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Impact of flour particle size and origin on the bread structure and the postprandial glycemic, insulinemic and appetite responses in healthy adults†

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Consumption of bakery products prepared with finely milled flour is associated with elevated postprandial glycemia, increased hunger, and reduced satiety. The milling process disrupts the plant cell walls of cereal grains and legumes, enhancing the accessibility of encapsulated starch to digestive enzymes. This study investigates the effects of flour origin (wheat and chickpea) and particle size in three wholemeal breads on physicochemical properties, postprandial glucose, insulin, glucagon-like peptide-1 (GLP-1) responses, and subjective appetite sensations in healthy individuals. In the test breads, 30% of refined wheat flour was substituted with cracked whole wheat (1.8–2.0 mm) to make whole grain bread (WGB), finely milled chickpea flour (CFM), or larger particle-chickpea flour (1.4–1.8 mm, CLP). Wheat bread (WB) served as the control. In all three test breads, 28% of refined wheat flour was substituted with wholemeal wheat flour. Compared to WB and WGB, CFM and CLP had a harder and more chewy texture, and a lower specific volume ($p < 0.05$). WGB, CFM, and CLP had reduced porosity and lightness (L^*) compared to WB ($p < 0.05$). In a randomized crossover study (RCT), fifteen normoglycemic individuals participated in four separate sessions. The glucose incremental area under the curve (iAUC) was lower for CLP compared to those of both WB and CFM ($p < 0.05$). While insulin responses were similar across all breads, GLP-1 iAUC was significantly higher following CLP consumption compared to WB ($p < 0.05$), whereas no significant differences were observed among the other test breads in the postprandial GLP-1 response. CLP consumption resulted in a lower iAUC for hunger and desire to eat, and a higher iAUC for fullness, as evaluated using Visual Analogue Scales (VAS), compared to WB ($p < 0.05$). Incorporation of large-particle chickpea flour into bread can effectively reduce postprandial glycemia, increase GLP-1 secretion and contribute to the enhancement of satiety. Such formulations may offer promising dietary strategies for glycemic control and appetite regulation.

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Introduction

The obesity epidemic constitutes a major public health challenge nowadays, with global rates nearly tripling since 1975, according to recent World Health Organization (WHO) data.[1] The prevalence of Type 2 Diabetes Mellitus (T2DM) has also significantly risen in recent decades.[2] Food processing, both primary (*e.g.* grain milling) and secondary (*e.g.* breadmaking), alters the natural microstructure of foods, and can potentially lead to increased glycemic responses and reduced satiety.[3] The excessive consumption of highly processed foods has been linked to elevated energy intake, ultimately contributing to weight gain and the impairment of glucose metabolism. So, the modification of food structures can be a promising strategy to tackle obesity.[4–9]

Wheat bread, exclusively made from refined wheat flour, has an open highly porous structure, with most of its starch being gelatinized, and its physical structure playing a crucial role in determining the postprandial glycemic response.[10] For bread-making flour, cereal grains are typically milled to achieve a particle size of about 180 μm. Reduction of particle size during milling disrupts plant cell walls, where starch gran-

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ules are encapsulated, thus enhancing the contact between starch and digestive enzymes (*e.g.* α-amylase).[11] According to geometric principles, flours with larger particle sizes have a lower specific surface area to volume ratio and contain a higher proportion of intact, unbroken cells, that restrict enzyme accessibility and diffusion. As a result, starch is more effectively encapsulated in these cells and thus is more resistant to digestion in the stomach and small intestine, which can lead to a milder glycemic response.[11–16] So, the degree of milling is of crucial importance when formulating functional bakery products with reduced starch digestibility.

Several studies have investigated the effect of flour particle size on the *in vitro* starch digestibility. In the case of wheat, an inverse relationship between particle size and starch digestion has been found, between coarse wheat farina (750–800 μm), fine farina (330 μm) and flour (85 μm).[11] In addition, it has been found that increasing durum wheat flour particle size from <0.21 mm to 3.15 mm led to a higher proportion of encapsulated starch and thus a reduced rate of starch digestibility.[17] In wheat porridges, a 50% reduction in the rate of sugar release was observed in coarse particles (1.95 mm) compared to that in finely milled flour (<0.11 mm).[18] In flour, intact wheat endosperm cells effectively slow down enzymic diffusion, even though their cell walls are permeable to α-amylase. In the case where wheat flour of larger particle size has been incorporated into bread, contradictory results have been found. Lin *et al.* (2020)[19] found that breads exclusively made from coarse (1325 μm) and medium flour (450 μm) were digested at a slower rate compared to a fine fraction (199 μm). However, no significant differences in starch digestibility were found with the incorporation of coarse farina particles at inclusion rates of 10–80% by Korompokis and Delcour (2021).[20] Similarly, Tagliasco (2022)[13] obtained similar results by substituting small (<350 μm), medium (1000–1800 μm) and large (>1800 μm) particle size wheat particles, even though the cell wall integrity was maintained in a large fraction during bread processing steps. In terms of human studies, no significant differences in glycemic and insulinemic responses were found in acute postprandial protocols, when either healthy individuals or those with risk factors for T2DM consumed breads (with identical amounts of available carbohydrates), in which larger particle size flours had been incorporated, compared to the respective finely milled whole meal flours.[21–23] However, in the case of diabetic subjects, a significant effect of flour particle size and degree of milling on postprandial glycemia has been found by several studies.[14,15,24,25]

Studies on the pulse flour particle size have shown that, for chickpeas, increasing the flour particle size more effectively reduces the rate and extent of starch digestibility compared to wheat flour of similar particle size, after they have undergone hydrothermal processing.[26] Chickpea cotyledons, which are storage cells located in the seed leaves of legumes, are rich in starch and protein that are naturally encapsulated within their thick cell walls. Their plant cell walls are thicker, less permeable, and compositionally different from wheat endosperm cell walls, as they are rich in pectin and xyloglucans. Intact chickpea cells tend to separate under hydrothermal treatment, making them more resistant to digestion in the stomach and small intestine. Thus, beyond particle size, the botanical origin plays a crucial role in digestibility.[12,27–31] Conventionally milled pulse flour mainly consists of ruptured plant cells, which lead to increased digestibility when they undergo hydrothermal processing.[32,33] In gluten-free bread, substituting 20% of rice flour with larger particle-sized legume flour (>200 μm) significantly reduced starch hydrolysis by nearly half, likely due to the presence of intact cells.[34] In another clinical trial it was shown that replacing 30% and 60% of refined wheat flour with chickpea flour containing intact cells in bread led to improved postprandial metabolic responses, specifically in glucose regulation, insulin levels, and gut hormone responses.[35]

The aim of the present study is to investigate how the particle size and botanical origin of flour—specifically, the structural differences between wheat and legumes—affect both the physicochemical properties of bread and postprandial metabolic responses in healthy humans. Breads were developed using finely milled or coarser particles from two botanical sources, hard durum wheat and chickpeas, to assess differences in glycemic, insulinemic, and GLP-1 responses following consumption. This research study explores the effects of the degree of flour milling and plant tissue structure on the postprandial metabolic responses of healthy individuals. To our knowledge, no previous study has directly compared breads prepared with different chickpea flour particle sizes to their quality attributes along with the postprandial metabolic responses. In the present study, the aforementioned effects of bread made with coarser chickpea flour were evaluated in comparison with bread containing the same proportion of finely milled chickpea flour, with both flours derived from the same chickpea source.

