ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Technol. Aliment. 23(4) 2024, 451–466
www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 http://doi.org/10.17306/J.AFS.001247

ORIGINAL PAPER

Received: 04.06.2024
Accepted: 10.09.2024

EVALUATION OF BIOAVAILABILITY AND POTENTIAL FUNCTIONAL ACTIVITY OF COMPOSITE CEREAL MEAL REPLACEMENT

Minghuan Du[1], Jiaqi Liu[1], Yiting Zheng[1], Lili Feng[3], Jing Zhang[3], Yahong Wang[1], Hongli Zhou[1]✉, Ke Sun[2]

[1]School of Chemistry and Pharmaceutical Engineering, Jilin Institute of Chemical Technology
Chengde Road no. 45,132022 Jilin, Longtan District, **China**
[2]Department of Neurology, Jilin Central General Hospital
Jilin Jilin, 132011, **China**
[3]Changchun Haobang Health Technology Co., Ltd
Changchun, 130052, **China**

ABSTRACT

Background. Cereal products have significant health benefits, and current research on them has focused on formulation processes and functionality. However, studies on the bioavailability and potential functional activities using digestive modeling combined with chemometrics have not been reported.

Materials and methods. The bioaccessibility of the composite cereal meal replacement (CCMR) was evaluated by establishing the *in vitro* digestive model, using field emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), in combination with chemometric methods such as dual indicator sequence analysis (DISA) and Pearson correlation analysis; potential functionality assessments included *in vitro* hypoglycemic potential, glycemic index (eGI) value, and predicted duration of satiety in humans.

Results. The total digestibility of CCMR was 51.34 ±0.52%, with significant characteristics of variation in FESEM, FTIR and DISA before and after digestion; the total polyphenol content, total flavonoid content and antioxidant activity of CCMR were increased ($p < 0.05$) after digestion; the inhibition rate of α-amylase was 7.95 ±0.21%, α-glucosidase was 9.95 ±0.34%, eGI was 45.10 ±0.52, and the duration of satiety was 3.50 ±0.25 h.

Conclusion. CCMR can be used as a low GI food for weight loss, diabetic patients and special medical population, the paper can enrich and extend the methodology for evaluation of cereal products by using digestive modeling, FESEM, FTIR and chemometrics methods.

Keywords: cereal beverage, bioaccessibility, functionality, infrared spectrum, field emission scanning electron microscopy

INTRODUCTION

The consumption of cereals is becoming increasingly popular and whole grain products are considered to be health foods. Studies have shown that long-term, regular consumption of whole grain products not only satisfies in terms of satiety (Masisi et al., 2016), but also serves as a preventive and control measure for disease, as well as helping with weight management (Kirwan et al., 2016), which is partly due to the

✉zhouhongli@jlict.edu.cn, tel.+86 432 62185246

anti-oxidative stress effects of phytochemicals, such as phenolic compounds in grains (Masisi et al., 2016). The types of whole grain phenolics and their antioxidant activity have been widely recognized in recent years (Gong et al., 2019).Phenolic substances are plant active substances, including phenolic acids, flavonoids, tannins, phytic acids, etc. (Masisi et al., 2016), which are the main contributors to the antioxidant properties of cereals, among which phenolic acids and flavonoids are common phenolic substances in whole grains (Pešić et al., 2019).

During food processing and formulation, polyphenols can interact with and bind to biomolecules in the food matrix, such as starches, proteins and lipids (Hui et al., 2021), and these interactions may affect the antioxidant activity (Sahu et al., 2021), digestibility and glucose-lowering activity of the final cereal product (Bao et al., 2016). Polyphenols have the ability to inhibit the activity of α-amylase and α-glucosidase, which potentially slow down the breakdown of sugar absorption in our body and improve insulin resistance, resulting in improved glycemic levels (Yang et al., 2023).

The bioavailability of polyphenols is a key factor in expressing potential for health-promoting characteristics such as antioxidant, hypoglycemic (Pešić et al., 2019). Glycemic index (GI) value is an indicator to differentiate the glycemic ability of food (Fontanelli et al., 2022).Studies have found that long-term consumption of low-GI foods significantly improves glycemic control and insulin sensitivity in diabetics. Highly satiating foods curb calorie intake, which reduce the risk of being overweight and developing type 2 diabetes.

Composite cereal meal replacement (CCMR) was made from corn, millet, rice and oats compounded with skimmed milk powder, based on whole grains, using a multi-grain, multi-hybrid model (Du et al., 2023). Field Emission Scanning Electron Microscopy (FESEM) is commonly used to observe the morphology and composition of ultrastructure on the surface of matter (Kalhori et al., 2022); Fourier Transform Infrared Spectroscopy (FTIR) has become commonly used and indispensable tool in modern structural chemistry and analytical chemistry, which is used to identify the structural composition of the substance or to determine the chemical groups (Durazzo et al., 2018). Dual-indicator sequence analysis (DISA) can reveal the relationship between different samples by calculating the co-peak rate and the variant peak rate in the infrared spectra, which is widely used in the study of Chinese herbal medicine (Liu et al., 2021), although with less application in the food field.

Currently, the evaluation of whole grains is more concerned with the changes in the physicochemical properties of single grains before and after processing, and multigrain products have been evaluated mainly in terms of functionality, with fewer studies on bioavailability. Composite cereal products have great potential, but their composition is complex. High performance liquid chromatography (HPLC) can only be used to detect a few components of flavonoids and polyphenols, which are not representative of the overall functional composition. The method of *in vitro* digestion mode combined with FESEM, FTIR and DISA analyses to evaluate the bioavailability of cereal products has not been reported yet, which has the advantage of not requiring complex sample pretreatment through overall non-destructive detection.

In this study, the bioavailability and potential functional activities of CCMR were evaluated by utilizing the *in vitro* digestion model and chemometrics. FESEM can intuitively characterize the digestive properties of CCMR, while FTIR and DISA can do so efficiently, which can be used for evaluating the digestive properties of food products. This paper adds to the evaluation method of cereal beverages and will provide a theoretical basis for developing and applying functional cereal beverages.

MATERIALS AND METHODS

Materials and chemicals

The materials and chemicals used in the study were as follows: the corn was Northeast yellow corn purchased from Jilin City (Jilin, China); rice, oats and millet from the Northeast Golden Dragonfish brand from the Baicheng Yihai Kerry (Panjin) Grain and Oil Industry Co., Ltd (Jilin, China); skim milk powder from the Yili brand, purchased in New Zealand; commercially available corn cereal drink branded as Tujia Wugu, purchased from Enshi Maohe Food Co., Ltd (Hunan, China); Pepsin (3000 U/mg), trypsin (250 U/mg), α-amylase (10000 U/g), α-glucosidase (50 U/mg), glycosylase (100000 U/g), forinol reagent, gallic

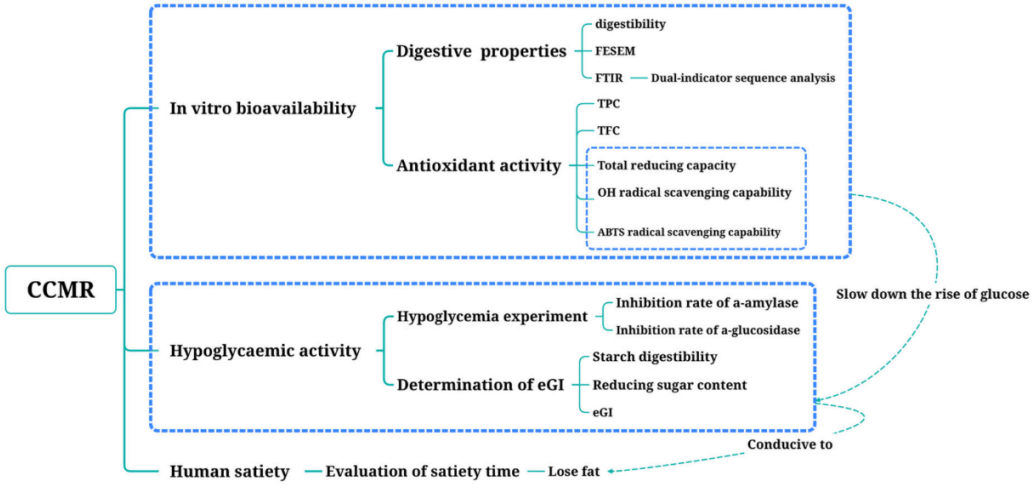

Fig. 1. Technical route

acid purchased from Shanghai Maclean Biochemical Co., Ltd (Shanghai, China); 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{+}$) was purchased from Xi'an Jingbo Biotechnology Co., Ltd (Shanxi, China); rutin and catechin were purchased from Fuzhou Feixing Biotechnology Co., Ltd (Fujian, China). All the above reagents and chemicals were of analytical reagent grade.

