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Effect of *in vitro* gastrointestinal digestion on the antioxidant properties of fruit and vegetable powdered smoothies reinforced with WPC80

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ABSTRACT

The development of novel powdered smoothies obtained by spray-drying (SD) technology can contribute to revaluing fruits and vegetables from seasonal surplus. The aim of this study was to evaluate the effect of *in vitro* gastrointestinal digestion (GID) on the retention, activity and bioaccessibility of key antioxidants of two smoothies elaborated with fruits (apple, orange and banana) or vegetables (pumpkin, apple and carrot), fortified with whey protein concentrate 80 g/100g (WPC80) and spray-dried. α-Tocopherol, ascorbic acid, polyphenol and flavonoid contents, and antioxidant capacity were determined in the smoothies reconstituted with water or semi-skimmed milk. Bioaccessibility and remaining antioxidant capacity after digestion was calculated. This study demonstrated contained smoothies were rich in sugar, protein and fibre. These smoothie powders retained most antioxidant properties intact. The reconstitution of smoothies with semi-skimmed milk means higher flavonoids and vitamin E content. *In vitro* GID showed a positive effect on the antioxidant properties of these smoothies by improving the bioaccessibility of TPC and favouring the scavenging ABTS radical, although the content of flavonoids and tocopherol was decreased. These results confirmed that powdered smoothies show high nutritional values and considerable antioxidant activity as a health-enhancing fortified food. Digestive simulations provide information on the potential bioaccessibility of antioxidants.

1. Introduction

Fruits and vegetables are characterized as low-calorie foods, but rich in carbohydrates, fibre, minerals, water-soluble vitamins (such as vitamin B complex and vitamin C), as well as bioactive compounds like flavonoids, carotenoids, tannins, among others (Dantas et al., 2018; Razola-Díaz et al., 2022). Numerous studies have supported that a balanced diet including fruit and vegetables not only reduces risk of cardiovascular disease, diabetes, metabolic syndrome (Silveira et al., 2015), but also increases life expectancy (Razola-Díaz et al., 2022). The World Health Organization (WHO) recommends consuming at least 400 g of fruit and vegetables per day (WHO, 2003). However, the adoption of unhealthy habits, such as lack of exercise, consumption of ultra-processed foods and low fruit and vegetable intake, is contributing to the increase in chronic non-communicable diseases (Dilrukshi & Senarath, 2021).

In recent years, there has been an increase in demand for new and convenient food products with nutritional properties easily accessible to consumers. Fruit and vegetable smoothies have gained popularity among ready-to-drink beverages, due to their sensory qualities, nutritional benefits and simplicity of preparation and consumption, making them suitable for people of all ages (Cano-Lamadrid et al., 2018). In addition, smoothies can represent the main meal, breakfast or as a healthy snack between meals, depending on the portion (Dilrukshi & Senarath, 2021). However, fruit and vegetable smoothies lack nutrients such as protein or essential fatty acids, which has prompted the development of fortified smoothies. Protein products are now among the leading trend products. The range of these is becoming increasingly versatile (Bäuerle & Kühn, 2022). For instance, smoothies enriched with flax seeds (high in protein, fibre and omega-3 fatty acids) (Shahada et al., 2024); amaranth seeds (high in lysine) and milk (Upadhyay et al., 2023); folic acid and iron (addition of spinach) (Qalbi et al., 2023); kefir with spinach

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and Kale (Yilmaz-Ersan et al., 2023); cow's milk and yoghurt (Neelissen et al., 2023); mother forest extracts (Waszkiewicz et al., 2023); microalgae extracts (*Chlorella vulgaris* and *Dunaliella salina*) (Sahin & Ozturk, 2021) or with plant extracts such as sage and rosemary (Gérard et al., 2019; Ivanišová et al., 2015).

Each year, around 50 million tons of fruit and vegetables grown in Europe are discarded due to unacceptable size and for being misshapen according to current market requirements (Porter et al., 2018). Products not meeting these standards are lost from the food supply chain. According to United Nations data, food waste is a growing concern. Around 10–50% of that food waste is made up of root crops, fruits and vegetables (FAO, 2013). Currently, the food industry is faced with the efficient management of perishable products such as fruits and vegetables (Dilrukshi & Senarath, 2021), the production of smoothies could contribute to a decrease in food waste. However, fresh smoothies have a limited shelf life, therefore, extending shelf life while preserving their nutritional and sensory characteristics remains a major challenge for the food industry (Dantas et al., 2018). It is therefore desirable to make smoothie powders in order to obtain stable and easily formulated food (addition of nutrients) that can be easily reconstituted in water/milk.

Drying techniques have been used since ancient times to extend the shelf life of food products. Freeze-drying and spray-drying are the most used drying techniques for obtaining powdered food. Freeze-drying has been used to freeze-dry fruit and vegetable smoothies (Dilrukshi & Senarath, 2021), pumpkin (Nur Dirim, & Çaliçkan, 2012), apple (Rajkumar & Devi Ganesan, 2021). Powder has been obtained from various fruits by pulverization, including avocado (Dantas et al., 2018), blackberry (Fazaeli et al., 2012), apple (Qadri et al., 2023) among others. Powdered products, at the industrial level, offer some advantages such as microbiological and oxidative stability and reduced volume or weight. In addition, reduced packaging and easier handling facilitate storage and transportation of large quantities without the need for chilling and extending the shelf life of perishable products (Fazaeli et al., 2012). In powdered smoothies, homogenization and drying with hot air can degrade some thermosensitive and easily oxidizable components from fruit and vegetables, including antioxidants such as vitamin C, with the consequent nutritional detriment, although dehydration prevents rapid loss of antioxidants that occur in fresh smoothies (Picouet et al., 2016).

Analysis of GID on the composition and antioxidant activity of processed foods has contributed to understanding of the influence of processing on health. *In vitro* digestion models are widely used to study the digestibility, release and bioaccessibility of food compounds by simulating gastrointestinal conditions; moreover, they lack the ethical concerns of *in vivo* assays (Ribeiro et al., 2019b). The availability in the body of bioactive compounds present in food depends on their bioaccessibility, resistance to digestion and intestinal absorption capacity, since only compounds which resist gastrointestinal conditions can have beneficial effects on the human body (Caponio et al., 2022). Several studies have detected the presence of antioxidants (including polyphenols) in the bioaccessible fraction of fresh drinks or smoothies (Buniowska et al., 2019; Ribeiro et al., 2019b). Nevertheless, scarce information is available on powdered smoothies and on the effects of reconstitution method before consumption on the antioxidant properties of specific compounds such as α-tocopherol after *in vitro* digestion. INFOGEST is a static digestion method which, using fixed proportions of food sample and digestive fluids, simulates digestion processes without the need for animal or human experiments. The main advantage of this method is that it allows the definition of standardised experimental conditions and procedures based on physiological data as close as possible to *in vivo* conditions, while keeping the method simple to reproduce. Other advantages include reproducibility, robustness, simplicity, relatively low cost and easy evaluation of each phase of digestion. In fact, *in vivo* comparison shows a good correlation with the INFOGEST method at the endpoints of each digestion step. However, this method also has limitations as it cannot mimic the dynamics of the digestion process and the physiological interactions with the host (it can lead to failure of probiotic survival); for example, for the gastric phase, the gradual addition of gastric fluid and gradual gastric emptying is missing (Brodkorb et al., 2019).

The aim of the present study was to evaluate the effect of *in vitro* digestion on the retention, activity and bioaccessibility of key antioxidants in two powdered smoothies elaborated with fruit or vegetable and reinforced with WPC80. The influence of the reconstitution method (with water or semi-skimmed milk) was also studied.

