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Development of a synbiotic snack for gut–brain axis health

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Snacks are ideal vehicles to intervene in the diet of consumers as they are becoming a staple in the modern diet. This study aimed to develop a synbiotic snack capable of delivering both prebiotics and live probiotics with a potential positive impact on supporting the gut–brain axis system by incorporating chickpea and red lentil flour as a source of protein and fibre, specialty fibres as sugar and fat/saturated fat substitutes, and dark chocolate enriched with a probiotic formulation (SLAB51®) with proven neuroprotective properties in a mouse model. Compared with a conventional wheat snack, the developed snacks presented not only better nutritional composition (high in proteins, fibres, and unsaturated fatty acids and low in sugars and total/saturated fats) but were also characterised by high prebiotic potential towards the SLAB51® multi-strain probiotic formulation, low glycaemic index, and the ability to induce slightly increased satiety. The developed snacks had a good shelf life with minimal chocolate blooming and high probiotic viability after six months of storage at both 25 °C and 4 °C (41.7% and 88.0% survival, respectively). Positive consumer response was observed among the senior population (>65 years old), with moderate acceptability and high willingness to buy in the senior population upon disclosure of pulse ingredients and potential health benefits. This research provides comprehensive scientific evidence for developing nutritional and healthy food products with a potential synbiotic effect tailored to an ageing population, without neglecting the pleasure of treating yourself to a good chocolate-coated cookie.

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1. Introduction

Snacks, "all foods and drinks with calories consumed between or outside the three main meals",[1,2] are becoming a staple in the modern diet, contributing to approximately 20% of energy intake in Western countries.[3,4] The snack market expansion has been taken as an opportunity to contribute to health and well-being, and several food manufacturers have shifted from conventional, unhealthy products towards healthy snacks.[3]

Snacks can also be a useful tool to address and mitigate specific societal issues, including age-related neurological diseases, which are among the most urgent challenges in modern society. Neurodegenerative conditions have been associated with an alteration of the gut–brain axis, a bidirectional communication system between the central nervous system and the gastrointestinal tract. The correlation between a healthy lifestyle, eating habits, and the correct development and functionality of the gut bacterial component is well established, with prebiotics and probiotics playing an important role in preventing and counteracting intestinal dysbiosis, which, in turn, could influence the onset of neurodegenerative diseases.[5,6]

The authors have developed a nutritionally improved cookie[7] that replaces traditional wheat with pulse flours and incorporates specialty fibres (Meltec® and WF fibre®) to lower both sugar[8,9] and fat/saturated fat.[10–12] This short-dough cookie has been shown to have high fibre and protein content, reduced sugar and saturated fat content, a lower predicted glycaemic index, and higher protein digestibility compared to a standard wheat short-dough cookie.[7] Specialty fibres used for sugar reduction (Meltec®) are expected to also have prebiotic activity, as measured in a dietary intervention study with a *Drosophila melanogaster* model system.[13] This prebiotic cookie presents several nutritional advantages, making it a suitable

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snack option to be included in a healthy diet for the elderly. The prebiotic cookies were further coated with probiotic-enriched dark chocolate to create a synbiotic snack. Live probiotics can be delivered in this synbiotic snack by encapsulating them in a chocolate carrier, which protects the probiotics until consumption.[14,15] This approach allows for easy integration with the prebiotic cookie if it is used as a coating. Among the various probiotic formulations, the SLAB51® formulation (which includes eight different live bacterial strains: *Streptococcus thermophilus* DSM 32245, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, and *Lactobacillus brevis* DSM 27961) was chosen due to its neuroprotective properties shown in an animal model.[16–18] Research has shown that in mice, SLAB51® altered the gut microbiota and its metabolic products, favourably influencing inflammatory cytokines, gut hormone levels, and proteolysis. This resulted in improved cognitive function, suggesting its potential in preventing and counteracting the progression of Alzheimer's disease.[16] Furthermore, this innovative probiotic formulation has shown *in vivo* and *in vitro* efficacy in combating neuroinflammation and oxidative stress, key features of Parkinson's disease, by restoring specific underlying molecular mechanisms to their baseline levels in treated mice.[18] However, to the best of the authors' knowledge, a synbiotic snack, produced with prebiotic-enriched cookies and a chocolate probiotic mix, has never been developed, characterized and sensory evaluated for a specific group of consumers (>65 years old).

This work aimed at designing, developing, and characterizing a synbiotic snack that simultaneously delivers prebiotics and live probiotics. The goal was to develop a snack with expected health benefits on the gut–brain axis while ensuring the snack is enjoyable for seniors (individuals over 65 years old). This snack is designed for individuals aged 65 and older because, whether the efficacy in supporting cognitive health was proven in a clinical trial, they are the population segment that can benefit the most from the consumption of this product. The developed synbiotic snack consists of a prebiotic-rich, pulse-based cookie coated with probiotic-rich chocolate, which exhibits properties to support gut–brain axis health in a mouse model. This health effect in a mouse model was reported in a separate preclinical study, where a lentil-based synbiotic snack was found to be efficient in a preclinical platform for Alzheimer's disease.[19] In successive project phases, human clinical trials will be done to prove the expected positive effects on the gut–brain axis and the promotion of healthy ageing, objectives that go beyond the aim of this work.

2. Materials and methods

2.1. Materials

Wheat flour (Molino Bianchi, Osimo, AN, Italy), sugar, butter, eggs, and leavening agent (Lievito Pane degli Angeli, Desenzano del Garda, BS, Italy) were bought in a local supermarket, while pulse flours (red lentils and chickpeas), Meltec®, and WF fibre® were kindly donated by Martino Rossi SpA (Malagnino CR, Italy) and Hi-Food SpA (Pilastro di Langhirano, PR, Italy), respectively. SLAB51® is a commercially available probiotic formulation (Ormendes SA, Jouxtens-Mezery, Switzerland), including eight different live bacterial strains: *Streptococcus thermophilus* DSM 32245, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, and *Lactobacillus brevis* DSM 27961. Inulin, α-amylase from a hog pancreas, pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, and bovine bile were from Merck Life Science S.r.l. (Milano, Italy).

2.2. Prebiotic-rich cookie preparation

Pulse-based cookies were developed as previously reported.[7] Shortly, a control conventional wheat "pasta frolla" cookie (W) formula (wheat flour: 100 g; sugar: 40 g; butter: 28 g; eggs: 28 g; leavening agent: 1.6 g) was modified to include pulse flours (chickpeas [C] and red lentils [L]) and specialty fibres (Meltec®) to ease sugar[8] and fat/saturated fat (WF fibre®) to reduce butter.[12] WF fibre® was used to produce a sunflower oil structured emulsion (named "fat block": 55% high oleic sunflower oil, 37% water, and 8% Hi-FibreWF) as previously described,[12] which was used as a butter substitute. Recipes for the pulse and specialty fibre cookies were: pulse flour: 120 g; sugar: 28 g; fat block: 28 g; eggs: 28 g; Meltec®: 20; leavening agent: 1.6 g.[7] Cookies were prepared in the facilities of Frolla Microbiscottificio (Osimo, AN, Italy). Dry ingredients (flour, sugar, and leavening agent) were premixed for 2 min in a mixer (PietroBerto MIX 60, Morano Vicentino VC, Italy), and other ingredients (eggs, butter or fat block, Meltec®) were then added and mixed for an additional 8 min to obtain a homogeneous dough. The dough was then sheeted (Rol-Fix 300, Fritsch, Germany) to a thickness of 4 mm, and manually cut with a circular shape mould (5 cm diameter). Cookies were placed into baking trays, cooked in a rotary oven (RT68, Tagliavini, Noceto PR, Italy) at 170 °C for 17 (wheat) or 20 min (red lentil and chickpeas), extracted from the oven and allowed to cool at room temperature for 30 min before packaging into polyethylene bags. Cookies made from three different flours were named W, C, and L cookies, respectively.