Methods

Test food development and evaluation

Bread preparation and nutritional composition. The test breads were produced in the research and development laboratories of the Greek food company VENETIS S.A. Preliminary pilot baking trials were conducted to finalize the bread recipes. Four different types of bread were made: white bread (WB) using 100% conventional refined wheat flour with the bran and the germ removed (<210 μm), and three test breads in which 30% of the white wheat flour was replaced with either broken hard wheat grains (WGB) between 1800 and 2000 μm, finely milled chickpea flour (CFM) (<210 μm), or chickpea flour with a larger particle size (CLP). Particle size distribution of CLP bread is presented in detail in Table 1, and it was determined by sieve analysis. The percentage of flour substitution was identical in all bread formulations to be able to compare the test breads with each other. Additionally, all three test breads contained wholemeal wheat, comprising 28% of the total flour content Fig. 1 presents the detailed composition of

Table 1 Flour particle size distribution of large particle size chickpea flour

Chickpea particle size (mm)	(%)
>2	1.5 ± 2.7
>1.6	17.9 ± 2.0
>1.45	42.5 ± 0.7
>1.25	17.4 ± 0.5
>1	19.0 ± 0.6
>0.8	1.6 ± 0.4

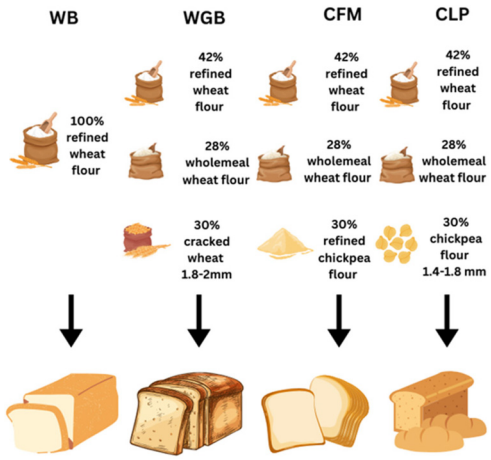

Fig. 1 Flour compositions of the four bread types tested in the study. WB: white bread (100% refined wheat flour); WGB: bread with 30% cracked whole wheat; CFM: bread with 30% finely milled chickpea flour; CLP: bread with 30% large particle size chickpea flour.

the test breads in terms of the different types of flours used. The remaining ingredients—dry yeast, sourdough, poolish, flour enhancer, water, and salt—were used in identical quantities across all recipes. All ingredients were sourced from the company's suppliers. For each bread recipe, the dry ingredients were weighed and mixed, followed by the addition of water. The mixing, kneading, rising, and baking times were consistent across all recipes. Specifically, the WB and CFM breads required 10 min of mixing, while the WGB and CLP breads required 12 min. The dough was allowed to rest for 20 min and then divided into 15 loaves, each weighing approximately 450 g. The dough was placed into baking pans (pan bread) and transferred to an incubator set at 37 °C with 75% relative humidity for 40–45 min. The loaves were then baked in an oven at 190 °C for 18–20 min, with 2.5 L of steam applied during baking. After baking, the loaves were cooled at room temperature for 2–3 hours (25 ± 2 °C) before being packaged in polyethylene bags and pasteurized. The packaged breads were then stored frozen at −20 °C. All bread preparation steps were conducted using the company's professional equipment, adhering to all necessary safety protocols to ensure the product is safe for human consumption. The production process replicated the company's standard commercial procedures for bread manufacturing. Fig. 1 presents images of bread slices taken from the center of each loaf for all four bread samples.

Regarding the chickpea flour of larger particle size used for CLP bread preparation, it was assessed with sieve analysis, as described elsewhere.[22] The whole chickpeas were first dehulled and then they were roller milled. A mechanical sieve shaker was used with sieves that captured particles of sizes of 700, 800, 1000, 1250, 1410, 1600, 2000, and 3150 μm. A 100 g sample of flour was placed on the top sieve (3150 μm), and the shaker was operated for fifteen min. The amount of chickpea flour retained on each sieve was then weighed to determine the particle size distribution. The results shown in Table 1 are expressed as a percentage of mass particles retained on each sieve. The process was repeated three times, and the average of these measurements was reported as the mean ± SD. For the finely milled chickpea flour, a commercially available variety from the same chickpeas was selected, with particle sizes small enough to pass through a 70-mesh sieve, which confirms that all flour particles were <210 μm based on the conversion chart mesh to millimeters. The wheat grains, supplied by the food manufacturer's partners, were specified to have a particle size between 1800 μm and 2000 μm, produced by milling debranned hard durum wheat grains.

The detailed nutritional composition of each test bread is presented in Table 2. All baked and frozen samples were analyzed regarding their composition. Protein content (Nitrogen: Nx6.25) was measured using the Kjeldahl method according to ISO 1871,[36] while fat was determined through Soxhlet procedures. Saturated and unsaturated fatty acids were analyzed according to the ISO 5509 method.[37] Total dietary fiber was determined by the AOAC method 991.43.[38] Available carbohydrates were calculated by difference, using the following equation: Carbohydrates (%) = 100 − (moisture + protein + fat + ash + fiber); the total starch was determined *via* enzymatic reaction and total sugars by GC-FID (gas chromatography with flame ionizing detector). The moisture content was measured by loss on drying. Energy content (kJ) was calculated using the equation: Energy (kJ) = 17 (g protein + g carbohydrate) + 37 (g fat) + 8 (g total dietary fiber).

Bread quality assessment. From each bread type, three bread rolls from each separate batch were subjected to analysis regarding their quality attributes. Test breads were thawed in their packaging at room temperature (25 ± 2 °C) for 10–12 hours before conducting quality assessments (overnight, as provided to the study participants in the clinical trial). These evaluations included texture analysis, specific volume and density, crumb grain structure evaluation and crumb color. The tests were performed in a consistent order for each treatment and completed within 2 hours to minimize quality variation, due to starch retrogradation and/or moisture loss.