2. Material and methods

2.1. Powdered smoothies manufacturing

Two novel powdered smoothies were manufactured in the Pilot Plant of IRTA, Monells, Girona, Spain. Both were formulated using surplus fruits and vegetables. The fruit smoothie was elaborated (g/100g) with apple 42.8, orange 34.2, banana 8.6 and whey protein concentrate 80% (WPC80) 14.4. The vegetable smoothie (g/100g) with pumpkin 39.5, apple 29.7, carrot 19.8 and WPC80 11. (Pumpkin: *Cucurbita pepo*, carrot: *Daucus carota* var. *Nantaise*, L., oranges: *Citrus sinensis* var. *Navellate*, apples: *Pyrus malus* var. *Golden Delicious*, bananas: *Musa cavendishii* var. *Pequeña Enana*). Fruits and vegetables were purchased in a local market and WPC80 was provided by Induxtra de Suministros, (Banyoles, Girona). Fresh smoothies were mixed with WPC80 and then powdered in a spray-drying equipment pilot model (evaporation capacity of 0.5–6 kg/h; Minor Mobile, GEA Niro, Denmark). Drying conditions were air temperature condition of 80 °C outlet temperature with the respective inlet temperature of 220 °C. The feed (flowrate: 48.3 g/min) was atomized by using 2 mm nozzle and compressed air at 3 bar. Finally, the powders were kept for up to 1 month at room temperature in darkness until their characterisation or reconstitution.

2.2. Assessment of powders

2.2.1. pH, water activity and colour

The pH was measured by homogenising 3 g powder in 12.5 ml distilled water using a GLP21 pH meter (Crison, Barcelona, Spain) equipped with a combination electrode, Cat. No. 52-21 (Ingold Electrodes, Wilmington, North Carolina, USA). Water activity was measured by weighing 5 g powder using a LabMaster hygrometer (Novasina AG, CH-8853 Lachen, Switzerland). Instrumental colour was directly measured in triplicate on the powder surface using a CR-200/08 Chroma Meter II (Minolta Ltd., Milton Keynes, UK) with a D65 illuminant, an observation angle of 2 and an aperture of 50 mm. Results were expressed as CIELab values: lightness (L*) redness (a*) and yellowness (b*).

2.2.2. Proximate composition

Total protein content was determined by the Kjeldahl method (AOAC reference No. 955.04, 2000) using a K-435 digestion unit (Buchi Ibérica S.L.U, Barcelona, Spain), a KT-200 Kjeltec distillation unit (Foss Analytical Co., Ltd., Hillerod, Denmark) and an Automatic Titrino 702 SM (Metrohm AG, Herisau, Switzerland). Total ash content was determined by gravimetry according to AOAC reference No. 923.03, (2000) using a muffle furnace (Forns Hobersal, Caldes de Montbui, Barcelona, Spain). Moisture content was determined with an infrared thermobalance (model MA 50.R, RADWAG®, Radom, Poland). 1 g powder was distributed evenly over dish surface heated in a temperature ramp during reading (5 °C/min up to 120 °C). Total lipids were determined according to the AOAC reference No. 920.85; (1990) using a 4002841 Soxhlet equipment (Selecta, Barcelona, Spain). Total carbohydrates were estimated by weight difference according to specifications published by (FAO, 2003).

2.2.3. Total dietary fibre (TDF)

TDF was determined according to procedure No. 985.29, described by Prosky et al. (1984). 1 g powder, 50 mL 0.05M phosphate buffer (pH 6), and 200 μL thermostable α-amylase A-3306 (Sigma Aldrich, Milan, Italy) were mixed and placed in a shaking bath (95 °C; 30 min). Samples were then placed on ice until reaching room temperature (20 °C) and the pH was adjusted to 7.5 with NaOH (0.171 N). Protease P-3910 (Sigma Aldrich) (0.001 g/mL) was next added and placed in a shaking bath (60 °C; 30 min). Subsequently, samples were cooled to 20 °C and the pH was adjusted to 4.5 (optimum for amyloglucosidase) with H_3PO_4 (0.205 M). 300 μL of amyloglucosidase A-9913 (Sigma Aldrich) was added to samples and placed in a shaking bath (60 °C; 30 min). To the cold samples, 280 mL of 95% ethanol was added and allowed to stand for 12 h (fibre precipitation). Samples were filtered using a Fibertec CSF6 equipment (VELP Scientifica Srl, Monza, Italy) and crucibles with the sample were placed in the oven for 12h. Ash and protein analyses were carried out using methods described above. TDF percentage was calculated using equation (1). Where: R1 and R2: weight of residues of duplicate samples; P: protein content; C: ash content; M1 and M2: weights of duplicate samples.

$$FDT\ (\%) = \frac{\frac{R1+R2}{2} - (P - C)}{\frac{M1+M2}{2}}\ x\ 100 \qquad \text{(Equation 1)}$$

2.2.4. Soluble sugars

Glucose, sucrose and fructose sugars were determined according to the modified method of Hurtado et al. (2019). 0.1 g powder were dissolved with MiliQ water in a 10 mL flask and placed in an ultrasonic cleaner KM (Terrassa, Barcelona, Spain) at 20 °C for 10 min. Samples and standards were filtered through a 0.25 μm Millipore filter and injected into an Agilent Technology 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) with refractive index detector (RI) and CarboSep CHO 682 Pb column 200 mm (Transgenomic, Elancourt, France). Operating conditions were: injection volume 20 μL; flow rate 0.4 mL/min; mobile phase: ultrapure water; analysis duration: 30 min; and column oven temperature: 80 °C. Sugars were identified by comparison of retention time and spectra of D-(+)-Glucose 47249, D-(−)-Fructose SLCN4265 (Sigma Aldrich) and D-(+)-Sucrose (ACROS, New Jersey, EEUU) standards. Quantification was performed with calibration lines obtained: Glucose (R^2 = 0.9949); Fructose (R^2 = 0.9969); Sucrose (R^2 = 0.9991). Results were expressed as g/100 g smoothie.

2.3. Reconstitution and in vitro digestion of smoothies

Fruit and vegetable powdered smoothies were alternatively reconstituted by stirring with water or semi-skimmed milk (12.5 g/100g dry extract concentration) at room temperature, simulating the usual conditions of consumption. *In vitro* digestion of reconstituted samples was performed following the INFOGEST methodology, without using the enzyme in the oral phase, standardized by (Brodkorb et al., 2019; Minekus et al., 2014). In the oral phase, 5 g smoothie, 4 mL simulated salivary fluid (SSF), 25 μL CaCl2 (0.3M) and distilled water (final volume 10 mL) were added. Samples were then placed in a shaking bath (model brand) (37 °C; stirring at 120 rpm; 2 min). As samples were starch-free, α-amylase was not used (Brodkorb et al., 2019). In the gastric stage, 8 mL of simulated gastric fluid (SGF) and 5 μL of $CaCl_2$ were added to the above mixture. Subsequently, pH was adjusted with HCl (3M and 0.1M), 0.5 mL of pepsin P6887 (Sigma Aldrich, Milan, Italy) (previously dissolved in distilled water) and distilled water (final volume of 20 mL) were added. Samples were incubated in a shaking bath (37 °C; 120 rpm; 2 h). In the intestinal phase (ID), to the above mixture, 8.5 mL of simulated intestinal fluid (SIF), 40 μL $CaCl_2$ (0.3M) was added and adjusted to pH 7, with NaOH (6M and 0.3M). Then, 5 mL of pancreatin P1750 solution (Sigma Aldrich) (previously dissolved in FIS), 2.5 mL of B8631 bile solution (Sigma Aldrich) (previously dissolved in SIF) and distilled water (final volume of 40 mL) were added. Samples were placed in a shaking bath (37 °C; 120 rpm; 2 h). After the GD and ID stages, samples were placed on ice to interrupt digestion and centrifuged (4 °C; 4554 × *g*; 20 min) (separate sediment from the soluble chyme fraction). The supernatant of GD and ID was stored at −80 °C until further analysis.

