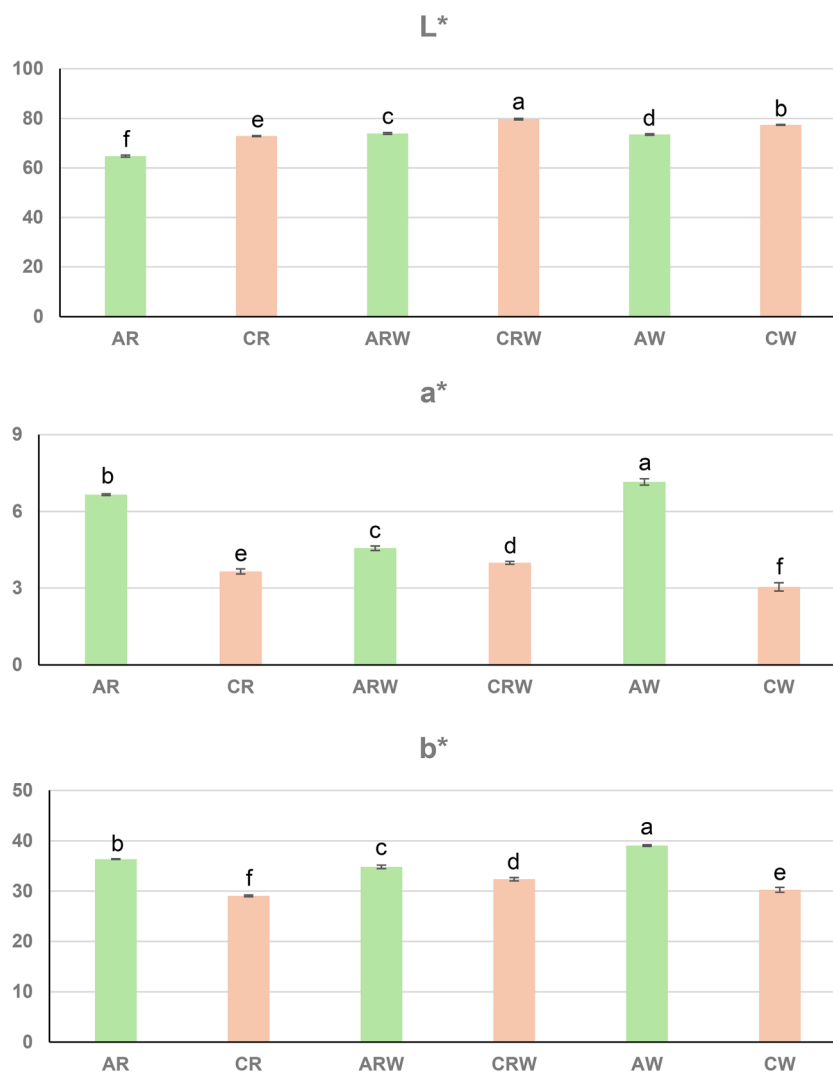


Fig. 3 The color parameters of smoothies: ( $L^*$ ) lightness, ( $a^*$ ) balance between redness and greenness, ( $b^*$ ) balance between yellowness and blueness. Results are expressed as average  $\pm$  standard deviation ( $n = 10$ ), followed by a letter. Different letters mean significantly different results (Tukey's HSD;  $p \leq 0.05$ ). AR is apple pomace and rice beverage; CR is carrot pomace and rice beverage; ARW is apple pomace with rice beverage and whey; CRW is carrot pomace with rice beverage and whey; AW is apple pomace and whey; and CW is carrot pomace and whey.

water activity, ranging from 0.966 (CW) to 0.971 (ARW and CRW), consistent with values reported for high water content beverages.<sup>49</sup> These results indicate that none of the formulations are shelf-stable at room temperature, without additional preservation strategies, such as pasteurization and refrigeration, likely due to their high-water activity, which promotes microbial growth.<sup>49,50</sup>

**3.2.3 pH.** The pH of foods is an important parameter that dictates taste, microbial stability, and ingredient interactions in food systems.<sup>51</sup> In this study (Fig. 2), the pH values of the smoothies were statistically different and varied from 5.21 (CR) to 3.73 (AW), reflecting the individual influence of the incorporation of the different pomaces and the addition of whey. Rice beverage-based smoothies (AR and CR) exhibited higher pH values, ranging from 5.07 to 5.21, consistent with the relatively neutral character of rice-based beverages. CR, containing carrot pomace, showed the highest pH value (5.21). Conversely, the incorporation of whey led to a considerable reduction in pH

that was expressed in both ARW (pH 4.36) and CRW (pH 4.45). The lowest pH values were recorded in AW (3.73) and CW (3.97), suggesting that the presence of the whey had an impact on the final pH, resulting in more acidic smoothies.

