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Agreeability and gastrointestinal motility responses to fully characterized experimental pasta enriched in wheat by-products

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ABSTRACT

Pasta, a Mediterranean diet staple, enhances well-being when enriched with healthy ingredients like durum wheat-germ (WG) and wheat-bran (WB). We studied the nutritional and clinical responses to four experimental pastas (EP1-EP4) made with de-oiled WG, WB, and microencapsulated durum wheat-oil (mWO), compared to a control pasta of water and semolina dough. WG addition significantly boosted total phenols and radical scavenging activity. Simulated colonic fermentation showed WG-enriched pasta enhanced short-chain fatty acids production.

The clinical response to pasta was studied in 70 healthy subjects by semiquantitative scales of sensory perception, functional ultrasonography of gastric and gallbladder kinetics, and breath test for orocecal transit time. Sensory analysis revealed differences in odor, aftertaste, and overall pleasantness, especially in EP2. Gastrointestinal motility was similar across pastas, but EP3 had a shorter transit time and higher colonic fermentation. This study suggests that wheat by-products enriched pastas offer nutraceutical benefits, agreeability, digestibility, and sustainability.

1. Introduction

Pasta is a cereal-based food widely available within the Mediterranean diet (MD) and has high palatability and nutritional quality. Especially in Italy, pasta is ideal for easy and quick meals, and its popularity has increased worldwide.

The current literature encourages the application of healthy dietary patterns, such as the MD, to counteract the rising burden of non-communicable diseases (NCDs) including obesity, diabetes, metabolic syndrome, and cardiovascular diseases (Caprara, 2021). MD is highly enriched in bioactive components such as fibers, polyphenols, and unsaturated fatty acids with potential beneficial effects on oxidative stress, inflammation, mitochondrial activity, and gut microbiota (Khalil et al., 2022; Schwingshackl et al., 2020).

Since durum wheat semolina is considered the most suitable raw material for pasta-making, its mixture with water is the most used to produce pasta. The different degrees of wheat milling led to a different flour gross composition, and, in fact, refined pasta is rich in starch with defects in vitamins, minerals, and phenolic compounds (Jalgaonkar et al., 2018). During conventional wheat milling, the endosperm, from which the white flour is obtained, is separated from both the wheat bran (WB) and embryo layers (i.e., wheat germ – WG) leading to an excess of them as important by-products of the flour milling industry.

WB is the outer layer of the wheat kernel representing around 13–14 % of the total seed weight. Acting as a concentrated source of dietary fiber (33.4–63.0 % of dry matter) (Curti et al., 2013), WB also provides additional bioactive compounds with well-known healthy properties, such as minerals, vitamins B6 and E, antioxidants, and phenolic

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compounds (Tosi et al., 2018). Although it represents a lesser quantity compared to WB as far as it constitutes only around 2–4 % of the total kernel weight, WG remains an important by-product of wheat milling due to the conspicuous presence of biological compounds that can offer significant beneficial effects to humans. Similarities with WB consist in the presence of fibers (although in a lesser quantity than WB, i.e., 17.7 % (Tosi et al., 2018)), minerals, and phenols. As the major difference compared to WB, WG is a concentrated source of lipids. Mostly accounting for the presence of triglycerides (linoleic, palmitic, and oleic acids) corresponding to around 57 % of total lipids, WG is also characterized by the presence of sterols, mono−/di-glycerides, phospholipids, and glycolipids (Tosi et al., 2018). However, by WG cold processing, it is possible to extract 50–90 % of oils allowing to recover of de-oiled WG featured by a high protein content (30–32 %) with a balanced amino acid profile (Tosi et al., 2018).

In humans, fibers act as bio-available substrates to gut microbes for fermenting processes, which mainly result in the metabolism of short-chain fatty acids (SCFAs) (Portincasa et al., 2022). These low-chain metabolites are, thus, bio-available for humans and exert a broad spectrum of health benefits. They support the gut by serving as an energy source for colon cells, strengthening the gut barrier, reducing inflammation, and fostering a balanced microbiome(Shin et al., 2023). SCFAs also have anti-inflammatory properties that can modulate immune responses, which may help alleviate conditions like inflammatory bowel disease(Shin et al., 2023). Metabolically, SCFAs improve insulin sensitivity, regulate glucose levels, and potentially reduce the risk of type 2 diabetes, with propionate specifically playing a role in decreasing glucose production in the liver(Zheng et al., 2024). They also aid in appetite regulation and weight management by influencing hormones contributing to control the appetite. Butyrate has been shown to inhibit the growth of colon cancer cells by promoting cell differentiation and programmed cell death, potentially lowering the risk of cancer (Shin et al., 2023). Additionally, acetate and propionate contribute to cardiovascular health by helping to reduce cholesterol levels and blood pressure(Nogal et al., 2021). Emerging research suggests SCFAs may also support brain health through the gut-brain axis, potentially protecting against mental health disorders by influencing neurotransmitter production. Polyphenols, instead, are plant-derived compounds with powerful antioxidant and anti-inflammatory properties, offering, as SCFAs, numerous health benefits. Polyphenols help to protect cells from oxidative stress, reducing the risk of chronic diseases like heart disease, cancer, and neurodegenerative disorders. In cardiovascular health, polyphenols improve blood vessel function, support blood pressure regulation, and lower cholesterol levels, which together reduce the risk of heart disease(Khurana et al., 2013). Polyphenols also benefit gut health by promoting the growth of beneficial bacteria, increase the production of beneficial metabolites (i.e. SCFAs), and enhance epithelial gut integrity(Khalil et al., 2024). Certain polyphenols are linked to improved brain function and memory, as they help protect neurons from oxidative damage and support brain-derived neurotrophic factor, a protein associated with learning and memory (Uddin et al., 2020). In metabolic health, polyphenols improve insulin sensitivity and regulate blood sugar levels, which can be beneficial for managing type-2 diabetes. They also play a role in weight management by potentially increasing fat oxidation and reducing fat accumulation(Aloo et al., 2023). Therefore, WB and WG, rich in fibers and polyphenols, appear to be optimal candidates to provide a broad pattern of functional nutrients by their intake.

Considering its composition, WB-based physiological effects in humans can be flagged as “nutritional effects” due to the available nutrients, “mechanical effects” on the gastrointestinal tract provided by the fiber content, and “antioxidant effects” determined by the polyphenolic compounds (Stevenson et al., 2012). As a result of this broad spectrum of beneficial effects, the number of WB-containing foods is increasing despite its greatest limitation resulting from the conspicuous presence of the antinutritional factor phytic acid (Prückler et al., 2014; Stevenson et al., 2012).

As an additional field of research, the increasing demand for food due to the growing global population is connected directly with an increase in food waste. However, during cereal processing and manufacturing in industrialized countries, the Food and Agriculture Organization of the United Nations (FAO) noticed that about 35 % of cereal production is lost or wasted (e.g., wheat straw, rice husk, and general cereal waste) (Cederberg & Sonesson, 2011). A study on pasta production revealed that for each kilogram of pasta made, ~2 kg of waste is produced (Principato et al., 2019). Based on such information, times have come to consider the pivotal role of circular and green economies as the best drivers “*to adapt or transform the current economy towards a more sustainable one*” (Santeramo, 2022). For this, food wastes can be transformed into raw materials (e.g., bio-fuels, bio-fertilizers, biomass, or chemical compounds) to be further used for different purposes allowing for new opportunities to meet circular economy (Belc et al., 2019). The application of these concepts in the agri-food system aims to produce more and safer foods while improving environmental conditions (Pretty & Bharucha, 2014).

Herein, we can intend the novelty of this research where we combined the interest in supporting human well-being through the broad consumption of pasta in the Mediterranean area and, in the meanwhile, moving towards the direction of educating the audience of consumers and manufacturing with circular and sustainable habits. Thus, our objective was to develop a strategy for incorporating WB and WG by-products into innovative food products, such as pasta which serves as an excellent vehicle for delivering macro and micronutrients in its final (Romano et al., 2021).

Research into modifying durum wheat semolina pasta has explored various ingredients, like dietary fiber, legume flour, rice, corn, emmer, and even cricket flour, to enhance its nutritional profile. However, incorporating dietary fiber, such as WB, can negatively impact pasta quality, affecting its cooking attributes, sensory characteristics, and appearance (Alzuwaid et al., 2020), as well as gastrointestinal responses in vivo. Fiber alters dough consistency, disrupting the gluten network’s formation through physical or chemical interaction (Zhou et al., 2021). Soluble fibers like oat and barley β-glucans, inulin-type fructans, and psyllium compete with starch for protein binding, reducing starch-protein interactions and dehydrating gluten due to their water-binding capacity (Nocente et al., 2019; Peressini et al., 2020). This substitution significantly changes dough properties and water absorption when soluble dietary fibers replace durum wheat semolina, often reducing the overall acceptance by consumers (Romano et al., 2021).

After the chemical characterization of the chosen ingredients, we aimed to evaluate how different types of experimental pasta (EP) enriched with fibers, polyphenols, and antioxidants naturally occurring in different by-products (i.e., WB and WG) can improve the nutraceutical properties of this MD-staple food in terms of radical scavenging activity and gut microbial modulation (in vitro).

Besides their nutritional value and sustainability, the influence of by-product-enriched pasta on the consumers’ perceptions, acceptance, and gastrointestinal safety presents challenges for food technology production. In this context, EPs were tested in vivo to assess how the WB and WG supplementation modulated domains of food perception and gastrointestinal motility. A control pasta (with durum wheat semolina only) was chosen to compare perception and physiological response with EPs. Healthy volunteers underwent organoleptic perceptions and full gastrointestinal motility studies including stomach and gallbladder by ultrasonography, and orocecal transit by hydrogen breath test in response to the consumption of four types of pasta, as compared to control pasta.

2. Materials and methods

2.1. Types of pastas

Three different ingredients have been provided by the flour-milling industry *Molino Casillo* (Corato – 70,033; Italy), i.e., de-oiled durum wheat germ (dWG, particle size 30 mm), de-oiled durum wheat bran (dWB, particle size 150 mm), and durum wheat oil (WO). The WO was added to the related pasta dough in a microencapsulated form (mWO). Oil microencapsulation preserves quality by preventing rancidity and oxidation while converting liquid oil into a solid powder for easier dispersion in solids like flour. The machine used for mWO production is a Sray Fluid Bad Dryed. The solid support that "adsorbs" and protects the oil is maltodextrin. In our experiment, fine micronized wheat fiber (PSD 100 mm approximately) was used precisely to allow aggregation with the oil.