2.3.1. Determination of antioxidants and antioxidant activities in reconstituted smoothies

2.3.1.1. Vitamin C. Ascorbic acid content was determined according to (Kravets et al., 2024) with some modifications. 1.5 g smoothie and 4.5% (*w/v*) metaphosphoric acid was added to a final volume of 10 mL, placed in ultrasound (5 °C; 5 min) and then centrifuged (5 °C; 4554 × *g*; 5 min). The supernatant was collected and analysed in two parts. 2 mL was filtered at 0.25 Millipore SAS filter (Molsheim, France) and injected directly into the HPLC system for analysis. The other, 1.5 mL, to which 2 mL of 1.4 Dithiothreitol (DDT), a reducing agent that converts dehydroascorbic acid (DHA) to ascorbic acid, was added and stored in darkness (20 °C; 2 h). Afterwards, it was filtered and the procedure was as described above. 20 μL of samples and standards were injected into an Infinity II HPLC-DAD-1260 Series system (Agilent Technologies, Santa Clara, CA, USA). A Brisa LC2C18 column (25 × 0.46 cm) with a pore size of 5 μm (Teknokroma, Barcelona, Spain) was used. Chromatographic conditions were: mobile phase: H_2O-Mili-Q which with the help of H_2SO_4 was brought to a pH = 2.5; flow rate: 0.8 mL/min; pressure: 9.8 Mpa; (IV) mode: isocratic; (V) detection wavelength: 254 nm; and column oven temperature: 35 °C. Identification was performed by comparison of retention times and their spectra and quantification was determined with linear regression models of standard dilution (R^2 = 0.9995). A calibration line from 0.05 to 0.25 μg/mL was used for L-ascorbic acid (Merck, Darmstadt, Germany). Results were expressed as μg Eq ascorbic acid/100 g smoothie.

2.3.1.2. α-tocopherol. α-Tocopherol content was determined as described by (Kravets et al., 2024), with some modifications. 1 g smoothie and a mixture of hexane: acetone: ethanol (2:1:1 *v:v:v*) were added to a final volume of 10 mL and placed in ultrasound (5 °C; 5 min).Next, 2 mL distilled water was added and kept in darkness for 10 min. The non-polar phase was evaporated in a Hei-vap rotary evaporator (Heidolph, Schwabach, Germany) at 35 °C and 300–350 mPa. The residue was resuspended in 2 mL of a MeOH: TBME mixture (1.1 *v:v*) and stored at −80 °C until analysis. Samples and standards were filtered at 0.22 μm Millipore SAS filter (Molsheim, France) and 20 μL were injected into an Infinity II HPLC-DAD-1260 Series system. A Brisa LC2 C18 column (25 × 0.46 cm) with a pore size of 5 μm (Teknokroma, Barcelona, Spain) was used. Chromatographic conditions were: mobile phase: methanol; flow rate: 1 mL/min; mode: isocratic; detection wavelength: 204 nm; and column oven temperature: 27 °C. A calibration line from 0.0625 to 1 μg/mL was used. α-tocopherol was quantified using the equation (R^2 = 0.9966). Results were expressed as μg α-tocopherol equivalents/100g.

2.3.1.3. Total phenol content (TPC). Phenolic antioxidant activity was determined as TPC using a modified version of the Folin–Ciocalteu method (Cedeño-Pinos et al., 2023). 0.5 g sample (US, GD or ID) and 1 mL methanol (Merck KGaA, Darmstadt, Germany) were mixed, placed in ultrasound for 5 min and then centrifuged (4 °C; 4554 × *g*; 5 min). The supernatant containing sample extract was collected and stored at −80 °C until further analysis. 250 μL sample extract, 800 μL Folin-Ciocalteu reagent and 5 mL distilled water were added to a 10 mL volumetric flask. After 8 min, 1.2 mL aqueous sodium carbonate solution (1:5 *w/v*) was added to the above mixture and kept in a water bath (20 °C; 2 h). Absorbance was measured at 760 nm using a UV–VIS spectrophotometer (Genesis 180, Madison, WI, USA). Gallic

acid (GA) (Merck KGaA, Darmstadt, Germany) was used as standard for quantification, using a calibration line (R^2 = 0.9989) from 0.1 to 6 μg GA/mL. Results were expressed as mg gallic acid equivalents (GAE)/100 g.

2.3.1.4. Total flavonoid content (TFC). Flavonoid antioxidant activities were determined as TFC according to (Lu et al., 2019), with some modifications. 0.5 smoothie mixed with 2 mL of ethanol (85%) and HCl (0.1%). This mix was placed in ultrasound for 10 min and centrifuged (4 °C; 4554 × *g*; 5 min). Supernatant containing sample extract was collected and stored at −80 °C until further analysis. The reaction was performed with 500 μL sample extract 1.5 mL of a mixture of ethanol (95%), 100 μL $AlCl_3$ (10 %), 100 μL CH_3CO_2K and 2.8 mL distilled water. After mixing, sample was incubated (20 °C; 30 min in darkness) and then absorbance was measured at 415 nm. TFC were quantified from a calibration line (R^2 = 0.9991) made with a standard solution of quercetin (Sigma Aldrich) at different concentrations (3–80 μg/mL). Results were expressed as mg quercetin equivalents (QE)/100 g.

2.3.1.5. 2.2-diphenyl-1 picrylhydrazyl (DPPH). DPPH radical scavenging activity was determined as described by (Cedeño-Pinos et al., 2023). 3.5 mg DPPH reagent (Sigma-Aldrich) was dissolved in 10 mL methanol and kept in darkness for 30 min. Absorbance (517 nm) of the DPPH solution was adjusted to 1.0 with methanol. 30 μL sample extract (see TPC) and 970 μL DPPH solution was mixed, incubated in darkness for 10 min, and absorbance (517 nm) was then measured. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma Aldrich) was used as standard for quantification, using a calibration line (R^2 = 0.9964) from 0.05 to 0.5 mg Trolox/mL. Results were expressed as mg Trolox equivalents (TE)/100 g.

2.3.1.6. Ferric Reducing Antioxidant Power (FRAP). Assay FRAP capacity was determined as described by (Cedeño-Pinos et al., 2023). To prepare the FRAP reagent, 300 mM acetate buffer (pH 3.6); 10 mM 2,4,6-tripyridyl-triazine diluted in 40 mM HCl, and 20 mM FeCl3 6H2O were mixed in a 10:1:1 ratio (*v/v/v*). 40 μL sample extract (see TPC) and 1.2 mL FRAP reagent were mixed and incubated in a water bath (37 °C; 2 min). $FeSO_4$ x $7H_2O$ was used as standard (Panreac, Barcelona, Spain). Absorbance was measured at 593 nm. A calibration line (R^2 = 0.9981) from 0.1 to 0.7 mM Fe^{2+} ($FeSO_4$ x $7H_2O$) was used for quantification. Results were expressed as mol Fe^{2+} or $FeSO_4$ equivalents/100 g.

2.3.1.7. 2.20-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). ABTS radical cation decolourisation was determined as described by (Cedeño-Pinos et al., 2023). 10 mL aqueous solution of ABTS (Sigma Aldrich) (7 mM) was prepared. The $ABTS^+$ radical cation was formed by reaction of ABTS with 1 mL potassium persulfate (2.45 mM). The mixture was first kept in darkness (25 °C; 16 h) and absorbance (734 nm) was then adjusted to 0.7 using distilled water. 970 μL of the ABTS solution was mixed with 30 μL aliquot of sample extract (see TPC) and incubated at 37 °C for 6 min in darkness. Absorbance was measured at 734 nm. A calibration line (R^2 = 0.9995) from 0.1 to 0.6 mg Trolox/mL was used for quantification. Results were expressed as mg TE/100 g smoothie.

2.4. Bioaccessibility of phenols and flavonoids

The percentage of bioaccessibility of TPC and TFC (mg/100g) was calculated as described by (Chait et al., 2020), which represents the percentage of phenolic compounds released after simulated GID that can be absorbed into the systemic circulation. Bioaccessibility was calculated using equation (2),

$$\% \text{ Bioaccessibility} = (A/B) \text{ x } 100 \quad \text{(Equation 2)}$$

where: A is the total TPC and TFC content in samples after *in vitro* GID and B is the total TPC and TFC content in samples before *in vitro* GID.

2.5. Remaining antioxidant capacity (RAC)

RAC was determined according to (Ribeiro et al., 2019b) as the percentage of AC available after *in vitro* GID. The RAC was calculated using equation 3

$$\% \text{ RAC} = (A/B) \text{ x } 100 \quad \text{(Equation 3)}$$

where: A is the AC of digested samples and B is the AC of undigested samples.