**3.2.4 Color measurements.** Color is a key sensory attribute that strongly influences consumer perception and acceptance of food products. The CIE  $L^*a^*b^*$  color system that measures the three dimensions of color, lightness ( $L^*$ ), red-green balance ( $a^*$ ), and yellow-blue balance ( $b^*$ ) was used to identify the color attributes of the smoothie samples (Fig. 3).

$L^*$  values ranged from 64.79 (AR) to 79.75 (CRW), indicating notable differences in the lightness of the smoothie samples. Apple pomace with rice beverage (AR) produced the darkest smoothie, which may be associated with oxidation of phenolic compounds during processing.<sup>52</sup> In contrast, CRW (carrot pomace with rice beverage and whey) exhibited the highest  $L^*$  value, indicating a lighter and more reflective appearance.



**Table 5** Sugar composition of smoothies. The results are the average of triplicates, shown as the average  $\pm$  standard deviation,<sup>a</sup> and are reported as g L<sup>-1</sup>

Smoothies	Maltotriose	Maltose	Xylose	Fructose
AR	1.42 $\pm$ 0.01 <sup>b</sup>	1.42 $\pm$ 0.01 <sup>b</sup>	0.29 $\pm$ 0.03 <sup>d</sup>	21.22 $\pm$ 0.16 <sup>a</sup>
CR	1.67 $\pm$ 0.03 <sup>a</sup>	1.66 $\pm$ 0.03 <sup>a</sup>	nd	13.28 $\pm$ 0.04 <sup>c</sup>
ARW	0.94 $\pm$ 0.02 <sup>c</sup>	0.93 $\pm$ 0.02 <sup>c</sup>	3.00 $\pm$ 0.55 <sup>c</sup>	21.58 $\pm$ 0.02 <sup>a</sup>
CRW	0.37 $\pm$ 0.01 <sup>d</sup>	0.37 $\pm$ 0.01 <sup>d</sup>	4.21 $\pm$ 0.02 <sup>b</sup>	15.38 $\pm$ 1.02 <sup>b</sup>
AW	nd	nd	6.81 $\pm$ 0.11 <sup>a</sup>	21.50 $\pm$ 0.49 <sup>a</sup>
CW	nd	nd	6.54 $\pm$ 0.03 <sup>a</sup>	12.98 $\pm$ 0.48 <sup>c</sup>

<sup>a</sup> Different letters mean different significant results (Tukey's HSD;  $p \leq 0.05$ ).

The  $a^*$  values, which reflect the red–green color balance, varied significantly depending on the type of pomace used. AW (7.16) and AR (6.66), both containing apple pomace, showed the most intense red coloration—likely due to the presence of polyphenolic compounds that undergo browning or oxidation during processing.<sup>53</sup> In contrast, CW (3.05) and CR (3.65), which included carrot pomace, had lower  $a^*$  values, indicating less red hue, consistent with the carotenoid-rich profile of carrots.<sup>54</sup> All the smoothie samples showed positive  $b^*$  values, which indicated that yellow color was dominant. AW (39.07) and AR (36.38) had the highest  $b^*$  values, which indicated high yellow color, possibly intensified by the apple's color pigments.<sup>55</sup> The content of whey could also be responsible for the increased yellowish hue since it is a by-product from cheese manufacturing.<sup>56</sup> CR (29.06) and CW (30.26) had lower  $b^*$  values, which indicated a less intense yellow color.