Before proceeding with their combination in different experimental pasta (EP) formulations, all ingredients were subjected to chemical characterization as detailed in Subsection 2.2. The formulations were carefully developed to balance nutritional enhancement with the sensory and structural integrity of the pasta. According to the manufacturer's internal testing, this specific combination was identified as optimal for preserving the desired texture and sensory quality of the pasta, avoiding issues such as fragility or unappealing taste often associated with higher levels of functional ingredients. This formulation reflects a commitment to creating a health-focused product without compromising consumer acceptability.

Thus, four different EP (EP1–4) and one control pasta were manufactured by *Molino Casillo* industry as follows:

- EP1, refined semolina and water dough containing 30 % of dWG
- EP2, refined semolina and water dough containing 30 % of dWB
- EP3, refined semolina and water dough containing 27 % of dWG and 6 % mWO
- EP4, refined semolina and water dough containing 27 % of dWB and 6 % mWO
- The control pasta (CP) consisted of refined semolina and water dough, with the addition of an integral-like coloring agent. This was done to minimize visual disparities during sensory analysis compared to EPs, and it was also included in the study design.

All pastas were produced following the AACC 66–41 method (AACC, 2000) using a plant (FAVA Italy) Artisan pasta factory in Bari (MastroPastaio S.r.l.). The plant is composed of a press, with a working capacity of 15–20 kg and a PC-controlled drying system that works with temperature/moisture equilibrium curves. The press is equipped with a dough vacuum mixing chamber and an extruder and presents a cooling-water system to keep the temperature of the extruding system below 50 °C (90 bar). The drier is composed of a ventilation unit, which controls the temperature, and a moisture regulation unit. The drying process was carried out at 85 °C for 5 h followed by a period of 5 h at 65 °C.

All pasta samples (CP and EP1–EP4) were shaped as "*Tortiglioni*" with a bromatological profile detailed in Subsection 2.3. All ingredients being part of this study agreed with the current European Community (EC) legislation in terms of genetically modified organism requirements, food hygiene, contaminant, and pesticide Regulations.

The optimum cooking time (OCT) was determined by the international standard ISO 7304-1 (ISO, 2016) in the Casillo laboratory. The pasta was squeezed between two glass slides during cooking at regular intervals of 30 s, to check whether the central core of the strand disappeared. The evaluation of firmness (resistance to cutting between the teeth), liveliness (inter-strand sliding ability, which depends on the degree of adhesion), and stickiness (release of starch of cooked pasta) were carried out following the ISO 7304-1 standard (ISO, 2016) by a group of ten assessors, who awarded grades from 0 (very low quality) to 100 (very good quality). The average of the individual scores awarded represents the overall score for cooking quality. Pasta with an overall score lower than 40 was classified as of poor or mediocre quality; between 40 and 50 as not completely satisfactory; between 50 and 70 as fair; from 70 to 80 as good; and higher than 80 as excellent.

2.2. Chemical characterization of ingredients

All the ingredients were characterized in terms of nutritional values, mineral content, amino acid composition, and microbiological analysis for both de-oiled durum WG and WB (Suppl. Table S1) and in terms of chemical profile, total tocopherols content, fatty acid compositions, metals, and pesticide residue for durum WO (Suppl. Table S2).

2.3. Pasta bromatological profiling

Based on different combinations of the aforementioned ingredients all five types of pastas were characterized for their bromatological composition including energy value (Kcal/100 g) and the content (g/100 g) of carbohydrates, fibers, proteins, fats, and ashes (Table 1).

2.4. Total phenol content and antioxidant activity of pasta

2.4.1. Extraction of phenolic compounds

The extraction was carried out according to the protocol described by Gu et al. (Gu et al., 2006) with slight modifications. Briefly, aliquots (10 g) of each pasta sample were cooked (8 min) in 100 mL of boiling tap water. The cooking water was discarded and replaced with an equal volume (100 mL) of hydroalcoholic solution (ethanol/water 80:20 *v*/v). After stirring (100 ×g for 10 min) at room temperature, the sample-containing solutions were sonicated (15 min at room temperature) by using a Bendelin Sonorex (Berlin, Germany) and centrifuged (10 min, 10,000 ×g, at 25 °C). Supernatants were recovered using a sterile 0.2 μm filter. Pellets were further processed twice following the same steps described above until resulting in a single hydroalcoholic solution containing an equal mixture of the three different extraction phases.

Table 1
Bromatological composition (mean ± standard deviation) of four experimental pasta (EP1–4) and control pasta (CP) samples.

Nutritional composition	CP	EP1	EP2	EP3	EP4
Energy value (Kcal)	346 ± 8.7^{a}	323 ± 8.1^{ab}	322 ± 8.1^{ab}	332 ± 8.3^{a}	311 ± 7.8^{b}
Carbohydrates (g)	79.5 ± 2.0^{a}	65.5 ± 1^{b}	66.6 ± 1.7^{b}	65.8 ± 1.7^{b}	60.8 ± 1.5^{c}
of which sugars (g)	3.8 ± 0.1^{a}	2.8 ± 0.1^{b}	2.8 ± 0.1^{b}	2.7 ± 0.1^{b}	2.6 ± 0.1^{b}
Total Fibers (g)	3.5 ± 0.1^{c}	11.3 ± 0.3^{b}	14.8 ± 0.4^{a}	10.8 ± 0.3^{b}	13.5 ± 0.3^{a}
of which arabinoxylans (g)	2.0 ± 0.1^{d}	5.9 ± 0.1^{c}	8.2 ± 0.2^{a}	5.0 ± 0.1^{c}	6.2 ± 0.1^{b}
of which insoluble fiber (g)$^{\#}$	0.1 ± 0.1^{c}	0.8 ± 0.1^{b}	3.0 ± 0.2^{a}	1.0 ± 0.1^{b}	2.8 ± 0.2^{a}
Proteins (g)	15.8 ± 0.4^{b}	19.1 ± 0.5^{a}	16.3 ± 0.4^{b}	18.0 ± 0.5^{a}	15.8 ± 0.4^{b}
Fats (g)	0.50 ± 0.1^{b}	0.62 ± 0.1^{b}	0.55 ± 0.1^{b}	2.25 ± 0.1^{a}	2.2 ± 0.1^{a}
Ashes (g)	0.7 ± 0.1^{c}	3.5 ± 0.1^{a}	1.8 ± 0.1^{b}	3.5 ± 0.1^{a}	1.9 ± 0.1^{b}

Legend: CP, control pasta; EP, experimental pasta; EP1, 30 % de-oiled durum wheat germ (dWG); EP2, 30 % "bran" de-oiled wheat (dWB); EP3, 27 % de-oiled wheat "germ" (dWG) plus 6 % microencapsulated durum wheat "oil" (mWO); EP4, 27 % de-oiled wheat "bran" (dWB) plus 6 % microencapsulated durum wheat "oil" (mWO).
($^{a-d}$) different superscript letters indicated a significant difference ($P < 0.05$) between samples (Tukey's test).

2.4.2. Total phenol quantification

The total phenolic content was assessed by using the *Folin-Ciocalteu* reagent as described by Ainsworth & Gillespie(Ainsworth & Gillespie, 2007). Specifically, 100 μL of *Folin-Ciocalteu* reagent and 800 μL of a 7.5 % Na_2CO_3 solution were added to 100 μL hydroalcoholic extracts and incubated for 60 min at room temperature. As carried out by Difonzo et al.(Difonzo et al., 2017), the absorbance was read at 750 nm using the Ultrospec 3000 lab spectrophotometer (Pharmacia Biotech, Milan, Italy) and results were expressed as mg of gallic acid equivalents (GAE) *per* mL of hydroalcoholic pasta extract.

2.4.3. Radical scavenging activity

The antioxidant activity featuring the hydroalcoholic pasta extracts was assessed as the scavenging activity against the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, as previously detailed(Brand-Williams et al., 1995). Both, a positive (synthetic antioxidant Butylated Hydroxytoluene – BHT) and negative control (ethanol-water solution – blank) were used. After incubation in the dark (30 min at 25 °C), the absorbance was measured at 517 nm and, as detailed by Limongelli et al. (Limongelli et al., 2023)the scavenging activity was calculated as the difference to the negative control expressed as a percentage of scavenging activity against the DPPH free radical.

2.5. Simulated pasta digestion and colonic fermentation

2.5.1. In vitro simulation of gastrointestinal digestion

Simulated pasta digestion was carried out in vitro by using different solutions mimicking the oral, gastric, and intestinal fluids(De Angelis et al., 2021). Briefly, cooked pasta samples (5 g) were manually ground (particle size <3 mm), mixed with distilled water (25 mL), and homogenized for 3 min using a lab stomacher (Bag Mixer, Interscience International; Roubaix, France). The oral phase accounted for the addition of 10 mg of porcine α-amylase (Sigma-Aldrich; Merk Life Science S.r.l.; Milan, Italy) dissolved in 3.125 mL of 1 mM $CaCl_2$ solution and an incubation step for 30 min at 37 °C under stirring conditions (50 rpm). The simulated gastric fluid contained 1.35 g of porcine pepsin (Sigma-Aldrich) dissolved in 12.5 mL of 0.1 M HCl (at pH 2.0 by adjusting it with a 6 M HCl solution) and, after its addition, samples underwent the second incubation step for 3 h at 37 °C under stirring conditions (150 rpm). Finally, the simulated intestinal fluid, which contained 280 mg of porcine pancreatin (Sigma-Aldrich), and 1.75 g of bile salts dissolved in 62.5 mL of 0.1 M $NaHCO_3$ (at pH 7.0 by adjusting with a 6 M NaOH solution), was added. At the end of the third incubation (3 h at 37 °C under stirring – 150 rpm – conditions), the enzymatically digested samples were used for the fecal microbiota-based experiments as hereinafter detailed.

2.5.2. In vitro colonic fermentation of digested pasta

As described(Vacca et al., 2023), the digested samples constituted part of the fecal media. To obtain fecal media, a fecal pool equally containing stool samples from 3 healthy donors belonging to the research lab who had not taken drugs or probiotics for at least the last three months, mixed in equal amounts and added, in a ratio 1:5 (*w/v*) to distilled water. The suspension, homogenized for 3 min with a lab stomacher in bags with a filter (250 μm), was centrifuged at 10000 ×*g* for 10 min to recover supernatants. The digested pasta samples were added in ratio 1:4 (*v*/v) to supernatants and supplemented with K_2HPO_4•2 g/L, $C_2H_3NaO_2$•5 g/L, $C_6H_{17}N_3O_7$•2 g/L, $MgSO_4$•0.2 g/L, $MnSO_4$•0.05 g/L, glucose•2 g/L, inulin•4 g/L, fructo-oligosaccharides•4 g/L, Tween 80 polysorbate•1 mL/L before to be sterilized (121 °C for 20 min). A cold-sterilized cysteine HCl (0.5 g/L), haemin (0.02 g/L), and vitamin K1 (10 μL/L) solution was finally added to constitute five different fecal media. Each fecal medium was inoculated with a fecal slurry (32 % w/v) containing a fecal microbial suspension obtained by processing, within 1 h, fresh feces delivered from one healthy donor(Vacca et al., 2021). The simulated colonic fermentation in fecal batches (i.e., fecal medium *plus* fecal slurry inoculum) accounted for a single-step anaerobic 42 h-incubation at 37 °C under stirring conditions (150 rpm).