2.3. Prebiotic-rich cookie characterisation

2.3.1 Glycaemic index determination. The glycaemic index (GI) determination was conducted based on the ISO-26642 guidelines. The Institutional Review Board of the Department of Agricultural, Food, Environmental, and Animal Sciences of the University of Udine (protocol number: 0006480 of 11/12/2023) approved the study, and all participants signed informed consent forms following the Helsinki Declaration on Human Rights before starting the test. The inclusion and exclusion criteria followed for recruiting volunteers were reported by Tagliasco *et al.* (2025).[20] A total of 12 participants

were recruited to ensure that at least 10 would complete the test, which aligned with the ISO standard. Of these, 11 participants completed the study by attending five testing sessions over a two-month period. The sessions included three for cookies and two for the glucose solution, which served as the food reference, and were conducted in a randomised order (Smart Sensory Solutions S.r.l., Sassari, Italy). The test food (cookies) was portioned to contain 25 g of available carbohydrates. Available carbohydrates were determined as the sum of two components: the glucose produced after 120 min of *in vitro* digestion, as reported by Englyst *et al.* (2018),[21] using an enzyme mix of pancreatin (P7545, from porcine pancreas), invertase (I4504, from baker's yeast), and amyloglucosidase (A7095, from *Aspergillus niger*), and the free glucose, defined as the native free glucose plus the glucose derived from sucrose after incubation with invertase (I4504), as reported by Englyst *et al.* (2000).[22] All the enzymes were purchased from Merck Life Science S.r.l. (Milano, Italy). The cookies were served along with 250 mL of room-temperature water. The reference meal, glucose monohydrate, was prepared 24 h in advance by dissolving 27.5 g of glucose monohydrate powder (Farmalabor s.r.l., Italy) in 250 mL of water to complete glucose mutarotation (ISO-26642, 2010). Participants were asked to consume the test food within 10–12 min from the first bite (time 0), and blood glucose was collected six times after they started eating (15, 30, 45, 60, 90, and 120 min). Capillary blood was sampled using a 21G × 1.8 mm ACCU-CHEK Safe-T-Pro Plus lancet (Roche, Switzerland) and collected in Microvette® CB 300 fluoride/heparin tubes (SARSTEDT AG & Co., Nümbrecht, Germany). Blood glucose concentration was immediately determined using the YSI 2500 Biochemistry Analyzer (Yellow Springs Instrument Company, Chicago, USA), and the concentration was expressed in mmol L^{-1}.[20] The GI value of the tested samples was calculated as the incremental area under the curve (IAUC), defined geometrically following the trapezoid rule, excluding the area beneath the baseline, resulting from the consumption of the tested cookie samples and expressed as the percentage of the mean IAUC elicited by the consumption of the two-glucose solution in the same subject. During the test, participants reported their feelings of satiety and hunger using a self-reported questionnaire at specific time points: before food consumption ($t0$), immediately after consumption, and at 15 min ($t15$), 30 min ($t30$), 60 min ($t60$), and 120 min ($t120$) after starting to consume the test food on a scale from 0 to 10.[23] The overall nutritional composition of the portions of the three cookies used for the glycaemic index determination was calculated based on the nutritional information provided on the front packages of wheat flour as well as the technical sheets of chickpea and lentil flours, Meltec®, and WF Fibre®. The nutritional values for eggs, butter, and sugar were obtained from the "Food Composition Database for Epidemiological Studies in Italy" (BDA; bda-ieo.it, consulted December 2024). The nutrient calculation followed the EuroFIR regulation for the recipe calculation procedures.[24] Weight changes during cookie processing were accounted for by applying yield factors from Bognar's tables (2002).[25]

2.3.2 Lipid composition. Lipid fractions were extracted from 0.5 g of cookie powder added with a small amount of anhydrous sodium sulfate (Sigma-Aldrich, Milan, Italy) by performing two subsequent extractions, each with 10 mL of *n*-hexane (Sigma-Aldrich, Milan, Italy) with the help of a disperser (Ultra-Turrax, IKA) at 9500 rpm for 3 min. The extract solution obtained was filtered, dried under a nitrogen stream and weighted to obtain the lipid extract yield, and then reconstituted with 3 mL of hexane. The obtained lipid extract hexane solution was transmethylated to obtain fatty acid methyl esters (FAMEs) to be analysed by using a gas chromatograph (Agilent Technologies 6850 series II, Santa Clara, CA, USA) equipped with a 30 m 50% cyanopropylphenyl 50% polydimethylsiloxane coated column (DB-225, ID, 0.25 mm; film thickness, 0.25 mm; Agilent Technologies, Santa Clara, CA, USA) and a flame ionization detector (GC-FID), according to the procedure reported in a previous report.[26] Duplicates were analysed for each cookie sample.

2.3.3 Antioxidant activity. The antioxidant activity of each type of cookie was measured according to the procedure reported by Ajila *et al.* (2008)[27] with some modifications. Cookie powder (1 g) was mixed with 10 mL of 80% methanol and homogenized by using a disperser (Ultra-turrax, IKA) for 3 min; then, a further 10 mL of 80% methanol was added to wash the disperser wall. The obtained mixture (20 mL) was extracted at room temperature for 30 min using ultrasound (NEY Ultrasonik 19H), followed by centrifugation for 10 min and supernatant collection. After 1 h of incubation of the extract solution with 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Milan, Italy), spectrophotometric analysis was performed according to the procedure of Brand-Williams *et al.* (1995)[28] using a spectrophotometer UV-2700 I (Shimadzu, Japan) at a wavelength of 515 nm and the absorbance was recorded at 25 °C.

2.3.4 Prebiotic activity. This study investigates the prebiotic activity of cookies and their ingredients, including wheat, lentils, and chickpeas, which were subjected to heating to simulate the baking process. The prebiotic inulin (Merk Life Science S.r.l., Milano, Italy) was used as a control. Samples were subjected to the internationally standardized INFOGEST *in vitro* digestion protocol[29] and digested residual materials were used as nutritional substrates for SLAB51®. An aliquot of 50 µL of each suspension was sown on de Man–Rogosa–Sharpe agar (MRS, Liofilchem®, Italy) following the spread plate procedure by using a sterile 'L' spatula and incubated at 37 °C for 48 h, anaerobically (GasPak™ EZ Anaerobe Container System BD, distributed by Liofilchem®, Italy). All analyses were carried out in duplicate. The viable bacteria were counted (CFU per g) and identified by MALDI-TOF MS (Bruker Daltonics, Germany). Following the manufacturer's instructions, standard operating procedure – direct transfer procedure was used for isolate identification. After adding 1 µL of α-cyano-4-hydroxycinnamic acid matrix solution (Bruker Matrix HCCA) on the bacterial colony transferred in a MALDI-TOF MS target plate, the mass spectra were processed using Flex Analysis (version 3.4; Bruker Daltonics, Germany)

and BioTyper software (version 3.1; Bruker Daltonics, Germany) and a bacterial test standard (BTS; Bruker Daltonics, Germany) was used as a calibrator for quality control. The spectra obtained were compared with those present in the Biotyper database and log (score) ≥ 2.0 was considered as secure genus identification and a highly probable species-level identification, as recommended by the manufacturer (Bruker Daltonics, Germany). Results were reported as $\log_{10}$ CFU per g.

2.3.5 Sensory acceptability. Sensory perception and acceptability of freshly produced cookies were assessed using a consumer test (30 judges, 43% males and 57% females, 18–62 years old). Cookies were individually placed into plastic bags and coded with 3-digit random numbers. Each judge received the three cookies (W, L, and C) with the instructions on how to consume the cookies and the order in which to taste the samples, ensuring randomization and balanced analysis. Consumers were asked to rinse their mouths between tastings to avoid the influence of the former sample. Judges were asked to assess cookies' overall acceptability and key sensory attributes, using a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like, 8 = like very much, and 9 = like extremely).

2.4. Probiotic-rich chocolate production

Dark chocolate (72%) was enriched with SLAB51® neuroprotective probiotics in the research and development facilities of a chocolate manufacturer (Socado s.r.l., Verona, Italy). The probiotic mix was added as a lyophilised powder into liquid-tempered "72%" dark chocolate (28–32 °C), which was then molded into 100 g chocolate bars. The probiotic mix was dosed into the chocolate as reported by previous research[16,18] (4.0×10^{10} CFU, C–P) in the daily chocolate portion (6 g) present in the assembled snack. Chocolate without probiotic addition was taken as a control (C–C). Chocolate bars were packaged by wrapping them in aluminium foil and then a paper sheet immediately after production.

2.5. Probiotic-rich chocolate characterisation

2.5.1 Prebiotic activity. The prebiotic activity of 72% dark chocolate before the addition of probiotics was assessed using the same method described in section 2.3.4.

2.5.2 Probiotic vitality. Probiotic viability was analysed in triplicate immediately after production and weekly for 68 weeks of storage at room temperature (25 °C) and at 4 °C in chocolate bars (control C–C and probiotic-enriched C–P). Samples (25 g) were homogenised with 225 mL of sterile 0.1% peptone water, serially diluted, and plated onto de Man–Rogosa–Sharpe (MRS) selective medium. Bacterial counts were determined using the pour plate technique in MRS agar (Liofilchem®, Italy) after anaerobic incubation at 37 °C for 48–72 hours (GasPak™ EZ Anaerobe Container System, BD, distributed by Liofilchem®, Italy). Results were reported as $\log_{10}$ CFU per g.[30]

2.5.3 Chocolate sensory acceptability. The sensory analysis and acceptability of freshly produced chocolates with and without probiotics were assessed using a consumer test (30 judges, 43% males and 57% females, 18–60 years old), as described in section 2.3.5, with a few modifications. Chocolate bars were divided into 1.5 × 5 cm portions, individually wrapped into aluminium foil and coded with a 3-digit random number. Each judge received the two chocolate samples (C–C and C–P) along with the instructions to carry out the test and the order to taste the samples to ensure randomization and balance of sample analysis. Judges were asked to assess chocolate's overall acceptability and liking of key sensory attributes using a 9-point hedonic scale.