The mechanical properties of the bread crumb samples were evaluated using Texture Analysis with a TA-XT2 plus Texture Analyzer (Stable Microsystems), equipped with a 5 kg load cell and using modification of the AACC method 74–09 (bread firmness by universal testing) (AACC, 2000). Each sample underwent two double-compression tests using a

Table 2 Nutritional composition of the four test breads per serving

Sample	Energy content (kJ)	Serving size (g)	Available CHO (g)	Fat (g)	SFA (g)	MUFA (g)	PUFA (g)	Protein (g)	Total dietary fiber (g)	Total starch (g)	Total sugars (g)
WB	1069	100	50	1.1	0.4	0.2	0.6	9.0	3.2	39.7	3.6
WGB	1157	114	50	1.5	0.5	0.2	0.8	11.7	7.6	39.4	2.6
CFM	1215	126	50	2.4	0.5	0.6	1.2	14.5	8.01	41.8	3.5
CLP	1305	136	50	2.7	0.7	0.6	1.4	15.5	11.7	39.7	2.8

SFA: saturated fatty acids; MUFA: monosaturated fatty acids; and PUFA: polysaturated fatty acids.

cylindrical stainless-steel probe (25 mm diameter), to perform 50% penetration tests at the center of each slice. The slices had a uniform thickness of 1.2 cm, matching the thickness typically found in commercially available bread. The test protocol included a pre-test speed of 10 mm s^{-1}, a test speed of 5 mm s^{-1}, and a post-test speed of 1 mm s^{-1}. A total of eight measurements were taken per sample, focusing on the central slices of the loaf to ensure consistent results. Crumb hardness was defined as the maximum force required to achieve 50% compression of the breadcrumb. Stable Micro Systems software was used to calculate textural attributes, such as cohesiveness, chewiness, and resilience as defined by Szczesniak (2002).[39] Specific volume (cm^3 g^{-1}) and density (g cm^{-3}) were assessed as the average of three loaves per recipe. The volume was determined using a volumetric displacement method, adapted from the rapeseed displacement technique, with 2 mm solid-glass beads, following the approach of Tsatsaragkou (2017).[40] The mass of the bread (g) was also measured to complete the calculations on a laboratory scale.

Crumb grain structure analysis was performed on four 1.2 cm-thick slices taken from the center of each loaf. The slices were scanned using a flatbed scanner (HPDeskjet 5200, Hewlett Packard, USA) with an analysis of 600 dpi. Image analysis was conducted with Image ProPlus 7 software (Media Cybernetics, USA). Key parameters such surface porosity (%), number of pores (1 × 1 cm), average cell size (mm^2), and cell density (cells per cm^2) were calculated.[40] Instrumental measurement of color was evaluated on eight different slices using a Minolta spectrophotometer (Chroma Meter CM-5, Konica Minolta, Japan), calibrated with white and black reference plates, with the CIE L^*, a^*, b^* model. The total color difference (ΔE^*) was calculated using the CIE76 formula (eqn (1)). Additionally, chroma (C) and hue angle (h) were also calculated using the eqn (2) and (3):

$$\Delta E = \sqrt{(L - L_o)^2 + (a - a_o)^2 + (b - b_o)^2} \quad (1)$$

$$C = \sqrt{a^2 + b^2} \quad (2)$$

$$H = a \tan\left(\frac{b}{a}\right) \quad (3)$$

The CIELAB color space is defined by three coordinates: lightness (L^*), where a value of 0 represents black and 100 represents white; the red-green axis (a^*), with negative values signifying green and positive values signifying red; and the yellow-blue axis (b^*), where negative values correspond to blue and positive values correspond to yellow.

Acute postprandial protocol

Study participants. Fifteen healthy, non-diabetic volunteers (6 males and 9 females) aged 20–35 years, with a BMI (Body Mass Index) of 18.0–24.9 kg m^{-2}, participated in the study. Exclusion criteria included the presence of type I or II diabetes mellitus, cardiovascular disease, gastrointestinal, renal, hepatic, inflammatory, thyroid, or psychiatric disorders. Dietary supplement intake, potential allergy or sensitivity to wheat, excessive exercise habits, a history of alcohol and/or drug use were also exclusion criteria. Pregnant or breastfeeding women could not participate in the study. The body weight, BMI, fasting plasma glucose and lipid concentrations as well as the liver function of study participants were evaluated and confirmed to be within the normal range during a screening visit, 1 week prior to the beginning of the study protocol, to assess their eligibility for participation. During the study, volunteers should have a relatively stable weight and should not have significantly changed their eating habits and physical activity levels.

Participants were recruited through posters around the campus of Harokopio University and Athens Medical School, online advertisements, and direct communication with them. Prior to enrollment, they received detailed written information about the study protocol and provided voluntary, informed written consent for participation. The study was conducted in accordance with the Helsinki Declaration and the protocol was reviewed and approved by the Institutional Review Board/Ethics Committee of Harokopio University of Athens and Laiko General Hospital. The study is registered under the number: ClinicalTrials.gov: NCT050691686. The flowchart of the study is depicted in Fig. 2.

Study design. The current protocol was an acute single-blind controlled cross-over trial (RCT) with three test breads (WGB, CFM, CLP) and a control WB. Breads were provided with an in-between wash-out period of at least 3 days. The randomization process was carried out using a computer-generated schedule (**https://www.randomizer.org**, Research Randomizer, version 4.0, accessed on 20 February 2022). The primary outcome of the current acute study was a 10% reduction in the glucose incremental area under the curve (iAUC) after consumption of the breads (WGB, CFM, and CLP) compared to the iAUC after consumption of the reference food (WB). Secondary outcomes

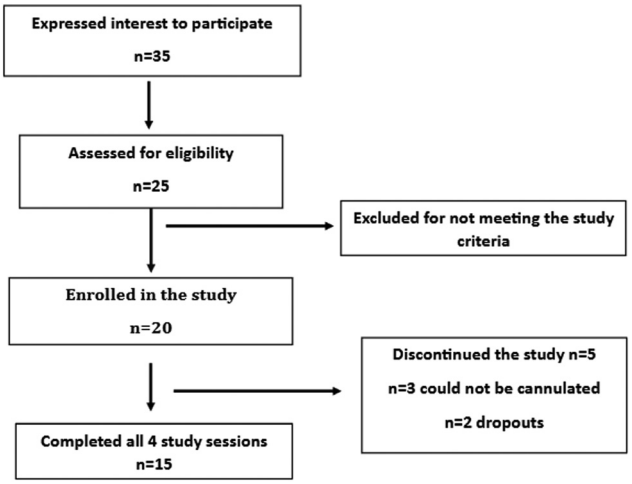

Fig. 2 Study flow chart.

were changes in the postprandial response of insulin, GLP-1 and subjective appetite sensations, as described by their respective iAUCs, as well as changes in specific time points.

The acute postprandial protocol was conducted in the Diabetes Laboratory of the 1st Department of Propaedeutic and Internal Medicine of Laiko University Hospital. Subjects arrived after overnight fasting between 8:00–9:00. Participants underwent clinical examinations, which included assessments of body weight and composition, at their first visit to the laboratory. The body weight was measured with an electronic scale, while body composition—specifically fat and lean mass—was determined using bioelectrical impedance analysis (Tanita BC-418, Tokyo, Japan). The height was measured with a stadiometer (Seca Mode 220, Hamburg, Germany) while participants stood in a relaxed position, without shoes, and with arms hanging naturally. The waist circumference was measured at the midpoint between the lower edge of the last palpable rib and the top of the iliac crest, with participants standing and at the end of a gentle exhalation. Hip circumference was measured at the widest part of the buttocks, with both measurements taken twice using a fiberglass tape. After a 10-minute rest period, participants completed the first set of visual analogue scale (VAS) questions, which assessed hunger, fullness, and desire to eat. An intravenous catheter was then inserted into a forearm vein, and baseline blood samples were collected. Participants were given one of the four test meals, which they were required to consume within 15 min. The meals consisted of a quantity of bread providing 50 g of available carbohydrates (approximately 3–4 slices), served with 250 mL of tap water. The test breads were stored frozen at −20 °C and defrosted overnight at room temperature (25 °C) prior to each test day.

