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High-fiber breakfast cereals using only carrot and cereal by-products†

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Dietary fibre intake remains below the recommended levels set by both the FAO and EFSA, limiting its well-established health benefits. Breakfast cereals are widely consumed globally but typically require nutritional improvements, particularly in reducing sugar and sodium content while increasing fibre. This study aimed to develop high-fibre breakfast cereals without added sugar, using only two ingredients: carrot flour and wheat or rice bran. Cold dough extrusion followed by drying and roasting was the production process chosen as a strategically simple and mild process. The final cereal formulations contained at least 40% fibre, with a soluble to insoluble dietary fibre (SDF/IDF) ratio of 1 : 3 for rice bran-based formulations and 1 : 5 for wheat bran-based formulations. The dietary fibre profile of the ingredients comprised pectins, β-glucans, galactans, arabinogalactans, soluble and insoluble arabinoxylans, cellulose, and lignin. The produced breakfast cereals had no added sugars and exhibited significant antioxidant and antidiabetic properties, attributed to the presence of phenolics, carotenoids, and vitamins A and E. This study demonstrates the feasibility of creating nutritious, high-fibre breakfast cereals from two simple ingredients using mild processing techniques that preserve or enhance bioactive compounds and associated health benefits.

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Sustainability spotlight

Global dietary fibre intake remains below recommended levels, while agri-food systems continue to generate vast amounts of underused by-products. This work addresses both challenges by creating high-fibre breakfast cereals using only carrot and cereal brans—side streams from vegetable and flour industries—processed with energy-efficient, mild extrusion and roasting. This innovation supports waste valorization, promotes healthy diets, and reduces reliance on refined ingredients or added sugars. The approach contributes to a circular economy and aligns with multiple UN Sustainable Development Goals (SDGs), notably SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), and SDG 12 (Responsible Consumption and Production). It demonstrates how simple, scalable interventions can generate nutritious food while minimizing environmental impact.

1. Introduction

Breakfast cereals products (BCP) are widely consumed across the globe. The average consumption in kg per capita may vary from less than 1 (Asia and Africa) to almost 7 (North America). Regarding countries, the higher consumption of BCP is found in France (16 kg per capita), United States and United Kingdom (9 kg per capita).[1] Approximately 50% of the population in developed countries consumes BCP regularly, with even higher percentages observed among children and adolescents.[1]

The popularity of BCP is primarily driven by convenience and taste.[2] However, multiple studies have shown that BCP often require nutritional improvement, particularly in reducing sugar, sodium and fat content. These concerns have been raised globally, including in the USA,[3] Australia,[3,4] Austria,[5] Belgium,[6] Canada,[3,7] France,[5] Italy,[8] Portugal,[9] Spain, United Kingdom,[3,10] Romania,[5] and New Zealand.[11,12] Notably, BCP targeting children frequently contain excessive amounts of sugar, saturated fats, and salt, and insufficient dietary fibre.[3,4,9,13] This nutritional gap underscores the need for reformulated BCP that meet modern health standards and dietary recommendations.

The importance of dietary fibre (DF) in promoting health is well established. Both EFSA and FAO recommend a minimum dietary fibre intake of 25 g per day [14] and U.S. Department of Health and Human Services recommend 33.6 g per day for men between 19–30 years and 28 g per day for women of the same age.[15] However, the actual intake amount is still under the recommendations in EU countries[14,16] and USA.[17] When taken at the recommended amount, DF reduces the risk of obesity, elevated waist-to-hip ratio, coronary heart disease, stroke, hypertension, type 2 diabetes, and various gastrointestinal disorders. Fibre contributes to the regulation of blood pressure,

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blood lipid profiles, glycaemic response, and inflammation markers levels.[18,19] Importantly, certain types of soluble dietary fibre (SDF) exhibit prebiotic properties that support the growth of beneficial intestinal microbiota, thereby contributing to gut health and systemic immune modulation. Insoluble dietary fibre (IDF), on the other hand, supports bowel regularity and may help prevent colorectal cancer.[20] All these health benefits of high fibre intake (≥28.5 g per day) result in lower mortality related to circulatory, digestive, and non-cardiovascular non-cancer inflammatory diseases.[21]

Despite the false believe of some consumers that BCP are healthy and a source of dietary fibre,[2] very few BCP have more than 10% of fibre[9] providing less than 3 grams of fibre per portion of 30 grams of product, which means that a meal of BCP usually gives less than 12% of the daily recommended dosage of 25 g per day. With these dosages, consumers are not able to achieve the dietary fibre dosage that prevents diseases, and the global population health is compromised. Therefore, increasing the fibre content in commonly consumed foods such as BCP presents a strategic opportunity to improve population health.

Fruit, vegetable and cereals industries generate significant quantities of by-products each year. More than 20% of fruit and vegetable are lost along the supply chain before arriving the retail level, and approximately 50% of by-products are generated in food industry.[22,23] These by-products can be transformed into flours which are rich in dietary fibre and other phytochemicals with health benefits[24] and can be used in the production of BCP.[1]

Wheat and rice brans are by-products from the production of wheat and rice flours, respectively. They are massively produced worldwide and have been used for animal feed or biogas production.[25,26] However, they can contribute positively to human health because of their content in bioactive compounds, such as fibre, lipids, vitamin E and phenolics.[25,26] Rice bran has been pointed out as underused given its proven health benefits coming from dietary fibre, essential fatty acids, γ-oryzanol, tocopherols and tocotrienols.[27]

This study aimed to develop high-fibre, sugar-free breakfast cereals using only two ingredients: carrot flour and cereal bran (wheat or rice), both sourced from food industry by-products. The use of cold extrusion and mild drying techniques was selected to preserve the functional and nutritional properties of the raw materials.

Several studies have shown that consumer is not always willing to sacrifice taste to get health benefits,[2,28] so, this study was divided into two parts: (i) first part aimed to evaluate the sensory performance of these products and reformulate accordingly for the second part; (ii) evaluate nutritional performance and potential health benefits of the formulations.

The specific objectives of this study were:

(i) to evaluate the sensory acceptability of formulations composed solely of carrot flour and bran;

(ii) to assess the nutritional composition, with a focus on fibre quantity and quality;

(iii) to determine the bioactive compound content and antioxidant and antidiabetic activities of the final products.

By leveraging food by-products and optimizing fibre content, this study contributes to both nutritional improvement and sustainable food processing.

2. Materials and methods

2.1. By-products flours production

All ingredients used in the production of BCP were sourced as by-products from the food industry. Each formulation only included two ingredients: carrot by-product flour and wheat or rice bran. Wheat and rice brans were kindly provided by Germen SA (Matosinhos, Portugal), while the carrot by-products, comprising non-compliant baby carrots (*Daucus carota* subsp. *sativus*), were supplied by Vitacress Portugal SA (Odemira, Portugal). Carrot by-product flour was prepared as previously described.[24] Briefly, the carrots were washed with tap water to remove residual organic matter, followed by disinfection using sodium hypochlorite solution (150 ppm, 5 L kg^{-1} vegetable), for 15 min. After rinsing to eliminate chemical residues, the carrots underwent two steps for water removal: (1) the carrot juice was removed using a juice machine (model MES1020 of 380 W, Bosch) that separates solid part from the liquid; (2) the solid parts were dried at 55 °C until humidity lower than 5%, using a convection dryer (STI Lda, Lisbon, Portugal) equipped with a temperature and humidity probe (model QFA3171, Siemens). Finally, the dried pomace was turned into powder using a blender-type machine (model TM5, Vorwerk, Germany). The flours were stored at room temperature in polyethylene bags, under vacuum conditions and protected from light until further use.

Flours were sieved through 2 mm and 100 μm sieves, and the final flours used into the cereals' formulations consisted of particle sizes in the range 0.1 to 2 mm.

2.2. Breakfast cereals production

BCP were produced according to the method described before.[1] The two flours (carrot flour and wheat or rice bran) were mixed followed by gradual addition of water corresponding to 50% of formulation weight. The mixtures were homogenized and left to stabilize in covered containers at 4 °C, overnight.

The moisture content was determined before extrusion using a moisture analyser (KERN, Germany) and ranged between 36 and 39%.

Cold dough extrusion was performed in a cold extrusion equipment (Nudelmaschine PN 100, HAUSSLER, Deutschland) with a single-screw and a 59 mm diameter die plate, which had twenty 2 × 3 mm oval shape die holes. The extrudates were cut into desired lengths using the attached cutting mechanism (Emma PN 100, HAUSSLER, Deutschland).