Preparation of Composite Cereal Meal Replacement (CCMR)

CCMR was prepared by mixing corn, rice, millet, skimmed milk powder and oats in a quantity ratio 55:10:10:5:15. 360 mL of distilled water was added and boiled for 1 min to obtain a total volume of 330 ml of CCMR with a grain content of 10%. It was then homogenized for 10 min at 20 MP, and sterilized for 15 min at 121°C (Du et al 2023). The technical route of this study is shown in Figure 1.

Simulated digestion *in vitro*

The preparation of simulated salivary solution included 0.238 g of Na_2HPO_4, 0.019 g of KH_2PO_4 and 0.8 g of NaCl, which were dissolved in water. The pH value of the solution was adjusted to 6.75, and 0.02 g of α-amylase was added, dissolved and diluted to 100 mL (Minekus et al., 2014). The preparation of the simulated gastric solution included 2 g of NaCl, 3.2 g of pepsin, and 7 ml of 36.5% hydrochloric acid (v/v) was dissolved in distilled water to 100 mL. The preparation of the simulated intestinal solution included 0.45 g of trypsin and 3 g of porcine bile salt. 6.25 g of $NaHCO_3$ were dissolved in distilled water to 500 mL and stored at 4°C (Huang et al., 2019). 20 mL centrifuge tubes were numbered 1 to 10, to which 1g of the sample and 0.5 mL of simulated saliva were added and shaken at 37°C for 5min. 7 mL of 0.9% saline (v/v) and 1.6 mL of gastric fluid were then added to them. The pH was adjusted to 2.0 with 36.5% concentrated hydrochloric acid, with water then being added to 10 mL. Centrifuge tubes were shaken at 37°C to simulate gastric digestion for 0, 30, 60, 90, 120 min, then centrifuged at 4500 r/min for 10 min to obtain the supernatant and precipitates. Based on 2 h of gastric digestion, 3 mL of 0.9% saline, 0.5 mol/L $NaHCO_3$, were added to centrifuge tubes No. 6–10, and then the pH was adjusted to 7.5. 5 mL of the intestinal fluid and water were added to 20 mL, digested at 37°C for 30, 60, 90, 120, and 150 min, then centrifuged at 4500 r/min for 10 min to obtain the supernatants and precipitates (Qin et al., 2022).

Digestive properties *in vitro*

The process of determining the *in vitro* digestibility of CCMR and pure maize powder (PMP) included the digestive fluid from 2 hours of gastric digestion and 3 hours of intestinal digestion. The digestive fluid was

poured into a pouch made of 150 Mm diameter microporous filter membrane and put into a beaker with NaCl for filtration. When the solution did not diffuse outwards, it was dried, weighed and the mass difference calculated before and after digestion (Kamiloglu et al., 2014).

In FESEM measurement, an appropriate number of samples before and after digestion were fixed on a sample stage with conductive adhesive and subjected to (3 times) sputter-coated gold spraying treatment. The morphology was then observed using a scanning electron microscope (Tokyo Electron Ltd, JSM-7610F plus, Japan) (Abd El-Lateef et al., 2020). Observation magnification was 1000 and 3000 times, respectively.

The FTIR measurement was that the samples before and after digestion were mixed with KBr powder in a ratio of 1:150 and measured in the Fourier infrared spectrometer (Harbour East Technology Development Ltd., Tianjin, China). The scanning range was 4000~500 cm^{-1} at a resolution of 4 cm^{-1} and the number of scans was 32, with the smoothing factor for the second order derivative being 21 (Cao et al., 2022).

In DISA, the common and variant peak ratios in the infrared fingerprints can reflect the differences between before and after digestion to a certain extent (Liu et al., 2021). The DISA of the common peak ratio P refers to the percentage of the infrared spectral peaks that appear both before and after digestion (the number of common peaks) to the total number of all independent peaks; the variant peak ratio P_{va} refers to the percentage of the variant peaks in the infrared profile before digestion to the number of common peaks; and the variant peak ratio P_{vb} refers to the percentage of the variant peaks in the post-digested profile to the number of common peaks.

Determination of total polyphenol content (TPC) and total flavonoid content (TFC)

The TPC in supernatants with different digestion times was determined by the Folin Phenol Reagent Colorimetry (Boateng et al., 2023); TFC was determined by means of the aluminum chloride-sodium nitrite colorimetric method (Mkaouar et al., 2018).

Determination of antioxidant activity

The antioxidant activity of the supernatants with different digestion times was determined. When determining the total reducing capacity, 200 μL of the sample was mixed with 200 μL of PBS and 200 μL of 1% potassium ferricyanide, then held in a water bath at 50°C for 20 min. After this, 200 μL of 10% TCA was added and shaken well, centrifuged at 3000 r/min for 5 min, and the supernatant removed. 200 μL of supernatant was mixed with 500 μL of distilled water and 100 μL of 0.1% ferric chloride, then allowed to stand for 8 min, after which absorbance was measured at 700 nm (Akhtar et al., 2022). Equation (1) is used to calculate the total reducing capacity:

$$\text{Total reducing capacity} = A_1 - A_0 \quad (1)$$

where:

A_1 is the absorbance of the sample

A_0 is the absorbance of the negative control.

To determine the OH radical scavenging activity, 150 μL of 7.5 mmol/L Fe_2SO_4, 300 μL of 8 mmol/L salicylic acid, 2 mL of sample and 300 μL of 7.5 mmol/L H_2O_2 were mixed well, and the absorbance was measured at 510 nm after shaking well for 45 min (Hernández-García et al., 2022). Vitamin C (Vc) (0.1, 0.2, 0.4, 0.6, 0.8 mg/mL) was used as the positive control. Equation (2) is used to calculate OH radical scavenging activity:

$$\text{OH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

where:

A_0 is the absorbance of the negative control

A_1 is the absorbance of the sample.

When determining $ABTS^+$ scavenging activity, 100 μL of sample was mixed with 900 μL of $ABTS^+$ working solution, and after a 10-minute reaction, the absorbance was measured at 734 nm (Qin et al., 2022). Vc (0.25, 0.5, 1, 2, 4 mg/mL) was used as the positive control. Equation (3) is used to calculate $ABTS^+$ scavenging activity:

$$\text{ABTS}^+ \text{ radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

where:

A_0 is the absorbance of the negative control

A_1 is the absorbance of the sample.

Measurement of hypoglycemic activity

The CCMR was centrifuged at 3000 r/min for 5 min and the supernatant was used to determine the inhibition of α-amylase. 0.5 mL of 10 μ/L α-amylase was added to 0.5 mL of the sample and reacted at 60°C for 10 min, 1 mL of 1 mg/mL starch solution was added, followed by a water bath at 60°C for 15 min, and 0.5 mL of 0.1mol/L HCl and 2 mL of dilute iodine solution were added as the control group. Acarbose (0, 1, 2, 4, 6, 8 mg/mL) was used as a positive control (Adisakwattana et al., 2012). The calculation is shown in Equation (4):

$$\alpha\text{ – amylase inhibition rate (\%)} = \frac{A_0}{A_1 - A_2} \times 100 \quad (4)$$

where:

A_0 is the absorbance of the sample
A_1 is the absorbance of the negative control
A_2 is the absorbance of the blank control.

When determining the inhibition rate of α-glucosidase, 100 μL of sample and 300 μL of 1 mg/mL α-glucosidase were added to 600 μL of PBS, and then 100 μL of 0.74 mg/mL PNPG was added for a 15-min water bath at 37°C. 4 mL of Na_2CO_3 were added and reacted for 20 minutes of a water bath at 37°C, and the absorbance was measured at 400 nm (Adisakwattana et al., 2012). Acarbose (0.005, 0.01, 0.02, 0.04, 0.06 mg/mL) was used as a positive control. The calculation is shown in Equation (5):

$$\alpha\text{ – Glucosidase inhibition rate (\%)} = \frac{(A_0 - A_1) - (A_2 - A_3)}{A_0 - A_1} \times 100 \quad (5)$$

where:

A_0 is the absorbance of the negative control
A_1 is the absorbance of the blank control
A_2 is the absorbance of the sample
A_3 is the absorbance of the background control.