### 3.3. Sugars and organic acids

According to Table 5, maltotriose and maltose appeared to be present only in the rice beverage-containing formulations (AR, ARW, CR, and CRW), but not in whey-only formulations (AW and CW). The highest concentrations were in the carrot pomace–rice beverage product (CR), where 1.67  $\pm$  0.03 and 1.66  $\pm$  0.03 g L<sup>-1</sup> of maltotriose and maltose were present, respectively. The presence of these disaccharides and trisaccharides, products of starch hydrolysis, ascertains the predominant contribution of the rice beverage.<sup>57</sup> Inclusion of whey in rice beverage formulations (ARW and CRW) resulted in a significant decline ( $p < 0.05$ ) in maltose and maltotriose content. For

instance, ARW had only 0.94  $\pm$  0.02 g L<sup>-1</sup> of maltotriose as compared to 1.42  $\pm$  0.01 g L<sup>-1</sup> in AR.

Xylose concentration varied noticeably among samples, with the highest amounts found in whey formulas without the rice beverage (AW: 6.81  $\pm$  0.11 g L<sup>-1</sup>; CW: 6.54  $\pm$  0.03 g L<sup>-1</sup>). Xylose is a pentose sugar and a major structural component of plant cell walls.<sup>58,59</sup> It mainly originates from xyloglucans and water-insoluble hemicelluloses, particularly xylans, which are constituents of the insoluble dietary fiber fraction of pomace.<sup>60</sup> The higher xylose content observed in AW and CW may suggest that whey influenced the release or extractability of hemicellulose-derived sugars from apple and carrot pomace. Because no targeted mechanistic analyses were performed, this observation should be interpreted with caution. The differences among formulations may reflect matrix-related effects associated with the physicochemical environment of the smoothies, including pH and composition, rather than a specific hydrolytic mechanism.

Interestingly, the addition of the rice beverage was associated with lower xylose levels, whereas formulations containing whey showed higher xylose concentrations. These results indicate that the liquid matrix influenced xylose occurrence in the final products, although the underlying mechanism remains to be elucidated.<sup>61–63</sup>

Interestingly, addition of the rice beverage appeared to suppress xylose release. No xylose was detected in CR (carrot pomace + rice beverage), whereas in AR it was present only at low levels (0.29  $\pm$  0.03 g L<sup>-1</sup>). With addition of whey (ARW and CRW), xylose content increased considerably to 3.00  $\pm$  0.55 and 4.21  $\pm$  0.02 g L<sup>-1</sup>, respectively.

The highest fructose content was found in all apple pomace smoothies (AR, ARW, and AW), between 21.22  $\pm$  0.16 and 21.58  $\pm$  0.02 g L<sup>-1</sup>, which confirms apple pomace as the primary source of this monosaccharide. Carrot pomace smoothies (CR, CRW, and CW), however, had notably lower contents of fructose ( $p < 0.05$ ), ranging between 12.98  $\pm$  0.48 and 15.38  $\pm$  1.02 g L<sup>-1</sup>. These receive support in the literature in which carrot pomace was found to have lower fructose content compared to apple pomace.<sup>13,45,60</sup>

Table 6 presents the organic acid profile across the six smoothie formulations, revealing different patterns according to ingredient type.

Oxalic acid was remarkably low in all samples (0.01–0.04 g L<sup>-1</sup>), without significant differences ( $p > 0.05$ ) between the

**Table 6** Organic acid composition of smoothies. The results are the average of triplicates, shown as the average  $\pm$  standard deviation,<sup>a</sup> and are reported as g L<sup>-1</sup>

Smoothies	Oxalic	Citric	Lactic	Acetic	Total organic acid
AR	0.01 $\pm$ 0.01 <sup>a</sup>	0.75 $\pm$ 0.01 <sup>c</sup>	0.29 $\pm$ 0.03 <sup>d</sup>	0.09 $\pm$ 0.00 <sup>d</sup>	1.14
CR	0.01 $\pm$ 0.00 <sup>a</sup>	1.02 $\pm$ 0.02 <sup>c</sup>	nd	0.07 $\pm$ 0.01 <sup>d</sup>	1.1
ARW	0.03 $\pm$ 0.01 <sup>a</sup>	1.86 $\pm$ 0.19 <sup>ab</sup>	4.21 $\pm$ 0.02 <sup>b</sup>	0.36 $\pm$ 0.13 <sup>c</sup>	6.46
CRW	0.03 $\pm$ 0.02 <sup>a</sup>	1.51 $\pm$ 0.01 <sup>b</sup>	3.00 $\pm$ 0.55 <sup>c</sup>	0.91 $\pm$ 0.20 <sup>b</sup>	5.45
AW	0.04 $\pm$ 0.01 <sup>a</sup>	2.36 $\pm$ 0.04 <sup>a</sup>	6.81 $\pm$ 0.11 <sup>a</sup>	0.93 $\pm$ 0.01 <sup>b</sup>	10.14
CW	0.02 $\pm$ 0.00 <sup>a</sup>	2.02 $\pm$ 0.03 <sup>ab</sup>	6.54 $\pm$ 0.03 <sup>a</sup>	1.38 $\pm$ 0.04 <sup>a</sup>	9.96

<sup>a</sup> Different letters mean different significant results (Tukey's HSD;  $p \leq 0.05$ ).