Blood samples were taken at 30, 45, 60, 90, 120, and 180 min postprandially. Subjective satiety was evaluated using VAS booklets provided to participants. These questionnaires were completed prior to the consumption of each test bread (t = 0 min) and at 30, 60, 90, 120, and 180 min postprandially, immediately before blood sample collection. Participants responded to three questions: "How hungry do you feel?", "How full do you feel?", and "How strong is your desire to eat?". Responses were recorded on a 100 mm linear scale, ranging from 0 ("not at all") to 10 ("extremely"), reflecting the lowest and highest possible intensity of the sensation, respectively. Participants were instructed to indicate their current feelings with a vertical mark on the scale, without discussing their responses with others or referring to prior responses. The results were quantified by measuring the distance (mm) from the left end of the scale to the participant's mark for each question. Participants were instructed to consume a meal of their choice, of no more than 400 kcal, with minimal fat and dietary fiber content, the evening before each study session and to repeat the same meal prior to each subsequent session. Twenty-four-hour dietary recalls and a physical activity questionnaire were collected for the day preceding each study visit. None of the participants engaged in regular exercise, and they were strongly encouraged to maintain their usual lifestyle habits throughout the entire experimental period.

Blood analyses. Blood samples were collected in K_3EDTA-coated vacutainers and immediately centrifuged at 1000g for 10 min at 4 °C to separate the plasma. For serum collection, blood was drawn into plain tubes, left to clot at room temperature for 30 min, and then centrifuged at 1000g for 10 min at 4 °C. Both plasma and serum were stored at −80 °C until further analysis.

Serum glucose concentrations were measured at 0, 30, 45, 60, 90, 120, and 180 min postprandially. Glucose, as well as the other biochemical measurements (total cholesterol, HDL-c, LDL-c, and triglycerides) were performed in serum, at the beginning of the study, on an automated biochemical analyzer (Medilyzer, Medicon Hellas S.A., Athens, Greece) using commercially available diagnostic kits. Insulin levels were assessed in serum at 0, 30, 45, 60, 90, 120, and 180 min postprandially *via* an enzyme-linked immunosorbent assay (ELISA) using a Human Insulin ELISA kit (Merck-Millipore, Burlington, MA, USA). Additionally, total GLP-1 levels were measured in plasma at 0, 30, 60, 90, 120, and 180 min using sandwich ELISA (Human GLP-1 ELISA, Ansh Labs, Webster, TX, USA). All measurements were carried out using a Multiskan™ FC Microplate Photometer (Thermo Scientific, Waltham, MA, USA).

Statistical analysis. A power analysis was conducted to calculate the necessary sample size for detecting a statistically significant 10% reduction in the $iAUC_{180}$ of glucose following the intervention. The analysis aimed for a power of 80% at a significance level of α = 5% (Type I error). Based on these parameters, a minimum of 16 participants was required to detect the desired reduction in $iAUC_{180}$ with sufficient statistical power. The recruitment aim was at least 18 participants, to account for a dropout rate of 10%. Finally, 15 participants were included in the calculations.

The iAUC for postprandial glucose and insulin responses were calculated using the trapezoidal rule, excluding any area below the baseline, following the method described by Yanni

et al. (2022).[41] For GLP-1, the iAUC was calculated based on the increase from pre-prandial levels, considering only the area above the *x*-axis. Similarly, the iAUC for VAS measurements, representing changes in subjective appetite sensations from pre-prandial values, was determined using the same approach. The Homeostasis Model Assessment of Insulin Resistance HOMA-IR for the study subjects was calculated using the following formula:[42]

$$\text{HOMA-IR} = \left(\text{fasting glucose}\,(\text{mg dL}^{-1}) \times \text{fasting insulin}\,(\mu\text{U mL}^{-1})\right)/405 \quad (4)$$

Descriptive statistics are reported as absolute numbers and percentages (%), while the numerical results are presented as mean ± SEM. Bread quality was analyzed using IBM SPSS statistics 22 with parametric tests, specifically one-way ANOVA, followed by Tukey's *post hoc* test for comparisons.

A general linear model with repeated measures ANOVA followed by Bonferroni's *post hoc* test was applied to evaluate postprandial changes in blood variables and subjective appetite ratings across treatments, identifying significant differences at specific time points and between iAUC values. A *p*-value of <0.05 was considered the threshold for the statistical significance in all analyses (ESI, Table S1†).

Results

Bread quality evaluation

The particle size distribution, nutritional composition and physicochemical properties of the four test breads are presented in Tables 1, 2 and 3, respectively. In terms of nutritional composition, the energy content of the test bread was slightly higher than that of the control, which was expected, as larger portions of the test bread were consumed to achieve 50 g of available carbohydrates. The WB and WGB had lower protein and total dietary fiber content compared to the CFM and CLP bread, whose contents were very similar. All samples exhibited a similar total starch content, while the moisture content was slightly higher in bread prepared with chickpea flour. Regarding the particle size distribution, most of the chickpea particles (62%) were higher than 1.45 mm, and almost all of them were higher than 1 mm (>80%).

The physicochemical properties of the test breads were evaluated, focusing on their textural and color attributes. The texture analysis of breads revealed that the three test breads (WGB, CFM and CLP) were significantly harder compared to the WB ($p < 0.05$, Table 3), which is mainly attributed to their higher dietary fiber content and the use of coarser flours. Additionally, WGB exhibited significantly lower hardness compared to both chickpea flour-enriched breads (CFM and CLP). Chewiness values were similar in WB and WGB, both of which had significantly lower chewiness than CFM and CLP ($p <$ 0.05, Table 3). Cohesiveness and resilience were comparable across all samples, with no significant differences between

Table 3 Physical characteristics of the four test bread samples

	Hardness (N)	Cohesiveness	Chewiness	Resilience	*L**	*a**	*b**	Δ*E*	*C*	Hue (rad)
WB	6.3 ± 0.7^{a}	0.6 ± 0.1^{a}	3.7 ± 0.4^{a}	0.2 ± 0.0^{a}	77.4 ± 1.2^{a}	1.2 ± 0.2^{a}	24.0 ± 0.3^{a}	—	24.0 ± 0.25^{a}	1.52 ± 0.01^{a}
WGB	7.3 ± 0.4^{b}	0.6 ± 0.1^{a}	4.5 ± 0.7^{a}	0.3 ± 0.0^{a}	64.1 ± 0.8^{b}	3.9 ± 0.3^{b}	17.8 ± 0.3^{b}	14.9 ± 1.0	18.22 ± 0.35^{b}	1.35 ± 0.01^{b}
CFM	8.9 ± 0.4^{c}	0.6 ± 0.1^{a}	5.5 ± 0.9^{b}	0.3 ± 0.3^{a}	67.6 ± 0.7^{c}	4.9 ± 0.2^{c}	20.4 ± 0.3^{c}	11.1 ± 1.6	20.93 ± 0.27^{c}	1.34 ± 0.01^{c}
CLP	8.3 ± 0.7^{c}	0.7 ± 0.1^{a}	5.5 ± 0.6^{b}	0.3 ± 0.1^{a}	66.8 ± 0.7^{d}	4.7 ± 0.1^{c}	19.1 ± 0.5^{c}	12.1 ± 1.1	19.62 ± 0.45^{d}	1.33 ± 0.0^{c}