Measurement of eGI

The process of determining starch digestibility included CCMR and PMP containing 1 g of carbohydrate (Englyst et al., 2003) which were added to 10 mL of deionized water in a boiling water bath for 15 minutes, with 7 mL of 0.1 mol/L phosphate buffer, 1 mL of (2.5 g/L) amylase and 6 μL of pepsin-guar gum mixture then being added to the samples. The pH was adjusted to 1.5 with HCl, and then subjected to a 37°C water bath for 30 min. 10 mL of phosphate buffer was added and the pH was adjusted to 6.9 with NaOH. A 125 μL of MgCl-$CaCl_2$ solution and 125 μL of trypsin were added, along with 400 uL of starch to glucosidase, made up with water to 50 mL and incubated for 3 h. 1 mL of the sample was taken at 0 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min and the enzyme inactivated in 4 mL of anhydrous ethanol, then centrifuged to extract the supernatant (Oñate Narciso and Brennan, 2018). The 3,5-dinitrosalicylic acid (DNS) method was used to determine the glucose content. 1 mL of supernatant and distilled water, and 1.5 mL of DNS solution were taken into a 25 mL stoppered test tube. After boiling in a water bath for 15 min, distilled water was added to 25 mL, and absorbance was measured at 550 nm (Bekele et al., 2020). The eGI values were determined according to previous methods (Ren et al., 2021). White bread was used as the reference standard and its hydrolysis rate was defined as 100 (Lawal et al., 2022). The protein, fat, total dietary fiber and moisture in CCMR and PMP were determined using the Kjeldahl method, Soxhlet extraction, enzyme gravimetric method and direct drying method, respectively, and carbohydrates were determined by calculation (Yang et al., 2023). The nutrient composition is shown in Table 1. Formula (6) and (7) was calculated as follows:

$$HI = AUC_1/AUC_2 \times 100\% \quad (6)$$

$$eGI = 0.862\ HI + 8.198 \quad (7)$$

where:

AUC_1 is the area under the hydrolysis curve of the sample
AUC_2 is the area under the hydrolysis curve of white bread.

Table. 1. Nutritional analysis of PMP and CCMR

Nutrient composition	Carbohydrate g	Protein g	Fat g	Total dietary fiber, g	Energy kJ
PMP, 100 g	81.83	6.94	1.25	6.25	1 610.21
CCMR, 100 g	74.61	10.58	1.05	7.79	1 546.64

Satiety measurement

Volunteers (15 men and 15 women) were recruited and selected with average age 20–40 years, an average weight index of 21.15 ±1.75 kg/m^2, without respiratory, digestive and endocrine system diseases or relevant family history, without bad habits, drugs or weight loss within the last month (Ni et al., 2021). Before the experiment, volunteers were trained according to the Visual Analog Scale (VAS), with 0 meaning "not at all" and 10 meaning "very much". Volunteers were required to make marks on the satiety VAS scale at appropriate locations, which represented their sensory levels at the time of empty stomach (0 min), 15 min, 30 min, 45 min, 60 min, 90 min, 120 min, 180 min, and 240 min (Evenson et al., 2022). The experimenter scored the volunteers' results based on the distance measured from point 0 to the volunteers' markers. The samples were CCMR and a commercially available cereal drink and glucose with equal energy (300 kcal).

Statistical processing

All experiments were performed three times and with results expressed as mean ± standard deviation. SPSS statistical software and origin version 2021 were used for data analysis, and the analysis of variance between groups of data was performed using the Tukey experiment. Statistical significance was designated $*p < 0.05$, $**p < 0.01$.

RESULTS AND DISCUSSION

In vitro digestive characteristics

The digestibility rate of CCMR was 30.13 ±0.17%, and of PMP it was 19.25 ±0.21% after gastric digestion; after intestinal digestion these rates were 24.62 ±0.35% and 17.18 ±0.15%, and total digestibility was 51.34 ±0.52% and 31.47 ±0.40%, respectively. The results are presented in Fig 2. CCMR has a higher digestibility than PMP, and the main factors influencing the digestibility of CCMR were antinutritional factors such as tannins, phytase and enzyme inhibitors in the grain ingredients (Nikmaram et al., 2017). Corn contains phytase, which competes for the mineral cofactor required by peptidase to interfere with protein digestibility (Gupta et al., 2015); Millet has a high tannin content, which reduces the hydrolytic activity of

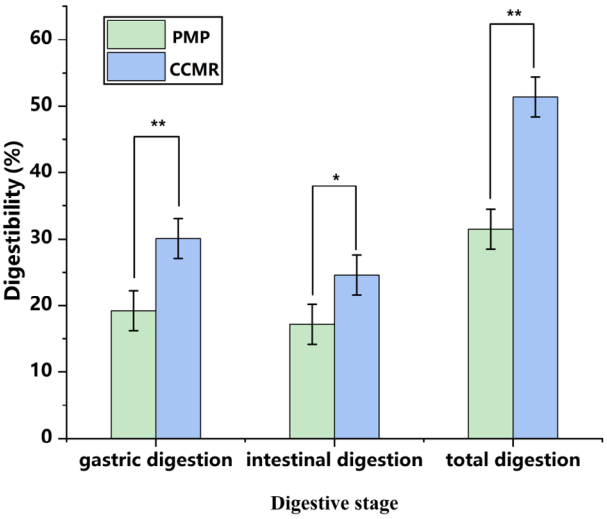

Fig. 2. *In vitro* digestibility
Note: n = 3, significant differences from the control group were shown as $*p < 0.05$ and $**p < 0.01$

CCMR by interacting with proteins (peptidases and protein substrates) and minerals in an aqueous environment, forming complexes and precipitating (Annor et al., 2017); CCMR is rich in dietary fiber, which can increase the viscosity of the gastrointestinal tract and affect the rate of digestibility and absorption rate by influencing the diffusion of hydrolytic enzymes to their substrates. The study shows that there was no single effective method can remove completely all the antinutritional factors present (Weerasooriya et al., 2018). Firstly, pretreatment processes such as dehulling, crushing and milling reduce phytase content in CCMR, and also exposure of the protein matrix to the environment increases hydrolysis as the cellular structure has been torn (Wu et al., 2017). Secondly, simple heat treatments such as boiling can partially inactivate anti-nutritional factors in CCMR such as trypsin inhibitors (Joye, 2019). The digestibility of CCMR reached 50%, indicating that CCMR could be well absorbed and promoted as a nutritional product.

In FESEM analysis, the microstructure of CCMR before digestion is shown in Figure 3 (A, B). It can be seen that the microstructure of CCMR was irregular in shape, with irregular elevations, a flaky whole, without voids on the surface, and was relatively rough. After digestion, as shown in Figure 3 (C, D), it can lead

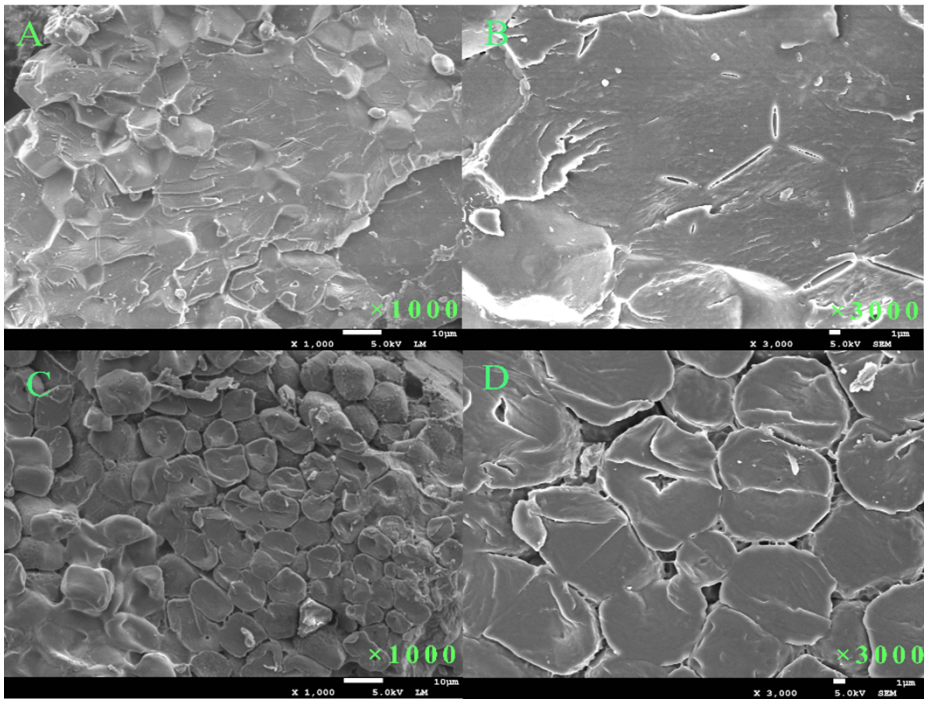

Fig. 3. FESEM micrographs of CCMR before digestion (A) 1000×; (B) 3000×; After digestion (C) 1000×; (D) 3000×

to the disruption of the cell structure, and digestion might lead to the decomposition of CCMR into microspherical particles with smooth surfaces and increased surface area, thus affecting the physical and chemical properties of CCMR.