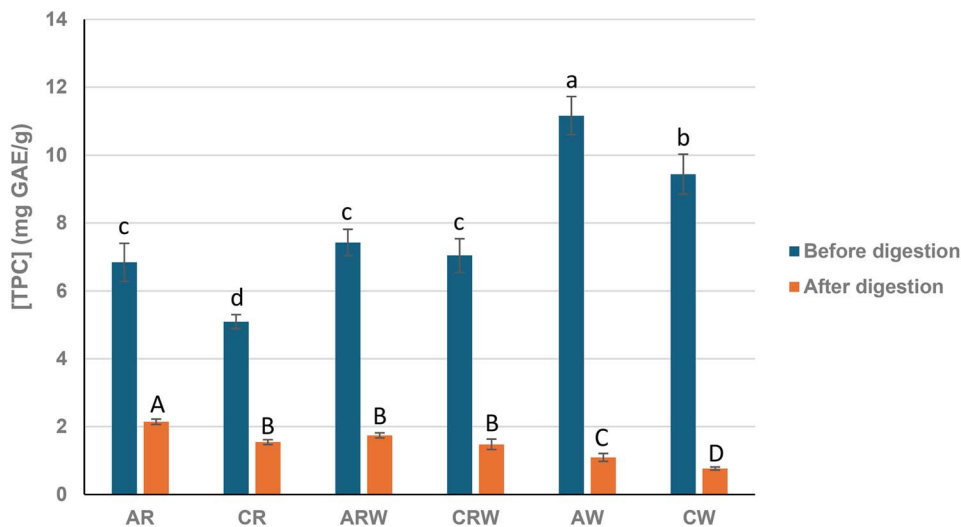


Fig. 4 Total phenolic content of smoothies, expressed as mg GAE per g. Results are expressed as average  $\pm$  standard deviation ( $n = 3$ ), followed by a letter. Different letters mean significantly different results (Tukey's HSD;  $p \leq 0.05$ ). AR is apple pomace and rice beverage; CR is carrot pomace and rice beverage; ARW is apple pomace with rice beverage and whey; CRW is carrot pomace with rice beverage and whey; AW is apple pomace and whey; and CW is carrot pomace and whey.

formulations. Oxalic acid, a compound commonly found in plants, can chelate minerals and inhibit their absorption.<sup>64,65</sup> Therefore this acid is considered an antinutritional organic compound, alongside tannins, saponins, protease inhibitors, and phytic acid.<sup>66</sup>

Citric acid contents varied significantly, from  $0.75 \pm 0.01 \text{ g L}^{-1}$  in AR to  $2.36 \pm 0.04 \text{ g L}^{-1}$  in AW. Whey-based formulations (ARW, CRW, AW, and CW) contained higher levels of citric acid ( $1.51\text{--}2.36 \text{ g L}^{-1}$ ), as citric acid is naturally present in dairy products.<sup>36,67,68</sup> The highest level observed in AW ( $2.36 \text{ g L}^{-1}$ ) supports this, indicating that the whey is the predominant contributor. Also, apple pomace contributes to citric acid content to a higher extent than carrot pomace.<sup>69</sup>

The concentration of lactic acid was greatly influenced by whey. It reached a maximum of  $6.81 \pm 0.11 \text{ g L}^{-1}$  in AW and  $6.54 \pm 0.03 \text{ g L}^{-1}$  in CW. These high values could be due to the occurrence of lactic acid fermentation in the whey by which sugar molecules were converted into lactic acid.<sup>68,70</sup> Intermediate levels of ARW and CRW ( $4.21$  and  $3.00 \text{ g L}^{-1}$ , respectively) support this presumption. The highest content of acetic acid was found in CW ( $1.38 \pm 0.04 \text{ g L}^{-1}$ ) and AW ( $0.93 \pm 0.01 \text{ g L}^{-1}$ ), possibly due to the fermentation of lactose during cheese production.<sup>71</sup>

The total concentration of organic acids in the smoothies is consistent with the measured pH values (Fig. 2), highlighting the significant contribution of the whey's acid profile, primarily dominated by lactic acid, to the overall acidity.