2.6. Statistical analysis

A randomised design was performed for the experiment. The main treatments were formulation (2), digestion levels (3) and reconstitution methods (2). Sample size for powdered smoothies was 12 (2 formulations x 6 replicates). Sample size for reconstituted smoothies was 72 (2 formulations x 3 digestion levels x 2 reconstitution methods x 6 replicates). Treatment effects on dependent variables were determined by one-way (powdered samples) or two-way ANOVA (reconstituted samples). The Tukey's homogeneity range test was used to compare group means ($p < 0.05$). Correlation among dependent variables were expressed as Pearson coefficient. Data were analysed using the Statistix 8 for Windows program (Analytical Software, Tallahassee, FL, USA).

3. Results

3.1. Physical and nutritional characterisation of powdered smoothies

The physical and compositional data of powdered smoothies are presented in Table 1. There were ($p < 0.05$) some physical differences between fruit and vegetable powders. The pH was slightly more acidic in the fruit (5.63) than in the vegetable powder (5.91). Water activity was very low, as is the norm with a dehydrated powder, being somewhat higher in the fruit (0.23) than in the vegetable powder (0.17). Both powders showed a brown hue with slight chromatic differences. Lightness was higher in the fruit (92.44) than in the vegetable powder (89.30), while brownness (higher b* and lower a*) was higher in the latter. Protein content was very high, due to the addition of WPC80, being similar for both powders with values of 33.8 g/100g and 33.61 g/

Table 1
Physical and compositional assessment of fruit and vegetable powdered smoothies.

	Smoothies				
	Fruit		Vegetable		
	Mean		Mean		SEM
Physical assessment					
pH	5.63	b	5.91	a	0.014
Water activity	0.23	a	0.17	b	0.002
Lightness (CIE units)	92.44	a	89.30	b	0.055
Redness (CIE units)	0.99	b	7.54	a	0.492
Yellowness (CIE units)	13.33	b	26.29	a	0.085
Proximate composition (g/100g)					
Proteins	33.80		33.61		1.209
Lipids	0.08		0.10		0.008
Ashes	4.16	b	5.27	a	2.120
Moisture	4.13		3.64		0.513
Fibre	21.12	b	24.40	a	0.060
Carbohydrates	36.71	a	32.99	b	1.388

Abbreviations: CIE: Commission Internationale de l'Éclairage); SEM: Standard Error of the Mean.
[a, b] Smoothie formulation effects ($p < 0.05$).

100g, for fruit and vegetables respectively. By contrast, lipid content was very low in both powders, showing percentages of 0.08% and 0.10% for fruit and vegetables respectively while residual moisture was similar in both with total content of 4.13% and 3.64% for fruit and vegetables respectively Both ashes as total fibre contents were lower in the fruit (4.16 and 21.12 g/100g) than in the vegetable powders (5.27 and 24.40 g/100g), while estimation of total carbohydrates (excluding fibre) was higher in the fruit (36.71 g/100g) than in the vegetable powder (32.99 g/100g). As for soluble sugar composition (Fig. 1), fructose (15.27 g/100g) predominated over glucose (10.06 g/100g) and sucrose (4.79 g/100g) in fruit powders, while glucose (12.72 g/100g) predominated over fructose (10.14 g/100g) and sucrose (8.64 g/100g) in vegetable powders.

3.2. *Effects of in vitro GID on the antioxidant properties of reconstituted smoothies*

The effects of *in vitro* GID on the phenolic antioxidant activities of the reconstituted smoothies are provided in Table 2. Interactions were found ($p < 0.001$) between the effects of reconstitution and digestion methods for all variables studied. GID increased ($p < 0.05$) the TPC of smoothies, particularly when semi-skimmed milk was used. The highest TPC corresponded to the GD samples in both fruit (40.95 mg GAE/100g) as vegetable smoothies (44.24 mg GAE/100g) with milk, representing an increase of more than double compared to the UD samples. Further ID led to some detriment in the TPC reached after GD. In contrast, most total flavonoids gradually degraded after GD and ID, especially when reconstituted with water, despite both smoothies containing over 12 mg QE/100g before digestion. Reconstitution with milk increased the initial and final TFC of fruit (from 24.5 to 7.3 and 8.6 mg QE/100g) and vegetable smoothies (from 79.1 to 6.9 and 1.1 mg QE/100g). Therefore, using semi-skimmed milk to reconstitute smoothies improved dietary levels of total phenols and, to a largely lesser degree, of total flavonoids, providing certain protection against GID. GID enhanced bioaccessibility of total phenols (118–222%), while having a detrimental effect on the bioaccessibility of total flavonoids (0.5–35%) (Figs. 2–3).

The effects of *in vitro* GID on of α-tocopherol levels are shown in Fig. 4. Reconstitution with milk again increased α-tocopherol content in UD samples from fruit (from 40 to 50 μg/100g) and vegetable smoothies (from 120 to 165 μg/100g), meaning that semi-skimmed milk also provided this vitamin. However, a drastic reduction ($p < 0.05$) in α-tocopherol content was observed after *in vitro* digestion for both types of smoothies, with concentrations above 5 μg/100g in all samples. Bioaccessibility of α-tocopherol strongly reduced after GD and ID in both smoothies. Thus, vitamin E was extensively degraded under the *in*

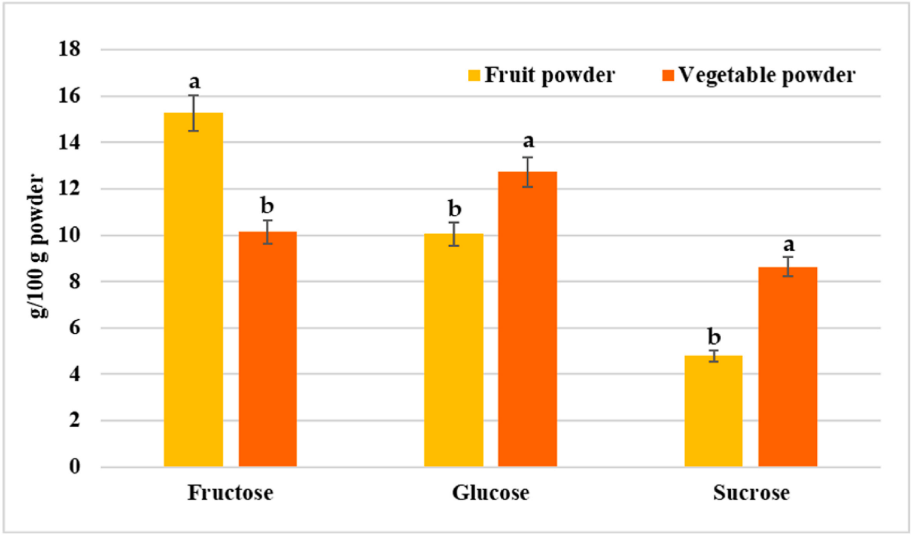

Fig. 1. Soluble sugars content of fruit and vegetable powdered smoothies.
[a,b] Smoothie formulation effects ($p < 0.05$).

Table 2
Phenol and flavonoid total contents after *in vitro* digestion of fruit and vegetable reconstituted smoothies.

		Fruit smoothies					Vegetable smoothies				
		Water		Milk			Water		Milk		
		Mean		Mean		SEM	Mean		Mean		SEM
TPC	UD	18.18	d	18.24	d	1.097	18.46	e	20.36	de	2.731
(mg GAE/100 g)	GD	29.63	c	40.59	a		35.84	b	44.24	a	
	ID	27.55	c	33.97	b		27.34	cd	32.50	bc	
TFC	UD	12.47	b	24.46	a	0.758	36.64	b	79.15	a	0.521
(mg QE/100 g)	GD	0.45	d	7.30	c		0.00	e	6.94	c	
	ID	0.00	d	8.57	c		0.00	e	1.07	d	

Abbreviations: TPC: Total Phenolic Content; TFC: Total Flavonoid Content; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; UD: Undigested; GD: Gastric digestion; ID: Intestinal Digestion; SEM: Standard Error of the Mean.
[a-e] *In vitro* digestion x reconstitution effects ($p < 0.05$; Tukey test) for each smoothie formulation.
There was significant ($p < 0.001$) interaction between digestion and reconstitution effects for all variables.