The FTIR spectra in 4000–500 cm^{-1} before and after digestion, and the SD-IR in the 3000–500 cm^{-1} of CCMR were shown in Figure 4 (A, B). The strong broad absorption peaks 3437.13 cm^{-1} and 3451.20 cm^{-1} were O-H and N-H stretching vibrations of intermolecular hydrogen bonds and absorption peaks from the bending vibrational peaks outside the O-H surface can be observed around 765.51, 754.6, 707.23 and 697.33 cm^{-1} (Liu et al., 2021). The peaks near 2952.17 and 2871.04 cm^{-1} belonged to the asymmetric stretching vibrations of $–CH_3$. $–CH_3$ bending vibration peaks can be found near 1420.19, 1405.99, 1373.28, 1367.82 cm^{-1} (Liang et al., 2023). Near 2923.86, 2840.02 cm^{-1} were the asymmetric stretching vibrations of CH_2 (Yuan et al., 2015). The C=O stretching vibration peak was observed near 1728.11 cm^{-1} and near 1647.68 and 1627.91 cm^{-1}, corresponding to the stretching vibrations of C=C. The peaks near 1534.84, 1506.21, 1460.06, 1451.33 cm^{-1} came from the stretching vibration of the C=C in the benzene ring. The peaks were around 881.06, 857.32, 850.04, 785.61, 763.51, 707.23 and 697.33 cm^{-1}, which corresponded to the out-of-plane bending vibrations of C-H in the benzene ring. Absorption peaks near 1291.46, 1237.26, 1155.17,1095.80, 1082.47, 1038.54 and 1007.52 cm^{-1} were from C-O stretching vibrations (Liang et al., 2023). There were many peaks in the spectral frequency in the region of 1650–1400 cm^{-1} and below 1300 cm^{-1}. The CCMR before and after digestion had the structure of aryl ring (C_6) and more O-H groups, indicating that CCMR contained phenolic acid compounds such as ferulic acid (Holser, 2012). The peaks near 3437.13, 2923.86, 2871.04,1728.11, 1534.84, 1506.21, 1460.06, 1451.33, 1291.46, 1237.26, 1155.17,1095.80, 1082.47, 1038.54 and 1007.52 cm^{-1} were reduced after digestion, which showed that the peak intensity of CCMR in the characteristic functional groups of polyphenols decreased. The content of polyphenol compounds in the precipitates of CCMR

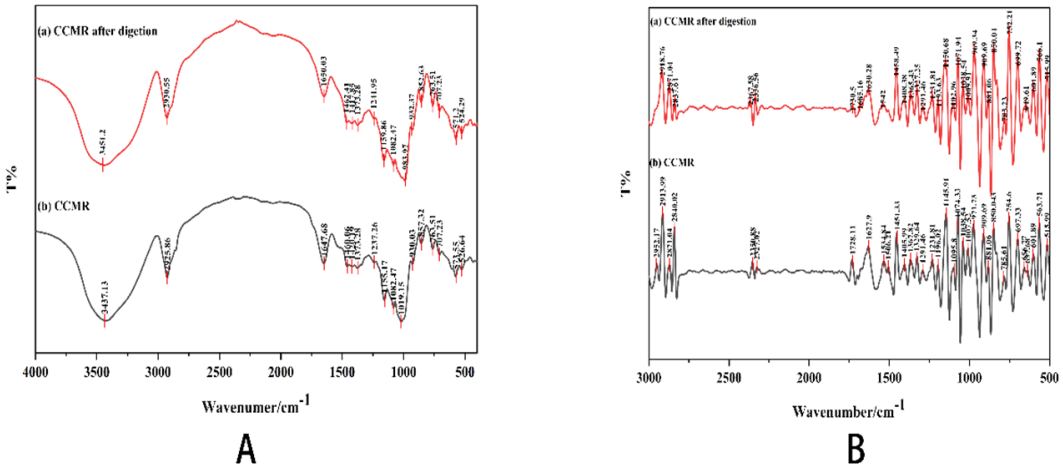

Fig. 4. A FTIR spectra of CCMR after digestion (a) and before digestion (b); B SD-IR spectra of CCMR after digestion (a) and before digestion (b)

was reduced, and digestion may promote the release of relevant active substances into the supernatant.

The DISA results of the SD-IR of CCMR before and after digestion show that the absorption peaks near 2952.17, 1506.21 and 656.77 cm^{-1} disappeared after digestion, and a new peak appeared at 1685.16 cm^{-1}, which showed that digestion could lead to changes in the content of C=C-containing compounds and in the internal structure of compounds. There were 31 common peaks before and after digestion, which were near 2913.99, 2871.04, 2840.02, 2350.88, 2327.02, 1728.11, 1627.91, 1534.84, 1451.33,1451.33, 1405.99, 1367.82, 1329.64, 1291.46, 1231.81, 1196.02, 1145.91, 1095.80, 1074.33,1038.54, 1007.52, 971.73, 909.69, 881.06, 850.04, 785.61, 754.6,0, 697.33, 637.68, 601.89, 563.71, and 515.99 cm^{-1} with the ratio of P: P_{va}: P_{Vb} = 88.57:9.68:3.22. The proportion of common peaks was larger, which indicated that CCMR compositions before and after digestion were similar. Combined with FTIR results, digestion might contribute to the decomposition and transformation of some substances to be released into the supernatant, mainly in changes in the peak intensity of the characteristic peaks of phenolic compounds. This was in agreement with the microspherical results of FESEM.

In vitro antioxidant activity of CCMR

The variations in the TPC and TFC of CCMR and PMP during digestion are shown in Fig. 5A. The standard curve of gallic acid in TPC determination was Y = 0.8055*x* + 0.073, R^2 was 0.9991. The TPC of CCMR was 3.21 ±0.13 mg/g and PMP was 1.67 ±0.11 mg/g before digestion and gradually increased to 6.45 ±0.16 mg/g and 3.24 ±0.14 mg/g, respectively, during gastric digestion and reached 6.74 ±0.10 mg/g for CCMR and 3.11 ±0.15 mg/g for PMP at the end of digestion. During the digestion process, the trend of TPC of CCMR and PMP was firstly increased ($p < 0.05$) and then basically remained constant ($p > 0.05$). The standard curve of catechin in the determination of TFC was Y = 1.8657*x* – 0.014, R^2 was 0.9995. Before digestion, the TFC of CCMR was 0.50 ±0.07 mg/g and PMP was 0.32 ±0.09 mg/g. During gastric digestion, the TFC increased initially and then continued to decrease, but generally showed an upward trend to reach 1.13 ±0.10 mg/g and 0.51 ±0.06 mg/g, respectively. During the intestinal digestion, the TFC increased firstly and then remained basically stable to reach 1.86 ±0.15 mg/g and 0.83 ±0.11 mg/g, respectively.

The high content of polyphenols in digestive was due to the destruction of the chemical bond between polyphenols and proteins by pepsin and trypsin, which led to the release of phenolic substances (Choi et al., 2017). The TFC decrease during gastric digestion may be caused by the acidic environment of gastric digestion and the inhibitory effect of pepsin on the release of flavonoid (Choi et al., 2017). The increased content of flavonoids in intestinal digestion may be due to the