2.6. Fecal microbiota characterization

2.6.1. Enumeration of cultivable microorganisms

Aliquots (5 g) from each fecal batch were added to 45 mL of sterile NaCl (0.9 % w/v) solution and a serial 10-fold dilution was carried out subsequently. To inspect the viable microbiota, Plate Count Agar (*PCA*); Wilkins-Chalgren anaerobe agar (*WCAn*); de Man, Rogosa and Sharpe (*MRS*) agar; M17 agar; Violet Red Bile Glucose Agar (*VRBGA*); and modified Bifidobacterium agar (*mBifA*) were used as culture media for total aerobes microbial (TAMC), total anaerobes microbial (TANMC), lactic acid bacteria (LAB); l*actococci/streptococci*; total coliforms; and fecal *Bifidobacterium* counts, respectively. Exception made for *mBifA*, which was purchased by Becton Dickinson GmbH (Heidelberg; Germany), all other media were purchased by Oxoid Ltd. (Basingstoke, Hampshire, England). *WCAn* and *mBifA* were anaerobically incubated, while other media were incubated aerobically. Time and temperature of incubation followed those defined by the related manufacturer.

2.6.2. Total DNA extraction and qPCR analysis

The total DNA was extracted starting from 500 μL of each fecal batch. Phosphate buffered saline (PBS) *plus* EDTA (phosphate buffer 0.01 M, pH 7.2, 0.01 M EDTA) solution (1 mL) and centrifugation (14,000 ×*g* for 5 min, 4 °C) were carried out twice to wash samples preliminarily. The FastDNA® Pro Soil-Direct Kit (MP Biomedicals, CA., USA) was used for DNA extraction according with manufacturer's instructions. The DNA quality was spectrophotometrically checked at 260, 280, and 230 nm wavelength by a NanoDrop® 2000c Spectrophotometer (ThermoFisher Scientific Inc., Milan, Italy).

Fecal microbiota was profiled by investigating specific microbial taxa, which were listed in Supplementary Table S3 providing details of the related primer also, by Real Time PCR (qPCR) analysis. The Applied Biosystems 7300 Real-Time PCR System (ThermoFisher Scientific Inc., MI., Italy) was used for qPCR reactions. The reaction mix (25 μL) contained 12.5 μL of SYBR Green Mix (# 1725271, Bio-Rad Laboratories S.r.l., Milano, Italy), 0.1 μL of 0.2 μM of primer, 11.4 μL of DNase and RNase-free water, and 1 μL of DNA template at a concentration of 40 ng. Each reaction was performed in duplicate. The amplification set-up consisted of 1 cycle of 95 °C for 2 min, 40 cycles of 95 °C for 5 s, appropriate annealing temperature (Supplementary Table S3) for 30 s, and 1 cycle of 72 °C for 35 s. Absolute quantifications were elaborated based on specie-specific standard curves. Thus, based on DNA concentration and amplicon length, the Copy Number (CN) was calculated.

2.7. Fecal batch volatile organic compound (VOC) analysis

An aliquot (1 g) of each fermented fecal batch supernatant was analyzed for volatile organic compound (VOC) using a Clarus 680 (Perkin Elmer, Beaconsfield, UK) gas chromatograph equipped with an Rtx-WAX column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Restek), and coupled to a Clarus SQ8MS (Perkin Elmer). The used protocol was previously detailed(Portincasa, Celano, et al., 2022). As an internal standard, 10 μL of 4-methyl-2-pentanol (final concentration of 1 mg/L) was added. Chromatograms and peaks were identified using the National Institute of Standard and Technology 2008 (NIST) library as a reference. A peak area threshold of >1,000,000 and 85 % – or greater – probability of match was set for VOC identification, followed by manual visual inspection of the fragment patterns when required. Internal standard was used to quantify VOCs by interpolation of the relative areas versus the internal standard area. For short chain fatty acids (SCFAs), standard curves were elaborated to obtain data of their absolute concentration in samples.

2.8. Clinical protocols

2.8.1. Subjects

A total of 70 healthy adult lean subjects (mean age 28.2 ± 0.6 years, range 20–44 years; 32 M: 38F, BMI 22.8 ± 0.3 Kg/m^2) joined the study. The group consisted of 5 subgroups with $N = 14$ subjects per group as the minimum sample size needed to obtain a power of 0.80, when the effect size is medium and a significance level of 0.05 (calculated with R software, package "pwr"). We ensured rigorous randomization of subjects across all five subgroups, maintaining no statistically significant differences in gender distribution, age, and BMI within each group. Examinators and most of the enrolled subjects had participated previously in different similar studies for the examination of food perception and gastrointestinal motility of our group (Caponio et al., 2020; Diella et al., 2018; Rizzello et al., 2019).

Subjects underwent full medical history assessment and physical exam to rule out major functional or organic diseases. Subjects were selected based on a set of inclusion and exclusion criteria to ensure homogeneity and reliability of the results. Inclusion criteria included: healthy lean adults (≥18 years old, BMI range 18.5–24.9 kg/m2). Exclusion criteria included: a history of current or prior gastrointestinal diseases (e.g. inflammatory, neoplastic diseases, gastrointestinal surgery, irritable bowel syndrome, functional constipation), pregnancy, recent use of medications that could alter sensory perception or gastrointestinal motility, and probiotics, symbiotic, postbiotic or antibiotics intake in the last 3 months.

Ensuring the accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), the study received the approval from Local Ethics Committee (study n. 7394, approved on 8th March 2023), and all subjects signed the informed consent. The test started at 8 a.m. and all subjects had to be fasting for at least 12 h before. Both subjects and operators were blinded with respect to the composition of each type of pasta. Subjects were randomly assigned to receive a different sequence of pasta to be scored. Each subject had to eat 80 g of each pasta sample cooked for 10 min in boiling salt-free water (200 mL) according to the manufacturer's instruction (optimal coking time Section 2.1). After each type of pasta test, the subject had to rinse the mouth with 200 mL of water and complete the questionnaire. All subjects completed the study without reporting side effects.

2.8.2. Sensory studies

Most of the participants in this study had prior experience participating in similar studies on food perception, which provided them with a foundational understanding of sensory evaluation by semiquantitative scales for sensory perception. Given the familiarity with pasta as a staple in Italy, no additional training or calibration sessions were conducted. Subjects were blinded to the type of pasta and the ingredients.

The visual Analogue Scales (VAS) were used to quantify different domains of perception according to previous studies of our group (Vitellio et al., 2019). The VAS consisted of a 100 mm horizontal line ranging from 0 (i.e., no perception) to 100 (i.e., max perception). Subjects had to mark the horizontal line at each time point, and the value in mm was recorded for each subject, scoring each domain and type of pasta. Domains of perception consisted of five categories and specific subcategories, i.e., i) visual aspect (colour; homogeneity); ii) odor (intensity; pungent; strong; bitter; sour); iii) taste (pungent; spicy; aftertaste); iv) chewing (particle break-down; hardness; tooth packing; cohesion); v) global pleasantness (taste and texture).

Subjective responses were controlled by ensuring a blinded experimental design. Additionally, to minimize bias and enhance the reliability of sensory evaluation, standardized testing conditions were maintained for pasta cooking procedures including water, temperature and ambient conditions.

2.8.3. Motility studies

All enrolled subjects underwent the gastrointestinal motility study by simultaneous combined measurements of gastric, gallbladder emptying, and orocecal transit time in response to the ingestion of pastas for two hours following the meal ingestion.

For the stomach and gallbladder kinetics, time-dependent changes of fasting postprandial antral areas (cm^2) and gallbladder volumes (mL) were measured simultaneously by ultrasound scans using a Sonoscape E2 Ultrasound machine equipped with a convex probe (3.4-5Mhz) to previously validated techniques(Di Ciaula et al., 2012; Portincasa et al., 2004). Transabdominal ultrasonography is a non-invasive, reliable technique used to assess gastric and gallbladder motility. Using a standard ultrasound probe, serial measurements of the antral cross-sectional area also provide an indicator of gastric emptying, and sagittal and transversal scans of the gallbladder indicate gallbladder volume.

Antral area and gallbladder volume were measured in the fasting subjects at baseline (−5 min), after meal ingestion (0 min), and every 15 min until 120 min. To ensure accuracy, these measurements were repeated at least 2 times at each time point to confirm the reliability. Gastric emptying was expressed as time-dependent changes of absolute (cm^2) and percentage (%) antral areas. Measurements were taken at baseline (−5 min), after maximal antral dilatation at 0 min, and every 15 min during the 120 min emptying process. Further indices included the Area Under the emptying Curve (AUC, cm^2 × 120 min) and the half-emptying time ($T_{1/2}$, min). The $T_{1/2}$ was calculated from linear regression analysis of the descending part of the emptying curves and expressed as "true" or estimated $T_{1/2}$ looking at the intercept between the regression line and the horizontal line corresponding to the 50 % decrease of antral area. Gallbladder emptying indices were obtained from gallbladder volumes expressed as mL and % at baseline and at regular 15 min intervals during 120 min after meal ingestion. The fasting volume was the gallbladder volume measured following at least 8 h of fasting. The minimum postprandial volume (mL and %) after meal ingestion was the residual volume. Additional indices included the AUC (mL × 120 min), the rate of gallbladder emptying and refilling (mL/min), the volume of ejected bile (expressed as mL and % of basal), and the half-emptying time ($T_{1/2}$). The $T_{1/2}$ was calculated by linear regression analysis from the descending part of the emptying curves and expressed as "true" or estimated $T_{1/2}$, looking at the intercept between the regression line and the horizontal line corresponding to the 50 % decrease of gallbladder volume. Gallbladder time to residual volume was the time requested to reach the postprandial residual volume.

The orocecal transit time (OCTT) and colonic fermentation were measured by the lactulose H_2-breath test following guidelines(Di Ciaula et al., 2012; Portincasa et al., 2004). In detail, breath samples were systematically collected to monitor changes associated with lactulose fermentation and H_2 production. The collection process began with a baseline sample taken immediately before meal ingestion. Following the meal, additional breath samples were collected at regular intervals of 10 min, continuing until 120 min post-ingestion. This schedule provided a comprehensive temporal profile of H_2 production. To ensure the reliability and reproducibility of the measurements, each breath sample was analyzed twice. If the results from the two measurements exhibited significant variability beyond a pre-defined threshold, a third measurement was conducted. The final recorded value for each time point was the average of all valid measurements taken (two or three, depending on the consistency of the initial pair).