The extrudates were then dried at 50 °C in a circulating air stove until moisture below 5%. For roasted variants, half portion of each batch was additionally roasted at 180 °C for 4 min in a circulating air oven.

All BCP samples were stored in polyethylene flexible bags at −20 °C until analysis and at −80 °C for analysis of bioactive compounds and bioactivities.

Table 1 Ingredients proportions and application of roasting process used in each breakfast cereal product for assessing the effect of formulation and production process on nutritional and bioactive composition and biological properties

Formulation	Carrot by-product flour (g)	Wheat bran (g)	Rosted
A	70	30	No
B	40	60	No
AR	70	30	Yes
BR	40	60	Yes
	Carrot by-product flour (g)	**Rice bran (g)**	
C	70	30	No
D	40	60	No
CR	70	30	Yes
DR	40	60	Yes

2.3. Formulations

2.3.1. Formulations for part 1 – sensory evaluation. To determine the sensory acceptability of these BCP containing only two ingredients, the tested two formulations consisted of 80% of carrot flour/20% wheat bran flour and 80% of wheat bran/20% carrot flour. To reduce the number of tastes, this test was performed only with wheat bran (rice bran was only introduced in the second part of this work). Therefore, two dried formulations were prepared, and half of the amount was roasted, ending with four samples to evaluate by consumer in a Focus Group (FG) meeting.

2.3.2. Formulations for part 2 – analysis of the effect of processing on health functionality and texture quality. FG results determined the formulations for the second part of this work, as discussed in the results section. The formulations used in this part are presented in Table 1. Four formulations were produced, two with wheat bran and two with rice. Additionally, half of the amount produced for each formulation was roasted, thus there was a total of eight cereals products.

2.4. Focus group

To understand consumers' preferences, opinions, and attitudes towards the new BCP formulations, a FG interview was performed. This FG was performed with 9 female participants, with ages between 25 and 35 years old from the university community. All participants signed informed consents and agreed with the recording of the meeting. Four samples were presented to the participants corresponding to two formulations and the correspondent roasted samples, as presented in Fig. 1.

The discussion topics were concerned to appearance, odour, taste, texture, and aftertaste of the samples. For appearance and odour, samples were evaluated dry. Taste, texture, and aftertaste were discussed in the dry form and also with milk. After the dry tasting, participants were asked to add milk (at room temperature) to the cup, and taste the cereals using a spoon.

After the meeting, all opinions were transcribed to text by topic. Afterwards, the information was analysed and organised by topics, highlighting the main conclusions, and pointing out all the different participants' ideas.

2.5. Proximate composition

Before any analysis, samples were ground in a coffee bean grinder (Caso® Design, Germany) and weighted in triplicate for all analysis.

Total protein was determined by Kjeldahl (Kjeltec system 1002 distilling unit (Tecator; Hoganas, Sweden), conversion factor was 6.25). Total fat content was determined by a Soxhlet method, using petroleum ether as the extraction solvent. Moisture and ash contents were determined according to the Association of Official Analytical Chemists (AOAC). All results were expressed as g/g dry weight (DW). Total carbohydrates were obtained by calculation, by the difference between the dry sample mass and the mass corresponding to proteins, fat and ash.

Total starch was determined by enzyme-spectrometric AOAC Method 996.11 using K-TSTA-100A Megazyme kit (Megazyme, Neogen). Before weighting, samples were sieved to particle size bellow 500 μm. First, D-glucose and matodextrins were removed with ethanol, then dimethyl sulphoxide (DMSO) were used to solubilise resistant starch. Next, the enzymatic degradation of the starch was conducted using α-amylase and amyloglucosidase sequentially. Finally, after dilution, an aliquot was mixed with glucose oxidase/peroxidase (GOPOD) reagent and let to react at 50 °C, for 20 minutes and then the absorbance was measured against the blank at 510 nm.

2.6. Dietary fibre, resistant protein and monosaccharides

Total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) were determined using the enzyme-gravimetric AOAC 991.43 method with slight modifications according to.[29] Resistant protein was determined for both SDF and IDF. As indicated by the AOAC 991.43 method, one of the duplicates was used to perform protein analysis on the fibre residue using Kjeldahl method and the 6.25 factor to convert

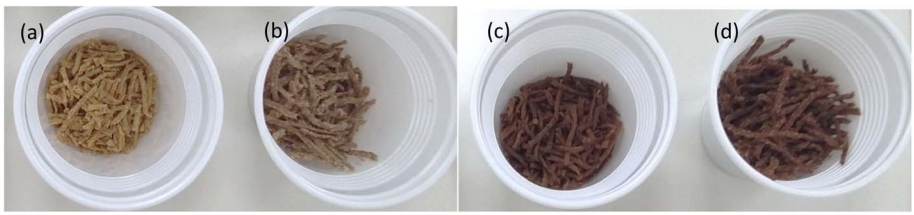

Fig. 1 BCP samples presented to focus group participants. (a) – carrot/wheat bran (80 : 20), (b) – carrot/wheat bran (20 : 80). (c) and (d) correspond to (a) and (b) roasted samples.

nitrogen to protein content. IDF and SDF monosaccharides were also determined using the methodology previously described by.[29] All measurements were done in triplicate and expressed as g/100 g DW.

2.7. Free sugars

Free sugars were extracted with 80% ethanol (20 mL for 1 g of sample, repeated three times), followed by homogenization with a high-performance dispersing instrument (T25 digital ULTRA-TURRAX® with a S18N-19 G dispersing tool, IKA, Germany), ultrasound bath for 10 minutes and centrifugation at 5000 rpm for 5 minutes. Supernatants were collected together, and the total volume was reduced below 10 mL using a rotatory evaporator (R-114, BüCHI, Flawil, Switzerland). The final volume was then corrected to 10 mL using a volumetric flask. Then, the extracts were evaluated by HPLC after being filtered (0.45 μm, Orange Scientific, Brain-l'Alleud, Belgium), using a Beckman Coulter System Gold HPLC (Knauer, Berlin, Germany) coupled to an Aminex® HPX-87P column (Bio-rad, Berkeley, USA) and a RI detector. Ultrapure water was used as the mobile phase (flow rate: 0.5 mL min^{-1}) and the measurements were performed at 85 °C. The quantification was achieved using standard calibration curves (0.3–20 mg mL^{-1}).

2.8. Minerals composition

For minerals composition evaluation, a microwave acid digestor was used (MARS ONE, 240/50, CEM, USA) according to producer instructions. Each sample (0.5 g) was introduced into the digestion vessel (Mars Xpress, 75 mL) and 10 mL of 65% nitric acid was added. The digestion programme was initiated by a temperature ramp (20 min) to 210 °C, and hold at that temperature for 15 min. At the end of digestion, the extracts were diluted up to 50 mL and evaluated by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Optima, 7000 DV ICP-OES, PerkinElmer, USA). Calibration curves with standard solutions were used for quantification.

2.9. Bioactive compounds and bioactivities

Total phenolic content (TPC), antioxidant capacity and antidiabetic capacity were determined after extraction of bioactive compounds according to[30] with slight modifications.

2.9.1. Extraction of free and bound phenolics for TCC, antioxidant activity, and antidiabetic activity analysis. A methanolic aqueous solution (25 mL, 80% v/v) was mixed with 2.5 g of flour/BCP (triplicates) using a high-performance dispersing instrument (T25 digital ULTRA-TURRAX® with a S18N-19G dispersing tool, IKA, Germany), at 24 000 rpm for 30 seconds. The mixture was then left for extraction in an orbital shaker for 30 min, at 300 rpm, room temperature, dark conditions.

After centrifugation (4480*g*, 10 min, 4 °C), supernatants were collected and concentrated below 10 mL volume using a rotary evaporator (R-114, BüCHI, Flawil, Switzerland). Final volume was corrected to 10 mL with ultrapure water using volumetric flasks. Finally, the extracts were filtered (0.45 μm, Orange Scientific, Brain-l'Alleud, Belgium) and stored at −80 °C in 2 mL aliquots until their analysis (in 2 days maximum).

The sediments (pellets) were stored at −20 °C and further used to extract bound phenolics. 2 M NaOH solution (20 mL) was added to the extraction tube and the headspace was flushed with N_2 to remove the air. Samples were stirred in an orbital shaker (200 rpm) for 4 h at room temperature and dark conditions. Afterwards, 6 M HCl was used to acidify the solutions up to pH 1.5–2. The extraction of liberated phenolics was achieved by shaking samples with 60% ethanol in an orbital shaker for 30 min at room temperature, dark conditions. Finally, samples were centrifuged (4480*g*, 10 min, 4 °C), the supernatant collected and evaporated to reduce the volume below 10 mL (corrected to 10 mL at the end with ultrapure water in a volumetric flask), filtered by 0.45 μm and stored at −80 °C until their analysis.