2.6. Synbiotic snack production

Synbiotic snacks were produced at the Frolla Microbiscottificio facilities (Osimo, AN, Italy) by coating prebiotic cookies (L and C, made as described in section 2.2) with tempered probiotic-rich chocolate (72%, 28–32 °C) to cover approximately half of the cookie. Before coating, the liquid-tempered chocolate was mixed with the SLAB51® probiotics (6.7×10^9 CFU per g of chocolate). Each assembled snack weighed approximately 10 g (8 g of cookie + 2 g of chocolate, with a 10% variability), in order to provide 4.0×10^{10} CFU per serving size (30 g, 3 snacks). The snacks were allowed to cool at room temperature, and then packaged in polyethylene bags and stored in a dry place at both 4 °C and 25 °C.

2.7. Synbiotic snack characterization and their stability during storage

The moisture content, hardness, and chocolate bloom were assessed at different time points: day 1 (fresh snack), and after 1, 2, 3, 6, and 12 months, to evaluate changes during storage at two different temperatures, 4 °C and 25 °C.

2.7.1 The nutritional composition. The nutritional composition of the three snacks was calculated as described in section 2.3.1. The nutritional values for dark chocolate were obtained from the "Food Composition Database for Epidemiological Studies in Italy" (BDA; bda-ieo.it, consulted in December 2025).

2.7.2 Moisture content. The moisture content (MC; %: g of water/100 g of sample) of the snack (fraction without chocolate) was measured by weight loss after drying in a forced air oven (Etuve air concept, FIRLABO, France) at 105 °C to constant weight. At least three repetitions for each snack type were analysed.

2.7.3 Hardness. The hardness of the synbiotic snacks was determined with a cutting test using a texture analyzer (TA1 Texture Analyzer, AMETEK, USA) equipped with a 1000 N load cell.[7] Snacks were placed on two holders (stainless steel equilateral triangular prisms, 15 cm long, 3 cm triangle side, height 2.6 cm; 3.5 cm gap distance) and were simultaneously cut across the cookie and chocolate fractions. Hardness (N) was evaluated by cutting samples at 2 mm s^{-1} (trigger force = 0.1 N) using a flat blade (FG/WBJ) and recorded as the maximum force at break (N). At least eight measurements were taken for each snack type.

2.7.4 Chocolate bloom stability. The chocolate bloom stability was assessed through colour analysis using a Minolta colorimeter (CR-400, Minolta Co., Osaka, Japan) equipped with a standard observer. Colour was measured on the chocolate fraction of each snack piece in 3 predetermined positions on at least 5 pieces of each snack. Colour coordinates (L, a, and b) were collected and used to calculate the whiteness index (WI) according to the equation: $WI = 100 - [(100 - L)^2 + (a^2) + (b^2)]^{0.5}$.[31]

2.7.5 Prebiotic activity. The prebiotic activity of the snacks (produced with dark chocolate with no added probiotic) was assessed using the same method described in section 2.3.4.

2.7.6 Sensory analysis. The sensory perception and liking were assessed on fresh pulse-based synbiotic snacks using a consumer panel of individuals aged over 65, as previously reported,[7] with some modifications. Briefly, the test was conducted following the ISO 11136:2014 guidelines in a central location (ISO 8589:2007 compliant laboratory) of Intertek Italia Spa (Matelica, MC, Italy). The panel consisted of 60 participants (30 males and 30 females), all habitual consumers of sweet baked products (more than once per week). The test was conducted in a "blind" format, with samples coded on disposable plastic plates (three-digit numerical codes) and presented to consumers using sequential monadic randomisation. Consumers were asked to rate the overall liking and liking of key sensory attributes (appearance, smell, flavour, chocolate flavour, texture and aftertaste) of the synbiotic snacks using 9-point hedonic scales and 5-point "just about right" scales to assess both liking and the appropriateness of specific sensory characteristics. Consumers were also asked to express their intention to purchase, providing additional insights into their perception of the product's composition. Evaluations were collected using a multimedia questionnaire (SI S2) uploaded onto a tablet. The data obtained from the evaluation were analysed statistically as previously reported.[7]

2.8. Statistical analysis

All data were subjected to statistical analysis. Data for GI determination are presented as mean ± standard error of the mean (SEM), and statistical differences were assessed using IBM SPSS Statistics for Windows version 29.0 (IBM Corp., Armonk, N.Y., USA) with the general linear model for repeated measures, followed by the Bonferroni *post-hoc* test for multiple comparisons ($p < 0.05$) after checking normality using the Kolmogorov–Smirnov test. When the Mauchly test of sphericity was significant, the Greenhouse–Geisser correction for degrees of freedom was applied.[20] Data related to prebiotic activity were analysed using GraphPad Prism 10 statistical software for MacOS, version 10.1.1-270 (GraphPad Software Inc., San Diego, CA, USA). All other data were processed using XLSTAT (Addinsoft, New York, USA). Normal and lognormal distributions for all other data were assessed using the Shapiro–Wilk test. Differences between means ± standard deviations were evaluated by one-way ANOVA, and Student's t-test at $\alpha = 0.05$. Multivariate analysis of variance (MANOVA) was performed to analyse the synbiotic snack with storage temperature, storage time, and flour type as fixed factors to identify their effect on snack moisture, hardness, and chocolate whitening index.

3. Results

3.1. Prebiotic-rich cookie characteristics

For the determination of GI, the portion of the three cookies was standardised to contain 25 g of available carbohydrates, and the calculated nutritional profile of each cookie portion is detailed in Table 1. Notably, the standardised portions of C (70 g) and L (69 g) cookies provided a higher amount of fibre (7.9 g and 6.3 g) and protein (9.7 g and 9.8 g) compared to the portion of cookie W (Table 1). Furthermore, cookies C and L contained lower saturated fat levels (1.1 g and 0.9 g) compared to cookie W (3.2 g).

Eleven healthy volunteers, comprising 8 females and 3 males, with an average age of 26.1 ± 3.4 years (mean ± standard deviation) and a body mass index of 21.0 ± 2.1 kg m^{-2}, were enrolled for the GI determination of cookies. Incremental blood glucose curves over 120 minutes following consumption of the three cookies are presented in Fig. 1A, with glucose included as a reference. Peak glucose concentrations were observed after 30 minutes for all samples. The increase in blood glucose was significantly higher after consuming the glucose solution than after consuming the cookies. The GI values for the cookies were: 46.9 ± 6.8 for W, 17.6 ± 3.6 for C, and 29.0 ± 3.3 for L (mean ± standard error of the mean; Fig. 1B). Statistical analysis revealed a significantly higher GI ($p < 0.05$) for the control cookie (W) compared to cookie C, which was made with chickpea flour, the sugar substitute Meltec®, and the butter substitute fat-block. Cookie L, made with red lentil flour, Meltec®, and fat-block, exhibited a lower GI, though not significantly different compared to W, and a slightly higher GI than C. No significant differences were observed among the cookies regarding hunger and satiety feelings, whereas differences were reported between the cookies and the glucose solution.