Time-dependent changes of H_2 in expired breath were studied using a pre-calibrated, portable hydrogen-sensitive electrochemical device (EC60Gastrolyzer; Bedfont Scientific, Medford, NJ, USA). Results were expressed as H_2 excretion in parts *per* million (device's accuracy ±2 ppm). The OCTT (min) was defined as a rise of 10 ppm above the baseline on two consecutive measurements. This test was also considered a measure of the fermentative capacity of the pasta meals. The rise of H_2 was observed in all subjects.

2.9. Statistical analyses

Data were expressed as means with standard deviations, median with ranges, or percentages, and unless explicitly stated, experiments were conducted based on biological triplicates. Based on Shapiro-Wilk's test, variables were assessed for their normality distribution and allowing for choosing the appropriate parametric or non-parametric statistical test to be used. For continuous variables, comparisons accounted for two-tailed *t*-test, Tukey's test, or Mann-Whitney *U* test corrected for multiple comparisons (Dunn's statistics). The analysis of variance was carried out by two-ways ANOVA test. For all comparisons, differences were considered as significant reaching a *P*-value (*P*) <0.05. Subsequently to normalization (*Z*-score), multivariate analyses were carried out by running a principal component analysis (PCA) or hierarchical clustering (based on Ward's metrics and Euclidean distance) in R-environment by running the "FactoMineR" package (Multivariate Exploratory Data Analysis and Data Mining) version 2.4 available in the CRAN repository.

3. Results

3.1. Bromatological characterization of pasta samples

First of all, pasta samples were studied for their bromatological composition. The CP provided the highest energy intake, also supported by data of both carbohydrates and sugars reaching the significance threshold in CP compared with all EPs (Table 1). By contrast, CP provided the lowest ($P < 0.05$) amount of total fibers and related subfractions (i.e., arabinoxylans and insoluble fiber). As expected, the highest content of proteins was found in WG-containing EP (i.e., EP1 and EP3) whereas, due to the mWO supplementation, the highest fat content was found in EP3 and EP4. Concerning ashes, the highest concentration was found in both EP1 and EP3 while no difference emerged between both EP2 and EP4 to CP. These findings highlight the distinct nutritional profiles of the experimental pastas, showcasing their potential for tailored dietary applications based on specific nutritional goals.

3.2. Total phenol quantification and radical scavenging activity

Starting from hydroalcoholic pasta extracts, the total phenolic content and the radical scavenging activity were evaluated using the *Folin-Ciocalteu* reagent and DPPH free radical assay, respectively. Expressed as GAE, CP displayed the lowest phenol content (Fig. 1A). In detail, while no difference ($P > 0.05$) existed between CP compared to both EP2 and EP4, the significance threshold ($P < 0.05$) was reached when CP was compared to EP1 and EP3. Data describing the potential antioxidant activity provided by hydroalcoholic pasta extracts were comparable to those observed for the phenolic content. In fact, CP showed the lowest scavenging activity against the DPPH free radical (Fig. 1B), and no difference emerged between CP and both EP2 and EP4. Instead, a higher ($P < 0.05$) scavenging activity featured both EP1 and EP3 compared to CP. These results underline the enhanced antioxidant potential of EP1 and EP3, likely attributable to their higher phenolic content, further supporting their value as functional food options.

3.3. Fecal microbiota characterization after simulated colonic fermentation

Simulated digestion was carried out in vitro to inspect how the different pasta ingredients had an impact on the intestinal microbiota. After fecal medium fermentation, data relying on viable microbiota counts were visualized based on principal components analysis (PCA), of which the first two principal components (PC1 and PC2, 77.7 and 14 %, respectively) explained 91.7 % of total variance between samples (Fig. 2). Negative values of the PC1 led to the clustering of mWO-containing samples (i.e., EP3 and EP4) based on high scores of viable TAMC, TANMC, coliforms, and *lactococci/streptococci*. Based on positive PC1 values, the LAB population was mainly found in batches containing CP or EP2. Bifidobacterial cells, instead, appeared extremely linked to EP1 samples according to positive values of the PC2.

To better inspect the microbiota composition in fecal batches at the lower taxonomic levels, a large panel of microbial genera and species (Suppl. Table S3) was investigated by qPCR analysis (Fig. 3A and B). Relying on a hierarchical clustering based on genus copy number (CN) *Z*-scores (Fig. 3A), 2 different microbial clusters (flagged "a" and "b") were identified while *Bifidobacterium* and *Bacteroides fragilis* group were outliers. The genus plot showed how positive scores of *Cl. leptum* group, *Desulfovibrio,* and *Prevotella* (i.e., the cluster-b) featured WB-containing samples (i.e., EP2 and EP4) while not other samples. The cluster-a showed intermediate scores of the belonging genera within all pasta samples, while *Bacteroides fragilis* group showed positive values in EP1 fermented samples only. At the species level (Fig. 3B), instead, the plot provided 3 different microbial clusters (I–III) and *Bif. bifidum* that acted as an outlier. Species included in the cluster I did not differentiate pasta samples. Species of cluster II were the most representative of WB-containing samples (i.e., EP2 and EP4). Furthermore, by fractioning cluster II in two different sub-clusters (IIa and IIb), the heatmap identified species included in the sub-cluster IIa (3 LAB and 3 *Bifidobacterium* species) were mostly representative of EP2 while those included in the sub-cluster IIb (i.e., 4 LAB and 3 species belonging to *Bacillus* (1) and *Enterococcus* (2) genera) mostly representative of EP4 samples. Within cluster III, *A. muciniphila* reported negative scores in samples EP2 and EP4, while other species (i.e., *Bif. infantis*, *S. thermophilus*, *Ll. curvatus* and *Lc. casei*) reported intermediate scores among all samples.These findings demonstrate the significant impact of specific pasta compositions on the modulation of gut microbiota at both genus and species levels, with notable distinctions observed in WB- and mWO-containing samples, suggesting potential for targeted dietary microbiota modulation.

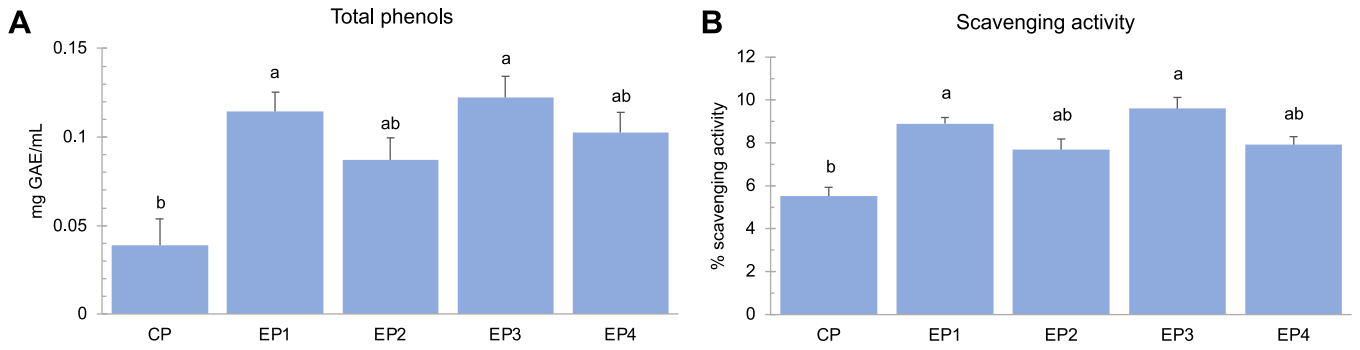

Fig. 1. Total phenols, expressed as mg of gallic acid equivalents (GAE9/ mL of extract, and radical scavenging activity against the free DPPH radical (expressed as percentage) featuring hydroalcoholic extracts of four experimental pasta (EP1–4) and control pasta (CP) samples. ($^{a\text{-}b}$) Different superscript letters on bars showed a significant difference (*P*-value; $P < 0.05$; two-tailed Student's *t*-test).

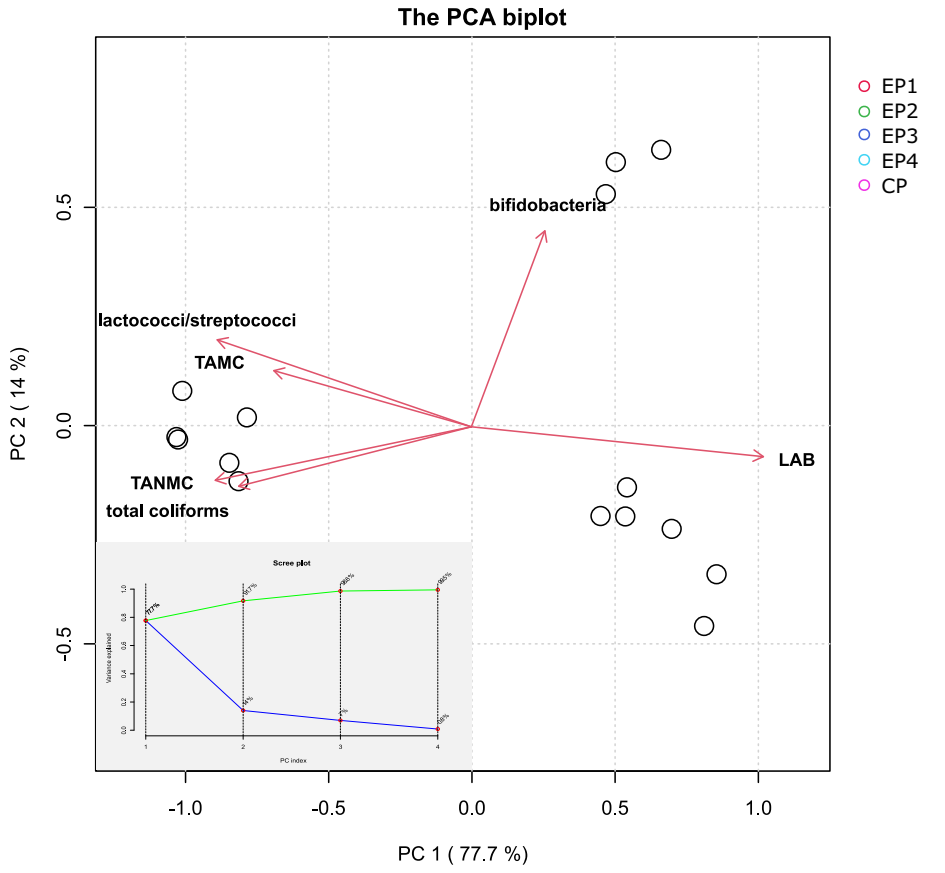

Fig. 2. Principal component analysis (PCA) of microbiota plated counts after simulated colonic fermentation of four experimental pasta (EP1–4) and control pasta (CP) samples. The single and cumulative contribute to variance of the first four PCs (PC1 to 4) was also shown in the gray-background panel. Abbreviations: TAMC, total aerobes microbial count; TANMC, total anaerobes microbial count; LAB, lactic acid bacteria.