TPC of flours and BCP was determined according to the Folin-Ciocalteu spectrophotometric method,[31] performed in a 96-well microplate according to.[32] The previous extracts of free and bound phenolics (30 μL) were mixed with 100 μL of Folin-Ciocalteu reagent (20% v/v) and 100 μL of anhydrous sodium carbonate solution (7.4% m/v). After shaking thoroughly and incubating for 30 min at 25 °C, the absorbance was measured at 765 nm using a Multidetection plate reader (Synergy H1, Vermont, USA) operated using the Gen5 software (BioTek Instruments). Gallic acid (0.025–0.200 mg mL^{-1}) was used as standard and results were expressed in milligrams of gallic acid equivalents per g of sample DW (mg_{GAE} g_{DW}^{-1}). The measurements were performed in triplicate for each extract replicate, also performed in triplicate.

The antioxidant capacity was measured using the free and bound phenolics extracts by ABTS, DPPH and ORAC scavenging assays according to the methods previously described.[33–35]

ABTS stock solution was obtained by reacting 7 mM ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) diammonium salt solution (Sigma-Aldrich, St. Louis, MO, USA) with 2.45 mM potassium persulfate (Merck, Kenilworth, NJ, USA) in dark, at room temperature for 16 hours. The ABTS assay was performed in a 96-well microplate, by adding 20 μL of the extract to 180 μL of $ABTS^{\cdot+}$ working solution, which was obtained by filtering (0.22 μm) the ABTS stock solution and diluting it with distilled water to an absorbance of 0.700 ± 0.020 at 734 nm. The absorbance of the test was read after 6 min of reaction at room temperature.

For DPPH assay, 25 μL of extract is added 175 μL of 90 μM $DPPH^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich, St. Louis, MO, USA) methanolic solution. The mixture was incubated at 25 °C and the absorbance was measured at 515 nm after 30 min of reaction.

For both assays, samples were diluted when needed in order to achieve inhibition percentage between 20–80%. Standard Trolox solution (25–250 μM) were used for the calibration curves. Measurements were performed in triplicate for each extract replicate.

For ORAC assay, a black 96-well microplate was used and the solutions were prepared in 75 mM phosphate buffer (pH 7.4). The extract (20 μL) and fluorescein (120 μL; 70 nM final concentration in well) solutions were placed in the well of the microplate and the mixture was preincubated for 10 min at 37 °

C. After this time, AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride) solution (60 μL; 12 mM, final concentration in well) was added rapidly using a multichannel pipet. The microplate was immediately placed in the reader and the fluorescence recorded at intervals of 1 min over a period of 80 min. Phosphate buffer blanks and eight calibration solutions using Trolox (1–8 μM, final concentration in well) as antioxidant were also analysed in each assay.

For all assays, incubation and absorbance measurements were performed on the Multidetection plate reader (Synergy H1, Vermont, USA) operated using the Gen5 software (BioTek Instruments).

Antidiabetic capacity was measured by the ability to inhibit the enzyme α-glucosidase (EC 3.2.1.20).[36] Acarbose (10 mg mL^{-1}) was used as a positive control and 5 mM *p*-nitrophenyl-α-D-glucopyranoside as substrate. Both were prepared with 0.1 M phosphate buffer (pH 6.9). The buffer was used as negative control. The analysis occurred in a 96-well microplate and a multiscan microplate reader (Synergy H1; BioTek Instruments, Winooski, VT, USA) according to the method of.[37] The phenolic extracts (50 μL) were mixed with 100 μL of 1.0 U mL^{-1} α-glucosidase (prepared with the phosphate buffer) and the mixture was pre-incubated at 25 °C for 10 min. Then, it was added 50 μL of substrate (or positive or negative control) and the absorbance was recorded at 405 nm during 5 min incubation at 25 °C. The inhibitory ability was calculated following the equation presented below and expressed as percentage inhibition.

$$\alpha\text{-Glucosidase inhibition}(\%) = \frac{\Delta\text{Abs (negative control)} - \Delta\text{Abs(sample)}}{\Delta\text{Abs(negative control)}} \times 100$$

where ΔAbs (negative control) and ΔAbs (sample) correspond to the absorbance variation of the negative control and the sample, respectively.

2.9.2. Carotenoids, retinol and tocopherols extraction and quantification. Carotenoids, retinol and tocopherols isomers were extracted (triplicates) as described before[38] with modifications according to.[39] Flours or ground BCP (100 mg) were mixed with 26 mg of ascorbic acid and 3 mL of ethanol using vortex for 10 seconds and then the extraction occurred under 85 °C for 5 min in a water bath. Saponification occurred by adding 380 μL of 5 M KOH, mixing by vortex for 10 seconds and reacting in a water bath (85 °C, 10 min, vortex after 5 min). Then tubes were cooled and kept in ice during extraction. 3 mL of 1 M NaCl were added to each sample, the tubes were gently inverted 5 times and 4 mL of *n*-hexane (containing 25 μg mL^{-1} BHT) were added. Then tubes were vortexed for 10 seconds and centrifuged (1500*g*, 5 min, a 4 °C). Supernatants were collected, the extraction with hexane was repeated on more time and supernatants were collected.

Carotenoids were identified and quantified using an HPLC-DAD system (Beckman System Gold®, 508 Autosampler, 126 Solvent Module and 168 Detector) with a reverse-phase column (Kromasil 100-5-C18, 4.6 mm I.D. × 250 mm) and the detector at 454 nm. The mobile phase contained acetonitrile, methanol, dichloromethane, hexane and ammonium acetate (55 : 22 : 11.5 : 11.5 : 0.02 v/v/v/v/w) and was used under isocratic conditions at 1 mL min^{-1} flow rate for 20 min, 30 °C.[30] The injected sample volume was 50 μL. Lutein, β-cryptoxanthin, lycopene, α- and β-carotene were quantified using pure standard calibration curves.

Tocopherols isomers and retinol were identified and quantified according to[40] using a HPLC (Beckman System Gold®) linked to a Waters™ 474 Scanning Fluorescence Detector (excitation wavelength of 295 nm and 244 nm for tocopherols and retinol, respectively, and emission wavelength of 325 nm and 472 nm for tocopherols and retinol, respectively) and a Varian ProStar Model 410 AutoSampler. The column was a normal-phase silica column (Kromasil 60-5-SIL, 250 mm, 4.6 mm ID, 5 μm particle size) and the mobile phase was 1% v/v isopropanol in *n*-hexane with a flow rate of 1 mL min^{-1}. The total run time was 20 min and the injection volume was 20 μL. Standard curves were used for each compound quantification.

For total carotenoids content (TCC) the absorbance of the carotenoids extracts at 454 nm was measured with a UV min 1240 spectrophotometer (Shimadzu, Tokya, Japan). A calibration curve (0.005–0.030 mg mL^{-1}) of a pure β-carotene standard (Sigma-Aldrich, St. Louis, MO, USA) was used to quantify TCC.

2.10. Statistical analysis

Data followed a normal distribution (which was evaluated by Shapiro–Wilk test) and differences between samples were assessed by one-way analysis of variance (ANOVA), at a degree of significance of $p < 0.05$, followed by the Tukey's post-hoc multiple comparison test also at $p < 0.05$ significance level. All statistical analyses were performed utilising SPSS 22 software program (SPSS Inc., Chicago, IL, United States).

3. Results and discussion

3.1. Consumer preference and formulations selection

Initial formulations consisting of 80 : 20 and 20 : 80 ratios of carrot flour to wheat bran were evaluated in a focus group (FG) to assess consumer perceptions of sensory quality. Table 2 present the main conclusions retrieved from this discussion.

The FG revealed that formulations with higher carrot flour content had a more appealing visual appearance, while roasted versions generally had a better aroma, especially the one with a higher wheat bran proportion. In terms of flavour, the formulation containing 20% carrot flour was considered to have the worst flavour and the formulation with 80% carrot flour was considered to have an undesirable texture, described as "difficult to swallow". Roasting decreased taste quality and improve texture slightly. However, overall taste and texture were still considered unsatisfactory. Participants also reported a bitter aftertaste in the sample with 80% carrot flour.

Based on these findings, formulations using 70% and 40% carrot flour were selected for further development, as they presented more balanced sensory characteristics.