	Surface porosity (%)	Specific volume ($\text{cm}^3\ \text{g}^{-1}$)	Density (g cm^{-3})	Number of pores (1 × 1 cm)	Cell diameter (mm)	Min	Max	Size (length)	Size (width)
WB	44.4 ± 2.8	3.1 ± 0.1^{a}	0.3 ± 0.0^{a}	28.2 ± 3.0^{a}	0.8 ± 0.0^{a}	0.4 ± 0.0^{abc}	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}	0.8 ± 0.0^{a}
WGB	35.0 ± 0.4^{b}	2.8 ± 0.1^{a}	0.4 ± 0.0^{a}	26.3 ± 2.7^{a}	0.83 ± 0.0^{b}	0.9 ± 0.5^{b}	1.0 ± 0.6^{a}	1.4 ± 0.1^{a}	0.8 ± 0.1^{a}
CFM	37.3 ± 0.2^{c}	2.4 ± 0.1^{b}	0.4 ± 0.0^{b}	34.3 ± 2.4^{b}	0.74 ± 0.0^{a}	0.4 ± 0.0^{abc}	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	0.7 ± 0.0^{b}
CLP	38.0 ± 0.3^{bc}	2.4 ± 0.1^{b}	0.4 ± 0.0^{b}	33.3 ± 1.4^{b}	0.75 ± 0.0^{a}	0.4 ± 0.1^{c}	1.1 ± 0.3^{a}	1.2 ± 0.0^{a}	0.7 ± 0.0^{ab}

Values followed by different letters are statistically different at a significance level $p < 0.05$. WB: white bread; WGB: whole grain bread with 30% broken wheat kernels 1.8–2 mm; CFM: bread with 30% finely milled chickpea flour; and CLP: bread with 30% large particle size chickpeas.

CFM and CLP in any of the textural attributes examined (approximately 8–9 N).

In terms of color parameters, all test bread samples had significantly reduced lightness (L^*) values, as expected due to the inclusion of whole meal flour in their formulations. WGB had the lowest lightness value compared to the other samples ($p < 0.05$, Table 3). The a^* value, which represents the blue-yellow color spectrum, was significantly higher in all test breads compared to that on WB ($p < 0.05$, Table 3). As expected, all three test breads were less yellow (lower a^* values) compared to WB, which was made exclusively with white wheat flour. In terms of the b^* value, all test breads had a redder hue than green, with high positive values, and WB had the highest value ($p < 0.05$). Chroma (C) and hue (h) values were also significantly higher in all test breads, with CFM and CLP differing only in their lightness (L^*) values, while all other color parameters were similar. The specific volume ($cm^3\ g^{-1}$) of chickpea breads (CFM and CLP) is significantly lower compared to wheat breads (WB and WGB). Conversely, the density ($g\ cm^{-3}$) of WB and WGB is significantly lower than that of CFM and CLP ($p < 0.05$, Table 3). The crumb of WB exhibited the highest surface porosity at 44.45 ± 2.81%, as determined by image analysis. CLP had similar porosity values to CFM and WGB, with the latter two significantly differing from each other ($p < 0.05$, Table 3). Chickpea breads had a significantly higher number of pores (28.18 ± 2.98 and 26.30 ± 2.73) compared to WB and WGB (34.30 ± 2.35 and 33.37 ± 1.40, respectively). The average pore diameter was significantly larger in WGB than in the other breads, as evident in the provided bread sample images. WGB had fewer but larger pores compared to the other breads. Additionally, larger wheat flour particles were visible and not all of them were fully embedded in the bread structure. In the case of CLP bread, all chickpea particles were not fully embedded in the bread structure, but it did not seem to significantly affect its textural attributes compared to CFM bread (Fig. 3).

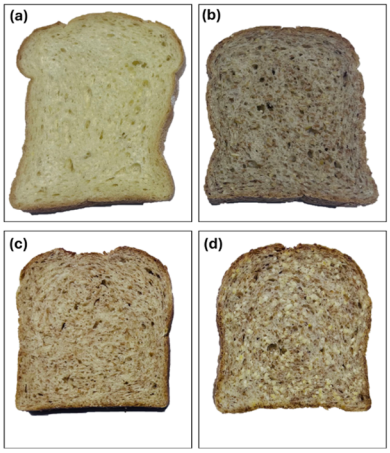

Fig. 3 Images of the four test breads: (a) wheat bread (WB), (b) whole grain bread (WGB), (c) chickpea finely milled (CFM), and (d) chickpea large particles (CLP).

Participants. All participants completed all four sessions of the study without experiencing any adverse effects. Table 4 provides an overview of the participants' characteristics, and the average values of classical biochemical parameters, such as glycemic and lipidemic profiles, which fell within the normal range. Participants followed the study guidelines, continuing their usual dietary and exercise routines, and maintained their body weight stable throughout the experimental phase.

Table 4 Anthropometric and biochemical characteristics of the study participants

Characteristic	Subjects
n	15
Sex (male/female)	(6/9)
Age (years)	23.3 ± 3.6
Body weight (kg)	61.6 ± 11.0
BMI ($kg\ m^{-2}$)	21.3 ± 2.4
Fat mass (%)	18.4 ± 5.1
Fat mass (kg)	11.2 ± 3.0
Lean mass (kg)	47.6 ± 14.9
Waist (cm)	72.8 ± 8.4
Hip (cm)	94.3 ± 8.4
Fasting serum glucose ($mg\ dL^{-1}$)	88.8 ± 5.0
Fasting serum insulin ($\mu U\ mL^{-1}$)	4.2 ± 1.9
HOMA-IR	0.9 ± 0.4
Total cholesterol ($mg\ dL^{-1}$)	144.3 ± 19.2
LDL-c ($mg\ dL^{-1}$)	86.9 ± 19.3
HDL-c ($mg\ dL^{-1}$)	47.3 ± 7.3
Triacyloglycerols ($mg\ dL^{-1}$)	48.6 ± 10.2

n, number of subjects; BMI, body mass index; and HOMA-IR: homeostatic model assessment of insulin resistance. Values are presented as mean ± SD.

Metabolic outcomes

Fig. 4 illustrates the postprandial glycemic, insulinemic, and GLP-1 responses following the consumption of the three test breads. The mean peak serum glucose concentration was attained 45 min after ingestion of WB, WGB, and CFM breads, while glucose peaked at 30 min after CLP bread ingestion. No significant differences were observed in peak glucose values or at any other time points throughout the 180 min period for any of the test breads. The WB bread exhibited the highest glucose iAUC, followed by CFM, WGB, and CLP, with the WB iAUC being significantly higher than that of CLP ($p < 0.05$, Fig. 3a). Additionally, the $iAUC_{180}$ for CLP was 26% lower compared to CFM ($p < 0.05$, Fig. 3a). Apart from this, no other significant differences were observed—neither between WGB and CLP, nor between WB and CFM, nor between CFM and CLP.