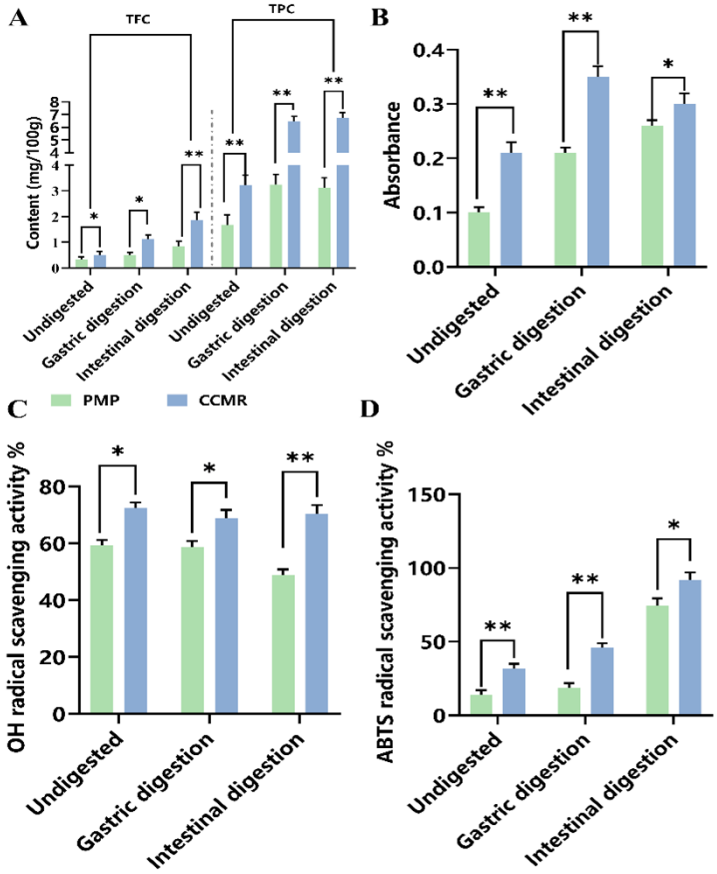

Fig. 5. Variations in TPC, TFC and antioxidant activity during simulated digestion. A variation in TPC and TFC; B variation in total reducing capacity; C variation in OH radical scavenging rate; D variation in ABTS radical scavenging rate
Note: n = 3, significant differences from the control group were shown as *$p < 0.05$ and ** $p < 0.01$.

increased pH and alkaline conditions, which lead to the hydrolysis and release of flavonoids originally deposited in an acidic environment, or the degradation of other phenolic compounds, such as phenolic acids and anthocyanins, and the transformation of polyphenol structure into more stable flavonoids (Sun et al., 2019).

Digestion can increase TPC and TFC because of the gradual destruction of the cell wall of the food matrix by pepsin and trypsin in the simulated digestive solution (Hettiarachchi et al., 2021), which was consistent with the results of FTIR analysis. Both TPC and TFC were higher in CCMR than PMP, which may be related to the large variety of cereals in CCMR. Rice, oats and millet all contain more phenolic compounds, and the synergistic effect between different types of cereals promoted the release of related active substances and increased bioaccessibility.

The digestion process promoted the release of the relevant active substances from the grains and antioxidant activity of CCMR increased compared to that before digestion. The results are shown in Fig. 5 (B, C, D).

As shown in Fig. 5B, the total reducing capacity of CCMR increased from 0.21 ±0.05 to 0.35 ±0.05, and the total reducing capacity of PMP increased from 0.10

±0.01 to 0.21 ±0.05 after gastric digestion. CCMR has greater reducing capacity than PMP and showed a trend of increasing and then remaining basically unchanged throughout the digestion period ($p < 0.05$).

The standard curve for the scavenging of OH radicals by Vc was Y = 1.0813x + 0.1452, R^2 was 0.9994. From Fig. 5C, the scavenging activity of OH radicals was 72.43 ±2.55 % for CCMR and was 59.20 ±3.21% for PMP before digestion, reaching 70.48 ±3.85% for CCMR ($p < 0.05$) and 48.84 ±3.27% ($p < 0.05$) for PMP after digestion.

The standard curve for the scavenging of $ABTS^+$ radical by Vc was Y = 21.622x + 0.0826, R^2 was 0.9995. From Figure 5D, the scavenging activity rate of $ABTS^+$ of CCMR was 31.89 ±1.53% and PMP was 13.93±1.74%; it gradually increased to 45.93 ±1.95% and 18.80 ±1.33% after gastric digestion and tended to increase initially and then remain basically unchanged, reaching 91.93 ±2.84% and 74.25 ±3.71% after intestinal digestion. The changes in total reducing capacity, OH radical scavenging activity and $ABTS^+$ radical scavenging activity were consistent with trends in TFC and TPC, respectively.

In addition, milk proteins in CCMR can be used in the gastrointestinal tract as carriers for delivering antioxidant compounds that protect the gastrointestinal tract itself from oxidative damage (Tagliazucchi et al., 2016). The larger percentage increase in antioxidant activity during gastric digestion was associated with a rapid increase in TPC and TFC, while during intestinal digestion TPC and TFC increased slowly and then remained essentially unchanged. The changes in antioxidant activity were consistent with them. In summary, the antioxidant activity of CCMR was higher than that of PMP, and the antioxidant potential active ingredients of CCMR were released and antioxidant activity increased after digestion.

Correlation of TPC and TFC with antioxidant activity

Attained through Pearson's correlation coefficient analysis, the results of the correlation analysis between TPC and TFC and antioxidant activity in CCMR and PMP during digestion are shown in Figure 6. The color bias towards blue and gray was a positive correlation, close to pink and purple was a negative correlation, while the larger the absolute value of the correlation coefficient, the greater the correlation between the factors. TPC and TFC in both CCMR and PMP exhibit some correlation with antioxidant activity. In CCMR, total

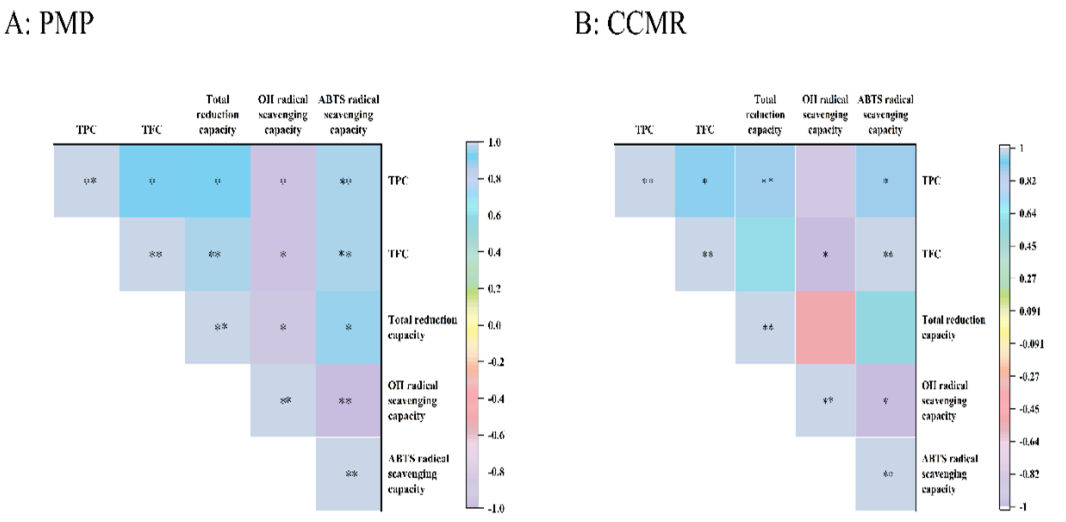

Fig. 6. Correlation analysis of TPC and TFC with antioxidant capacity in PMP and CCMR. A Correlation of TPC and TFC with oxidative capacity in PMP; B Correlation of TPC and TFC with oxidative capacity in CCMR

Note: n = 3, significant differences from the control group are shown as *$p < 0.05$ and **$p < 0.01$.

reducing capacity mainly had a good positive correlation with TPC ($p < 0.05$), and OH radical scavenging activity and $ABTS^+$ scavenging activity had a positive correlation with both TPC and TFC ($p < 0.05$). In PMP, antioxidant activity has a positive correlation with both TPC and TFC ($p < 0.05$). CCMR had a higher content of active substances such as TPC and TFC and stronger antioxidant activity than PMP ($p < 0.05$). The combination of cereals, miscellaneous grains and digestion can increase TPC, TFC and antioxidant scavenging activity of CCMR (Apea-Bah et al., 2016).

Polyphenols are a major contributor to the antioxidant properties of cereals. During gastric digestion, TPC and TFC in CCMR increased and there was a general increase in antioxidant activity. Gastric digestion may cause structural changes in the fibrous components of the cereal cell wall or break covalent bonds between polyphenols and cellulose, polysaccharides, and proteins, etc. The complexes are thereby hydrolyzed, facilitating the release or conversion of phenolics, and thus affecting the total antioxidant effect (Sahu et al., 2021). Polyphenols contain other phenolics such as tannins and phytic acid in addition to phenolic acids and flavonoids. In addition to polyphenols, grains contain other antioxidant active components, such as cellulose, polysaccharides and organic acids. The total reducing capacity, OH radical scavenging activity and $ABTS^+$ radical scavenging activity were the combined effect of multiple antioxidant components in CCMR. Correlation analysis showed that changes in TFC were highly correlated with changes in TPC, and the antioxidant activity of CCMR was directly correlated with polyphenol content.