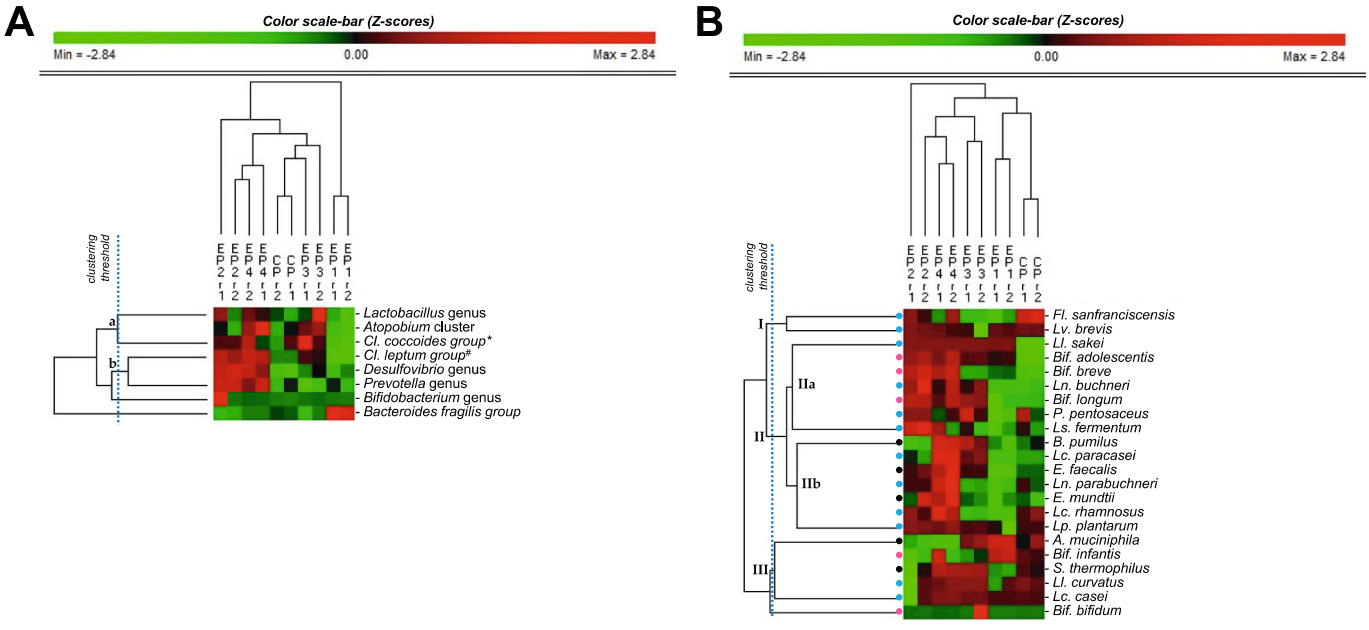

Fig. 3. Based on normalized data (Z-scores) of copy numbers (CN), heatmap (red to green for positive to negative Z-scores, respectively) with clustering (Euclidean distance) of bacterial genera (A) and species (B) found in fecal batches simulating the colonic fermentations of four experimental pasta (EP1–4) and control pasta (CP) samples. For each fecal batch, "r1" and "r2" distinguished the technical replicates. (*) clostridial cluster XIVa of Firmicutes. (#) clostridial cluster IV of Firmicutes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Analysis of fecal volatile organic compounds (VOCs)

Metabolomic profiles of fecal batches included 152 volatile organic compounds (VOCs), differently shared in the related chemical classes, i.e., alcohols (20), aldehydes (14), carboxylic acids (34), esters (23), hydrocarbons (27), indoles (3), ketones (8), phenols (6), pyrazines (3), and sulfur compounds (2) (Supp. Table S4). Additional 12 VOCs not belonging to any of the aforementioned classes were also identified.

Studying the sample distribution by plotting a PCA, the first two PCs explained 46.1 % of the total variance (PC1 27.96 % and PC2 18.04 %; Supp. Fig. S1). Variable contributions to score and loading plots of PC1 and PC2 were identified (Suppl. Table S5) and used to inspect which allowed for differentiating EP3 from other samples based on positive PC1 scores. The inspection allowed for assessing how the positive score of the PC1 mainly described a high incidence of carboxylic acids, hydrocarbons, and esters with respect to other chemical classes. Therefore, to deeply discriminate differences between samples, data provided by metabolite relative concentration were compared between samples. Thirty-two VOCs reached the significance threshold ($P < 0.05$), and these were used to perform hierarchical clustering (Fig. 4A). Being found three different clusters of metabolites (Cluster I–III), cluster I mainly included those metabolites found at the highest scores in EP4 pointing out a conspicuous incidence of esters. The cluster II encompassed a panel of metabolites with intermediate scores in all samples except for EP2. The cluster III included those metabolites found at the highest relative concentration in both WG-containing samples (i.e., EP1 and EP3).

In a second step of the workflow, a targeted analysis of short-chain fatty acids (SCFAs) was carried out (Fig. 4B). This investigation allowed for assessing how the entire panel of SCFAs displayed the highest absolute concentration when simulated colonic conditions fermented EP3. In detail, isovaleric, isobutyric, and propanoic acids were significantly enriched in EP3 than CP, while butanoic acid was higher ($P < 0.05$) in EP3 compared to EP4. In addition, isovaleric and isobutyric acids displayed a significantly higher concentration in EP3 than in both EP1 and EP4. For acetic acids, instead, no significant differences were found between samples. These findings highlight the distinctive metabolomic profile of EP3, characterized by enriched VOCs and SCFAs, which suggests its unique impact on metabolic activity during fermentation. This underscores the potential of EP3 for promoting specific metabolic outputs in the gut environment.

3.5. Cohort definition and sensory analysis

The sensory profile of all types of pasta is depicted for the five domains and relative sub-domains (Table 2). Differences between pastas existed for odor intensity, aftertaste, and overall pleasantness ($P < 0.05$). In particular, the EP2 compared to the other types had lower scores for odor intensity, after-taste, and pleasantness (taste). The control pasta also had also lower score for aftertaste, as seen for EP2 pasta.

3.6. Gastrointestinal motility

3.6.1. Gastric motility

Table 3 depicts the kinetic motility parameters of the stomach by functional ultrasonography. Basal antral area ranged from 4.0 to 4.4 cm^2, with no significant differences among all groups. Upon pasta ingestion by 5 min, maximal antral areas increased to 12.2–13.8 cm^2 without significant differences between groups. By 2 h, minimal antral areas decreased to 6.4–7.4 cm^2, i.e., 53–57 % of maximum. The estimated and "true" half-emptying times were comparable across groups. The AUC as absolute value was slightly smaller for CP than Eps. The time-dependent changes of normalized antral areas in response to meals were comparable and are depicted in Fig. 5A.

3.6.2. Gallbladder motility

Table 4 depicts the kinetic motility parameters of the gallbladder by functional ultrasonography. Fasting gallbladder volumes were comparable in all subgroups, ranging from 20.5 to 22.9 mL. The analysis of kinetic showed that each meal-induced comparable gallbladder contraction (ejection volume 50–60 %) and half-emptying times ranging from 20 to 25 min as true time to 35–50 min as estimated emptying time. In addition, the refilling rate was comparable between all pastas. At the end of the observation period, the gallbladder volume did not return to basal values in all cases, ranging from 78.7 % for EP1 to 84.9 % for CP of the basal (fasting) volume. The AUC of the time-related gallbladder

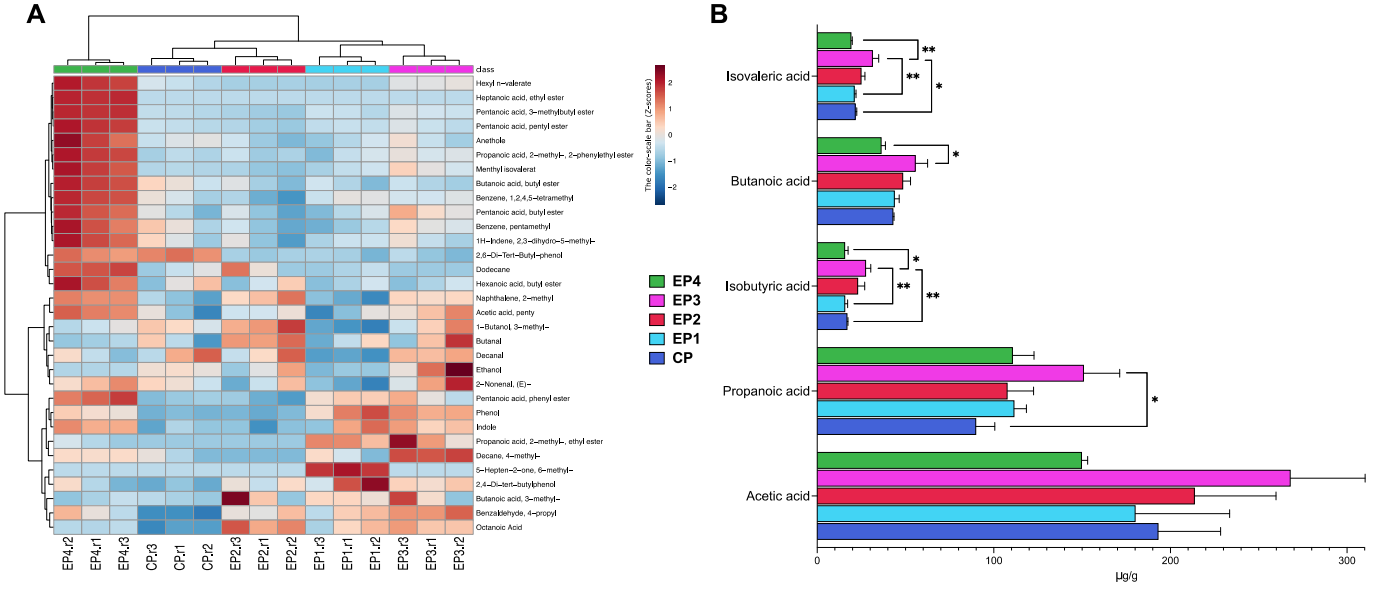

Fig. 4. (A) Heatmap (brown to blue for positive to negative Z-scores, respectively) with clustering (Ward's metrics) of untargeted volatile organic compound (VOC) relative concentrations and (B) absolute concentration of volatile short chain fatty acids (SCFAs) found fecal batches simulating the colonic fermentations of four experimental pasta (EP1–4) and control pasta (CP) samples. For each fecal batch, "r1", "r2", and "r3" distinguished the replicates. (*) Asterisk indicates a significant *P*-value ($P < 0.05$; corrected Mann-Whitney *U* test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Sensory profiles and features according to types of pasta.