The measured lipid profile of the cookies is presented in Table 2 and expressed in g per 100 g. W cookies exhibited a

Table 1 Nutritional composition of the cookie portions for glycemic index determination (25 g of available carbohydrates; W – wheat cookie, control, L – red lentil cookie, and C – chickpea cookie)

	W	C	L
Portion (g)	46	70	69
Energy (kcal)	665	894	821
Energy (kJ)	158	213	195
Total fat (g)	5.6	5.9	4.8
of saturated fat (g)	3.2	1.1	0.9
Available carbohydrates (g)	**25**	**25**	**25**
of sugars (g)	9.4	8.0	6.8
of total starch (g)	15.6	17.5	17.3
Dietary total fibre (g)	0.6	7.3	6.3
Total protein (g)	2.9	9.7	9.8
Salt (g)	0.02	0.05	0.04

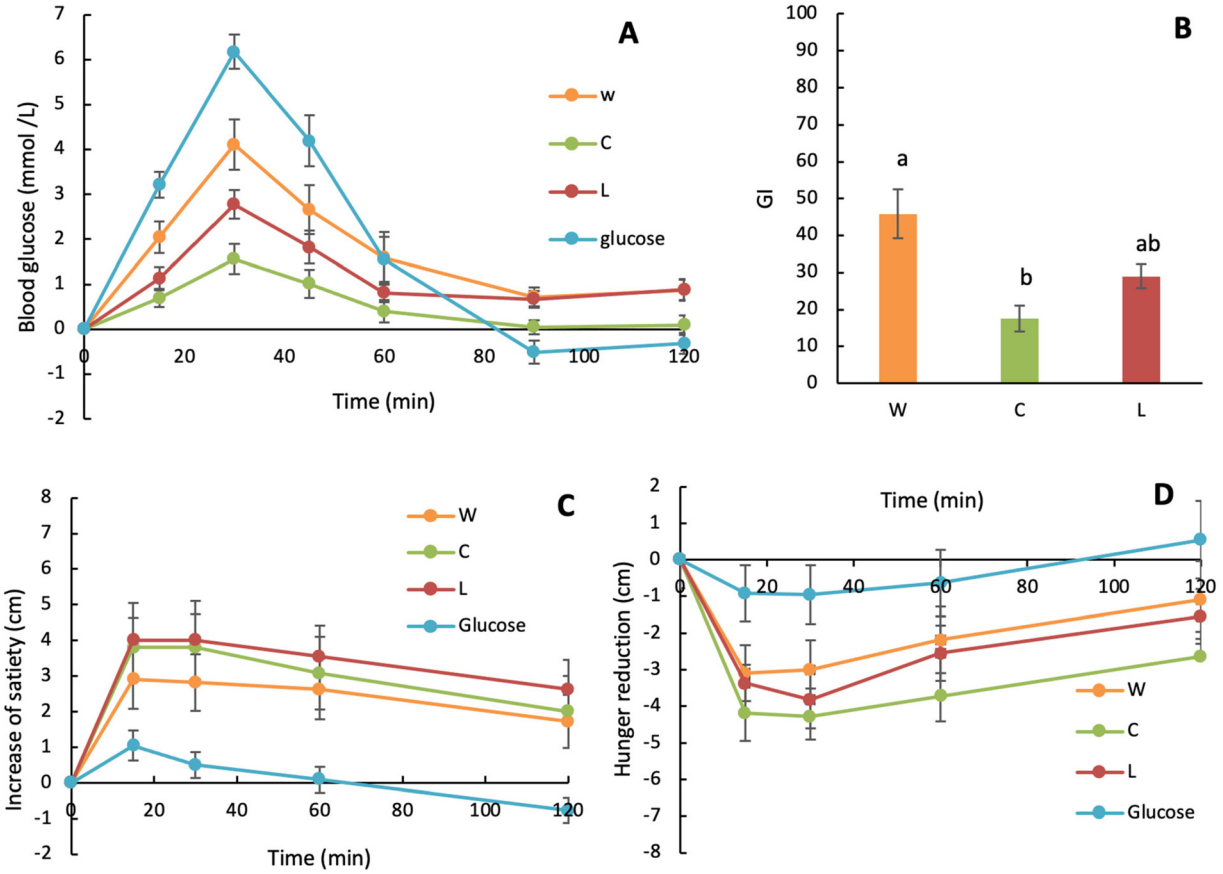

Fig. 1 Incremental blood glucose curves during 120 min after consumption (A), glycemic index (GI; B), satiety over time (C), and decrease of hunger over time (D) after consuming the cookies (W – wheat cookie, control, L – red lentil cookie, and C – chickpea cookie) and glucose solution. Data are expressed as means ± standard error of means (n = 11). The different letters above the columns of GI (B) indicate significant differences among the samples (general linear model for repeated measures, followed by a *post-hoc* test, Bonferroni $p < 0.05$ at a 95% confidence level).

Table 2 Fatty acid composition and antioxidant capacity of the cookies (W – wheat cookie, control, L – red lentil cookie, and C – chickpea cookie)

		Fatty acid composition (% of total)			
Cookie	Total fat (g per 100 g)	SFAs	MUFAs	PUFAs	Antioxidant capacity (% inhibition, DPPH radical assay)
W	11.2 ± 0.4^{a}	68.1 ± 1.3^{a}	27.4 ± 1.4^{b}	4.5 ± 0.1^{c}	2.4 ± 1.7^{b}
C	7.5 ± 0.7^{b}	11.7 ± 0.1^{b}	67.1 ± 1.1^{b}	21.2 ± 1.1^{a}	12.5 ± 1.2^{a}
L	7.1 ± 1.0^{b}	11.5 ± 0.9^{b}	75.4 ± 1.3^{a}	13.1 ± 0.5^{b}	11.8 ± 1.1^{a}

SFAs – saturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids. Data are reported as mean values ± standard deviations (n = 2). Means followed by different superscript letters in the same column indicate significant differences among the cookie samples (one-way ANOVA, followed by a *post-hoc* test, Tukey's test, $p < 0.05$ at a 95% confidence level).

significantly higher lipid content than C and L cookies ($p < 0.05$). Furthermore, W cookies had a higher saturated fatty acid (SFA) content (68.1%) compared to C and L, which contained approximately 11.5%. Conversely, unsaturated fatty acids (monounsaturated [MUFA] and polyunsaturated [PUFA]) constituted approximately 90% of the lipid content in C and L cookies. In detail, L cookies showed a higher proportion of MUFAs (75%) and a lower proportion of PUFAs (13%) compared to C cookies (67% and 21%, respectively). The individual fatty acid compositions revealed further differences between W cookies and pulse flour cookies (Table S1). L and C cookies exhibited lower levels of palmitic acid (C16:0) and stearic acid (C18:0) but higher levels of unsaturated fatty acids ($p < 0.05$), such as oleic acid (C18:1, ω-9) and linoleic acid (C18:2, ω-6), which is an essential fatty acid for human health.

The antioxidant activity of the cookies, produced by substituting wheat flour with pulse flour, was assessed in terms of DPPH radical inhibition and is shown in Table 2. The results revealed that the C and L cookies exhibited significantly higher antioxidant activity (12.5% and 11.8% inhibition,

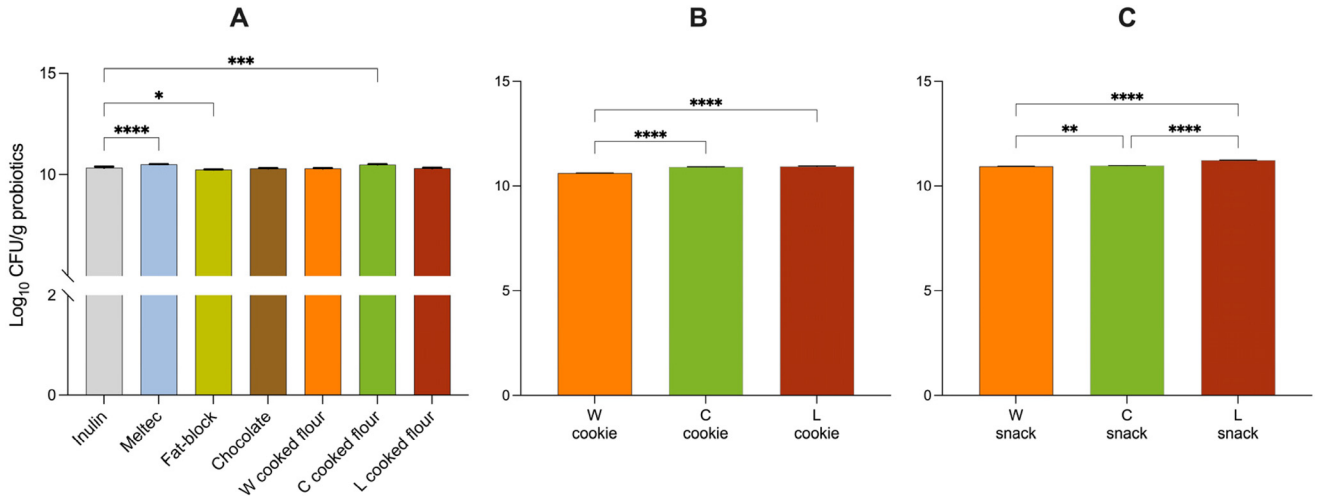

Fig. 2 Prebiotic activity (log_{10} CFU per g substrate) of ingredients (inulin, Meltec®, fat block, 72% dark chocolate), cooked W, C, and L flours (A), cookies (B) and snacks (C) in the presence of SLAB51® (W: wheat, C: chickpea, L: red lentil). CFU: colony-forming unit. Data are expressed as means ± standard deviation (n = 3). The asterisk on the figure represents the significance between the samples (*post-hoc* test, Holm–Šidàk *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 at a 95% confidence level).

respectively) compared to the W cookies (2.4% inhibition, p < 0.05; Table 2). No significant differences were found between the C and L cookies.