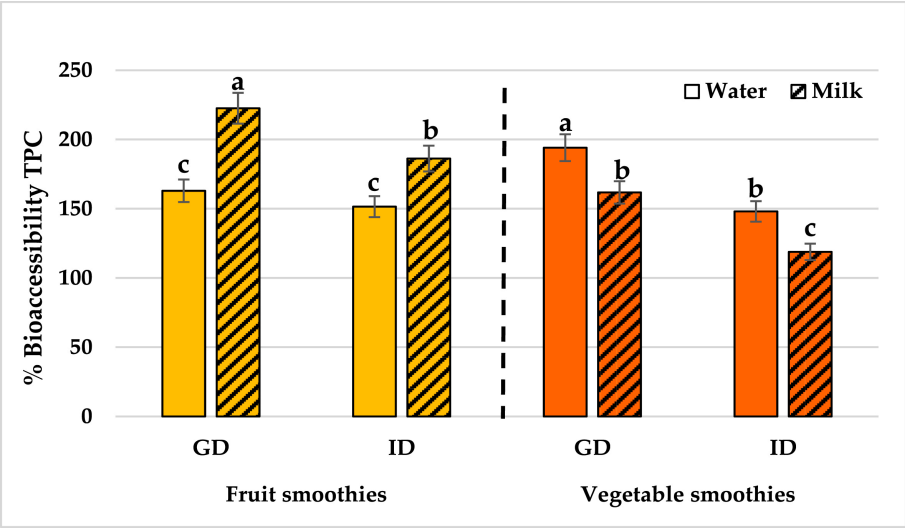

Fig. 2. Total phenol bioaccessibility after *in vitro* digestion of reconstituted smoothies.
Abbreviations: TPC: Total Phenolic Content; GD: Gastric digestion; ID: Intestinal digestion.
[a-d] *In vitro* digestion x reconstitution effects ($p < 0.05$) for each smoothie formulation.

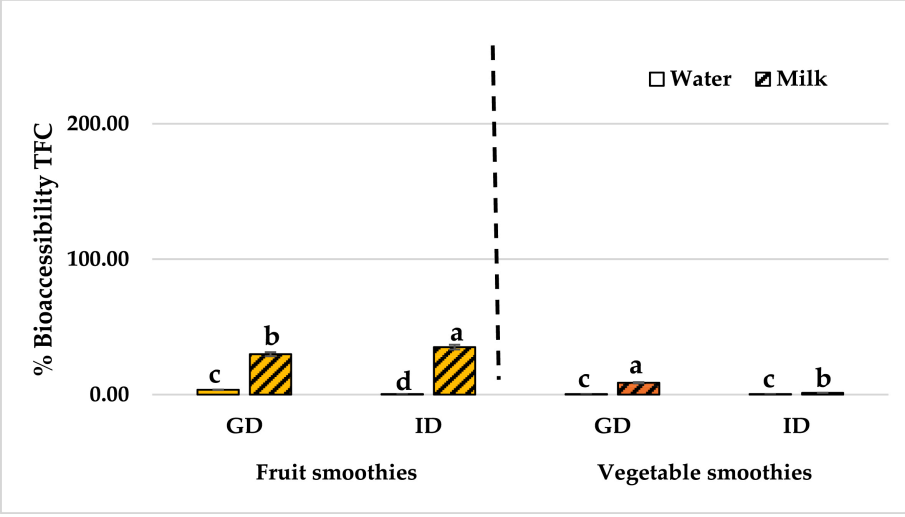

Fig. 3. Total flavonoid bioaccessibility after *in vitro* digestion of reconstituted smoothies.
Abbreviations: TFC: Total flavonoid content; GD: Gastric digestion; ID: Intestinal digestion.
[a-d] *In vitro* digestion x reconstitution effects ($p < 0.05$) for each smoothie formulation.

vitro digestion conditions tested. In addition, the vitamin C content of UD smoothies was residual. In the fruit smoothie, concentrations of ascorbic and dehydroascorbic acids were 1.76 and 7.02 μg/100g, respectively, while their concentrations in the vegetable smoothies were 2.52 and 6.76 μg/100g, respectively. This reveals vitamin C practically degraded during elaboration of powders, therefore, possible effects of GID on this vitamin can be considered irrelevant and therefore, data were not shown.

The effects of *in vitro* digestion on the AC of reconstituted smoothies are provided in Table 3. Once more, interactions ($p < 0.001$) were observed between effects of reconstitution and digestion methods for all variables studied. Digestion effects on AC practically reproduced in both smoothies. The UD samples showed intermediate ABTS + values (40–50 mg TE/100 g) compared to GD (30–35 mg TE/100 g) and ID (53–55 mg TE/100 mg). Thus, their AC decreased after GD and then recovered during ID. In contrast, the DPPH values of UD samples (15–26 mg TE/100 g) clearly decreased after GD (< 4.6 mg TE/100 g) and after ID (< 4 mg TE/100 g), indicating that most of this AC would not be recoverable in the intestine. Similar findings were observed for FRAP values. UD samples again showed the highest reduction of ferric ion (Fe^{3+}) in both fruit (49–64 μM Fe 2+/100 g) and vegetable smoothies (55–91 μM Fe 2+/100 g). In these samples, reconstitution with milk had a positive effect on the AC. First GD and then ID led to gradual decreases in DPPH values until reaching less than 28 and 20 μM Fe 2+/100 g for fruit and vegetable smoothies, respectively. Therefore, GID had a detrimental effect on the antioxidant status of both smoothies assessed as FRAP and DPPH radical scavenging activities, while in contrasts it improved ABTS radical scavenging activities.

Results concerning the %RAC after digestion varied according to the assay used (Figs. 5–7). The %RAC for ABTS was particularly high, being lower in GD (61–85%) than in ID samples (84–135%), the %RAC for DPPH was small with higher or lower values in GD (9–30%) or ID

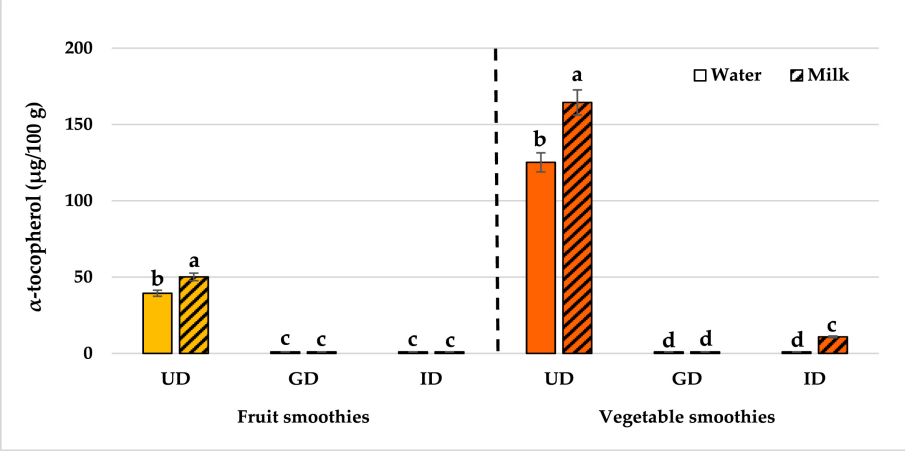

Fig. 4. α-Tocopherol content after *in vitro* digestion of fruit and vegetable reconstituted smoothies.
Abbreviations. UD: undigested; GD: Gastric digestion; ID: Intestinal digestion.
[a-d] *In vitro* digestion x reconstitution effects ($p < 0.05$) for each smoothie formulation.

Table 3
Antioxidant capacity after *in vitro* digestion of fruit and vegetable reconstituted smoothies.

		Fruit smoothies					Vegetable smoothies				
		Water		Milk			Water		Milk		
		Mean		Mean		SEM	Mean		Mean		SEM
ABTS (mg TE/100)	UD	44.56	b	43.00	b	0.754	50.46	b	40.55	c	0.672
	GD	32.04	d	35.92	c		31.02	e	34.44	d	
	ID	53.1	a	54.64	a		53.1	a	54.61	a	
DPPH (mg TE/100)	UD	19.36	b	26.04	a	0.773	14.77	b	19.02	a	0.979
	GD	2.56	c	1.93	c		4.56	c	3.75	cd	
	ID	3.96	c	3.64	c		2.25	cd	0.95	d	
FRAP (µM Fe $^{2+}$/100 g)	UD	49.45	b	63.93	a	3.818	54.80	b	91.35	a	3.128
	GD	45.72	b	39.26	bc		47.90	b	33.98	c	
	ID	22.17	d	27.66	cd		15.20	d	19.76	d	

Abbreviations: ABTS: 2.2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic); DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; FRAP: Ferric Reducing Antioxidant Power; TE: Trolox Equivalents; UD: Undigested; GD: Gastric digestion; ID: Intestinal digestion; SEM: Standard Error of the mean.
[a-e] *In vitro* digestion x reconstitution effects ($p < 0.05$) for each smoothie formulation.
There was significant ($p < 0.001$) interaction between digestion and reconstitution effects for all the variables.