Domains	CP (N = 14)	EP1 (N = 14)	EP2 (N = 14)	EP3 (N = 14)	EP4 (N = 14)	P-value
Visual aspect						
Colour	51.4 ± 5.2	52.1 ± 5.2	52.6 ± 4.4	57.1 ± 5.5	50.6 ± 5.5	NS
	50 (20–80)	50 (20–80)	50 (26–80)	62.5 (26–80)	45 (26–80)	
Not homogenous	10.5 ± 2.5	11.9 ± 4.7	13.0 ± 5.2	8.4 ± 3.0	10.5 ± 5.6	NS
	10 (0–30)	2.5 (0–60)	5 (0–70)	7.5 (0–40)	2.5 (0–80)	
Odor						
Intensity	45.9 ± 3.4^{a}	39.5 ± 5.2^{ab}	22.9 ± 5.6^{b}	38.1 ± 6.1^{ab}	44.4 ± 4.4^{a}	0.02
	40 (30–76)	40 (10–76)	25 (0–56)	37.5 (0–76)	40 (20–76)	
Pungent	29.4 ± 2.2	23.6 ± 1.7	16.1 ± 4.1	28.6 ± 3.8	23.8 ± 4.6	NS
	30 (10–40)	21.5 (10–33)	20(0–40)	30 (10–60)	21.5 (0–70)	
Strong	37.6 ± 4.4	32.6 ± 4.5	20.6 ± 5.8	34.8 ± 4.8	31.9 ± 3.6	NS
	40 (0–60)	30 (10–60)	20 (0–60)	31.5 (10–70)	30(20–60)	
Bitter	7.5 ± 3.2	8.9 ± 3.4	7.1 ± 2.9	14.6 ± 5.6	12.9 ± 0.5.4	NS
	0 (0–30)	0 (0–40)	0(0–30)	7.5 (0–60)	0 (0–60)	
Sour	8.6 ± 3.8	8.6 ± 3.3	7.1 ± 3.2	7.1 ± 4.2	7.1 ± 4.5	NS
	0 (0–30)	0(0–40)	0 (0–30)	0 (0–60)	0 (0–60)	
Chewing						
Particle break-down	27.4 ± 3.3	33.9 ± 1.7	34.0 ± 6.0	34.6 ± 4.5	28.9 ± 2.6	NS
	30 (0–41)	33 (20–41)	40 (10–60)	33 (10–60)	30 (10–41)	
Hardness	25.1 ± 2.2	30.9 ± 2.4	32.7 ± 3.5	25.9 ± 1.6	26.6 ± 2.2	NS
	22.5 (10–40)	30 (20–50)	28 (20–50)	24.5 (20–40)	22.5 (20–40)	
Tooth packing	28.1 ± 4.5	28.8 ± 4.3	27.0 ± 2.8	33.1 ± 6.8	29.0 ± 4.3	NS
	30 (0–51)	30 (0–51)	30 (10–45)	30 (0–80)	30 (0–54)	
Cohesion	49.9 ± 6.8	52.1 ± 6.6	51.9 ± 7.1	47.1 ± 5.8	52.5 ± 6.7	NS
	49 (20–80)	59.5(10–80)	59 (10–80)	40 (10–80)	60(10–80)	
Taste						
Pungent	38.2 ± 3.2	28.2 ± 5.1	23.4 ± 5.0	33.9 ± 3.3	28.4 ± 5.0	NS
	40 (20–60)	30(0–60)	25 (0–50)	30 (20–60)	30 (0–60)	
Spicy	6.2 ± 2.2	12.6 ± 4.3	6.4 ± 3.2	7.6 ± 4.5	6.9 ± 4.2	NS
	0 (0–20)	8.5 (0–50)	0 (0–40)	0 (0–60)	0 (0–60)	
After taste	14.1 ± 3.0^{a}	28.4 ± 4.6^{b}	15.1 ± 3.6^{a}	21.2 ± 3.7^{b}	24.4 ± 2.8^{b}	0.03
	16 (0–40)	30 (5–60)	10 (0–30)	25 (0–50)	25(5–40)	
Pleasantness						
Score	70.9 ± 2.8^{a}	71.4 ± 2.1^{a}	54.7 ± 3.4^{b}	69.3 ± 3.5^{a}	64.9 ± 2.9^{a}	0.001
	70 (40–80)	70 (60–90)	60 (30–70)	70 (45–90)	69.5 (46–80)	

Data are mean ± SEM (Standard Error of the Mean) and Median (Min-Max).
Legend: CP, control pasta; EP, experimental pasta; EP1, 30 % de-oiled durum wheat "germ"; EP2, 30 % "bran" de-oiled wheat; EP3, 27 % de-oiled wheat "germ" plus 6 %.
Different lower-case letters showed a significant difference ($P < 0.05$; Kruskal-Wallis Multiple-Comparison *Z*-Value Test and Dunn's Test for multiple comparisons) between samples. NS: not significant.

Table 3
Gastric motility in response to control and four experimental pastas.

	CP (N = 14)	EP1 (N = 14)	EP2 (N = 14)	EP3 (N = 14)	EP4 (N = 14)	P-value
Basal Area (cm^2)	4.0 ± 0.3	4.2 ± 0.2	4.1 ± 0.2	4.4 ± 0.3	4.3 ± 0.2	0.66
Maximal Area (cm^2)	12.2 ± 0.6	13.0 ± 0.4	13.2 ± 0.6	12.6 ± 0.6	13.8 ± 0.4	0.16
Minimal Area (cm^2)	6.4 ± 0.3	7.1 ± 0.3	7.4 ± 0.2	7.2 ± 0.3	7.3 ± 0.3	0.15
Minimal Area (%)	53.4 ± 2.3	55.4 ± 2.3	57.1 ± 2.4	58.5 ± 2.8	53.5 ± 2.7	0.57
$tT_{1/2}$ (min)	61.5 ± 4.7	61.1 ± 3.3	70.3 ± 2.7	68.0 ± 4.3	67.3 ± 2.7	0.07
$eT_{1/2}$ (min)	100.0 ± 10	98.3 ± 7.3	123 ± 6.1	109.2 ± 8.5	112.5 ± 9.3	0.10
AUC (cm^2 x 120 min)	1127 ± 61^{a,b,c,d}	1201^{a} ± 21.5	1372^{b} ± 53.9	1264^{c} ± 54.8	1304^{d} ± 61	0.009
AUC (% x 120 min)	9225 ± 268	9215 ± 160	9777 ± 206^{a}	9510 ± 172	9022 ± 308	0.08

Data are mean ± SEM. Legend: AUC, area under curve; $eT_{1/2}$, estimated half-emptying time; N, Number of subjects; $tT_{1/2}$, true half-emptying time; CP, control pasta; EP, experimental pasta; EP1, 30 % de-oiled durum wheat "germ"; EP2, 30 % "bran" de-oiled wheat; EP3, 27 % de-oiled wheat "germ" plus 6 %.
$^{(a\text{-}e)}$ Similar superscript lowercase letters indicated a significant difference ($P < 0.05$: Kruskal-Wallis Multiple-Comparison Z-Value Test and Dunn's Test for Multiple Comparisons) between the related values.

volume curves were comparable between all groups (Fig. 5B).

3.6.3. OCTT measurement

In exhaled air, basal H_2 concentration was comparable between groups and ranged between 1.9 and 2.3 ppm confirming optimal dietary preparation on the day before the test. The graphic profile of time-dependent changes in H_2 concentrations are depicted for each group in Fig. 6A. All pasta samples produced a time-dependent increase of H_2 with higher maximal levels after EP3 with a trend of shorter OCTT and significantly greater AUC compared to CP, EP1, and EP2, $P = 0,02$ (Fig. 6B,C).

Gastrointestinal motility findings highlight the similar gastric and gallbladder motility responses across all pasta types, while also suggesting that EP3 may have a more pronounced impact on intestinal transit, as indicated by the significant increase in H_2 production and shorter OCTT. This reinforces the potential of EP3 to influence gastrointestinal motility, which may have implications for digestive health.

4. Discussion

Global trends about the population increase necessitate a substantial and consistent rise in food production, estimated at 70 % or more within the next 30 years (Hodges et al., 2011). This surge in demand underscores the urgent need for exploring additional food sources for the future. Based on the volume of by-products from wheat milling, both WB and WG represent valid alternatives providing fibers, proteins, minerals, vitamins, and antioxidants, all nutrients that featured the MD and highly

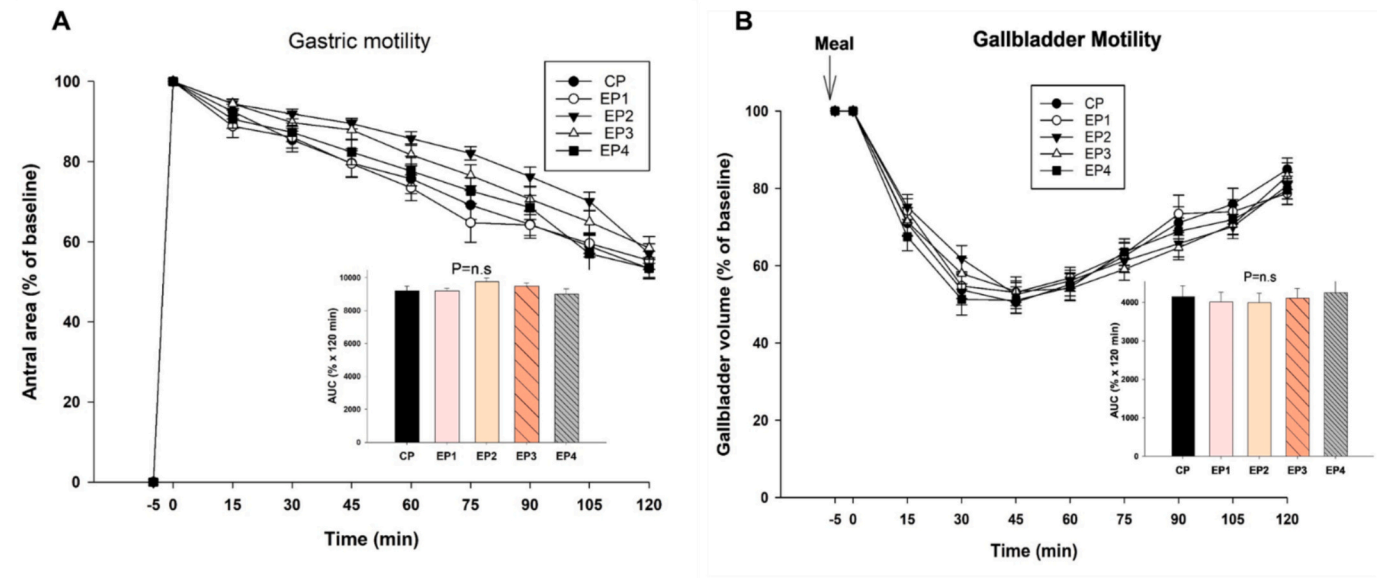

Fig. 5. (A) Time-related curves of antral area (%) and the area under curve (AUC) of the postprandial gastric emptying; (B) time-related gallbladder volume curves (%) and the area under curve (AUC). Significant difference was tested by Kruskal-Wallis Multiple-Comparison Z-Value Test and Dunn's Test for Multiple Comparisons). Abbreviations: CP, control pasta; EP, experimental pasta.