The prebiotic activity of cookies, their ingredients, and an inulin control, after being subjected to *in vitro* digestion (INFOGEST)[29] was evaluated based on their ability to stimulate the growth of SLAB51® probiotic strains (Fig. 2). Individual ingredients showed a prebiotic potential comparable to the inulin control, with the exceptions of Meltec® and cooked chickpea flour, which exhibited a higher potential, and fat-block, which, in contrast, showed slightly lower prebiotic potential compared to the control (Fig. 2A). Notably, all three cookies showed significantly higher prebiotic potential than their individual ingredients and the inulin control (p < 0.05; Fig. 2A and B). Moreover, pulse flour cookies (C and L) exhibited significantly higher prebiotic potential (p < 0.0001; Fig. 2B) compared to the control W cookie, after 48 h of incubation with the probiotics.

The acceptability and preference of the control cookies and pulse-based (L and C) samples were evaluated by a consumer panel (18–62 years old). Control cookies showed significantly higher overall acceptability (7.5 ± 1.0) compared to pulse-based cookies L (5.9 ± 1.7) and C (5.7 ± 1.3) (Fig. 3A). The significantly higher liking of W (p < 0.05) was primarily related to the attributes of texture, flavour, and aftertaste when compared with the two pulse flour cookies (L and C). No significant differences were found regarding the acceptability of the visual appearance and odour among the 3 cookies. The marked pre-

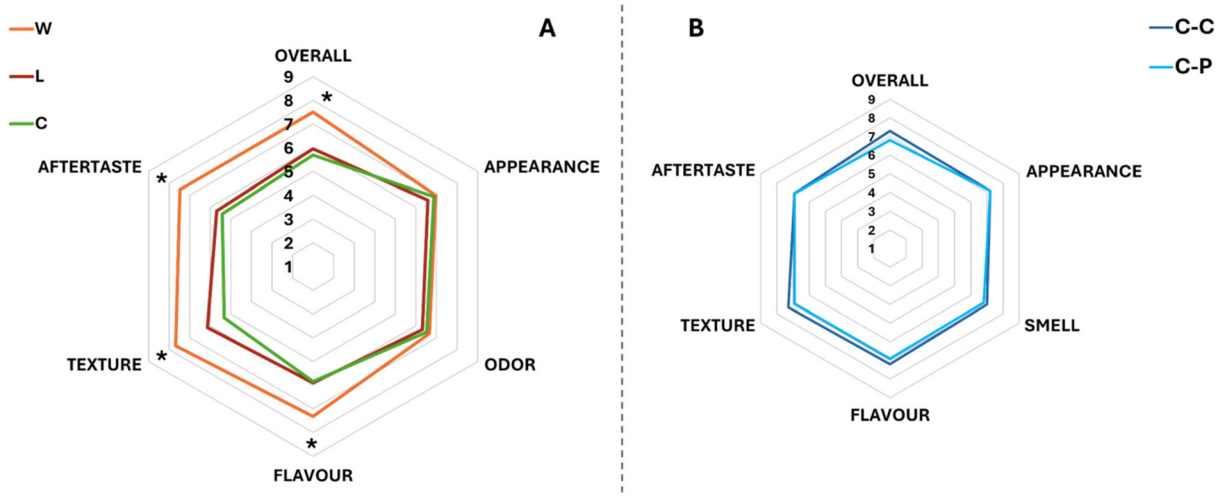

Fig. 3 Sensory acceptability results for (A) cookies (W, L, and C) and (B) chocolate without (C–C) and with probiotics (C–P). The asterisks on the figure (A) represent the significance among the samples (*post-hoc* test, Bonferroni *p < 0.05 at a 95% confidence level).

ference of the consumer for the W cookie was expected due to the familiarity of consumers with this traditional, widely consumed among Italians. To mitigate the potential negative impact of direct comparison with the highly familiar W cookie on the evaluation of L and C, an additional consumer test (n = 30, data not shown) was conducted to evaluate the sensory acceptability of only L and C cookies. This test revealed an improved overall acceptability for both pulse-based cookies (6.1 ± 1.5 and 6.5 ± 1.2 for L and C, respectively).

3.2. Probiotic chocolate characteristics

As shown in Fig. 2A, the ingredient chocolate exhibited a prebiotic activity comparable to that of the inulin control. Probiotic viability in chocolate enriched with SLAB51® stored at 25 °C and 4 °C was monitored over one year (Table 3). A gradual decrease in viability was observed over time, with a more pronounced decline in samples stored at 25 °C. After three months of storage at 25 °C, probiotic survival was above 80%. On the other hand, the viability remained at 88% even after six months of storage at 4 °C. At one year, 55.7% of probiotics were viable in samples stored at 4 °C, but none remained viable in samples stored at 25 °C. The final snack meets the FAO/WHO threshold throughout the shelf-life when stored at 4 °C.[32]

The key sensory attributes of tempered chocolate with the incorporated probiotics were evaluated to determine if potential alterations in chocolate perception, possibly due to fat crystallisation changes, occurred. Results from a consumer panel are presented in Fig. 3B. The addition of probiotics did not significantly affect the perceived sensory attributes. However, a slight, non-significant reduction in the overall acceptability, texture, smell, and flavour was observed (Fig. 3B).

3.3. Synbiotic snack characteristics

The calculated snack nutritional composition, which is reported in Table 4 in g per 100 g, aligns with the nutritional features discussed for the cookie fractions, as the chocolate coating contribution was comparable in all snacks. Indeed, snacks made with pulse flour had lower energy, saturated fats, and sugars, whereas they contained higher fibres and proteins than W snacks. Each serving of snacks (3 snacks) coated with chocolate also contained approximately 4.0 × 10^{10} CFU probiotics.

Table 3 Probiotic viability in chocolate (% residual SLAB51 viability in chocolate)

Probiotic viability (%)	25 °C	4 °C
Time (month)		
0	100.0 ± 6.8	100.0 ± 7.2
1	93.3 ± 7.5	108.2 ± 7.5
2	90.0 ± 6.8	111.5 ± 7.3
3	83.3 ± 6.3*	95.1 ± 6.7
6	41.7 ± 6.7*	88.5 ± 6.3
12	0.0 ± 6.0*	55.7 ± 6.0*

Data are reported as mean ± standard deviation (n = 2). The asterisk shows significant differences with the viability at t_0 in the same column (p < 0.05, paired Student's t-test).

Table 4 Nutritional composition of 100 g of snack (W – wheat cookie, control, L – red lentil cookie, and C – chickpea cookie)

	W-snack	C-snack	L-snack
Energy (kcal)	496.5	455.5	450.7
Energy (kJ)	2083.7	1906.5	1889.2
Total fat (g)	22.8	20.5	18.2
of saturated fat (g)	12.7	6.8	6.5
Total carbohydrates (g)	65.6	47.0	49.9
of sugars (g)	27.9	16.0	16.0
of total starch (g)	37.7	27.7	31.8
Dietary total fibre (g)	3.8	13.9	13.1
Total protein (g)	9.6	16.9	18.3
Salt (g)	0.07	0.09	0.07

The prebiotic activity of the final snacks obtained by coating prebiotic-rich cookies with dark chocolate is shown in Fig. 2C. With snack digesta as nutritional substrates for the probiotic mix, the L snack presented the highest prebiotic potential (p < 0.0001) after 48 h of incubation, followed by the C snack (p < 0.01), when compared to the W snack. As expected, when compared to their cookie counterparts, the addition of chocolate significantly boosted the prebiotic potential of all prebiotic-rich cookies (W: p < 0.0001, C: p < 0.01, L: p < 0.0001; Fig. 2B and C).