3.2. Fibre composition of the ingredients

Table 3 presents the fibre composition (both SDF and IDF) of the ingredients used in this study. Carrot flour had the highest SDF content and rice bran has the lowest IDF content.

Table 2 Summary conclusions of focus group meeting

Feature	Carrot/wheat bran 80 : 20				Carrot/wheat bran 20 : 80			
Appearance	Regarding appearance, participants mentioned that these BCP were similar to those on the market (All-bran®). When roasted, the appearance quality decreased comparing to the corresponding samples only dried at 50 °C							
Odour	Regarding odour, participants indicated that roasting improved odour and the roasted sample with 20% carrot flour was preferred							
Sensory analysis	Dry		With milk		Dry		With milk	
BCP finishing type	Dried	Dried + roasted	Dried	Dried + roasted	Dried	Dried + roasted	Dried	Dried + roasted
Taste	Tastes better than its odour. Tastes a bit as raw carrot	The worst flavour among roasted samples	Floral flavour	Tastes roasted	The worst taste of all. Very astringent	Roasting made it taste worse	Too bitter and astringent	The roasting didn't improve flavour nor texture
Texture	It created a lot of bolus in mouth and it is not pleasant, it's difficult to process	Almost no improvement, it still creates a lot of bolus	It keeps in mouth for too long. It gets like porridge super quick	The texture improves (maintains the crispiness for longer time) but is still bad, because is very dry	Low crispiness. It is soft and very dry	Roasting improved texture but it is still bad. It's still very dry	It makes a big bolus in mouth	
Aftertaste	The most intense and long aftertaste. Very bitter	Nothing pointed	Nothing pointed	Nothing pointed	Aftertaste very bitter	Nothing pointed	Nothing pointed	Nothing pointed

Carrot flour presented an SDF/IDF ratio of 1 : 1.3, while wheat and rice brans presented 1 : 7 and 1 : 3 SDF/IDF ratios respectively. Previous studiesdemonstrated that SDF/IDF ratio affects the health benefits of fibre, such as cation exchange capacity, glucose absorption capacity, and cholesterol absorption capacity. These studies also demonstrates that the ideal proportions for maximum health benefits involve higher SDF content.[41,42] Other study pointed that the most appropriate proportion of SDF and IDF for the best health benefits should be 30–50% and 70–50%, respectively.[43] Carrot flour is nearer this proportion, which is consistent with previous research showing that fruit and vegetable fibres generally have higher SDF content than cereal fibres.[44]

Carrot flour was rich in uronic acids (32%) and galactose (22% of fibre), consistent with the presence of pectins. Pectins are composed of galacturonic acid units linked to rhamnopyranose units from which occur side chains of galactose, mannose, glucose and xylose (it may contain galacturonans, rhamnogalacturonans, arabinans, galactans, and arabinogalactans[45]). Thus, carrot flour is the one richer in pectin as expected. As other SDF, because of its solubility and high gelling capacity, pectins have been reported to reduce cholesterolaemia, improve lipid metabolism, gastric emptying and glucose metabolism, and may act in the prevention or treatment of intestinal infections, atherosclerosis, cancer and obesity.[46]

Brans exhibited lower levels of uronic acids (1% and 0.3%) and higher contents of xylose and arabinose, indicartive of hemicellulose.[25,45,47] Glucose in SDF suggests the presence of β-glucans, which are another group of polysaccharides formed by glucose by β-(1 → 3) and β-(1 → 4) linkages. Wheat bran showed the highest β-glucan content, which is also expected given previous studies on wheat bran,[47] rice bran[25] and carrot.[45]

IDF in rice bran was primarily composed of cellulose, as expected,[48,49] while in carrot flour and wheat bran, hemicelluloses

Table 3 Fibre composition of the ingredients (g/100 g of fibre, except for fibre content)

		Carrot flour		Wheat bran		Rice bran	
		SDF	IDF	SDF	IDF	SDF	IDF
Type of fibre (g/100 g ingredient DW)		24.67 ± 2.62	31.26 ± 1.30	4.90 ± 0.31	33.60 ± 1.94	7.97 ± 0.00	23.73 ± 0.48
Monosaccharides	Glucose	0.69 ± 0.17	8.82 ± 0.39	4.00 ± 0.26	17.81 ± 0.37	0.58 ± 0.16	19.37 ± 0.37
	Xylose	0.00 ± 0.00	0.65 ± 0.14	6.69 ± 0.44	21.28 ± 1.02	1.34 ± 0.17	10.80 ± 0.51
	Galactose	21.65 ± 1.80	10.34 ± 0.39	2.00 ± 0.30	2.11 ± 1.30	1.34 ± 0.05	5.59 ± 2.16
	Arabinose	5.55 ± 1.10	6.47 ± 1.55	5.69 ± 0.38	6.18 ± 1.88	2.44 ± 0.19	4.97 ± 2.53
	Mannose	0.00 ± 0.00	0.00 ± 0.00	1.39	0.00 ± 0.00	1.11 ± 0.27	0.00 ± 0.00
	Fructose	1.45 ± 0.70	5.57 ± 0.87	0.63	1.52 ± 0.33	0.00 ± 0.00	0.69 ± 0.03
Uronic acids		31.46 ± 0.37	11.17 ± 0.20	1.14 ± 0.02	2.22 ± 0.13	0.27 ± 0.01	3.41 ± 0.03
Klason lignin		—	25.65 ± 0.19	—	6.20 ± 2.44	—	15.44 ± 5.50
Resistant protein		15.87	7.21	35.00	10.47	20.32	16.50

and lignin were more prevalent. Lignin content was especially high in carrot flour (27%), contributing to faecal bulk and reduced gut transit time, although its role in cancer prevention remains debated.[50,51] Rice bran presented hemicellulose composed mostly of xylose (11%), galactose (6%), arabinose (5%) and mannose (1%). It is known that rice bran IDF is composed of cellulose, arabinoxylan, galactan and uronic acids,[25] which is in accordance with the results obtained. For wheat bran, arabinoxylan (6% arabinose and 21% xylose) is the principal IDF, as expected, followed by cellulose (18% glucose) and lignin (6%).[52,53] Water insoluble arabinoxylans promote the growth of probiotics from genus *Bacteroides*, which produces more propionate, which in turn, inhibits cholesterogenesis and lipogenesis, thus reducing cholesterolaemia and cardiovascular diseases.[54] For carrot flour IDF there is mainly lignin (27%), arabinogalactans (7% arabinose and 10% galactose), uronic acids (11%) and cellulose (9% glucose), which is also in accordance to previous works.[55]

Resistant proteins were also quantified and were more abundant in the SDF fraction across all ingredients. Although their health effects are still under investigation,[56–60] their presence may contribute to gut fermentation processes.

3.3. Proximate composition of ingredients and cereals formulations

As shown in Table 4 cold extrusion and roasting did not significantly alter the proximate composition of the cereal formulations, as expected.

Carrot flour,[45] wheat bran[47,53] and rice bran[27] proximate composition were in accordance with previous works. The small differences observed for carrot flour regarding fibre content, when comparing to previous results, are expectable considering the probable differences in maturation of samples. It is known that during growing season, there is a decrease in fibre content.[61] Additionally, several studies have been proving that growing location, genotype, crop year, climate conditions and stresses influence TDF, SDF and IDF contents.[62]

Carrot flour presented the highest TDF content (55.9%) and lowest protein and total fat contents. Rice bran exhibited the highest total fat content (22.7%), while wheat bran had the highest starch content (36.4%). In general, all ingredients are potentially good sources of fibre to produce high fibre BCP, nevertheless carrot flour presented the highest TDF content (56%).

For all formulations, TDF content was at least 51% allowing a 30 g serving to deliver up to 15 g of fibre, equivalent to 60% of the daily recommended dosage. Worth to mention that dietary recommendations are only about the minimum amount that should be ingested,[14] so there is no recommended upper limit for dietary fibre.

3.4. Free sugars

Sucrose was the predominant sugar in all ingredients. It is well known that sucrose is by far the most important sugar reserve formed by carrot, followed by fructose and glucose.[61] Fructose was not detected in both brans (Table 5).

After extrusion, sucrose content was as expected according to the sucrose content of each ingredient, but for glucose and fructose the results were higher than expected (Table 5) most likely related to the degradation of starch.[63]

Roasting significantly decreased glucose content but did not affect the sucrose or fructose contents (Table 5). This is related to caramelization and Maillard reaction that occurs during roasting by heat action. Previous studies have been demonstrating that glucose is rapidly destroyed during roasting duet to high temperatures,[64] corroborating the results obtained in the present study.