Hypoglycemic activity of CCMR

The rate of inhibition of α- amylase by acarbose was $Y = 0.1034x - 0.026427$ and R^2 was 0.9994, and the inhibition rate of α- amylase for CCMR was 7.95 ±0.21%. Meanwhile, the inhibition rate of acarbose at 1 mg/mL was 7.88 ±0.21%. The rate of inhibition of α- glucosidase by acarbose was $Y = 1.2029x - 0.0985$, R^2 was 0.999, the inhibition rate of CCMR for α-glucosidase was 9.95 ±0.34%, and the inhibition rate for acarbose at 0.000748 mg/mL was 9.95 ±0.18%. There was no hypoglycemic activity detected from PMP.

The variations in starch hydrolysis rates of CCMR, PMP and white bread with time in the evaluation of glycemic index experiment *in vitro* are shown in Figure 7A. The area AUC of the hydrolysis rate curve for PMP was greater than AUC for CCMR ($p < 0.05$). White bread can be used as a reference food in the evaluation of eGI, where eGI value = 100 (Simsek and El, 2015). The eGI value for CCMR was 54.10 ±0.52<55.00 and for PMP it was 65.91 ±0.57, which was close to the value of 68 in the China Food Composition Tables (6th Edition), indicating that the data has validity. Firstly, oats and skim milk powder are rich in high-quality protein, which could reduce the digestibility of starch through mechanisms such as inhibition of amylase activity (Bao et al., 2023). Secondly, millet has lower starch digestibility, and its addition to other grains lowered the rate of glucose release from α-glucosidase and α-amylase, thereby reducing the glycemic index of the grains (Annor et al., 2017). Thirdly, the interaction between β-glucan and protein in oats could contribute to the change in the microstructure of CCMR by the starch-protein matrix, thus altering the starch digestibility of CCMR and reducing the starch hydrolysis rate (Nguyen et al., 2022), which in turn decreases the eGI value. β-glucan in oats could also adsorb glucose molecules, hinder glucose transport, and inhibit the activity of glycolytic enzymes (Zhang and Wang, 2016). Finally, the dietary fiber content of CCMR is 0.38 g/100g (Du et al,. 2023), negatively correlated with GI values (Bae et al., 2016), which can reduce the rate of glucose release by inhibiting the action of amylase to reduce digestibility.

Studies have shown that most natural antioxidants, such as polyphenols and flavonoids, can inhibit α-amylase and α-glucosidase even after digestion. This inhibition is eventually expected to show significant antidiabetic activity (Bao et al., 2016). Meanwhile, the interaction of phenolic compounds with carbohydrates can reduce the amount of postprandial glucose released from CCMR (Ajayi et al., 2021). Tannin's interaction with gluten in cereal foods creates a barrier that prevents digestive enzymes from entering the starch and enhances tannin's ability to slow starch degradation (Yang et al., 2023). Lower starch digestibility can inhibit the rate of postprandial blood glucose elevation and play a regulatory role in chronic diseases such as obesity and diabetes (Tagle-Freire et al., 2022). In contrast to drugs, CCMR has weak hypoglycemic

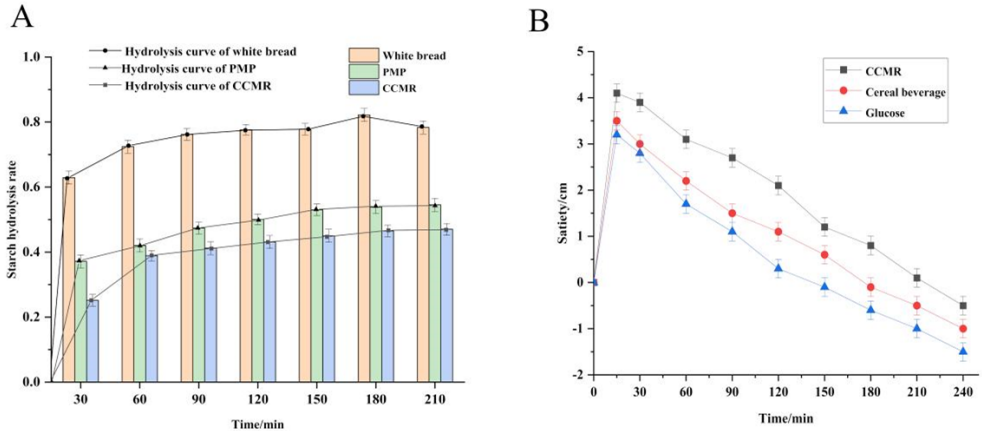

Fig. 7. A Starch hydrolysis rate; B Satiety curve (n = 3)

activity as a grain-based food. Thus, CCMR was a low GI food suitable for diabetics, with the characteristic of not raising glucose, which is available in a wide range of people.

Effect of CCMR on satiety

Based on the follow-up records of 30 volunteers who consumed 300 kcal of CCMR, a commercially available cereal drink and glucose between 0 and 240 minutes after the meal, the satiety score was used as an indicator score and the average score was calculated. As shown in Fig. 7 B, three samples revealed the same satiety trend, with the peak satiety occurring 15 min after the meal. The area under the satiety curve was greater of CCMR than the commercially available beverage, with a significant difference ($p < 0.05$). The satiety values for CCMR, the commercially available cereal beverage and glucose reached 0 at 210 min, 180 min and 150 min, respectively. The satiety index is 1 for glucose, the curve area showed a satiety index of 3.38 ±0.24 and 1.91 ±0.21 for CCMR and commercially available cereal drink, respectively. The duration of satiety of CCMR was 3.50 ±0.25 h, and of the commercially available cereal drink it was 3.00 ±0.25 h.

The reasons for the better satiety duration in CCMR are as follows: firstly, the higher viscosity of CCMR increases its satiety (Stribițcaia et al., 2022), and the high viscosity of β-glucan in oats is effective in attenuating the glycemic response, thereby slowing down gastric emptying and glucose absorption (Zaremba et al., 2018), thus prolonging satiety and lowering the glycemic index by increasing the viscosity of CCMR. Secondly, the high content of dietary fiber prolongs the satiety of CCMR (Du et al.,2023), which reduces the amount of food intake and achieves fat loss (Ye et al., 2015). Finally, dietary proteins provide satiety signals that influence CCMR intake through central and peripheral neurohumoral mechanisms (Luhovyy and Akbari, 2021), facilitating the regulation of satiety and thus body weight. CCMR had a higher satiety effect than high-glycemic foods or meals, which is conducive to postprandial blood glucose stabilization and weight control (Ni et al., 2021).

CONCLUSION

In this study, chemometrics-based methods, Pearson's correlation analysis and DISA combined with FESEM and FTIR were used to assess the digestive properties and potential functionality of CCMR *in vitro* and *in vivo*. The results showed that CCMR had higher digestibility, TPC, TFC and antioxidant activity than PMP, with better bioavailability and some hypoglycemic activity, and that satiety experiments proved the effectiveness of a reduced energy intake. CCMR can be assessed as a low GI food with good bioavailability and adjunctive hypoglycemic potential. Therefore, this study may provide a methodology for the quality assessment of meal replacements and provide a theoretical basis for better utilization of cereals and enhancement of the health values of compound cereal products.

DECLARATIONS

Data statement
All data supporting this study has been included in this manuscript.

Ethical Approval
Not applicable.

Competing Interests
The authors declare that they have no conflicts of interest.

OPEN ACCESS

REFERENCE

Abd El-Lateef, H. M., Mohamed, I. M. A., Zhu, J.-H., Khalaf, M. M. (2020). An efficient synthesis of electrospun TiO_2-nanofibers/Schiff base phenylalanine composite and its inhibition behavior for C-steel corrosion in acidic chloride environments. J. Taiwan Instit. Chem. Eng., 112, 306–321. https://doi.org/10.1016/j.jtice.2020.06.002

Adisakwattana, S., Ruengsamran, T., Kampa, P., Sompong, W. (2012). *In vitro* inhibitory effects of plant-based foods and their combinations on intestinal α-glucosidase and pancreatic α-amylase. BMC Complemen. Altern. Med., 12(1), 110. https://doi.org/10.1186/1472-6882-12-110

Ajayi, I. O., Otemuyiwa, I. O., Adeyanju, A. A., Falade, O. S. (2021). Vegetable polyphenols inhibit starch digestibility and phenolic availability from composite carbohydrate foods in-vitro. J. Agric. Food Res., 3, 100116. https://doi.org/10.1016/j.jafr.2021.100116