Snacks were stored at 25 °C and 4 °C for 12 months, and their shelf-life stability was monitored by measuring moisture content, hardness, and chocolate colour (Table 5). The moisture content of freshly produced snacks was comparable (p > 0.05), which was about 2–3%, as expected for this product category. After one month's storage at both 25 °C and 4 °C, L and C snacks became moister compared to the W snack (p < 0.05). The moisture content of the three snacks increased slightly over 12 months of storage, reaching up to 5.5%. The observed fluctuation in the cookie moisture content was relatively small. As observed, the moisture content in snacks made with wheat flour changed at a slower rate compared to the C and L snacks. However, even though the differences were small, as indicated by the MANOVA test, moisture content was found to be statistically influenced by both flour type (F = 89.5, p < 0.001), storage time (F = 76.5, p < 0.001), and the interaction of storage temperature and time (F = 5.9, p < 0.001). However, storage temperature did not significantly impact the moisture content (F = 0.3, p > 0.05; Table 5). The hardness of freshly produced W and L snacks was found to be softer than C snacks (p < 0.05; Table 5), with a strong impact of flour type (F = 25.1, p < 0.001). After six months of storage at 25 °C or 4 °C, the C snack was still the hardest one among the three snacks (p < 0.05). The hardness of all snacks gradually increased up to 6 months of storage (F = 6.4, p < 0.001), without a statistical difference due to storage temperature (F = 1.0, p > 0.05, Table 5). Consequently, the snack hardness was strongly influenced by the interaction of flour type with storage temperature (F = 18.9, p < 0.001), storage time (F = 3.4, p < 0.001), and by

Table 5 Moisture content (cookie fraction), hardness (snack), and whitening index (chocolate fraction) of the control (W), red lentil (L), and chickpea (C) snacks stored at 4 °C and 25 °C for 12 months

Snack	W-25 °C	L-25 °C	C-25 °C	W-4 °C	L-4 °C	C-4 °C
Time (month)						
Moisture (%)						
0	2.5 ± 0.3A^{e}	3.3 ± 0.7A^{d}	3.4 ± 0.2A^{d}	2.5 ± 0.3A^{d}	3.3 ± 0.7A^{b}	3.4 ± 0.2A^{d}
1	3.5 ± 0.1B^{c}	4.4 ± 0.1A^{bc}	4.6 ± 0.1A^{bc}	3.3 ± 0.0B^{c}	4.6 ± 0.1A^{a}	4.6 ± 0.1A^{bc}
2	4.3 ± 0.1B^{b}	5.0 ± 0.1A^{abc}	5.1 ± 0.1A^{ab}	3.9 ± 0.2B^{ab}	4.8 ± 0.0A^{a}	5.0 ± 0.3A^{abc}
3	4.3 ± 0.1B^{ab}	5.2 ± 0.1A^{ab}	5.1 ± 0.2A^{ab}	4.1 ± 0.2B^{a}	4.7 ± 0.2AB^{a}	5.1 ± 0.4A^{ab}
6	4.7 ± 0B^{a}	5.3 ± 0.1A^{a}	5.5 ± 0.2A^{a}	4.2 ± 0.1B^{a}	5.1 ± 0.2A^{a}	5.5 ± 0.1A^{a}
12	3.0 ± 0.1B^{d}	4.3 ± 0.0A^{c}	4.0 ± 0.3A^{c}	3.6 ± 0.1B^{bc}	4.7 ± 0.1A^{a}	4.4 ± 0.2A^{c}
Hardness (N)						
0	41.2 ± 3.6B^{bc}	43.6 ± 4.9B^{c}	66.4 ± 5.8A^{b}	41.2 ± 3.6B^{b}	43.6 ± 5.2B^{b}	66.4 ± 6.4A^{ab}
1	45.7 ± 5.2B^{bc}	57.1 ± 5.6B^{bc}	68.9 ± 8.3A^{b}	58.1 ± 5.2A^{ab}	47.1 ± 4.6B^{ab}	68.0 ± 4.9A^{ab}
2	49.8 ± 4.3B^{ab}	70.2 ± 9.0A^{a}	73.8 ± 6.4A^{ab}	60.7 ± 5.6A^{ab}	56.1 ± 7.1A^{ab}	68.9 ± 8.4A^{ab}
3	62.1 ± 8.3B^{a}	79.4 ± 7.3A^{a}	84.7 ± 5.7A^{ab}	65.4 ± 6.2A^{a}	63.8 ± 6.4A^{a}	77.0 ± 6.3A^{ab}
6	50.5 ± 7.1B^{ab}	71.2 ± 9.1A^{a}	90.8 ± 9.8A^{a}	60.1 ± 7.5B^{ab}	64.7 ± 8.3B^{a}	86.0 ± 8.9A^{a}
12	32.9 ± 10.6C^{c}	64.0 ± 10.8B^{b}	85.5 ± 9.7A^{ab}	57.0 ± 10.4A^{ab}	38.8 ± 12.9B^{b}	63.4 ± 11.6A^{b}
Whitening index						
0	28.9 ± 0.6A^{bc}	28.7 ± 0.4A^{ab}	28.8 ± 0.3A^{b}	28.9 ± 0.6A^{b}	28.7 ± 0.4A^{ab}	28.8 ± 0.3A^{b}
1	27.7 ± 0.4B^{c}	29.3 ± 0.8A^{ab}	28.8 ± 0.5A^{b}	27.2 ± 0.5A^{c}	27.4 ± 0.3A^{b}	27.9 ± 0.5A^{b}
2	29.6 ± 0.6A^{b}	29.8 ± 1.4A^{ab}	29.0 ± 0.4A^{b}	30.2 ± 0.5A^{a}	29.5 ± 0.6A^{a}	30.3 ± 0.8A^{a}
3	28.2 ± 0.6A^{bc}	28.3 ± 0.7A^{b}	29.6 ± 1.9A^{b}	28.1 ± 0.6A^{bc}	28.1 ± 0.4A^{ab}	28.3 ± 0.8A^{b}
6	28.7 ± 0.4A^{bc}	28.9 ± 0.6A^{ab}	28.9 ± 0.93A^{b}	29.5 ± 0.5A^{ab}	28.8 ± 0.7A^{ab}	28.7 ± 0.7A^{b}
12	30.7 ± 1.2A^{a}	30.2 ± 1.5A^{a}	31.7 ± 2.4A^{a}	28.9 ± 0.7A^{b}	28.4 ± 0.6A^{ab}	28.2 ± 0.5A^{b}

MANOVA	Moisture	Hardness	Whitening index
Storage temperature			
F	0.3	1	5.5
p	ns	ns	*
Storage time			
F	76.5	6.4	5.4
p	***	***	**
Flour type			
F	89.5	25.1	0.4
p	***	***	ns
Storage temperature × storage time			
F	5.9	2.2	23.4
p	***	ns	***
Storage temperature × flour type			
F	1.4	18.9	4.8
p	ns	***	*
Flour type × storage time			
F	1.7	3.4	3.3
p	ns	***	**
Storage temperature × flour type × storage time			
p	0.6	2.2	3
F	ns	*	**

Data are presented as mean ± standard deviation. Within each table section, means followed by different uppercase letters represent significant differences ($p < 0.05$) between the three snacks at the same storage temperature and time. Different superscript lowercase letters indicate significant differences for the same sample stored at the same temperature across different storage durations (*post-hoc* test, Bonferroni $p < 0.05$ at a 95% confidence level). ns: not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

both temperature and time ($F = 2.2$, $p < 0.05$). At 12 months of storage, most pulse-based snacks (8–10 pieces of each snack) were broken, and some intact pieces were relatively fragile, suggesting increased fragility of the products as reflected by the significantly decreased hardness and large standard deviation at 12 months of storage ($p < 0.05$).

Chocolate bloom stability was also studied as the addition of probiotics into the tempered chocolate might also affect fat crystallisation, therefore affecting its tendency to bloom (Table 5). Throughout 12 months, the whitening index of the chocolate coating from the three snacks was comparable at the same storage duration. The whitening index of chocolate coating was found to increase only slightly, even though significantly ($F = 5.4$, $p < 0.01$), up to 12 months of storage in snacks stored at both 25 °C and 4 °C, indicating good stability of the snacks during storage.

3.3.1 Sensory analysis and willingness to buy. The sensory analysis of two pulse flour snacks (C and L) was conducted through a consumer test to assess their perception and acceptability among older adults (>65 years old), including overall acceptability and key product attributes (Fig. 4). The overall liking of C and L snacks received scores of 6.1 and 6.0, respect-