Regarding the postprandial insulinemic response, the peak serum insulin values were detected in all test breads at 45 min postprandially, without significant differences between them to be observed. Moreover, no significant differences were observed between the test breads at any other time point. Additionally, the $iAUC_{180}$ of the four breads were similar (Fig. 3b). Total plasma GLP-1 concentrations increased after the ingestion of the CLP, compared to WB in 60, 90, 120 and 180 min postprandially ($p < 0.05$, Fig. 3c). CFM had also a sig-

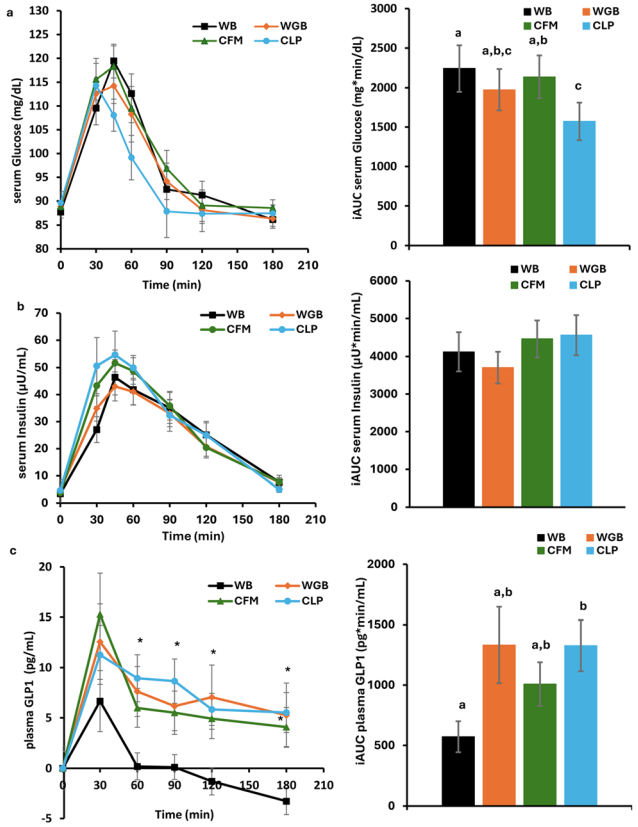

Fig. 4 Postprandial responses and the iAUC for (a) glucose, (b) insulin and (c) GLP-1. *p < 0.05 between WB and CFM or CLP. iAUC values followed by different letters are statistically different (p < 0.05).

nificantly higher GLP-1 concentration at 180 min, compared to WB (p < 0.05, Fig. 3c). The $iAUC_{180}$ for GLP-1 after CLP was significantly higher than after the control bread, by approximately 2.3 times (p < 0.05, Fig. 3c). Apart from this, no other significant differences were observed—neither between WGB and CLP, nor between WB and CFM, nor between CFM and CLP.

Subjective appetite sensations

Fig. 5 presents the differences in subjective appetite ratings from pre-prandial values over the 180 min period following the consumption of the three test breads and the reference bread (WB). Significantly lower hunger ratings were observed after the ingestion of CFM at 60 and 90 min, and after consuming CLP at 60, 90, and 120 min, compared to WB (p < 0.05, Fig. 4a). The iAUC for hunger was significantly lower for both CFM and CLP compared to WB (p < 0.05). CLP ingestion led to a significant increase in fullness compared to WB, while no significant differences in fullness were observed between WB and CFM, WB and WGB, or between CFM and CLP. Similar results were observed for the desire to eat, where CLP showed a significantly greater reduction (higher iAUC) compared to WB. Similar to fullness, no significant differences in the iAUC for the desire to eat were observed among the other test breads.

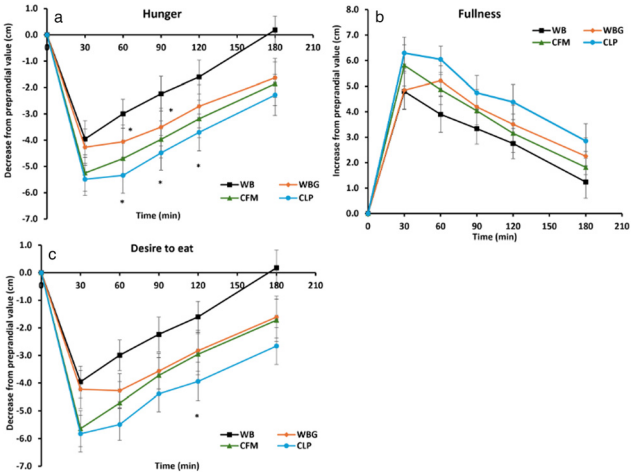

Fig. 5 Postprandial responses for subjected appetite sensations: (a) hunger, (b) fullness, and (c) desire to eat. *p < 0.05 between WB and WGB or CLP.

Discussion

The present study investigates whether modifying the food structure by reducing flour milling to create larger particle-size flour from two distinct botanical sources (wheat and chickpeas) can affect the postprandial metabolic responses of healthy individuals. These coarser flours, with higher proportions of intact plant cells and thus naturally encapsulated starch, partially replaced refined flour. We hypothesize that flour with significantly larger particle size contains a higher proportion of intact cells, which may influence glycemic response and appetite regulation by slowing starch digestion and glucose absorption in the gut—an effect that could be retained even after baking. The effect of particle size on the cell wall integrity has been previously observed in legumes (*e.g.* beans and chickpeas),[12,26,30,43,44] as well as cereal grains (*e.g.* wheat, sorghum and barley).[11,19,29]

To our knowledge, there are no *in vivo* studies comparing the postprandial responses to bread made with commercially available finely milled pulse flour *versus* bread containing coarser pulse flour from the same source. Similar to refined wheat flour, commercially available pulse flour predominantly consists of cells with disrupted cell walls, making their starch readily accessible to digestive enzymes following hydrothermal processing.[32,33] Based on the literature and our preliminary lab trials, a substitution rate of 30% refined wheat flour was selected for the three test breads, allowing for significant incorporation of coarser flours.[15,22,45] Although coarser flour incorporation has been shown to reduce *in vitro* starch digestibility due to limited enzymatic accessibility, this effect may not fully translate to the *in vivo* glycemic response, likely due to the complexity of physiological digestion and the food matrix.[23] The primary reason is that normal eating processes, such as increased oral processing and chewing, tend to break down coarser particles during ingestion. This enhanced masti-