Akhtar, W., Ali, G., Ashraf, N., Fatima, I., Kayani, W. K., …, Khames, A. (2022). Efficiency of Multiple Extraction Solvents on Antioxidant, Cytotoxic, and Phytotoxic Potential of *Taraxacum officinale* (L.) Weber ex F.H. Wigg. From Poonch Valley, Azad Kashmir, Pakistan. In: I. U. Haq (Ed), Evidence-Based Complementary and Alternative Medicine, 1–9. https://doi.org/10.1155/2022/5118553

Annor, G. A., Tyl, C., Marcone, M., Ragaee, S., Marti, A. (2017). Why do millets have slower starch and protein digestibility than other cereals? Trends Food Sci. Technol., 66, 73–83. https://doi.org/10.1016/j.tifs.2017.05.012

Apea-Bah, F. B., Minnaar, A., Bester, M. J., Duodu, K. G. (2016). Sorghum -cowpea composite porridge as a functional food, Part II: Antioxidant properties as affected by simulated in vitro gastrointestinal digestion. Food Chem., 197, 307–315. https://doi.org/10.1016/j.foodchem.2015.10.121

Bae, I. Y., Jun, Y., Lee, S., Lee, H. G. (2016). Characterization of apple dietary fibers influencing the in vitro starch digestibility of wheat flour gel. LWT – Food Science and Technology, 65, 158–163. https://doi.org/10.1016/j.lwt.2015.07.071

Bao, H., Liu, Q., Yang, Y., Xu, L., Zhu, K., Jin, Z., Jiao, A. (2023). Effects of rice protein, soy isolate protein, and whey concentrate protein on the digestibility and physicochemical properties of extruded rice starch. J. Food Sci., 88(3), 1159–1171. https://doi.org/10.1111/1750-3841.16458

Bao, T., Wang, Y., Li, Y., Gowd, V., Niu, X., …, Sun, C. (2016). Antioxidant and antidiabetic properties of tartary buckwheat rice flavonoids after in vitro digestion. Journal of Zhejiang University-SCIENCE B, 17(12), 941–951. https://doi.org/10.1631/jzus.B1600243

Bekele, M., Satheesh, N., Sadik, J.A. (2020). Screening of Ethiopian mango cultivars for suitability for preparing jam and determination of pectin, sugar, and acid effects on physico-chemical and sensory properties of mango jam. Scient. African, 7, e00277. https://doi.org/10.1016/j.sciaf.2020.e00277

Boateng, I. D., Mustapha, A., Daubert, C. R., Kuehnel, L., Kumar, R., …, Somavat, P. (2023). Novel Two-pot Microwave Extraction of Purple Corn Pericarp's Phenolics and Evaluation of the Polyphenol-rich Extract's Product Quality, Bioactivities, and Structural Properties. Food Bioproc. Technol., 16(11), 2668–2691. https://doi.org/10.1007/s11947-023-03072-7

Cao, D., Wei, E., Wang, Z., Hu, Z., Qi, L., Zhou, H., Zhao, J. (2022). Microwave-assisted extraction, structural elucidation, and *in vitro* anti-glioma and immunostimulatory activity of polysaccharide from *Panax ginseng* C. A. Meyer. Ind. Crops Prod., 189, 115729. https://doi.org/10.1016/j.indcrop.2022.115729

Choi, A. S., Bae, I. Y., Lee, H. G. (2017). Predicting Buckwheat Flavonoids Bioavailability in Different Food Matrices Under In Vitro Simulated Human Digestion. Cereal Chem. J., 94(2), 310–314. https://doi.org/10.1094/CCHEM-03-16-0054-R

Du, M., Zhao, L., Liu, J., Zheng, Y., Zhou, H., Sui, X. (2023). Development and stability optimisation of composite cerealbased meal replacement. Acta Scient. Polon. Technol. Alimen., 22(3), 243–256. https://doi.org/10.17306/J.AFS.2023.1150

Durazzo, A., Kiefer, J., Lucarini, M., Camilli, E., Marconi, S., …, Marletta, L. (2018). Qualitative Analysis of Traditional Italian Dishes: FTIR Approach. Sustainability, 10(11), 4112. https://doi.org/10.3390/su10114112

Englyst, K. N., Vinoy, S., Englyst, H. N., Lang, V. (2003). Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. Brit. J. Nutr., 89(3), 329–339. https://doi.org/10.1079/BJN2002786

Evenson, A., Zwack, C., Stovern, S., Brewer, K., Windsperger, A., Metelmann, L., …, Johnson, G. (2022). The Impact of Gluten and Inulin on Breath Hydrogen, Gastrointestinal Symptoms, Satiety, and Sensory Attributes in Health Adults. Curr. Dev. Nutr., 6, 509. https://doi.org/10.1093/cdn/nzac077.012

Fontanelli, M., Batista, L., Rogero, M., Micha, R., Mozaffarian, D., Fisberg, R., Sarti, F. (2022). Comparison of Different Approaches to Assess Carbohydrate Quality of Grain Foods: Glycemic Index Versus Carbohydrate Metrics. Curr. Dev. Nutr., 6, 482. https://doi.org/10.1093/cdn/nzac059.010

Gong, E. S., Gao, N., Li, T., Chen, H., Wang, Y., …, Liu, R. H. (2019). Effect of In Vitro Digestion on Phytochemical Profiles and Cellular Antioxidant Activity of Whole Grains. J. Agric. Food Chem., 67(25), 7016–7024. https://doi.org/10.1021/acs.jafc.9b02245

Gupta, R. K., Gangoliya, S. S., Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J. Food Sci. Technol., 52(2), 676–684. https://doi.org/10.1007/s13197-013-0978-y

Hernández-García, Y., Melgar-Lalanne, G., Téllez-Medina, D. I., Ruiz-May, E., Salgado-Cruz, Ma. D. L. P., …, Santiago Gómez, M. P. (2022). Scavenging peptides, antioxidant activity, and hypoglycemic activity of a germinated amaranth (*Amaranthus hypochondriacus* L.) beverage fermented by *Lactiplantibacillus plantarum*. Journal of Food Biochemistry, 46(7). https://doi.org/10.1111/jfbc.14139

Hettiarachchi, H. A. C. O., Gunathilake, K. D. P. P., Jayatilake, S. (2021). Effect of In-vitro Gastrointestinal Digestion and Dialysis Process on Phenolic Compounds and Antioxidant Capacity of Selected Underutilized Fruits in Sri Lanka. Trop. Agric. Res., 32(2), 212. https://doi.org/10.4038/tar.v32i2.8468

Holser, R. A. (2012). Principal Component Analysis of Phenolic Acid Spectra. ISRN Spectroscopy, 1–5. https://doi.org/10.5402/2012/493203

Huang, F., Liu, Y., Zhang, R., Bai, Y., Dong, L., …, Zhang, M. (2019). Structural characterization and in vitro gastrointestinal digestion and fermentation of litchi polysaccharide. Int. J. Biol. Macromol., 140, 965–972. https://doi.org/10.1016/j.ijbiomac.2019.08.170

Hui, X., Wu, G., Han, D., Gong, X., Stipkovits, L., …, Brennan, C. S. (2021). Bioactive compounds from blueberry and blackcurrant powder alter the physicochemical and hypoglycaemic properties of oat bran paste. LWT, 143, 111167. https://doi.org/10.1016/j.lwt.2021.111167

Joye, I. (2019). Protein Digestibility of Cereal Products. Foods, 8(6), 199. https://doi.org/10.3390/foods8060199

Kalhori, F., Yazdyani, H., Khademorezaeian, F., Hamzkanloo, N., Mokaberi, P., Hosseini, S., Chamani, J. (2022). Enzyme activity inhibition properties of new cellulose nanocrystals from *Citrus medica* L. pericarp: A perspective of cholesterol lowering. Luminescence, 37(11), 1836–1845. https://doi.org/10.1002/bio.4360

Kamiloglu, S., Pasli, A. A., Ozcelik, B., Capanoglu, E. (2014). Evaluating the in vitro bioaccessibility of phenolics and antioxidant activity during consumption of dried fruits with nuts. LWT – Food Sci. Technol., 56(2), 284–289. https://doi.org/10.1016/j.lwt.2013.11.040

Kirwan, J. P., Malin, S. K., Scelsi, A. R., Kullman, E. L., Navaneethan, S. D., Pagadala, …, Ross, A. B. (2016). A Whole-Grain Diet Reduces Cardiovascular Risk Factors in Overweight and Obese Adults: A Randomized Controlled Trial. The Journal of Nutrition, 146(11), 2244–2251. https://doi.org/10.3945/jn.116.230508

Lawal, O. M., Fogliano, V., Rotte, I., Fagbemi, T. N., Dekker, M., Linnemann, A. R. (2022). Leafy vegetables fortification enhanced the nutritional profile and reduced the glycemic index of yellow cassava pasta. Food Funct., 13(11), 6118–6128. https://doi.org/10.1039/D2FO00072E