Formulation C showed the higher sugar content, 21 g total sugars/100 g of product, and could have the nutritional claim "CONTAINS NATURALLY OCCURRING SUGARS" according to the European policies.[65]

3.5. Minerals composition

The results of mineral composition (Table 6) are as predicted for carrot flour,[61] wheat[47] and rice[66] brans. Previous works demonstrates carrots providing mainly potassium (K), sodium (Na), phosphorus (P), calcium (Ca) and magnesium (Mg) by 32, 6.9, 3.5, 3.3 and 1.2 mg g^{-1} respectively.[61] Wheat bran did not provide any especial amount of any mineral comparing to the other ingredients. Previous work presented similar minerals

Table 4 Proximate composition of cereal formulations and ingredients (g/100 g DW, except for moisture)[a]

Formulation	Moisture	Ash	Protein	Total fat	Total CH	Total starch	TDF	SDF	IDF
A	4.2 ± 0.0^{f}	6.8 ± 0.0^{c}	10.9 ± 0.2^{c}	1.9 ± 0.0^{a}	80.4 ± 0.2^{f}	2.4 ± 0.0^{b}	$53.5 \pm 0.8^{c,d,e}$	$19{,}8 \pm 0.3^{d}$	$33.8 \pm 0.4^{b,c,d}$
AR	2.5 ± 0.0^{c}	6.8 ± 0.0^{c}	$10.7 \pm 0.1^{b,c}$	2.0 ± 0.1^{a}	80.5 ± 0.1^{f}	2.5 ± 0.1^{b}	$53.5 \pm 0.9^{c,d,e}$	20.5 ± 0.4^{d}	$33.0 \pm 0.6^{b,c,d}$
B	4.2 ± 0.0^{f}	5.9 ± 0.0^{b}	14.0 ± 0.3^{e}	3.0 ± 0.0^{b}	77.2 ± 0.2^{e}	7.8 ± 0.0^{d}	$53.8 \pm 0.5^{c,d,e}$	14.4 ± 0.3^{c}	$39.4 \pm 0.8^{e,f}$
BR	2.0 ± 0.1^{b}	5.9 ± 0.0^{b}	13.8 ± 0.0^{e}	3.1 ± 0.0^{b}	77.2 ± 0.0^{e}	7.8 ± 0.0^{d}	$52.6 \pm 0.0^{c,d}$	15.1 ± 0.0^{c}	$37.9 \pm 0.4^{d,e,f}$
C	3.9 ± 0.0^{e}	7.5 ± 0.6^{d}	10.5 ± 0.1^{b}	8.2 ± 0.0^{c}	$73.8 \pm 0.7^{c.d}$	2.2 ± 0.0^{b}	51.1 ± 0.9^{c}	21.1 ± 0.5^{d}	30.1 ± 0.4^{b}
CR	2.1 ± 0.1^{b}	7.9 ± 0.0^{d}	10.5 ± 0.1^{b}	8.4 ± 0.1^{c}	73.3 ± 0.1^{c}	2.1 ± 0.1^{b}	$51.9 \pm 0.4^{c,d}$	$22.4 \pm 0.4^{d.e}$	29.5 ± 0.1^{b}
D	3.5 ± 0.0^{d}	8.6 ± 0.0^{e}	12.2 ± 0.0^{d}	14.5 ± 0.0^{d}	64.7 ± 0.0^{b}	5.7 ± 0.3^{c}	$53.2 \pm 2.3^{c,d,e}$	10.5 ± 0.7^{b}	$42.7 \pm 3.0^{f,g}$
DR	1.8 ± 0.1^{a}	8.7 ± 0.0^{e}	12.4 ± 0.1^{d}	14.5 ± 0.0^{d}	64.4 ± 0.0^{b}	5.7 ± 0.0^{c}	$54.3 \pm 1.4^{d,e}$	9.2 ± 3.1^{b}	45.2 ± 4.5^{g}
Carrot flour	9.3 ± 0.0^{h}	7.7 ± 0.0^{d}	8.6 ± 0.0^{a}	1.9 ± 0.0^{a}	81.8 ± 0.0^{g}	0.2 ± 0.0^{a}	55.9 ± 0.9^{e}	24.7 ± 1.9^{e}	$31.3 \pm 0.9^{b,c}$
Wheat bran	11.1 ± 0.1^{i}	4.5 ± 0.0^{a}	17.0 ± 0.2^{g}	3.7 ± 0.1^{b}	74.8 ± 0.2^{d}	36.4 ± 0.4^{f}	38.5 ± 1.6^{b}	4.9 ± 0.3^{a}	$33.6 \pm 1.9^{c,d,e}$
Rice bran	8.4 ± 0.1^{g}	10.2 ± 0.0^{f}	15.1 ± 0.1^{f}	22.7 ± 0.8^{e}	52.1 ± 0.9^{a}	14.1 ± 0.3^{e}	31.7 ± 0.5^{a}	8.0 ± 0.0^{a}	23.7 ± 0.5^{a}

[a] A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples. Moisture is presented as g/100 g of product and the following components are presented as g/100 g of dry weight of product for comparison. Means with different upper letter within the same column, differ (p-value < 0.05).

Table 5 Free sugars content (g/100 g of sample DW) on the cereal's formulations and ingredients[a]

Formulation/Ingredient	Sucrose	Glucose	Fructose	Total sugars
A	$11.98 \pm 3.30^{c,d,e}$	1.69 ± 0.48^{f}	3.69 ± 0.04^{c}	$17.36 \pm 3.79^{d,e,f}$
AR	$13.94 \pm 0.29^{d,e,f}$	$1.17 \pm 0.04^{d,e}$	3.88 ± 0.44^{c}	$18.99 \pm 0.76^{e,f}$
B	$5.87 \pm 0.13^{a,b}$	2.67 ± 0.10^{g}	$3.03 \pm 0.00^{b,c}$	$11.57 \pm 0.23^{b,c}$
BR	$5.80 \pm 0.35^{a,b}$	$1.12 \pm 0.05^{c,d,e}$	$2.76 \pm 1.04^{b,c}$	9.68 ± 1.41^{b}
C	$15.37 \pm 0.14^{e,f}$	$1.55 \pm 0.02^{e,f}$	4.05 ± 0.81^{c}	$20.97 \pm 0.95^{e,f}$
CR	15.85 ± 0.47^{f}	$0.69 \pm 0.02^{b,c}$	$2.92 \pm 0.17^{b,c}$	19.47 ± 0.64^{f}
D	$11.23 \pm 0.22^{c,d}$	$1.27 \pm 0.03^{d,e,f}$	$1.60 \pm 0.01^{a,b}$	$14.11 \pm 0.24^{c,d}$
DR	$8.37 \pm 2.75^{b,c}$	$0.40 \pm 0.12^{a,b}$	$1.68 \pm 1.26^{a,b}$	$10.45 \pm 2.09^{b,c}$
Carrot flour	$13.66 \pm 0.44^{d,e,f}$	$0.95 \pm 0.02^{c,d}$	1.82 ± 0.36^{b}	$16.42 \pm 0.10^{d,e}$
Wheat bran	2.62 ± 0.18^{a}	$0.34 \pm 0.08^{a,b}$	0.00 ± 0.00^{a}	2.96 ± 0.19^{a}
Rice bran	$5.28 \pm 0.05^{a,b}$	0.20 ± 0.02^{a}	0.00 ± 0.00^{a}	5.48 ± 0.06^{a}

[a] A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples. Means with different upper letter within the same column, differ (p-value < 0.05).

patterns with 11.8, 10.1, 6.1 mg g^{-1} of wheat bran for K, P, Mg respectively, 0.11 mg g^{-1} for both iron (Fe) and manganese (Mn), 0.7 mg g^{-1} of Ca and 0.02 mg of Na per g of wheat bran.[47] Rice bran was the richer in P, K, and Mg, which is in accordance with previous works that also demonstrates P, K and Mg as the main mineral in rice bran with ranges of 15–29 mg g^{-1} for P, 14–24 mg g^{-1} for K, 9–12 mg g^{-1} for Mg. For the other minerals it was also coherent with these studies: 0.1–0.9 mg g^{-1} of Mn, 0.1–1.3 mg g^{-1} of Ca and 0–0.3 mg g^{-1} of Na.[66]

Table 6 also shows the recommended daily allowance for each mineral found in the BCP.[67,68] In Table 6, minerals are presented by order of recommended daily amount, which also corresponds to the order of mineral content in carrot flour. Thus, the formulations with 70% of carrot and 30% of wheat bran are the most aligned with the recommended daily allowance.