### 3.4. Bioactive studies

**3.4.1 Total phenolic content.** As shown in Fig. 4, smoothies formulated with whey (AW:  $11.2 \pm 0.56 \text{ mg GAE per g}$ ; CW:  $9.4 \pm 0.59 \text{ mg GAE per g}$ ) exhibited the highest TPC. This can be due to the ability of whey to enhance solubilization and extractability of phenolic compounds from pomaces, resulting in higher yields of extraction.<sup>72</sup> Smoothies combining the rice

beverage with whey (ARW:  $7.4 \pm 0.39 \text{ mg GAE per g}$ ; CRW:  $7.0 \pm 0.49 \text{ mg GAE per g}$ ) also presented TPC values that were generally higher than the TPC values of those prepared with the rice beverage alone. In contrast, formulations only with the rice beverage (AR:  $6.8 \pm 0.56 \text{ mg GAE per g}$ ; CR:  $5.1 \pm 0.21 \text{ mg GAE per g}$ ) showed the lowest TPC values, suggesting that the rice beverage matrix is less efficient than whey in promoting phenolic extraction.

After *in vitro* digestion, a marked reduction in TPC was observed in all formulations, as expected, due to the degradation of phenolic compounds under acidic gastric conditions and enzymatic activity, which can limit bioaccessibility.<sup>73</sup> Interestingly, the relative decrease in AR ( $2.1 \pm 0.08 \text{ mg GAE per g}$ ) and CR ( $1.5 \pm 0.07 \text{ mg GAE per g}$ ) was less pronounced than that in whey-containing smoothies. This effect may be related to the buffering and protective role of dietary fiber against phenolic degradation during digestion.<sup>74</sup> Such protection may arise from non-covalent interactions, including hydrogen bonding, and covalent linkages between phenolic compounds and dietary fiber components, particularly pectin.<sup>75</sup> These interactions suggest that apple and carrot pomace can partially shield phenolic compounds in the gastrointestinal environment.<sup>73</sup> It should also be noted that the INFOGEST protocol is a static *in vitro* model and therefore does not account for intestinal absorption, colonic microbiota metabolism, or the dynamic physiological conditions of the human gastrointestinal tract.<sup>27</sup> Consequently, the results should be interpreted as an estimate of potential bioaccessibility rather than a direct measure of *in vivo* bioavailability.

**3.4.2 Antioxidant potential.** Before digestion, the highest DPPH radical scavenging capacity and ferric reducing antioxidant power (FRAP) were observed in whey-containing smoothies AW ( $18.0 \pm 0.92$  and  $26.5 \pm 0.47 \mu\text{mol TEAC per g}$ ) and CW ( $16.3 \pm 0.60$  and  $25.7 \pm 0.27 \mu\text{mol TEAC per g}$ ), respectively (Fig. 5