Liang, L., Yue, Y., Zhong, L., Liang, Y., Shi, R., …, Shu, Z. (2023). Anti-aging activities of Rehmannia glutinosa

Libosch. Crude polysaccharide in Caenorhabditis elegans based on gut microbiota and metabonomic analysis. Int. J. Biol. Macromolec., 253, 127647. https://doi.org/10.1016/j.ijbiomac.2023.127647

Lin, Y. A., Chen, K., Tu, D., Yu, X. N., Dai, Z. Y., Shen, Q. (2019). Characterization of dietary fiber from wheat bran (*Triticum aestivum* L.) and its effect on the digestion of surimi protein. LWT – Food Sci. Technol., 102, 106–112. https://doi.org/10.1016/j.lwt.2018.12.024

Liu, Y., Cao, D., Liang, Y., Zhou, H., Wang, Y. (2021). Infrared spectral analysis and antioxidant activity of *Dictamnus dasycarpus* Turcz with different growth years. J. Molec. Struc., 1229, 129780. https://doi.org/10.1016/j.molstruc.2020.129780

Luhovyy, B. L., Akbari, M. (2021). Food Proteins in Controlling Satiety. In: C. C. Udenigwe (Ed.), Food Chemistry, Function and Analysis (pp. 347–370). London: Royal Society of Chemistry. https://doi.org/10.1039/9781839163425-00347

Masisi, K., Beta, T., Moghadasian, M. H. (2016). Antioxidant properties of diverse cereal grains: A review on in vitro and in vivo studies. Food Chem., 196, 90–97. https://doi.org/10.1016/j.foodchem.2015.09.021

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., …, Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food – an international consensus. Food Funct., 5(6), 1113–1124. https://doi.org/10.1039/C3FO60702J

Mkaouar, S., Krichen, F., Bahloul, N., Allaf, K., Kechaou, N. (2018). Enhancement of Bioactive Compounds and Antioxidant Activities of Olive (*Olea europaea* L.) Leaf Extract by Instant Controlled Pressure Drop. Food Bioproc. Technol., 11(6), 1222–1229. https://doi.org/10.1007/s11947-018-2098-1

Nguyen, T. T. L., Flanagan, B. M., Tao, K., Ni, D., Gidley, M. J., Fox, G. P., Gilbert, R. G. (2022). Effect of processing on the solubility and molecular size of oat β-glucan and consequences for starch digestibility of oat-fortified noodles. Food Chem., 372, 131291. https://doi.org/10.1016/j.foodchem.2021.131291

Ni, D., Gunness, P., Smyth, H. E., Gidley, M. J. (2021). Exploring relationships between satiation, perceived satiety and plant-based snack food features. Int. J. Food Sci. Technol., 56(10), 5340–5351. https://doi.org/10.1111/ijfs.15102

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z. Z., Barba, F. J., Greiner, R., Oey, I., Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food products: An overview. Food Control, 79, 62–73. https://doi.org/10.1016/j.foodcont.2017.03.027

Oñate Narciso, J., Brennan, C. (2018). Whey and Pea Protein Fortification of Rice Starches: Effects on Protein and Starch Digestibility and Starch Pasting Properties. Starch – Stärke, 70(9–10), 1700315. https://doi.org/10.1002/star.201700315

Pešić, M. B., Milinčić, D. D., Kostić, A. Ž., Stanisavljević, N. S., Vukotić, …, Tešić, Ž. Lj. (2019). In vitro digestion of meat- and cereal-based food matrix enriched with grape extracts: How are polyphenol composition, bioaccessibility and antioxidant activity affected? Food Chem., 284, 28–44. https://doi.org/10.1016/j.foodchem.2019.01.107

Qin, W., Ketnawa, S., Ogawa, Y. (2022). Effect of digestive enzymes and pH on variation of bioavailability of green tea during simulated *in vitro* gastrointestinal digestion. Food Sci. Human Wellness, 11(3), 669–675. https://doi.org/10.1016/j.fshw.2021.12.024

Ren, F., Yang, X., Wang, L., Zhou, S. (2021). Effects of Different Processing Methods and Internal Components on Physicochemical Properties and Glycemic Index of Adzuki Bean Powder. Foods, 10(8), 1685. https://doi.org/10.3390/foods10081685

Sahu, R., Kundu, P., Sethi, A. (2021). In vitro antioxidant activity and enzyme inhibition properties of wheat whole grain, bran and flour defatted with hexane and supercritical fluid extraction. LWT, 146, 111376. https://doi.org/10.1016/j.lwt.2021.111376

Simsek, S., El, S. N. (2015). *In vitro* starch digestibility estimated glycemic index and antioxidant potential of taro (*Colocasia esculenta* L. Schott) corm. Food Chem., 168, 257–261. https://doi.org/10.1016/j.foodchem.2014.07.052

Stribiţcaia, E., Blundell, J., You, K.-M., Finlayson, G., Gibbons, C., Sarkar, A. (2022). Viscosity of food influences perceived satiety: A video based online survey. Food Qual. Prefer., 99, 104565. https://doi.org/10.1016/j.foodqual.2022.104565

Sun, Y., Tao, W., Huang, H., Ye, X., Sun, P. (2019). Flavonoids, phenolic acids, carotenoids and antioxidant activity of fresh eating citrus fruits, using the coupled *in vitro* digestion and human intestinal HepG2 cells model. Food Chem., 279, 321–327. https://doi.org/10.1016/j.foodchem.2018.12.019

Tagle-Freire, D., Mennah-Govela, Y., Bornhorst, G. M. (2022). Starch and protein hydrolysis in cooked quinoa (*Chenopodium quinoa* Willd.) during static and dynamic *in vitro* oral and gastric digestion. Food Funct., 13(2), 920–932. https://doi.org/10.1039/D1FO02685B

Tagliazucchi, D., Helal, A., Verzelloni, E., Conte, A. (2016). Bovine milk antioxidant properties: Effect of

in vitro digestion and identification of antioxidant compounds. Dairy Sci. Technol., 96(5), 657–676. https://doi.org/10.1007/s13594-016-0294-1

Weerasooriya, D. K., Bean, S. R., Nugusu, Y., Ioerger, B. P., Tesso, T. T. (2018). The effect of genotype and traditional food processing methods on *in-vitro* protein digestibility and micronutrient profile of sorghum cooked products. PLOS ONE, 13(9), e0203005. https://doi.org/10.1371/journal.pone.0203005

Wu, T., Taylor, C., Nebl, T., Ng, K., Bennett, L. E. (2017). Effects of chemical composition and baking on in vitro digestibility of proteins in breads made from selected gluten-containing and gluten-free flours. Food Chem., 233, 514–524. https://doi.org/10.1016/j.foodchem.2017.04.158

Yang, D., Lew, H. L., Mak, Y. Y., Ou, S. J. L., …, Liu, M. H. (2023). Incorporation of okra (*Abelmoschus esculentus* (L.) Moench) seed powder into fresh rice noodles with tapioca starch improves postprandial glycemia, insulinemia and satiety in healthy human volunteers. J. Funct. Foods, 100, 105382. https://doi.org/10.1016/j.jff.2022.105382

Ye, Z., Arumugam, V., Haugabrooks, E., Williamson, P., Hendrich, S. (2015). Soluble dietary fiber (Fibersol-2) decreased hunger and increased satiety hormones in humans when ingested with a meal. Nutr. Res., 35(5), 393–400. https://doi.org/10.1016/j.nutres.2015.03.004

Yuan, Q., Xie, Y., Wang, W., Yan, Y., Ye, H., Jabbar, S., Zeng, X. (2015). Extraction optimization, characterization and antioxidant activity *in vitro* of polysaccharides from mulberry (*Morus alba* L.) leaves. Carbohydr. Polymers, 128, 52–62. https://doi.org/10.1016/j.carbpol.2015.04.028

Zaremba, S. M. M., Gow, I. F., Drummond, S., McCluskey, J. T., Steinert, R. E. (2018). Effects of oat β-glucan consumption at breakfast on *ad libitum* eating, appetite, glycemia, insulinemia and GLP-1 concentrations in healthy subjects. Appetite, 128, 197–204. https://doi.org/10.1016/j.appet.2018.06.019

Zhang, H. H., Wang, S. (2016). Optimization of Total Polyphenols Extraction from *Vigna angularis* and Their Antioxidant Activities. Ind. J. Pharmac. Sci., 78(5). https://doi.org/10.4172/pharmaceutical-sciences.1000159