(5–22%) samples depending on the smoothie, while the %RAC for FRAP had intermediate values and was higher in GD (37–92%) than in ID samples (22–45%). GID generally enhanced, or at least maintained ABTS radical scavenging activity, but it also attenuated the FRAP, in particular, the DPPH radical scavenging activities. In most cases, reconstitution with milk had a positive effect on the %RAC.

4. Discussion

Food SD is a popular choice due to its low operating costs and flexibility compared to freeze drying; drying techniques that extend the shelf life of food products at room temperature by reducing water activity will negatively affect the spoilage of microorganisms and enzymes responsible for undesirable changes in foods (Fathi & Sefidkon, 2012; Razola-Díaz et al., 2022). As expected, the two powdered smoothies presented low water activity and an acidic pH, due to fruits and vegetables used in their elaboration. Spoiling microorganisms and enzymes from fruit and vegetables remain controlled in dehydrated matrixes, and in powdered smoothies, the time elapsed between reconstitution and consumption is often short, thus it is not necessary to control the microbiological quality of these products.

All powders showed a brown hue. The darker colour formation may be because homogenization and spray drying favour oxidation of fruit and vegetable pigments, due to release of tissue components by mechanical treatment, heating and exposure to drying air (Movahhed & Mohebbi, 2016; Li et al., 2023a). This leads to browning and, in the case of products rich in yellow and orange pigments, like flavonoids, intensifies browning, as observed. The increase of a^* value in vegetable powder denotes a redder chroma which might indicate the presence of carrot and pumpkin, for which redness is relatively constant in drying (Krokida et al., 2001). Other authors also observed that sugar content could contribute to browning of carrot powder (Movahhed & Mohebbi, 2016).

In addition to sugar content, these powders are rich in proteins and contain a large amount of fibre, especially vegetable powder. The moisture content of the analysed powders was around 4%. Tahsiri et al. (2017), reported a similar moisture content (4.79 g/100g) in a barberry milk smoothie powder also obtained by SD. This drying method basically reduces moisture content increasing the relative proportions of other food components. In homogenised food products with high sugar and fibre content, evaporation is modulated by water diffusion through hygroscopic molecules, which, in SD, is facilitated by the formation of fine drops which increase drying rate. It has been reported that shelf life and consumer acceptance of many powders can be influenced by moisture content above 5 g/100g (Goula & Adamopoulos, 2005). Proximate composition of fruit and vegetable powders with WPC80 was similar to those reported by Victor-Aduloju et al. (2023) who prepared powdered smoothies fortified with protein-rich cashews and breadfruit. Values which they obtained for protein varied between 22.70 and 33.75 g/100g and carbohydrates between 18.69 and 35.35 g/100g. However, in terms of lipid content (3.00–4.50 g/100g), our results were the lowest (0.08–0.10 g/100g). Their high level is likely due to the high lipid content of nuts, such as cashew nuts. The high fibre content of the vegetable and fruit smoothies analysed in this work should be noted, compared to the fibre values (0.75–1.35 g/100g) obtained by Victor-Aduloju et al. (2023). As for soluble sugars, glucose values agree with those obtained by (Li et al., 2023b) on Chinese citrus fruit smoothies dried by SD, ranging from 11.73 to 15.52 g/100g. Nevertheless, fruc-

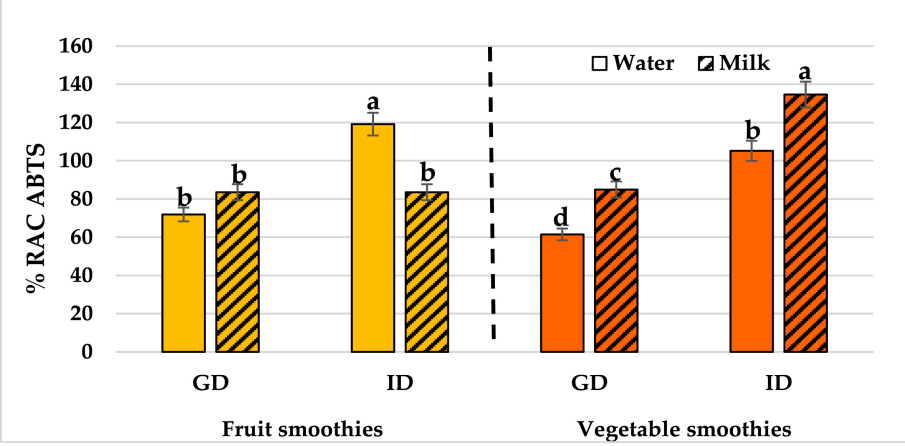

Fig. 5. Remaining antioxidant capacity for ABTS after *in vitro* digestion of fruit and vegetable reconstituted smoothies.
Abbreviations: RAC: Remaining antioxidant capacity; ABTS: 2.2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic); GD: Gastric digestion; ID: Intestinal digestion.
[a-c] *In vitro* digestion x reconstitution effects ($p < 0.05$) for each smoothie formulation.

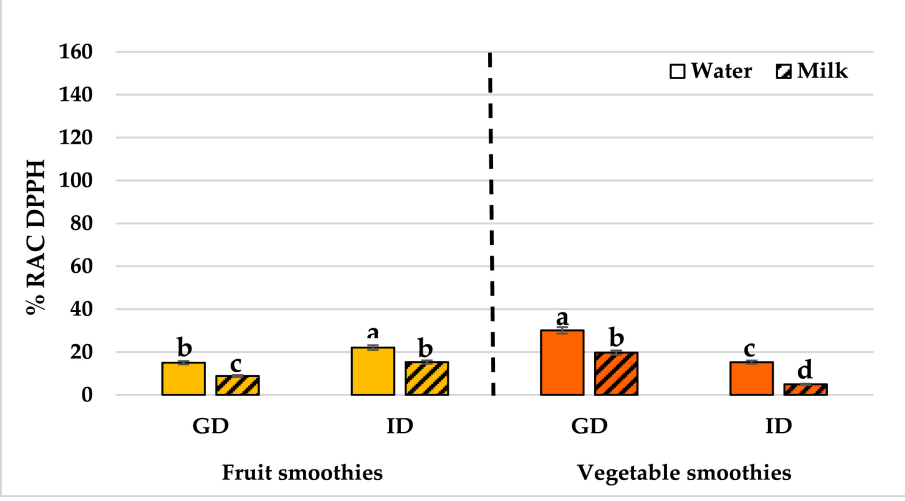

Fig. 6. Remaining antioxidant capacity for DPPH after *in vitro* digestion of fruit and vegetable reconstituted smoothies.
Abbreviations: RAC: Remaining antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; GD: Gastric digestion; ID: Intestinal digestion.
[a-c] *In vitro* digestion x reconstitution effects ($p < 0.05$) for each smoothie formulation.

tose and sucrose values were lower than those obtained in this work, sucrose having the lowest content of soluble sugars. Li et al., 2023b observed that SD fruit powder had the highest mineral elements and soluble sugar content.

Smoothies can lose thermolabile and oxidizable nutrients, including vitamins and/or antioxidants, during processing. The **vitamin C** content of the SD smoothies was notably lower than those produced via freeze drying and hot air drying (Li et al., 2023a), since vitamin C is very thermolabile and easily degraded by food processing (Razola-Díaz et al., 2022). This was confirmed by the degradation of vitamin C which occurred in SD smoothies from this study. Patras et al. (2009), analysed levels of ascorbic acid in unprocessed and thermally processed carrot purees, observing a 46% reduction following treatment. Our results concur with those by Hurtado et al. (2015, 2019), in fruit and vegetable fresh smoothies. These authors compared vitamin C retention in thermally and high-pressure treated smoothies, observing that most ascorbic acid degraded to dehydroascorbic acid. Vitamin C total content reported in these studies for fruit and vegetable smoothies ranged between 35-40 and 2–3 mg/100 mL, respectively. Vitamin C loss is considered a nutritional handicap in powdered smoothies, owing to the fact ascorbic and dehydroascorbic acids are antioxidants capable of reducing cellular oxidative stress, thus both forms are bioaccessible and functional in the organism (Gordon et al., 2020).