impacted Western-featuring NCDs, such as obesity, type 2 diabetes (T2D) and cardiovascular diseases (CVDs). The health benefits of whole wheat largely stem from its nutraceuticals and bioactive phytochemicals. Evidence suggests that consuming WB-foods can help reduce the risk of chronic conditions such as obesity, T2D, CVDs, and cancer due to their anti-inflammatory and antioxidant properties, their ability to improve immune function, and their influence on gut microbiota. A study noted that frequent consumption of whole wheat products, like bread, reduced T2D risk by 20–30 %(Gil et al., 2011). Additionally, studies have highlighted the potential role of alkylresorcinols (AR) – a group of amphiphilic phenolic lipids that occur in the bran fraction of wheat grain, including the hyaline layer and inner pericarp (Ross et al., 2003) – in diabetes prevention. In one Swedish cohort, a higher ratio of plasma AR-C17:0 to AR-C21:0 was linked to lower T2D risk and improved glucose tolerance (Horikawa et al., 2017; Sun et al., 2019).

Believing that this aspect holds industrial value in crafting diverse pasta types for targeted nutritional interventions, the primary goal of the present study was to enrich 4 different experimental pastas (EPs) with fibers, polyphenols, and antioxidants to inspect how the resulting bromatological c0.0000omposition of EPs exerted different effectiveness in vitro. Furthermore, we combined the in vitro analyses with in vivo organoleptic evaluations and gastrointestinal motility responses to provide novel, highly integrated, and physiologically relevant insights into digestion and food-gastrointestinal interactions concerning motility and gut microbiota.

The conspicuous content of minerals and vitamins in both dWG and dWB resulted in a significantly higher percentage of ashes in EPs than in CP. Therefore, the highest amount of vitamins with antioxidant activity detected may have influenced the scavenging properties assessed in vitro. In details, although slight differences were found between EPs, the higher content of vitamins with antioxidant activity in dWG coupled with the collected total phenolic concentrations allowed for reaching the significance threshold for both EP1 and EP3 to CP (Opara & Rockway, 2006). With respect to the total energy assessment, although significance was specifically observed between EP4 and CP, all EPs demonstrated lower kcal values. This trend aligned with the significantly lower values of sugar concentration observed in all EPs compared to CP, allowing us to flag EPs as healthier alternatives based on previous evidence discussing how excessive consumption of high-carbohydrate foods led to the development of insulin resistance as a risk factor for different cardio-metabolic diseases (Firth et al., 2020; Srilatha, 2011). The detailed bromatological composition provided in Table 1 showed a slight difference in nutritional value between control pasta (CP) and EP1–4. The nutrient composition varied following the enrichment ingredients, indeed, the 2-fold higher percentage between WG and WB resulted in the highest protein content found in WG-containing EPs (i.e., EP1 and EP3). By contrast, both EP3 and EP4 exhibited the highest fat content, attributable to the mWO supplementation. Besides the highest fiber content found in WB-enriched pasta, all EPs provided a significantly higher percentage of fibers than CP.

A previous crossover study noted that participants consuming whole wheat pasta showed better appetite regulation than those consuming refined wheat pasta according to findings suggesting that the whole wheat group, with a higher fiber intake (11 g vs. 3 g in refined wheat), had improved satiety(Costabile et al., 2018). This emphasizes additional potential beneficial effects of EPs, as the health benefits associated with dietary fibers have been extensively elucidated in the scientific literature (Joye, 2020). The insoluble fibers directedly act by promoting the movement of material through the intestinal tract and increasing stool bulk, the soluble fraction needs the intervention of the gut polymicrobial community to be completely exploited for its beneficial effects (Gidley & Yakubov, 2019). However, evidence has also been noticed that even insoluble fibers, such as wheat arabinoxylans, undergo transformation by the microbiota community (Calvete-Torre et al., 2023), challenging the binary classification between insoluble types as non-fermentable and soluble types as fermentable. This transformation sheds light on observed modifications in fermentations carried out in vitro, indicating that even within low soluble dietary fiber content in wheat, the viable microbiota-plated counts resulted in being affected. In the human gut lumen, due to the hosts' lacking specific enzymes, both types of fibers cannot be metabolized whereas, accounting for their great genomic power, gut microbiota can use them as substrates to synthesize different metabolites bioavailable for hosts (Tasse et al., 2010), such as SCFAs. As deeply introduced, SCFAs exert a broad spectrum of beneficial roles in humans because they are pivotally involved in the modulation of several metabolic pathways that are involved in the prevention of obesity, insulin resistance, and T2D onset while, at cellular – colonocytes – level, hence, favoring the intestinal homeostasis and function acting as trophic modulator and mucin secretion stimuli (Portincasa, Bonfrate, et al., 2022). Moreover, SCFAs also exerted bacteriostatic functions by

Table 4
Gallbladder motility in response control and experimental pastas.

	CP (N = 14)	EP1 (N = 14)	EP2 (N = 14)	EP3 (N = 14)	EP4 (N = 14)	P
Fasting Volume (mL)	22.9 ± 1.0	21.2 ± 1.1	21.8 ± 1.2	21.2 ± 1.3	20.5 ± 1.2	0.46
Residual Volume (mL)	10.4 ± 0.8	10.0 ± 1.1	10.2 ± 0.5	9.5 ± 0.5	9.1 ± 0.6	0.61
Residual Volume (%)	45.1 ± 1.9	46.8 ± 1.7	47.2 ± 2.1	45.5 ± 2.2	44.5 ± 2.3	0.87
Time to Residual Volume (min)	42.9 ± 3.1	45.0 ± 3.5	47.1 ± 2.1	47.1 ± 3.1	43.9 ± 4.0	0.73
Ejection Volume (mL)	12.5 ± 0.6	11.3 ± 0.6	11.6 ± 0.9	11.7 ± 1.0	11.4 ± 0.8	0.71
Ejection Volume (%)	55.0 ± 1.9	53.2 ± 1.7	52.8 ± 2.1	54.5 ± 2.2	55.5 ± 2.3	0.84
Emptying Rate (mL/min)	−0.33 ± 0.03	−0.34 ± 0.06	−0.27 ± 0.04	−0.25 ± 0.02	−0.33 ± 0.06	0.63
Emptying Rate (%/min)	−1.42 ± 0.14	−1.52 ± 0.28	−1.0 ± 0.16	−1.02 ± 0.20	−1.55 ± 0.28	0.26
Emptying $tT_{1/2}$ (min)	22.2 ± 2.4	20.5 ± 1.9	20.4 ± 1.2	25.0 ± 2.7	20.1 ± 2.1	0.46
Emptying $eT_{1/2}$ (min)	35.7 ± 4.2	40.3 ± 4.5	47.6 ± 5.0	44.9 ± 5.9	40.9 ± 4.9	0.55
Final Volume (mL)	18.7 ± 1.1	16.7 ± 1.0	16.5 ± 1.1	16.6 ± 1.1	16.0 ± 1.6	0.49
Final Volume (%)	84.9 ± 3.0	78.7 ± 2.9	80.7 ± 3.4	83.2 ± 3.4	80.8 ± 3.4	0.82
Refilling Rate (ml/min)	0.12 ± 0.01	0.11 ± 0.02	0.1 ± 0.01	0.11 ± 0.01	0.1 ± 0.01	0.89
Refilling Rate (%/min)	0.49 ± 0.05	0.44 ± 0.07	0.4 ± 0.04	0.5 ± 0.06	0.44 ± 0.04	0.69
Refilling $tT_{1/2}$ (min)	75.1 ± 4.7	77.4 ± 3.0	85.0 ± 2.3	86.9 ± 2.7	77.8 ± 2.6	0.07
Refilling $eT_{1/2}$ (min)	63.1 ± 5.0	52.8 ± 4.9	54.9 ± 5.2	59.8 ± 5.3	53.4 ± 6.2	0.41
AUC (mL x 120 min)	906 ± 77.5	855 ± 74	871 ± 92	875 ± 99	835 ± 64	0.98
AUC (% x 120 min)	4149 ± 281	4018 ± 246	4000 ± 236	4113 ± 247	4256 ± 283	0.96

Data are mean ± SEM. Legend: AUC, area under curve; $tT_{1/2}$, true half-emptying time; CP, control pasta; EP, experimental pasta; EP1, 30 % de-oiled durum wheat "germ"; EP2, 30 % "bran" de-oiled wheat; EP3, 27 % de-oiled wheat "germ" plus 6 %. Statistical difference was tested by Kruskal-Wallis Multiple-Comparison Z-Value Test and Dunn's Test for Multiple Comparisons.

contributing to the reduction of intestinal pH, a condition that, creating an environment less conducive to certain bacteria, influences the microbiota composition within the gut. For these reasons, microbes involved in the SCFA synthesis are generally recognized as health-promoting bacteria and include the mostly known lactobacilli and bifidobacteria (LeBlanc et al., 2017) as well as additional species belonging to the clostridial clusters IV (*Ruminococcaceae*) (Xie et al., 2022) and XIVa (*Lachnospiraceae*) (Vacca et al., 2020) of the Firmicutes phylum. However, it is important to discuss how, in our study, culture-based investigations did not provide a clear depiction. The highest bifidobacterial and LAB densities were observed in EP1 and EP2 simulated colonic fermentations, respectively. However, the overall synthesis of SCFAs relies on microbial cross-feeding mechanisms involving the entire gut microbiota community(Culp & Goodman, 2023). In line with this evidence, in fact, we found the highest SCFAs concentration in the fecal medium containing EP3 substrate, the same that displayed the highest culturomics-based densities of TAMC and TANMC. Various studies have shown previously that diets high in whole grains modify gut microbiota composition and stimulate the production of SCFAs and hormones related to appetite(Costabile et al., 2018). Overweight participants on a high whole wheat diet over six weeks showed greater weight loss and a rise in *Prevotella* genus abundance in gut (Christensen et al., 2019). Another study noted that whole wheat consumption in overweight adults was associated with changes in fecal butyrate concentrations, indicating positive effects on gastrointestinal health(Vuholm et al., 2017). Besides the microbial saccharolytic metabolism, VOC-metabolomics pointed out how the previously stated content of proteins in dWG ingredient (31 %) positively modulated the metabolism of branched-chain fatty acids (i.e., isovaleric and isobutyric acids) under simulated colonic fermentation of EP3 (Hald et al., 2016; Piero Portincasa, Leonilde Bonfrate, et al., 2022).