cation can compensate for the initial slower starch breakdown and diminish the beneficial effects on starch digestibility seen in lab settings. In addition to this, it has been stated that supplementation levels higher than 35% (of total flour) can adversely affect functional and sensory properties of bakery products.[16] The impact of chemical composition was also evaluated by the inclusion of bread in which a part of the wheat flour was replaced with finely milled chickpea flour, allowing us to explore whether any potential observed benefits are attributed to particle size, compositional differences, or both, given the distinct nutrient and structural profile of chickpeas compared to wheat. In all three test breads, whole-meal wheat flour was also added to the recipes.[23] It has to be noticed that although wheat flour is poor in soluble fiber which is known to affect glycemic response, the inclusion of 28% wholemeal wheat flour in the three test breads (WGB, CFM, and CLP) may have interfered with the results since it contains a considerable amount of fiber.[46] The characteristics and the quality parameters of the test breads were evaluated. Measurement of textural attributes is of crucial importance, since they directly affect disintegration behavior and sensory characteristics. A significant difference in hardness was observed between WB, and all other test breads, which as expected, was the softest, as in most similar research studies,[13,45,47] with no significant differences being observed between the chickpea breads (CFM and CLP). Chickpea bread (CFM and CLP) had lower values of chewiness ($p < 0.05$), compared to wheat breads (WB and WGB). Contrary to other similar study protocols, in which substitution of finely milled flour with rye flour,[48] wheat flour,[19] or legume flour[45] of higher particle size distribution, had an adverse effect on the cohesiveness and resilience of the test breads, in this study substitution of wheat flour with larger particle size flours did not have an impact. All samples had similar values for those textural parameters (cohesiveness and resilience), whose decreased values have been related to a higher rate of food disintegration and thus increased rates of starch digestibility.[48] CFM and CLP breads exhibited significantly lower surface porosity and bread volume than WB, mainly due to gluten network disruption, which reduced gas retention, and gas cell distortion from non-endosperm components like dietary fiber.[19,49] However, no significant differences were observed in bread volume and porosity between two chickpea breads indicating that substitution for larger particle size chickpea flour did not seem to adversely affect the structural properties of bread. WGB had significantly lower surface porosity than WB due to its higher fiber content, and although its specific volume was also lower, this difference was not statistically significant. Similar results have been recorded in another study as well, in which 85% of whole grain wheat flour was substituted with 85% broken wheat kernels.[21] The incorporation of 30% higher particle size wheat flour, finely milled chickpea flour and chickpea flour of larger particle size, induced a less porous crumb, due to a reduction in the gas retention capacity, in accordance with other studies.[34] The three test breads had significantly lower L^* (lightness) values, as expected, primarily due to the addition of whole meal flour, which darkens the bread color by including all parts of the wheat grain (*e.g.* wheat bran). All breads showed positive a^* values, indicating a yellow hue rather than blue. Chickpea bread had a more pronounced yellow color compared to wheat breads, primarily due to the addition of chickpea flour.

The postprandial glycemic response, measured by the iAUC, was significantly lower in CLP, compared to both WB and CFM ($p < 0.05$, Fig. 3a). In addition, the peak value of glucose after CLP occurred at 30 min and then glucose concentrations began to decrease. In all other breads, peak glucose values were observed at 45 min, with WB having the highest peak glucose value. It is suggested that the lower glycemic response after CLP ingestion compared to CFM and WB is attributed to a lower rate of glucose release in the gut. CLP contains chickpea flour with a considerably larger particle size, which results in a lower surface-to-volume ratio compared to the finely milled flour in CFM, highlighting the impact of higher chickpea particle size in the amelioration of postprandial glycemia. This limits the accessibility of digestive enzymes such as α-amylase and also reduces the degree of starch gelatinization.[10,26] Since blood glucose levels are strongly influenced by luminal glucose absorbance, lower starch digestion rate and extent may explain the significantly lower iAUC of CLP.[21,35] The similar glycemic responses observed between CFM and WB can be attributed to the use of conventional milling techniques, such as roller milling, which markedly reduce the particle size and disrupt plant cell walls. This structural breakdown facilitates the release of intracellular starch and results in substantial starch damage, thereby increasing susceptibility to enzymatic digestion. These findings highlight that the preservation of plant cell integrity may be crucial for achieving beneficial effects on postprandial metabolic responses.[50,51]

In the case of WGB, although larger particles were still visible, the thorough mixing during bread-making may have contributed to further structural breakdown. In addition, in the case of wheat, hydrothermal processing (*e.g.*, during bread-making) increases the porosity of endosperm cell walls, thereby enhancing the accessibility of encapsulated starch to digestive enzymes. This increase in porosity is partly due to thermal degradation and structural modifications of cell wall polysaccharides. In particular, the most affected molecules are β-glucans and arabinoxylans, which are subjected to changes in their molecular weight under high-temperature conditions. As a result, the protective barrier function of their cell wall is diminished, potentially offsetting the reduced starch digestibility that is typically associated when increasing the wheat flour particle size.[21,52] In other similar studies, where the postprandial glycemia of bread with a higher wheat flour particle size (coarser particles) was examined, no significant differences were found, both in glycemia and insulinemia in healthy participants, even at high inclusion rates of wheat particles (up to 85%).[21–23,53] Significant differences in the postprandial glycemia, in response to bread ingestion have been found in diabetic subjects, but it must be noted that they

already have compromised glycemic and insulinemic regulation.[14,15,25]

Chickpea cotyledon cells are thicker and more resistant, even after hydrothermal processing, compared to wheat endosperm cells, whose porosity increases after hydrothermal processing (*e.g.* breadmaking).[12,31] Hydrothermally treated legume cotyledon cells tend to separate during mastication, due to the weakening of their intercellular adhesions. That leads to intact cells forming the primary structural component of the food bolus as it enters the gastrointestinal tract (GIT). Cotyledon cells can also resist digestive conditions in the stomach and the upper small intestine (*e.g.* duodenum).[27,54] Studies on postprandial glycemia after consuming bread with larger legume particles yielded mixed results. For instance, incorporating 75% soybeans with particles >2.8 mm in bread showed no significant effect on postprandial glycemia. This outcome was attributed to the breakdown of the food structure during oral processing, which likely increased enzyme accessibility despite the larger particle size.[23] Another study found that adding 30% intact cellular powder to bread significantly reduced postprandial glucose levels. Increasing the substitution level to 60% did not provide additional benefits, suggesting that higher substitution of refined wheat flour does not further lower the glycemic response. The postprandial insulin response (iAUC) was significantly reduced only with a 60% incorporation of chickpea cellular powder, while a 30% incorporation, as also observed in the present study, did not significantly lower insulin levels. In the present study, no significant differences were observed in postprandial insulin responses. The postprandial insulin levels following the consumption of the two chickpea-based breads (CFM and CLP) were slightly higher than those observed for the wheat breads; however, these differences did not reach statistical significance. From these findings, it can be concluded that a lower glycemic response is achieved after CLP ingestion, compared to WB and CFM, without a significant change in insulin secretion.[35,45]

GLP-1, an anorexigenic incretin peptide, is secreted by L-cells in the gut in response to meals. These L-cells, particularly abundant in the distal colon, contain nutrient-sensing receptors like G-protein-coupled receptors that detect luminal nutrients and stimulate GLP-1 release. GLP-1 constitutes one of the gut peptides, which are responsible for glucose and appetite regulation, stimulation of insulin secretion and lowering the rate of gastric emptying.[55] The postprandial GLP-1 response, indicated by the iAUC after CLP ingestion, is significantly higher than that observed with WB. This enhanced response is primarily attributed to the ileal brake mechanism. It is suggested that the higher proportion of encapsulated starch in CLP bread results in a slower release of sugars (mono- and disaccharides) in the distal gut, where L-cells are abundant and actively secrete GLP-1.[35,56,57]