In vitro GID is a valuable tool in understanding the behaviour of food components during human digestion. Knowledge of the physicochemical changes which take place in foods during the digestion process would be useful for formulating functional foods that benefit consumer health (Lucas-González et al., 2018). Due to their formulation, based on fruits, vegetables and WPC80, the tested smoothies were rich in phenolic antioxidants, including flavonoids. Hurtado et al. (2015, 2019), reported that pasteurised fruit smoothies contain more phenols (40 mg GAE/100 mL) and flavonoids (7 mg QE/100 mL) than vegetable smoothies (11 mg GAE/100 mL and 1 mg QE/100 mL). In the present study, both reconstituted smoothies with WPC80 already achieved lower values for the above-mentioned antioxidants before digestion. Moreover, these smoothies with WPC80 did not result in a relevant dietary source of vitamin E, despite semi-skimmed milk increasing its content. As seen, both smoothies contained α-tocopherol and diges-

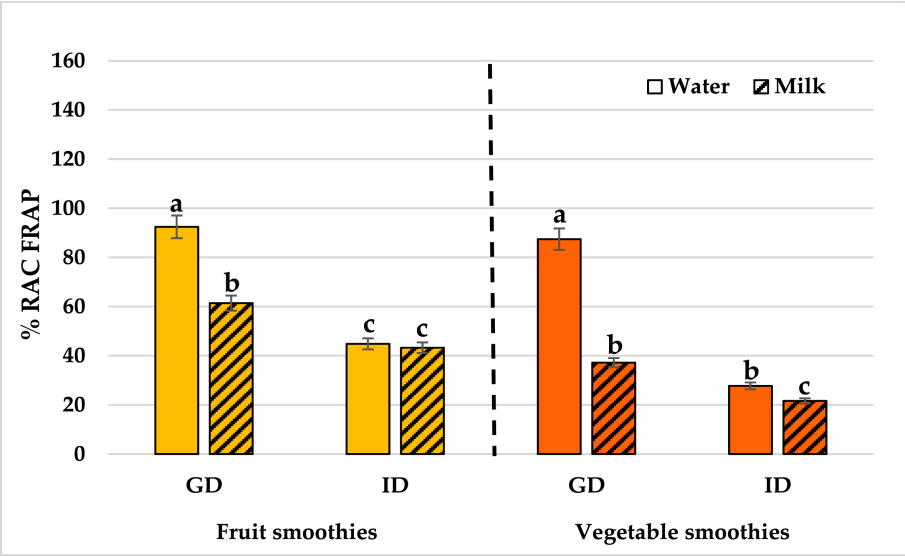

Fig. 7. Remaining antioxidant capacity for FRAP after *in vitro* digestion of fruit and vegetable reconstituted smoothies.
Abbreviations: RAC: Remaining antioxidant capacity; FRAP: Ferric Reducing Antioxidant Power; GD: Gastric digestion; ID: Intestinal digestion.
[a-c] *In vitro* digestion x reconstitution effects ($p < 0.05$) for each smoothie formulation.

tive preparations degraded it. The absence of vitamin E in the digested samples reveals this vitamin is prone to degrade under the action of digestive fluids. Minimal lipid oxidation during GID might also have occurred, affecting vitamin E bioaccessibility. Alberdi-Cedeño et al. (2020), observed that antioxidant phenolic compounds such as dodecyl gallate and hydroxytyrosol acetate could reduce oxidation of tocopherol during gastrointestinal *in vitro* digestion of olive oil. Rocha Torres et al. (2022), analysed the various factors which can affect bioaccessibility of vitamin E, one being *in vitro* digestion conditions. Rocha Torres et al. (2022), confirmed that methodological conditions used in simulation of GID, such as the addition of pancreatic lipase, concentration of bile extract and variations in gastric pH, are determinants in *in vitro* bioaccessibility of vitamin E. Another factor involved could be the physical-chemical interactions with polysaccharides of the cell walls of fruits and vegetables, since it has been shown these can modify the structure of gastrointestinal fluids, forming hydrogels which inhibit the action of lipase and hinder absorption of this vitamin (Tan & Mcclements, 2021).

Most polyphenols in food are in the form of esters, glycosides or polymers that cannot be absorbed in their native form; only aglycones and some glycosides can be absorbed in the small intestine (Manach et al., 2005). These substances must be hydrolysed by intestinal enzymes, such as a small intestinal brush border membrane glucosidase that catalyses the extracellular hydrolysis of certain glycosides. Some polyphenols with special structures cannot be absorbed until they reach the colon (H. Zhang et al., 2014). It has been observed that after *in vitro* digestion, the association between whey protein-polyphenol apple complexes can be partially broken by enzymatic hydrolysis, leading to an increase in polyphenol content. Studies have suggested that polyphenols can interact with globular proteins by binding the tryptophan residues of proteins, thereby improving the bioavailability and functionality of polyphenols (Li et al., 2022). Our results show that gastric digestion probably favoured the release of phenolic compounds in the smoothies, but not flavonoids, most of which came from the semi-skimmed milk. These results coincide with those of López de Dicastillo et al. (2019), who studied the bioaccessibility of açai fruit polyphenols, and with Rodríguez-Roque et al. (2014), who analysed a soy beverage and justified this behaviour as perhaps being due to the acid pH value of the stomach which plays an important role. Other factors such as interactions with bile sales also decrease the content of phenolic compounds (Viuda-Martos et al., 2018). Similarly, Luo et al. (2022) studied different varieties of *Kadsura coccinea* fruit, and also stated that the highest release of polyphenols occurred during gastric digestion. In contrast, during *in vitro* intestinal digestion, they observed that polyphenols were unstable due to small molecules formed by hydrolysis during digestion and degraded, likely because of the higher pH of the intestine (Soriano Sancho et al., 2015). During the gastric stage, some changes could have occurred in the phenolic compounds, such as modification of the chemical structure, reduction of their solubility due to pH and/or interaction with other compounds like iron, fibre, proteins or other minerals, thus producing a loss of active compounds (Gullon et al., 2015). Previous studies have shown that polyphenols exist in a free and complex state, especially with pectin components that form nanostructures. It has been described that several factors can affect the content and bioaccessibility of phenolic compounds upon *in vitro* digestion, including inhibition of digestive enzymes by polyphenols, oxidation of these compounds in an alkaline environment to quinones, changing their structural and biological properties, or oxidation and polymerization to chalcones, which are not bioaccessible (Simonetti et al., 2021).

Several studies observed that flavonoid content of vegetable samples lead to a significant reduction following the digestion process, potentially attributed to fluctuations in pH levels during the *in vitro* digestion, known to induce a decrease in TFC (Lin et al., 2014; Zhao et al., 2024). Similarly to Ydjedd et al. (2017), all digested fractions presented low total contents for flavonoids, thus showing low bioaccessibility in studied smoothies.

The AC is largely conditional on the chemical properties of food compounds and nature of the radical used. Regarding the disposition of the phenolic hydrogens, the AC of phenolic compounds depends on a number of OH-groups and their position. Such as, OH-groups on 3′-, 4′-, 5′- position of B-ring of flavonoids increase the AC of compounds in contrast to phenolics with one hydroxy group (Kopjar et al., 2015). The resulting AC of smoothies also depends on interactions among their antioxidants and other components involved in redox reactions, where digestive fluids contribute to chemical processes. ABTS, DPPH and FRAP are widely contrasted AC tests used in food samples containing phenolic antioxidants, though the information provided by each assay can vary. The ABTS test is widely applied to test food and natural water-soluble

phenolics. This method monitors the decay of the radical-cation ABTS produced by oxidation of ABTS caused by the addition of a phenolic-containing food. The $ABTS^{\cdot}+$ has low selectivity in reactions with H-atom donors since it reacts with any hydroxylated aromatics regardless of their real antioxidant potential. The DPPH test is based on the capability of stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with H-donors including phenolics. As DPPH shows a very intensive absorption in the visible region, it can be easily determined by the UV–Vis spectroscopy, though ESR determination is also acceptable. DPPH is likely more selective than ABTS + in reaction with H-donors. The FRAP assay is based on ferric–to–ferrous reduction in the presence of a Fe(II)-stabilizing ligand such as tripyridyltriazine (TPTZ). The coloured complex is formed at an acidic pH. Moreover, antioxidants detected by FRAP are limited to water-soluble compounds.