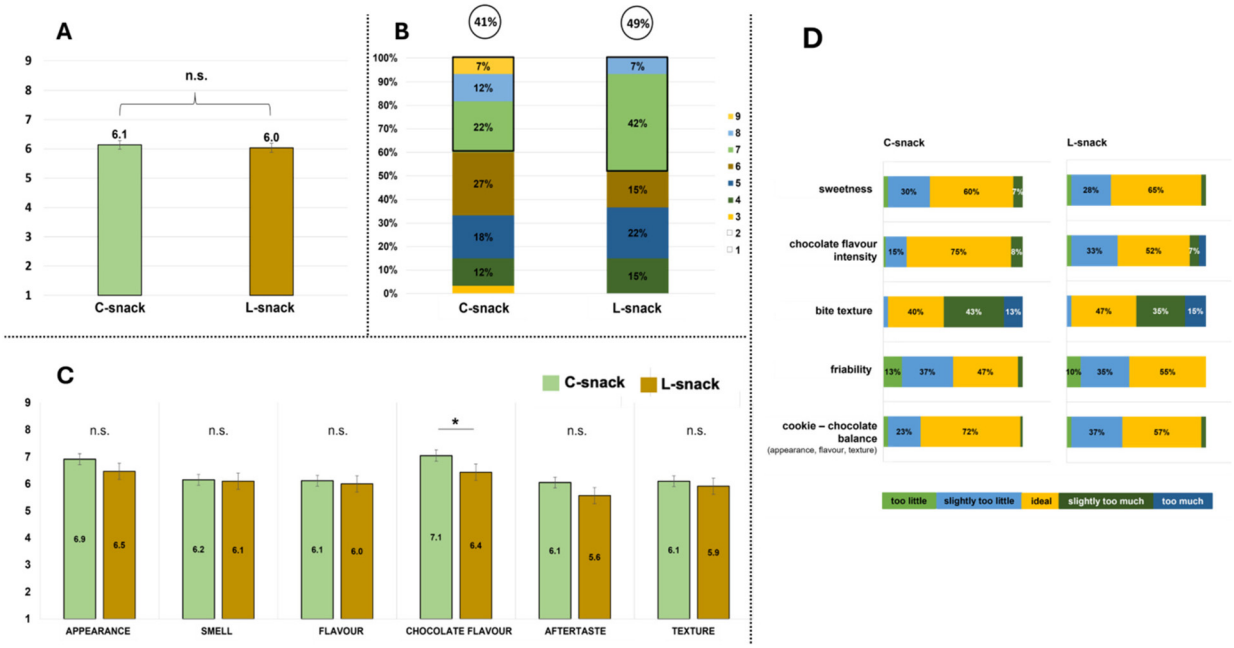

Fig. 4 Sensory analysis results ((A): overall liking; (B): top 3 boxes of overall liking [ratings between 7 and 9]; (C): key sensory attributes; (D): penalty analysis) of chickpea-chocolate and lentil-chocolate snacks as perceived by a >65-year-old consumer panel. Student's *t*-test, $*p < 0.05$, significantly different; n.s.: no significance.

ively (Fig. 4A). The top value of the three boxes revealed that the overall liking of the two snacks was comparable; however, the L snack had a better overall acceptance than the C snack (49% *vs.* 41%), although the C snack's liking was skewed towards higher liking grades. All key sensory attributes (Fig. 4C) were found to oscillate around a score of 6, and no significant differences were found between the two snacks, with the exception of chocolate flavour, which was significantly higher in the C snack than in the L snack. Both snacks were mainly penalised in terms of texture: snacks were perceived as slightly too hard/too hard (56% C snack and 40% L snack), and not friable/slightly not friable (50% C snack and 45% L snack). These findings clearly indicated that the product's optimization, necessary to bring them to the market, should focus primarily on physical properties (*i.e.*, texture) as they are important for enhancing its intrinsic sensory appeal. The most pronounced difference in perception between the two samples was related to the chocolate flavour intensity and, more

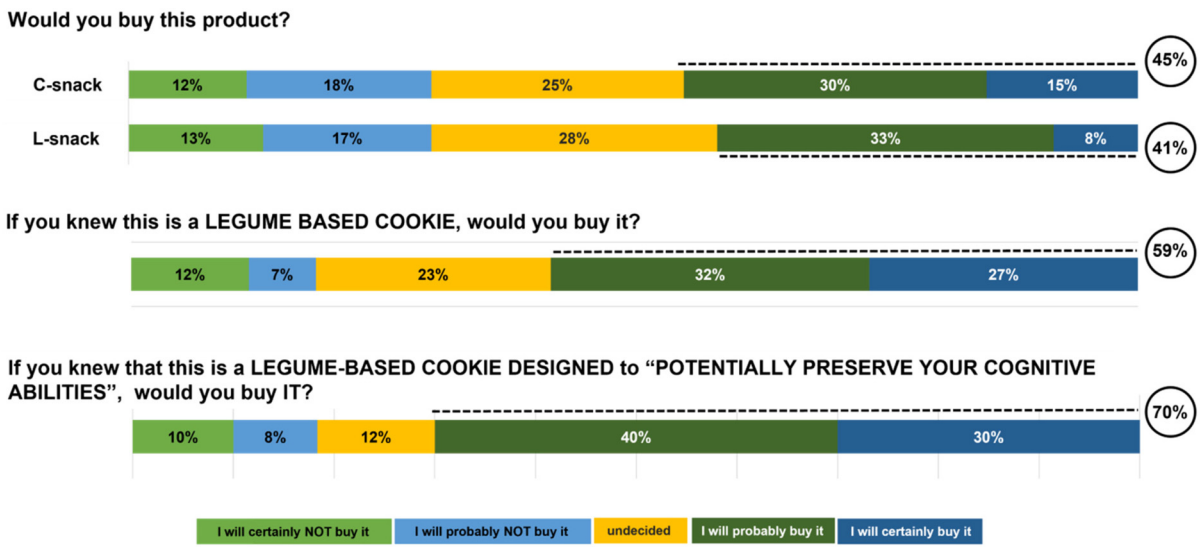

Fig. 5 The willingness to purchase chickpea-chocolate and lentil-chocolate snacks under different conditions in a >65-year-old consumer panel.

broadly, to the balance between chocolate and biscuit (Fig. 4D). In particular, for the L snack, there was an increase in penalties concerning the lower intensity of the chocolate flavour, and a higher number of consumers, compared to the C snack, considered the chocolate-to-biscuit ratio to be less balanced. The preference towards the C snack was mainly driven by the flavour, the greater ability to convey the chocolate flavour and, more generally, by a preference towards the aromatic olfactory characteristics of the product. In contrast, the consumers who preferred the L snack were mainly concerned with its texture characteristics.

Willingness to buy was also investigated and assessed at 45% and 41% in the C snack and the L snack, respectively, when no information about cookies was provided to the consumers (Fig. 5). Interestingly, willingness increased by 14% (to 59%) when consumers were informed that the snacks contained pulse flour, and this was further enhanced by an additional 11% (to 70%) upon disclosure of the potential health benefit ("potentially preserve your cognitive abilities"). Moreover, up to 70% of the panel considered this information highly relevant to their decision-making process, with 18% rating it as extremely important and 50% as very important.

4. Discussion

Synbiotics are combinations of an exogenous probiotic and a prebiotic, where the prebiotic serves as a specific substrate for the probiotic, promoting its growth and/or activity.[33] Cereal-based products have the potential to be developed into effective synbiotic food systems, as they can combine encapsulated probiotic strains with whole grains or pulse-enriched snacks, which can act as a valuable prebiotic source.[34] In this context, the authors developed synbiotic snacks by coating prebiotic-rich cookies with 72% chocolate enriched with SLAB51® for a specific group of consumers. The probiotic formulation used (SLAB51®) was selected because of its documented neuroprotective properties[16–18] and the neuroprotective properties of a lentil-based synbiotic snack observed in Alzheimer's disease mice.[19] This synbiotic snack was, thus, developed for individuals over 65 years old, as they are the population segment that can benefit the most from the consumption of this product, whether the efficacy in supporting cognitive health was proven in a future clinical trial.

We described step by step the development of these snacks by assessing the nutritional features of the cookie fraction, including its glycaemic index. We evaluated the prebiotic characteristics of the ingredients, which were found to be more prebiotic than the well-known prebiotic inulin. We also evaluated the stability of these snacks over a year at two different storage temperatures and carried out a sensory test with our target consumers to gauge their preference and willingness to buy these snacks. The results were very promising, as the developed synbiotic snacks possessed positive characteristics and were positively received by the target consumers, especially when they were aware of their potential to preserve cognitive abilities.