A more detailed discussion for each mineral can be found on ESI† file.

In general, it is observed that the mineral composition of theses BCP contribute to a balanced and healthy minerals intake.[69]

Table 6 Mineral composition (mg g^{-1}) of cereals formulations and ingredients[a]

Mineral	K	Na	P	Ca	Mg	Zn	Fe	Mn	Cu	Al
Recommended daily allowance (mg per day)[b]	3500	2400	800–1300	800–1300	200–400	8–11	8–18	2–11	1.0–1.6	—
Samples[c]										
A	$19.11 \pm 0.40^{c.d}$	6.35 ± 0.14^{e}	6.35 ± 0.14^{b}	3.26 ± 0.06^{f}	1.93 ± 0.04^{b}	$0.07 \pm 0.00^{e.f}$	0.07 ± 0.00^{d}	0.06 ± 0.00^{c}	0.01 ± 0.00^{f}	0.01 ± 0.00^{c}
AR	$19.71 \pm 0.09^{d.e.f}$	6.45 ± 0.11^{e}	6.48 ± 0.02^{b}	3.22 ± 0.01^{f}	1.94 ± 0.01^{b}	0.07 ± 0.00^{f}	0.07 ± 0.00^{d}	0.06 ± 0.00^{c}	$0.01 \pm 0.00^{f.g}$	0.01 ± 0.00^{c}
B	15.89 ± 0.30^{b}	3.41 ± 0.01^{b}	8.73 ± 0.08^{d}	2.34 ± 0.00^{e}	2.82 ± 0.03^{d}	0.09 ± 0.00^{h}	0.10 ± 0.00^{g}	0.12 ± 0.00^{f}	0.02 ± 0.00^{h}	0.01 ± 0.00^{c}
BR	16.31 ± 0.22^{b}	3.78 ± 0.09^{c}	6.45 ± 0.09^{b}	2.01 ± 0.08^{d}	2.30 ± 0.03^{c}	0.07 ± 0.00^{e}	0.08 ± 0.00^{e}	0.09 ± 0.00^{e}	$0.01 \pm 0.00^{g.h}$	0.01 ± 0.00^{c}
C	$20.08 \pm 0.19^{e.f.g}$	$6.25 \pm 0.12^{d.e}$	7.91 ± 0.04^{c}	2.39 ± 0.04^{e}	3.31 ± 0.00^{e}	$0.04 \pm 0.00^{a.b}$	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}	0.01 ± 0.00^{e}	$0.01 \pm 0.00^{c.d.e}$
CR	$20.46 \pm 0.19^{f.g}$	6.10 ± 0.10^{d}	7.98 ± 0.04^{c}	2.39 ± 0.05^{e}	3.30 ± 0.04^{e}	0.04 ± 0.00^{b}	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}	$0.01 \pm 0.00^{d.e}$	$0.01 \pm 0.00^{c.d}$
D	$19.54 \pm 0.65^{c.d.e}$	$3.57 \pm 0.04^{b.c}$	12.44 ± 0.21^{e}	1.65 ± 0.02^{c}	5.66 ± 0.08^{f}	0.04 ± 0.00^{a}	0.06 ± 0.00^{c}	0.08 ± 0.00^{d}	0.01 ± 0.00^{c}	$0.01 \pm 0.00^{d.e}$
DR	$19.95 \pm 0.06^{d.e.f.g}$	$3.61 \pm 0.02^{b.c}$	12.82 ± 0.20^{e}	1.66 ± 0.02^{c}	5.75 ± 0.02^{f}	$0.04 \pm 0.00^{a.b}$	0.06 ± 0.00^{c}	0.08 ± 0.00^{d}	$0.01 \pm 0.00^{c.d}$	0.01 ± 0.00^{e}
Carrot flour	20.79 ± 0.35^{g}	8.27 ± 0.09^{f}	4.37 ± 0.05^{a}	3.63 ± 0.02^{g}	1.19 ± 0.00^{a}	0.05 ± 0.00^{d}	0.04 ± 0.00^{a}	0.02 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{b}
Wheat bran	10.12 ± 0.05^{a}	0.03 ± 0.00^{a}	9.03 ± 0.14^{d}	1.00 ± 0.01^{b}	2.94 ± 0.03^{d}	0.08 ± 0.00^{g}	0.11 ± 0.00^{h}	0.13 ± 0.00^{g}	0.01 ± 0.00^{c}	0.00 ± 0.00^{a}
Rice bran	18.71 ± 0.35^{c}	0.09 ± 0.00^{a}	25.20 ± 0.59^{f}	0.70 ± 0.01^{a}	10.82 ± 0.18^{g}	0.05 ± 0.00^{c}	0.10 ± 0.00^{f}	0.16 ± 0.00^{h}	0.01 ± 0.00^{b}	$0.01 \pm 0.00^{d.e}$

[a] Means with different letters within the same column are statistically different (p-value < 0.05). [b] From ref. 67 and 68. [c] A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples.

Table 7 Total carotenoids content (TCC) (μg of β-carotene eq. g^{-1} DW) and α- and β-carotenes contents (μg g^{-1} DW) in ingredients and cereals formulations[a]

Formulation/ingredient	α-Carotene	β-Carotene
A	0.60 ± 0.06^{b}	20.68 ± 1.00^{c}
AR	0.18 ± 0.13^{a}	18.70 ± 1.94^{c}
B	b.l.o.q.[a]	8.16 ± 0.25^{b}
BR	b.l.o.q.[a]	6.11 ± 0.91^{b}
C	1.29 ± 0.04^{c}	27.85 ± 0.20^{d}
CR	1.08 ± 0.20^{c}	28.76 ± 2.15^{d}
D	$0.36 \pm 0.04^{a,b}$	20.06 ± 1.63^{c}
DR	0.14 ± 0.04^{a}	19.23 ± 0.34^{c}
Carrot flour	4.66 ± 0.28^{d}	61.25 ± 2.07^{e}
Wheat bran	n.d.[a]	n.d.[a]
Rice bran	n.d.[a]	n.d.[a]

[a] A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples. b.l.o.q., below limit of quantification. n.d., not detected. Means with different upper letter within the same column, differ (p-value < 0.05).

3.6. Bioactive compounds

3.6.1. Carotenoids. The results obtained for the amount of carotenoids are shown in Table 7. Lutein, β-cryptoxanthin, lycopene, α- and β-carotenes were identified in carrot flour and formulations, but only carotenes were above limit of quantification. Values reported as below the limit of quantification (b.l.o.q) were detected but their corresponding peak areas were lower than the lowest concentration level of the calibration curve and therefore could not be quantified with acceptable accuracy and precision. It is known that carrot is an excellent source of β-carotene[61,70] which was the most prominent carotenoid in these formulations, derived from carrot flour.

Roasting slightly decreased β-carotene in wheat bran-based formulation, and α-carotene in rice bran-based formulations, although it was not statistically different, possibly due to protective effects from rice bran higher fat content. Our results corroborates the results of a recent study which demonstrated that fat content may form a structure that stabilizes β-carotene against thermal treatment and ultraviolet light exposure.[71] As rice bran contains higher fat content than any other ingredient of this study, probably the fat content in rice bran explains the higher β-carotene content in the BCP formulations after processing. The same discussion and conclusions apply to α-carotene content.

3.6.2. Vitamin E and vitamin A. Carrot flour contained more retinol, while rice bran contributed significantly to tocopherol levels (Table 8).

Tocopherols content pattern in carrots vary depending on maturation phase, type of water supply and year, but in general α-tocopherol is the most prominent vitamin E vitamer.[72] Accordingly to previous works with the same carrot samples from the same source, reported α- and β-tocopherol as the most prominent followed by γ-tocopherol in lower quantities.[73] Regarding wheat bran, tocopherols and particularly α-tocopherol contents were in accordance with previous data reported in literature.[74] Rice bran was the richer in tocopherols and these results were also similar to those reported in literature.[66,75]

Consequently, BCP formulation with higher rice bran content presented the higher content in vitamin E vitamers. α-Tocopherol content did not decrease in wheat bran-based formulations comparing with carrot flour, but β- and γ-tocopherol did (Table 8). Nevertheless, the losses of tocopherols during processing were higher in the formulations of wheat bran than the formulations with rice bran. The same mechanism of protection by the fat content in rice bran must be the reason for the protection of tocopherols as it occurs for carotenoids,[71] as tocopherols are also liposoluble compounds.