In addition to monosaccharides, amino acids and peptides can also stimulate GLP-1 secretion by activating G-protein coupled receptors such as GPRC6A and calcium-sensing receptors (CaSR) expressed on enteroendocrine L-cells in the gut.[58] Therefore, we acknowledge that the higher postprandial GLP-1 response observed after CLP ingestion is not solely attributable to the slower rate of starch digestion and subsequent glucose release, but also to the significantly higher protein content (9 g in WB *vs.* 15.5 g in CLP). This protein is naturally encapsulated within chickpea cotyledon cells, a proportion of which becomes bioaccessible during digestion. The higher protein content is thus likely to contribute to the increased postprandial secretion of incretins such as GLP-1, both in acute and potentially longer-term metabolic settings.[35,59] However, it must be acknowledged that although the amount of CFM consumed contained a similar protein content to CLP (14.5 and 15.5 g) and higher than WB, no significant differences in GLP-1 response were observed between CLP and WB. Regarding the role of dietary fiber in incretin release, its contribution is generally mediated *via* the production of short-chain fatty acids (SCFAs) resulting from microbial fermentation of non-digestible carbohydrates in the colon. However, fermentation typically begins 2–4 hours after ingestion. Since our study evaluated responses only within a 3-hour postprandial period, at 180 min, it is possible that GLP-1 levels were slightly influenced by resistant starch fermentation; however, this is not the primary mechanism impacting the GLP-1 response in the current study. This is consistent with findings from other acute studies with similar durations, which also report a minimal impact of fiber fermentation on early gut hormone responses.[7,35] Postprandial GLP-1 responses following WGB and CFM ingestion were higher than that of WB, though not being significant. Moreover, no significant differences were observed in the iAUC of GLP-1 between CLP and WGB or between CLP and CFM, suggesting that the plant tissue type (wheat *vs.* legume) did not significantly influence the postprandial GLP-1 response, regardless of the flour particle size (coarse *vs.* fine). Additionally, GLP-1 levels for WGB, CFM, and CLP remained elevated at 180 min without returning to the baseline. GLP-1 levels remained elevated for a prolonged time following CLP ingestion and at higher levels compared to the other breads. Previous studies have shown that GLP-1 levels following starch-rich foods, including breads high in slowly digestible starch (SDS), can remain elevated for up to 300 min due to a secondary GLP-1 response.[60,61] A larger sample size or a longer postprandial study duration may have uncovered significant differences in GLP-1 responses.[21,62]

The results also show significant differences in subjective appetite sensations, particularly between WB and CLP, which is expected since apart from the addition of chickpeas of larger particle size (different origin and particle) different nutritional composition, particularly the higher protein and dietary fiber content, also has a crucial role in appetite regulation. Specifically, CLP consumption led to significantly lower hunger, higher fullness, and a reduced desire to eat, as measured by $iAUC_{180}$, compared to the WB ($p < 0.05$) Additionally, no significant differences were observed between the other breads. These findings agree with postprandial GLP-1 levels, which were significantly higher following CLP

consumption, both in terms of iAUC and at most of the measured time points. So, from these findings, it can be concluded that CLP bread ingestion substantially enhances satiety postprandially for the whole study duration.

This study has some limitations. The breads were frozen shortly after baking to maintain freshness throughout the experimental period, then thawed overnight at room temperature. This process, while practical, may have influenced postprandial glycemia and insulinemia, introducing factors beyond flour particle size. Freezing and thawing could also lead to the formation of resistant starch, potentially further reducing starch digestibility in all test breads. However, this storage method reflects real-life conditions, as people commonly refrigerate or freeze bread to extend the shelf life and reduce food waste. It must be noted that all bread samples were stored under the same conditions.[21,22] Additionally, it is well known that humans can have differences in their eating behavior, such as eating rate, chewing characteristics (bolus formation) and salivary amylase activity, which can influence the respective glycemic response. The increased oral processing of foods (*e.g.* breaking down and chopping) can destroy the native food structure (*e.g.* disruption of plant cell walls) and eliminate its protective effects. For instance, some participants may have swallowed the larger wheat and/or particles unchewed, whereas others may have broken them down into smaller pieces. However, it has been found elsewhere that the innate plant tissue structure has a greater influence on starch digestibility than oral processing behavior.[23,63] All these factors may increase variability in the results. In the current study protocol, 15 participants successfully completed all four study visits. According to the power analysis, a sample size of 16 participants was deemed sufficient to detect statistically significant differences in glucose response. Initially, 19 individuals enrolled in the study; however, 4 participants dropped out, primarily due to either difficulties in cannulation or discomfort with intravenous catheter. In addition, some were unwilling to complete, because they did not like some of the bread samples. Despite using a slightly lower number of subjects than initially determined, the experiment yielded statistically significant results. Other research protocols with a similar study design did not manage to recruit a higher number of participants as they also initially intended.[7,15] However, it must be acknowledged that this can potentially limit the strength of our conclusions. Moreover, the inclusion of 28% wholemeal wheat flour in the three test breads (WGB, CFM, and CLP), since it contains a considerable amount of fiber may have interfered with the results. In addition, there are also differences in the energy content, serving size and protein between the samples that may have influenced satiety responses independently of the flour type or particle size. However, matching all samples to provide 50 g of available carbohydrates was necessary for comparing the postprandial glycemic response, the primary outcome of this study. Future studies could explore the effects on blood glucose and appetite regulation in a subsequent meal, or investigate the effects of incorporating higher proportions than 30% of chickpeas in test breads.

Conclusions

In conclusion, this study shows that incorporating larger particle-sized chickpea flour in wheat bread formulations effectively lowers postprandial glycemia, compared to both WB and CFM. Following CLP ingestion, the postprandial GLP-1 response is significantly higher than that with WB and remains elevated up to 180 min. In contrast, the ingestion of WGB did not lead to significant changes in the postprandial metabolic response compared to both CLP and WB. However, a larger sample size or higher incorporation rate in bread might have revealed some beneficial effects. Moreover, in the context of this study, the inclusion of 30% finely milled chickpea flour also showed no significant effects on the examined parameters compared to that with WB. CLP ingestion also caused enhanced subjective appetite sensations compared to WB ingestion (reduced hunger and desire to eat and increased fullness). However, it must be acknowledged that CLP and WB also differed in their chemical compositions, particularly in terms of protein and dietary fiber content. This study highlights the importance of both the degree of milling and the botanical structure of ingredients in metabolic response modulation. Since bread is typically a high-GI, low-satiety food, reducing the degree of flour milling presents a promising way to improve its nutritional profile, by preserving starch encapsulation, thus enabling slower, more controlled nutrient release during digestion. Overall, this study provides additional information to that field regarding the effect of milling on postprandial metabolic responses. More studies are required in the future to support the current findings and thoroughly investigate the underlying mechanisms.

Author contributions

Conceptualization, A. E. Y. and V. T. K.; data curation, M. C. K. and A. E. Y.; funding acquisition, A. E. Y, V. T. K. and M. C. K.; investigation, M. C. K.; methodology, C. K. and I. A. A.; supervision, A. E. Y., N. T. and V. T. K.; resources, A. E. Y., N. T. and V. T. K.; writing – original draft, M. C. K.; writing – review & editing, A. E. Y., C. K., N. T., V. T. K.

Data availability

Data are available from the corresponding author upon request.

Conflicts of interest

There are no conflicts to declare.

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