The convenience of snack consumption has led to a significant increase over recent decades, now making up over 30% of daily energy intake, including for older adults who often face challenges in obtaining and preparing healthy food.[3,35] The developed synbiotic snacks produced with pulses and a fibre mix offer nutritional advantages compared to their standard wheat-based counterpart (W). They are a good source of fibre and protein, while containing less total fat and sugar than their wheat counterparts. Furthermore, they are rich in beneficial unsaturated fatty acids (*i.e.*, MUFAs and PUFAs) per 100 g (Table 2). They also exhibit strong antioxidant properties, which stem from the high fibre, protein, and bioactive compounds present in pulses.[31] Pulses, fibre, and PUFAs are nutritional components that are frequently deficient in the diets of elderly individuals.[36] For a standard 30 g serving (around 3 cookies per day), the developed cookie formulation provides approximately 4 g of dietary fibre and 5.5 g of protein, compared to 1.2 g of fibre and 3.2 g of protein typically found in conventional cookies.[37] These nutritional enhancements address two common concerns in the elderly population: inadequate fibre intake, which is often linked to constipation and other gastrointestinal dysfunctions, and insufficient protein intake, contributing to the loss of skeletal muscle mass, function, and strength.[38] Regular consumption of the formulated cookies may help increase dietary fibre intake, moving toward the European Food Safety Authority (EFSA) recommendation of 25 g per day, which is considered sufficient to promote normal laxation and overall gastrointestinal health.[39] In addition, these cookies may help older adults achieve the suggested protein intake of 1.1 g kg^{-1} body weight per day, particularly by providing vegetable protein, which is valuable in the context of a transition toward more plant-based diets.[40,41]

In addition, the use of pulse flours, together with the substitution of sugar and butter with a fibre mix (Meltec®) and the fat block, reduced the GI of these cookies compared to standard cookies (W) or commercial cookies, which typically exhibit a GI between 47 and 58.[42] This effect is probably due to the higher protein and fibre content, as well as the lower sugar content of the formulation. The high fibre content from pulses and the fibre ingredients may increase digestive viscosity by acting as a physical barrier in the small intestine, limiting contact between starch and amylolytic enzymes and leading to delayed gastric emptying.[43] Additionally, pulse proteins tightly bound to carbohydrates can further slow digestion by limiting starch accessibility. The high protein content has been linked to increased insulin release and lower blood glucose levels.[44,45] Interestingly, chickpea cookies exhibited a lower GI compared to lentil cookies. Based on existing literature, it can be hypothesised that this difference may be attributed to several factors, including starch granule size and the ratio of amylose to amylopectin. In general, depending on the cultivar, chickpea starch granules (15–30 μm) are larger than lentil granules (6–37 μm).[46] Moreover, chickpeas contain a higher amount of

amylose (33–40%), depending on the cultivar, whereas lentils contain 24–32% amylose.[46,47] The larger granule size and denser amylose structure in chickpeas may have limited starch hydrolysis, thereby slowing glucose release into the bloodstream. Additionally, the overall nutritional composition of chickpeas, including their relatively higher fat and fibre content along with proteins, might have contributed to slower carbohydrate absorption, resulting in a reduced GI.[48]

Incorporating pulse flour into cookies and snacks positively enhanced their prebiotic potential, likely due to the high dietary fibre content, which can stimulate probiotic growth and/or activity during fermentation.[49,50] Interestingly, no significant differences in prebiotic activity were observed among the individual ingredients and the cookies. We did not measure the probiotic activity of cookies coated with plain chocolate. Therefore, we cannot conclude that the addition of SLAB51® was the only reason for the increased probiotic activity in the snack, since, for example, the fibre in the chocolate itself might have boosted the prebiotic activity. However, a notable increase in probiotic activity was observed when chocolate enriched with SLAB51® was added. As reported in the literature, encapsulated probiotic strains in chocolate are well-protected from environmental stress, and their viability in the small intestine was three times higher than in dairy products, attributed to the cocoa butter lipid fraction. Furthermore, the high phenolic content of cacao in chocolate, thanks to its antioxidant capacity, may offer additional probiotic protection.[14,50] Consistent with the findings of Kemsawasd *et al.* (2016),[14] probiotic activity remained nearly unchanged for 6 months and decreased by half after 12 months at 4 °C, while at 25 °C, it decreased by half after 6 months but preserved high activity in the first 3 months, showing only minimal blooming even after one year of storage, unlike some other chocolate products.[51] Therefore, 4.0×10^{10} CFU of probiotics in one serving (30 g, 3 snacks) of our freshly produced synbiotic snacks decreased to 3.5×10^{10} CFU and 2.2×10^{10} CFU when stored at 4 °C for 6 and 12 months, respectively, while it decreased to 3.3×10^{10} CFU and 1.7×10^{10} CFU when stored at 25 °C for 3 and 6 months, respectively. Thus, one portion of the freshly produced synbiotic snacks contains the probiotics that meet the probiotic intake guidelines set by the FAO/WHO of 10^6–10^7 CFU per g or mL for probiotic health benefits.[52] Moreover, the developed snacks meet the FAO/WHO threshold throughout the studied shelf life (12 months) when stored at 4 °C, and within 6 months when stored at 25 °C. Probiotic consumption can improve inflammatory markers, modulate neurological signaling, and reduce oxidative stress. It is well documented in the literature that the combined effect of prebiotics and probiotics can have a beneficial impact on gut microbiome health, preventing endotoxins from entering the bloodstream, which can lead to diseases, such as diabetes, non-alcoholic fatty liver disease, and obesity.[53,54] In particular, probiotics can rebalance the gut microbiota and restore the pathways that link microbial activity with the host's metabolism.[55] Moreover, among others, the probiotic used in the development of these snacks may contribute to counteracting the onset and progression of neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, at least in mouse models.[16,18] We have recently demonstrated in a preclinical trial that long-term consumption of the lentil-based synbiotic snack affected the gut microbiota composition, preserved cognition, reduced amyloid load, improved glucose and lipid homeostasis and diminished oxidation and inflammation in Alzheimer's disease mice.[19]

From a consumer perspective, pulse flour synbiotic snacks received a score between 6 and 6.1 on a 1–9 scale. These scores indicate moderate acceptability, suggesting that consumer acceptance is currently limited by the product's sensory characteristics, such as its texture. This highlights a clear necessity for further sensory refinement, which is likely achievable through product optimization in an industrial setting to bring it to market. Interestingly, older consumers showed a strong interest in purchasing these snacks once they learned about their potential benefits for preserving cognitive abilities, with their willingness to purchase them increasing by up to 70%. Consistent with previous research, older adults tend to be cautious about unhealthy foods and are more inclined to purchase healthy foods that contain functional ingredients to address multiple age-related health concerns.[56,57] The pronounced impact of health-related information, especially when contrasted with moderate sensory acceptance, highlights the need for a dual strategy to ensure market viability. This involves, on one hand, the targeted improvement of the sensory profile to address the negative textural attributes that act as key barriers to consumer acceptance and, on the other hand, the development of a clear and compelling communication strategy that effectively conveys the expected cognitive health benefits of the product based on our promising *in vitro* study as the primary driver of purchase intent and a sign of strong market potential.

In conclusion, the developed synbiotic snack delivers prebiotic fibres along with live neuroprotective probiotics that might improve gut–brain axis health. This research provides robust scientific support for the development of functional food products tailored for an aging population. The formulation incorporated chickpea or red lentil flour as a source of both protein and fibre, with a significant reduction in sugar and saturated fat, due to the use of fibre syrup to partially substitute sugar and butter, and lower GI. The inclusion of probiotic-rich chocolate enhanced the functionality of snacks. Moreover, the developed snacks had a good shelf life with minimal chocolate blooming and high probiotic viability after three months of storage under both ambient (25 °C) and refrigerated (4 °C) conditions. The developed snacks showed moderate sensory acceptability among older adults, with texture emerging as the primary aspect requiring improvement. Moreover, the disclosure of health benefits substantially enhanced purchase intent, indicating that effective communication of functional attributes is critical for market success. Further experiments are necessary to optimise the snack's texture (possibly by partial replacement of the fat block with other fat sources or favouring product expansion/aeration

during processing) to meet the needs of elderly consumers. The current study successfully developed a snack with a promising composition (prebiotics and probiotics), providing a rationale for expecting gut–brain axis health benefits, as supported by findings from a separate preclinical study in a mouse model. However, it is pivotal to prove the effects of consuming the synbiotic snacks in a human intervention trial measuring gut microbiota changes and cognitive outcomes in the elderly population, which remains an area for future research. Such studies could confirm the potential benefits of the developed synbiotic snacks, paving the way for future advancements in functional foods targeting age-related health challenges.

Author contributions

Xinying Suo: data curation, formal analysis, investigation, visualization, and writing – original draft-lead; Marianna Tagliasco: data curation, formal analysis, investigation, visualization, and writing – original draft-lead; Laura Bonfili: conceptualization, funding acquisition-lead, formal analysis, and writing – review & editing; Francesco Maria Grasselli: investigation and data curation; Matteo Bonfini: data curation, formal analysis, and writing – review and editing; Lucia Bailetti: formal analysis and writing – review and editing; Dennis Fiorini: data curation, formal analysis, and writing – review and editing; Anna-Rita Attili: data curation, formal analysis, and writing – review and editing; Shaymaa B. Abdulrazzaq: formal analysis and writing – review and editing; Anna Maria Eleuteri: conceptualization, funding acquisition-lead, and writing – review and editing; Nicoletta Pellegrini: conceptualization, funding acquisition-lead, and writing – review and editing; and Elena Vittadini: conceptualization, funding acquisition-lead, project administration-lead, supervision-lead, visualization, and writing – review and editing.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

Data will be made available upon request.

Supplementary information (SI) is available (Table S1. Individual fatty acid composition). See DOI: https://doi.org/10.1039/d5fo02051d.

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