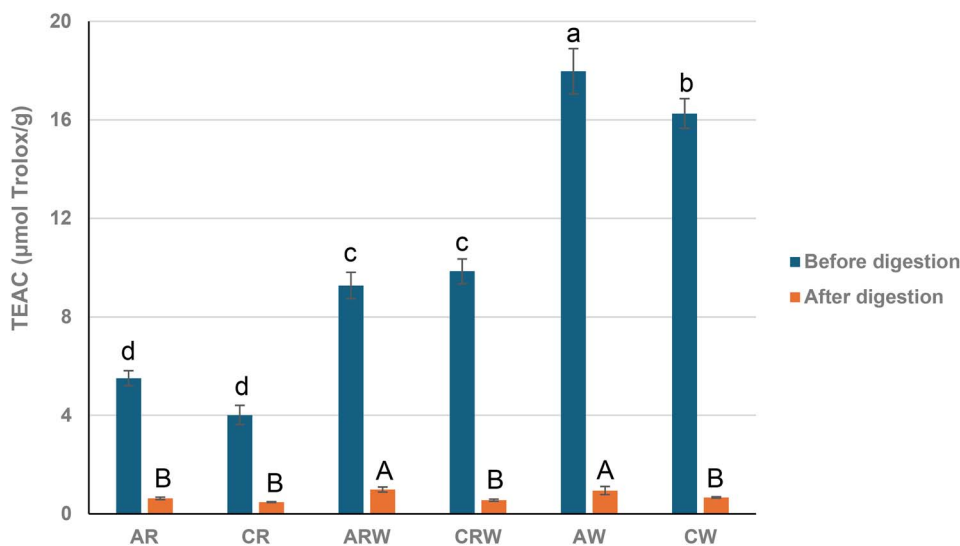


Fig. 5 Results of antioxidant potential DPPH assay of smoothies, expressed as  $\mu\text{mol TEAC per g}$ . Results are expressed as average  $\pm$  standard deviation ( $n = 3$ ), followed by a letter. Different letters mean significantly different results (Tukey's HSD;  $p \leq 0.05$ ). AR is apple pomace and rice beverage; CR is carrot pomace and rice beverage; ARW is apple pomace with rice beverage and whey; CRW is carrot pomace with rice beverage and whey; AW is apple pomace and whey; and CW is carrot pomace and whey.

and 6). These results suggest that whey enhances antioxidant potential, likely by improving the solubilization and release of phenolic compounds from pomace during extraction. Intermediate antioxidant activity was found in the mixed formulations (ARW:  $9.3 \pm 0.53$  and  $17.3 \pm 1.03$   $\mu\text{mol TEAC per g}$ ; CRW:  $9.8 \pm 0.50$  and  $16.8 \pm 1.17$   $\mu\text{mol TEAC per g}$ ) for DPPH and FRAP, respectively, highlighting the influence of the formulation matrix on extractability and antioxidant expression.

After *in vitro* digestion, all formulations experienced a marked decline in antioxidant potential, with the greatest reduction observed in whey-based smoothies: AW ( $0.9 \pm 0.17$

and  $1.0 \pm 0.15$   $\mu\text{mol TEAC per g sample}$ ) and CW ( $0.7 \pm 0.03$  and  $0.8 \pm 0.09$   $\mu\text{mol TEAC per g sample}$ ) for DPPH and FRAP, respectively.

Despite their initially high antioxidant activity, AW and CW lost most, probably due to degradation of sensitive compounds (e.g., polyphenols) under acidic gastric conditions.<sup>73</sup> In contrast, rice beverage-pomace based smoothies (AR and CR) retained a relatively higher proportion of their initial activity with 11.50% and 12.10% retention for DPPH, respectively (Table 7). A similar trend was observed for the FRAP assay (Fig. 6), where AR and CR showed  $9.9 \pm 0.98\%$  and  $12.3 \pm 0.33\%$  retention,