In the present study, higher ABTS radical scavenging activity in the intestinal phase compared to the gastric phase was noted. Similar results were observed by Tagliazucchi et al. (2010), who analysed grape extracts rich in phenolic compounds. Some factors involved could be stability under gastrointestinal conditions and release from the food matrix (Pérez-Vicente et al., 2002). Fibre can also retain a greater amount of antioxidant compounds as concluded by Yang et al. (2022), who like our study also used pumpkin in preparation of smoothies (Sui et al., 2014). concluded that the increase in the AC was directly proportional to that in pH. It must be stressed that though digested extracts have demonstrated relevant scavenger capacity against ABTS, the presence of other non-phenolic compounds, such as amino acids derived from the hydrolysis of proteins, may interfere with the antioxidant (Soriano Sancho et al., 2015).

In contrast to ABTS, during *in vitro* GID, DPPH radical scavenging activity was largely reduced (>80%) in smoothies. Similar to results obtained by Kashyap et al. (2022), who analysed cherry pomace extracts, and (Ribeiro et al., 2019b) who studied juçara smoothies. In contrast (Correa-Betanzo et al., 2014), observed DPPH scavenging activity did not change during simulated *in vitro* gastric digestion but was significantly reduced (<50%) during *in vitro* intestinal digestion of wild blueberry extracts. This could be due to the fact phenolic compounds can undergo structural changes during the gastrointestinal tract (Sinela et al., 2017). FRAP ferric ion reduction capacity also decreased after intestinal digestion, in agreement with (Kashyap et al., 2022; Lucas-González et al., 2018). This decrease in AC might also be attributed to the action of digestive enzymes and bile salts in the food matrix, resulting in the release of phenolic compounds bound to the digestive fluid (Zhang et al., 2017). Lucas-González et al. (2018), suggest that total dietary fibre content and nature of the raw material determine release of polyphenolic compounds from the matrix. In all likelihood, because the FRAP method is developed at acidic pH to promote electron transfer (Jin et al., 2016), AC in the gastric phase was better than in the intestinal phase.

Since measured AC of smoothies varied with the assay used, correlation coefficients between AC and total phenol/flavonoid content were calculated to explain the role of phenols and flavonoids in the antioxidant properties of smoothies. The TFC significantly correlated ($p < 0.05$) with the radical DPPH ($r = 0.621$) and FRAP ($r = 0.857$), indicating that flavonoids could contribute to scavenging of these radicals. Nevertheless, the inverse correlation coefficient of TPC with DPPH was observed ($r = -0.722$). These results mean that the antioxidant behaviour of the digested smoothies is variable, depending on the antioxidant radical tested, and it would be interesting to further study specific bioactive compounds and a wide range of antioxidant assays in the near future.

Reconstitution of smoothies with semi-skimmed milk improved the bioaccessibility of α-tocopherol and phenolic compounds, including flavonoids. In fact, Hayes et al. (2001), noted that the presence of milk increased vitamin E absorption. On the other hand, Rodríguez-Roque et al. (2015) concluded that the release of phenolic compounds after GID was improved in milk and fruit juice with milk, but not in fruit juice alone. Similarly, Stinco et al. (2022), reached the same conclusion in a mandarin beverage and milk.

The bioaccessibility and stability of phenolic compounds in the presence of the milk matrix could be explained by the interaction of these compounds with milk proteins (Helal et al., 2022) generally due to non-covalent hydrophobic bonds. It is mainly phenolic compounds with a higher number of hydroxyl groups which interact with proteins. This suggests that protein-polyphenol complexes are formed by multiple interactions between amino acid side chains and aromatic rings of polyphenols, indicating that the association is mainly a surface phenomenon. These interactions may sometimes be complemented by hydrogen bonds, which play an important role in reinforcing and stabilizing the complexes. The protective effect of dairy products with phenolic compounds during *in vitro* digestion may be due to binding to caseins that prevent the interaction of phenolic compounds with digestive enzymes, and/or which may protect these compounds from oxidation reactions. Subsequently, hydrolysis of the milk protein during digestion might favour the release of the bound phenolic compounds, which improves their absorption and bioaccessibility (Helal et al., 2022; Lang et al., 2021; Zhang et al., 2021). A further explanation could be the slower digestion rate of β-lactoglobulin, which may offer greater protection to polyphenols. The reason could be because of greater encapsulation of polyphenols in the protein structure, affording protection during digestion. (van de Langerijt et al., 2024). On the other hand, flavonoid-protein covalent bonding is mainly explained by the reaction between amine groups and quinones. In fact, interactions of milk proteins with flavonoids have been shown to give flavonoids increased stability, possibly because of the entrapment of the polyphenolic molecules and their protection against oxidation reactions (Oliveira et al., 2018).

Several studies have reported some discrepancy among bioavailability data determined *in vitro* and *in vivo* for different food antioxidants (Liang et al., 2021). As mentioned, the static model used to assess *in vitro* GID has some limitations such as the fact this model only measures endpoints and not kinetics; enzyme activity in each digestive phase remains constant regardless of food matrix. The intestinal phase is treated as a single phase and not as the sequential duodenal, jejunal and ileal phases, which have different dilutions, mineral content, pH, enzyme activities and microbial content. Although the *in vitro* models are commonly adopted to assess different compound bioavailability because of low cost and easy control, more research is needed to determine interactions which cause binding between polyphenols and proteins and whether bioavailability of compounds will increase when bound to proteins in cell model and/or clinical study. Regardless, *in vitro* GID tests, despite limitations, have proven a suitable tool in assessing antioxidant properties in powdered smoothies.

5. Conclusion

Fruit and vegetable powdered smoothies reinforced with whey milk protein show high nutritional values and considerable antioxidant activity as a health-enhancing fortified food. In addition, due to their high protein and fibre content, they can be considered a healthy alternative food and labelled as a source of fibre (Regulation (EC) No 1924/2006). The knowledge achieved in this manuscript will contribute to the development of new high-value products for valorisation of food waste such as surplus fruits and vegetables. These results are of great interest to the food industry and can contribute to addressing food waste and increase circularity in the food industry as well as achieving the SDG goal 2 (zero hunger).

The antioxidant properties of these powders would be related with the retention of phenol compounds after drying. The reconstitution of these powders with semi-skimmed milk instead of water increases flavonoids and tocopherol contents. *In vitro* GID may improve bioaccessibility of total phenols in detriment to flavonoids and tocopherol. As a

result, scavenging radical activities may improve. Digested smoothies retained good antioxidant values and might reinforce body antioxidant response. Digestive simulations provide valuable information on potential bioaccessibility beyond knowing antioxidant retention levels. Further nutritional studies will be necessary to establish whether digestion effects observed *in vitro* are also reproduced when these types of powdered smoothies are consumed.

Uncited references

FAO, 2003; FAO, 2013; Rocha Torres et al., 2022; Rodríguez-Roque et al., 2014; Victor-Aduloju et al., 2023., Li et al., 2023a, Li et al., 2023b

CRediT authorship contribution statement

Antonia M. Jiménez-Monreal: Writing – original draft, Methodology. **Cristina Cedeño-Pinos:** Writing – original draft, Methodology, Formal analysis. **Sancho Bañón:** Writing – original draft, Validation, Funding acquisition, Conceptualization. **Israel Muñoz:** Supervision, Funding acquisition, Conceptualization. **Maria Dolors Guardia:** Supervision, Funding acquisition. **Nisrine Tahori:** Methodology, Investigation, Formal analysis. **Magdalena Martínez-Tomé:** Supervision, Funding acquisition, Formal analysis.

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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.

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Data availability

No data was used for the research described in the article.

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