As expected, for the present formulations, the retinol content is relatively low (Table 8) and the main source of vitamin A would be the carotenoids previously discussed (Section 3.6.1). As α- and β-carotenes are closely connected to vitamin A content, it is understandable that retinol was higher for carrot flour (2.9 μg g^{-1} DW) than for brans (0.9–1.7 μg g^{-1} DW) (Tables 7 and 8). Consequently, formulations with higher carrot flour content (A, AR, C and CR) presented the highest retinol content. The other formulations presented a similar value to the content

Table 8 Tocopherols and retinol contents (μg g^{-1} DW) in ingredients and cereals formulations[a]

Formulation/ingredient	α-Tocopherol	β-Tocopherol	γ-Tocopherol	Retinol
A	4.29 ± 0.10^{a}	$2.40 \pm 0.11^{a,b,c}$	6.01 ± 0.43^{b}	$2.41 \pm 0.28^{c,d}$
AR	4.10 ± 0.04^{a}	$2.31 \pm 0.05^{a,b}$	5.89 ± 0.26^{b}	2.63 ± 0.19^{d}
B	3.24 ± 0.03^{a}	$3.53 \pm 0.25^{b,c,d}$	12.24 ± 0.28^{c}	0.94 ± 0.30^{a}
BR	3.43 ± 0.04^{a}	$3.30 \pm 0.06^{b,c,d}$	11.19 ± 0.50^{c}	$1.46 \pm 0.20^{a,b}$
C	10.99 ± 0.24^{b}	4.83 ± 0.13^{d}	19.34 ± 0.36^{d}	$2.24 \pm 0.26^{b,c,d}$
CR	11.17 ± 0.35^{b}	$4.58 \pm 0.07^{c,d}$	18.89 ± 0.72^{d}	$2.49 \pm 0.17^{c,d}$
D	17.59 ± 0.39^{d}	9.19 ± 0.97^{e}	36.50 ± 2.71^{f}	$1.67 \pm 0.24^{a,b,c}$
DR	18.31 ± 0.13^{d}	9.32 ± 0.09^{e}	37.92 ± 0.48^{f}	$1.74 \pm 0.03^{a,b,c}$
Carrot flour	4.78 ± 0.63^{a}	0.46 ± 0.06^{a}	b.l.o.q.[a]	2.86 ± 0.02^{d}
Wheat bran	14.84 ± 1.53^{c}	15.98 ± 2.28^{g}	33.12 ± 1.10^{e}	$1.68 \pm 0.77^{a,b,c}$
Rice bran	22.41 ± 0.70^{e}	13.53 ± 0.22^{f}	52.18 ± 0.69^{g}	0.93 ± 0.04^{a}

[a] A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples. b.l.o.q., below limit of quantification. Means with different upper letter within the same column, differ (p-value < 0.05).

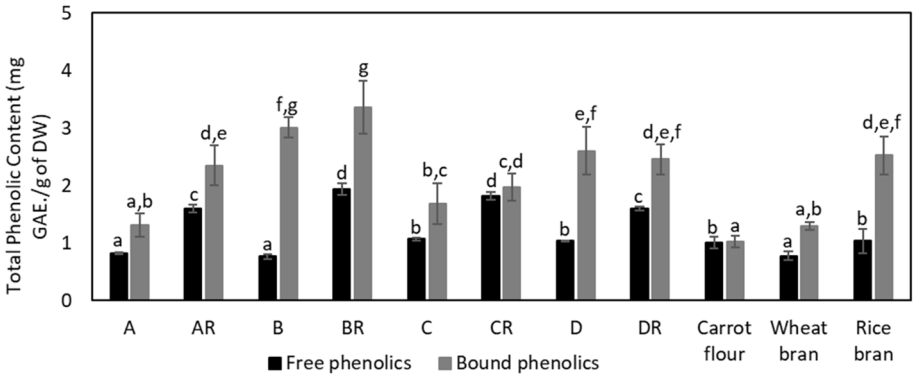

Fig. 2 Total phenolic content (TPC) of the phenolic extracts of the ingredients and cereals formulations. A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples. Means with different letter in each series, differ (p-value < 0.05).

in the brans. We can conclude that cold extrusion did not significantly affect retinol content.

3.6.3. Total phenolic content (TPC). Both free and bound phenolics were evaluated (Fig. 2).

Free phenolics ranged between 0.8 and 1.9 $mg_{GAE}\ g_{DW}^{-1}$ in BCP formulations and bound phenolics between 1.3 and 3.4 $mg_{GAE}\ g_{DW}^{-1}$ (Fig. 2). Carrot flour had a TPC of 1.0 $mg_{GAE}\ g_{DW}^{-1}$ for both free and bound phenolics which is in accordance to previous studies of baby carrots.[73] Wheat bran presented 0.8 and 1.3 $mg_{GAE}\ g_{DW}^{-1}$ for free and bound phenolics, respectively and rice bran 1.0 and 2.5 $mg_{GAE}\ g_{DW}^{-1}$ for free and bound phenolics, respectively. Accordingly, previous studies also found similar values and higher amounts of bound phenolics than free phenolics, for both wheat[76] and rice brans.[77]

Results indicate that rice bran phenolics (both free and bound) were the more consistent and less affected by processing (Fig. 2). Apparently, wheat bran phenolics increased with processing (formulation B, with 60% wheat bran). Ferulic acid is the predominant bound phenolic in wheat bran, which is ester-linked to arabinoxylans.[74] Cold extrusion processing may increase the ability of ferulic acid and other phenolics to be extracted from fibre,[78] thus explaining the observed results.

Roasting consistently increased free phenolics. This could be due to the effect of temperature on the release of bound phenolics, especially after the pressure applied during cold extrusion processing. It is known that during hot extrusion the combination of high temperature and pressure promotes the rupture of bonds between phenolic compounds and cell wall components.[1] Roasting also increased bound phenolics, especially for formulation A.

The evaluation of TPC on ingredients and formulations showed that cold extrusion maintains or increases the content of phenolic compounds in BCP, and thus their availability to be potentially absorbed in the gastrointestinal tract. Free phenolics are absorbed in the stomach and small intestine contributing to health benefits such as antioxidant activity on LDL-cholesterol and liposomes,[79] whereas bound phenolics typically survive stomach and intestinal digestion, being released in the colon through fermentation of the fibre by gut microbiota, where they may also exhibit health benefits, including prevention of colon cancer.[80]

3.7. Bioactivities

3.7.1. Antioxidant capacity. BCP formulations and ingredients are rich in several compounds with potential antioxidant activity, as discussed before. The antioxidant capacity was assessed by ABTS, DPPH, and ORAC assays and is presented in Fig. 3.

Different compounds deliver antioxidant capacity through different mechanisms. Phenolic compounds can donate a hydrogen atom from its hydroxyl group or chelate metal ions (iron and copper), thus inhibiting the oxidation of important biomolecules, such as LDL. Carotenoids antioxidant capacity is usually related to their capacity for electron donation, and they are characterized as excellent peroxyl radical scavengers. Retinol can act as antioxidant by donation of a hydrogen atom from its hydroxyl group; as an electron donor in the reaction with the hydroperoxyl radical ($HOO^{\bullet}$); and, mainly by radical adduct formation reaction between retinol and the $HOO^{\bullet}$ radical. Vitamin E is also a peroxyl radical scavenger which contributes to the maintenance of the integrity of long-chain polyunsaturated fatty acids in cell membranes maintaining their bioactivity.[81]

ABTS and ORAC assays mainly evaluate antioxidant capacity from hydrophilic and amphipathic compounds, and DPPH assay contemplates the contribution of lipophilic compounds.[82] Rice bran presented the highest antioxidant activity among ingredients, especially when assessed by DPPH, likely due to its high content in γ-oryzanol and tocopherols. γ-Oryzanol is a mixture of liposoluble steryl ferulates present in rice bran that exerts higher antioxidant activity.[27]

ABTS, DPPH and ORAC assays resulted in similar pattern of antioxidant activity comparing the formulations and ingredients, which complies with the pattern of TPC (Fig. 2). Additionally, ABTS results presented approximately two times higher antioxidant activity than the DPPH assay. So, one can expect a higher contribution of hydrophilic compounds (phenolics) for the total antioxidant activity of the samples than from lipophilic