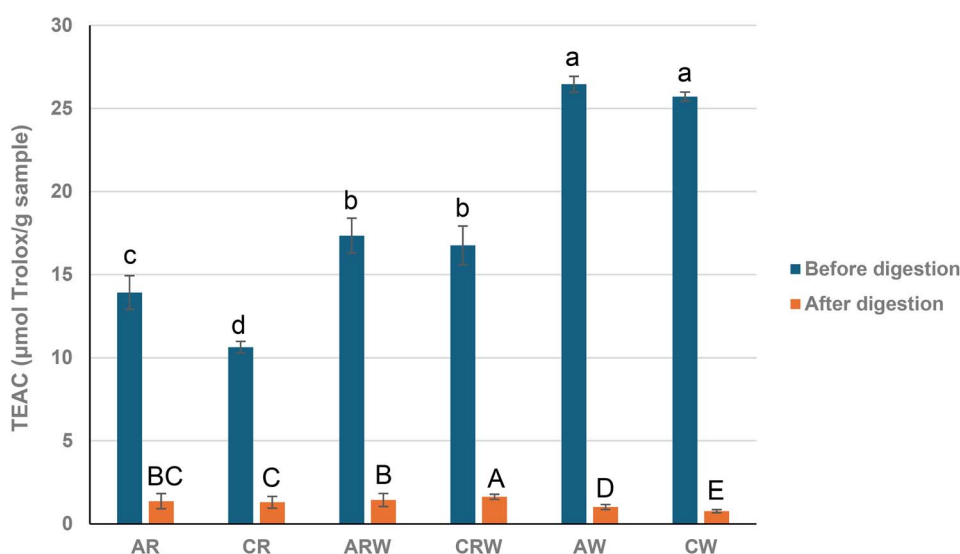


Fig. 6 Results of antioxidant potential from FRAP assay of smoothies, expressed as  $\mu\text{mol TEAC per g sample}$ . Results are expressed as average  $\pm$  standard deviation ( $n = 3$ ), followed by a letter. Different letters mean significantly different results (Tukey's HSD;  $p \leq 0.05$ ). AR is apple pomace and rice beverage; CR is carrot pomace and rice beverage; ARW is apple pomace with rice beverage and whey; CRW is carrot pomace with rice beverage and whey; AW is apple pomace and whey; and CW is carrot pomace and whey.



**Table 7** The retention percentage of TPC and antioxidant activity after *in vitro* digestion of smoothies<sup>a</sup>

Smoothies	TPC (%)	DPPH (%)	FRAP (%)
AR	31.4 ± 1.38 <sup>a</sup>	11.5 ± 1.43 <sup>a</sup>	9.9 ± 0.98 <sup>a</sup>
CR	30.3 ± 2.28 <sup>a</sup>	12.1 ± 1.53 <sup>a</sup>	12.3 ± 0.33 <sup>b</sup>
ARW	23.4 ± 0.99 <sup>b</sup>	10.7 ± 0.60 <sup>a</sup>	8.3 ± 0.35 <sup>bc</sup>
CRW	21.2 ± 3.45 <sup>b</sup>	5.7 ± 0.61 <sup>b</sup>	9.8 ± 0.83 <sup>c</sup>
AW	9.8 ± 1.30 <sup>c</sup>	5.3 ± 0.80 <sup>b</sup>	3.9 ± 0.10 <sup>d</sup>
CW	8.2 ± 0.85 <sup>c</sup>	4.1 ± 0.13 <sup>b</sup>	3.0 ± 0.03 <sup>d</sup>

<sup>a</sup> Different letters mean different significant results (Tukey's HSD; *p* ≤ 0.05).

respectively. This protective effect may be attributed to the dietary fiber in pomace and the buffering capacity of the rice beverage matrix, which can reduce phenolic degradation during digestion.

## 4 Conclusion

This study underscores the crucial role of ingredient selection in defining the nutritional, physicochemical, and functional attributes of clean-label smoothies. The whey-based smoothies contained more water, protein, xylose, and organic acids like lactic, citric, and acetic acids and were lower in pH. In contrast, rice beverage-based smoothies contained much higher levels of maltotriose, maltose, carbohydrates and dietary fiber. The pomace type also played a role. The inclusion of carrot pomace considerably increased viscosity, ash content, and mineral content (potassium, calcium, and magnesium). Apple pomace increased dietary fiber and fructose content, and, similar to whey, trended towards a lower pH. Apple pomace-based smoothies were darker and more brownish; however, the whey-based smoothies were all lighter in color.

Regarding stability of bioactive compounds, whey smoothies (AW and CW) had higher antioxidant potential but lower retention after *in vitro* digestion. This would suggest that whey proteins interact with phenolic compounds such that they become less bioaccessible. In contrast, rice beverage-based smoothies (AR and CR) lost a smaller proportion of bioactive compounds upon digestion. This improved stability is likely to be a result of the ability of the rice beverage as a buffer and the protective effect of dietary fiber, notably from carrot pomace.

In summary, the findings point towards a trade-off between initial antioxidant capacity and post-digestion bioaccessibility. While whey-based systems offer greater initial antioxidant capacity, rice-based smoothies may offer greater functional benefits upon digestion.

## Author contributions

Conceptualization, J. F., A. L. and I. S.; methodology, J. F., A. L. and I. S.; validation, J. F., A. L. and I. S.; formal analysis, S. S., P. C., J. F., A. L. and I. S.; investigation, S. S. and P. C.; data curation, S. S., P. C. and J. F.; writing – original draft preparation, S. S., P. C. and J. F.; writing – review and editing, J. F., A. L. and I. S.; visualization, J. F., A. L. and I. S.; supervision, J. F., A. L.

and I. S.; project administration, J. F. and I. S.; funding acquisition, I. S. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

All the data generated or analyzed during this study are included in the manuscript.

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