Therefore, by critically analyzing the chemical, antioxidant, and microbiological profiling, we concluded that both EP1 and EP3 allowed for obtaining innovative pasta samples with ameliorated nutraceutical properties than CP.

Still, because innovative and sustainable food products with possible nutraceutical properties, especially those made by by-products, are rarely consumed by large-scale populations due to their poor palatability (Fraga et al., 2019), our additional challenge was to ensure that nutraceutical ingredients did not impact the "innovative" food palatability, quality, and consumer appeal. Therefore, here takes place our secondary outcome of the study, which was comparing in vivo their palatability and sensory profile to a control pasta (CP) made by refined wheat believing that this integrated approach is the key to better disentangling the precise nutraceutical, functional, and gastrointestinal aspects of standard and novel foods in the "real life" environment. To reach this goal, we also inspected domains of perception in volunteer individuals.

Despite the minimal variability of nutrient composition, all types of EPs induced similar sensory perception compared to CP and were appreciated by consumers except for EP2, which resulted in the tasteless one. In addition, aftertaste was lower in EP2 compared to other EPs but was similar to CP. This means that the addition of enrichment ingredients, more than variability in nutrient composition, affects sensory perception.

The enrichment of pasta and bread with de-oiled durum wheat bran or processed wheat bran to enhance nutritional content without compromising sensory qualities is an active area of food science (Dziki, 2021). The overall results of the sensory profile agree with other studies that have evaluated the effects of including beneficial ingredients in foods on their palatability, as demonstrated by Jaworska et al., which found that the perceived liking of fresh pasta increased concomitantly with the oat β-glucan fiber addition (Jaworska et al., 2020), and by Namir et al. reporting a variation of palatability in different types of nutraceutical pasta depending on the different percentages of fibers contained (Namir et al., 2022).

Other studies showed that while the incorporation of wheat germ and wheat bran increased the nutritional and functional proprieties of pasta, a high amount of WB (15–20 %) had an undesirable effect on the sensory profile especially for odor and taste (Cankurtaran & Bilgiçli, 2019). Moreover, the addition of 15–20 % WB resulted in a decrease in the breakage susceptibility of WB-enriched pasta (Chillo et al., 2008). These effects could be attributed to β -glucans which are present in high amounts in WB and can affect food texture by increasing viscosity, creating a creamier or thicker consistency. This aspect influences sensory perception, enhancing or masking tastes(Krawęcka et al., 2020; Zhao et al., 2023).

The results based on gastrointestinal motility studies were very instructive confirming that the dynamic response to different EPs was comparable to those provided by CP. This aspect was observed along with the comprehensive evaluation of chemical composition and sensory properties. Although some differences of chemical and nutrient composition exist between EPs and CP, no significant difference is observed for gastric emptying kinetics. Similarly, all pastas induced comparable emptying responses due to similar fat content (13 g) which represented the appropriate *stimulus* for the neuro-hormonal response of the gallbladder (Wang et al., 2019).

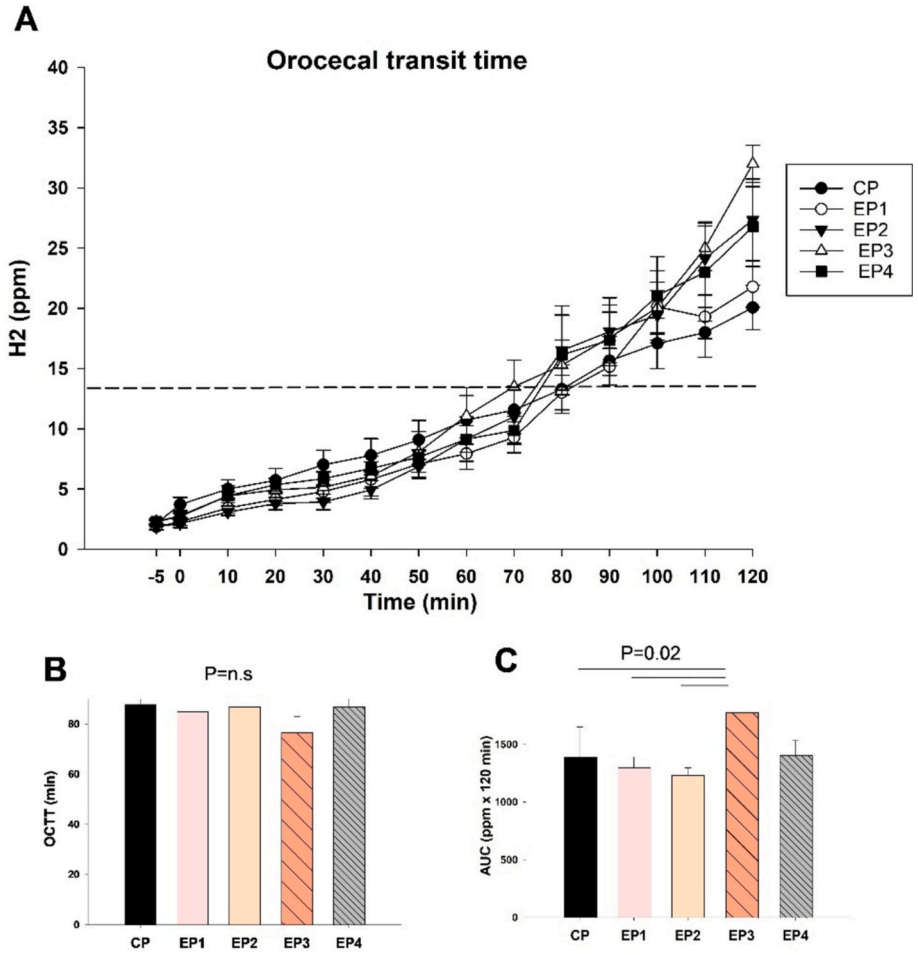

Fig. 6. A) Time-dependent curves of H_2 concentration as ppm in exhaled air; B) orocecal transit time (OCTT); C) area under the curves of time-dependent H_2 concentration curves. (*,#) Significant difference difference was tested by Kruskal-Wallis Multiple-Comparison Z-Value Test and Dunn's Test for Multiple Comparisons. Abbreviations: CP, control pasta; EP, experimental pasta; OCTT, orocecal transit time.

Of note, data on OCTT added information concerning the fermentation capacity of foods (Abdallah et al., 2023). Interestingly, the EPs, which featured by a high fiber content compared to CP, demonstrated a higher in vivo microbial fermentation, especially with EP3, as depicted by the higher production of H_2 compared to CP. This finding is in line with the in vitro outcomes in which we observed an increasing production of SCFAs in fiber-enriched EPs, especially for EP3, and the beneficial effects of SCFAs on hosts have been discussed on a recent paper from our group (Piero Portincasa, Leonilde Bonfrate, et al., 2022). This study provided relevant information regarding functional and physiological gastrointestinal motility and food agreeability when dealing with nutrients and foods. In addition, future research could incorporate microbiota profiling to explore further how individual baseline microbiota characteristics interact with dietary interventions and their potential role in shaping transit time and fermentation outcomes.

This study also emphasizes its relevance to the field of sustainability. Utilizing wheat by-products, such as WB and WG, provides multiple environmental benefits by promoting waste reduction, lowering emissions, and enhancing soil health. These by-products are often discarded or incinerated, generating waste and releasing significant greenhouse gases like carbon dioxide and methane (Koul et al., 2022). Here, we harnessed specific wheat by-products to improve the nutritional properties of pasta, supporting research into the critical role of diet in human health. However, this is just one way to reduce the loss of natural resources. Wheat by-products also have potential in sustainable bioenergy production, as they can be converted into bioethanol, biogas, and other renewable energy forms, reducing fossil fuel dependence and lowering the carbon footprint (Zuin & Ramin, 2018). In industrial applications, wheat by-products can replace wood and plastics by being processed into building materials, paper, or biodegradable packaging, offering renewable alternatives to logging and plastic manufacturing(Comino et al., 2021). Looking to the future, combining these research efforts to repurpose wheat by-products can further contribute to a circular economy by reducing pollution, promoting sustainable land and resource use, and establishing wheat by-products as valuable assets in sustainable agricultural and industrial practices.

5. Conclusions

In this study, the addition of three by-products in pasta production significantly lowered energy intake while increasing the fiber content. Moreover, incorporating these by-products introduced phenolic compounds, enhancing scavenging activity, and modulating culturable fecal microbiota composition and activity according to an increased SCFA metabolism. Sensory profile, palatability, and gastrointestinal motility evaluation scores showed that EPs have similar palatability and physiological responses to CP and, therefore, could be accepted by consumers. In conclusion, our findings support the incorporation of WG, WB, and WO as valuable sources of bioactive compounds in 'novel' pasta formulations. Given the nutraceutical benefits observed, future research could examine these enriched pastas for targeted clinical and public health applications, particularly for individuals with specific dietary needs, such as older adults or those with metabolic syndrome. These

groups could benefit from the higher fiber content, phenolic compounds, and improved gastrointestinal modulation offered by these enriched formulations. This study encourages further in vivo testing to explore these applications in diverse populations and evaluate potential health outcomes.

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CRediT authorship contribution statement

Mirco Vacca: Writing – original draft, Methodology. **Mohamad Khalil:** Writing – original draft, Methodology. **Antonio Rampino:** Investigation. **Giuseppe Celano:** Formal analysis, Data curation. **Elisa Lanza:** Methodology. **Giusy R. Caponio:** Methodology. **Felice Ungaro:** Investigation. **Alessandro Bertolino:** Investigation. **Agostino Di Ciaula:** Formal analysis, Data curation. **Maria De Angelis:** Supervision. **Piero Portincasa:** Writing – review & editing, Conceptualization.

Ethical statement

Ethical approval for the involvement of human subjects in this study was granted by Local Ethics Committee (study n. 7394, approved on 8th March 2023), and all subjects signed the informed consent.

Declaration of competing interest

M.G.M. is employed by Casillo Group, Via Sant'Elia Z.I. – 70,033 Corato, Italy. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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