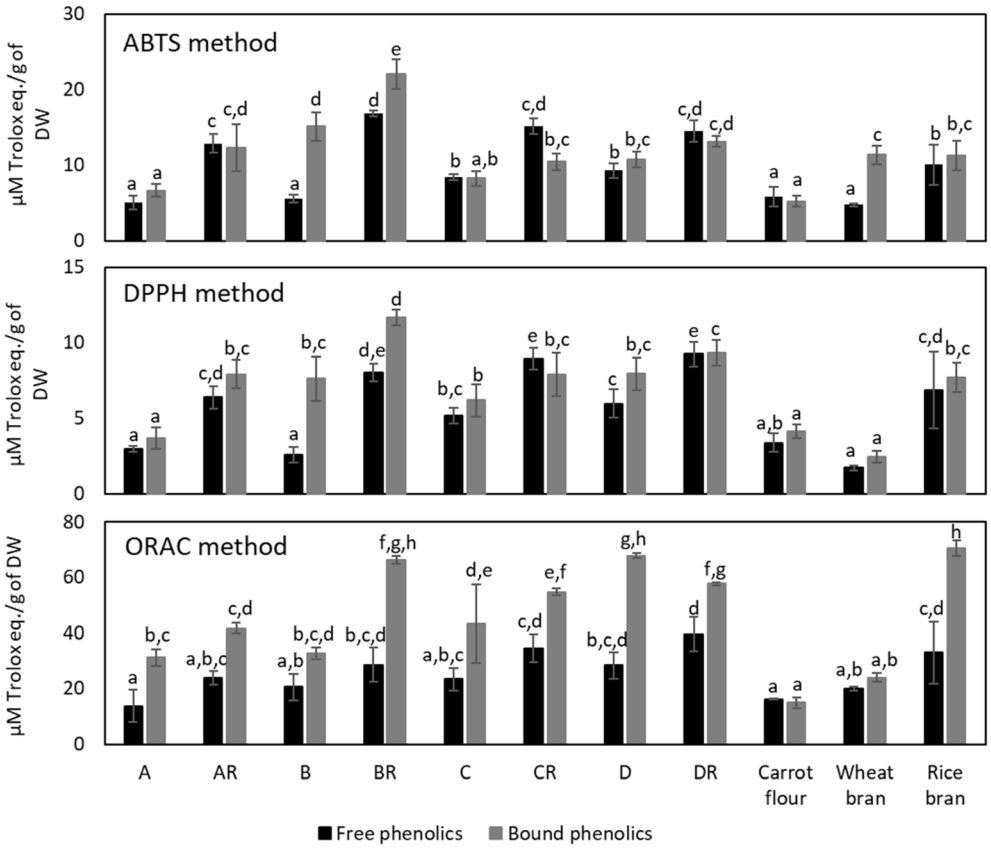

Fig. 3 Antioxidant activity of the phenolic extracts of the ingredients and cereals formulations. A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples. Means with different letter in each series, differ (p-value < 0.05).

compounds (carotenoids and vitamins E and A). These results and the those regarding TPC previously discussed, support that cold extrusion did not affect or positively affected antioxidant capacity, whereas roasting increased it.

3.7.2. Antidiabetic activity. Fig. 4 shows the antidiabetic activity measured for the phenolic extracts (free and bound) obtained from methanolic extraction (see Section 3.9.1). Antidiabetic activity of bound phenolics extracts was similar to acarbose (92–93%) with low differences among ingredients and formulations. Antidiabetic activity of the free phenolics extracts was lower than the bound phenolics and ranged between 18% (AR) and 46% (A) (Fig. 4). This is probably directly related to the amount of phenolics (the amount of bound phenolics was higher than the amount of free phenolics – Fig. 2). Despite

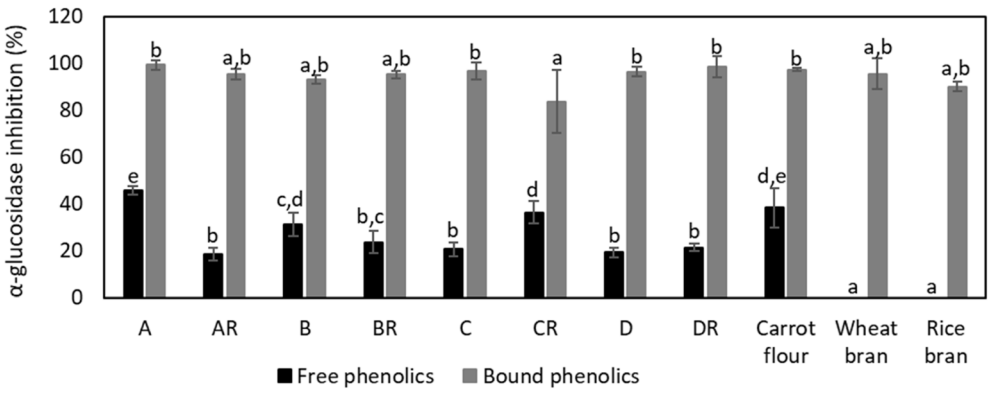

Fig. 4 Antidiabetic activity of the phenolic extracts of the ingredients and cereals formulations, in 250 mg of sample/mL of extraction solvent. A, carrot/wheat bran (70 : 30). B, carrot/wheat bran (40 : 60). C, carrot/rice bran (70 : 30). D, carrot/rice bran (40 : 60). AR, BR, CR and DR correspond to the A, B, C and D roasted samples. Means with different letter in each series, differ (p-value < 0.05).

the higher amounts of bound phenolics and their higher antidiabetic activity, the majority of these compounds are only released in colon after fermentation by the existing microflora and enzymatic rupture of the linkages between them and fibre.[80]

The free phenolic extracts of wheat and rice brans did not show antidiabetic activity. Thus, the free phenolics present in carrot flour will be the main responsible for the observed antidiabetic activity of the BCP. However, there was not a consistent relationship between the amount of carrot flour in the formulations and the observed antidiabetic activity, we can conclude that BCP developed from carrot flour and wheat or rice bran exhibited antidiabetic activity corresponding to inhibition of 18 and 46% of α-glucosidase in extracts of 250 mg of sample/mL of solvent.

4. Conclusions

In conclusion, it is feasible to develop BCP with at least 40% TDF using only two ingredients – carrot flour and wheat or rice bran – *via* cold extrusion, presenting antioxidant and antidiabetic activity, due to the presence of bioactive compounds such as phenolic compounds, carotenoids, vitamins E and A.

The resulting BCP can carry the claim "CONTAINS NATURALLY OCCURRING SUGARS" according to regulations and the formulations with 70% carrot flour and 30% wheat bran were also low-fat (less than 3 g of fat/100 g), as per[65] guidelines.

Cold extrusion proved to be an effective process for creating high-nutrient BCP, preserving proximate composition, minerals, and retinol content, while enhancing TPC and antioxidant activity. Although cold extrusion increased free sugars (glucose and fructose) and slightly reduced carotenoids and tocopherol levels, rice bran formulations exhibited better protection of carotenoid stability, likely due to the presence of rice bran fat. Similarly, retinol remained unaffected by processing, possibly due to protection from the cereal's fat content.

Roasting did not significantly alter the proximate composition, fibre, mineral content, or bioactive compounds (carotenoids, tocopherols, retinol) in the BCP, although it did reduce glucose levels due to caramelization and the Maillard reaction. Roasting also increased free phenolic content and antioxidant activity, as measured by ABTS and DPPH methods, but not by the ORAC method.

Comparing the two brans, rice bran contributed higher ash content, particularly macrominerals (K, P, Mg), total fat (which may protect liposoluble bioactive compounds), and SDF. Rice bran also had lower levels of total and digestible carbohydrates and IDF. Wheat bran, on the other hand, provided higher amounts of microminerals such as Ca, Fe, Zn, and Mn.

The developed BCP demonstrated potential antidiabetic properties, indicating their promise for contributing to health benefits, particularly in managing diabetes.

Despite the promising nutritional profile and bioactivity of the developed breakfast cereals, this study presents some limitations. Sensory quality was assessed under exploratory analysis, hence, consumer acceptance under sensory analysis techniques remains to be validated. The focus was primarily on compositional and *in vitro* analysis to predict potential health benefits. The antidiabetic and antioxidant properties were assessed only through extract-based assays, which do not account for potential interactions during digestion or absorption. In addition, some formulations presented flavour and texture limitations, and further work is required to identify strategies to improve the sensory profile. Future studies should include bioavailability and gut microbiota assessments, and shelf-life evaluation to better understand the health benefits and industrial applicability of these formulations.

Data availability

All data supporting the findings of this article are also presented in the manuscript tables and figures. No restrictions apply to the availability of these data.

Conflicts of interest

The authors of this article declare that they have no conflicts of interest of any kind.

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