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Research article

Crackers enriched with Savoy cabbage and *Annurca* apple by-products: DPPH radical-scavenging activity, enzyme inhibition and predicted glycaemic index

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Abstract: The growing demand for healthier and more sustainable food options is prompting the food sector to develop products that deliver nutritional benefits while reducing waste. This study examines a dual valorization strategy that integrates both vegetable and fruit by-products and underutilized seed oils into cracker formulations. Powders derived from *Brassica oleracea* var. *sabauda* (Savoy cabbage) by-products and *Malus domestica* var. *Annurca* peels were used to partially replace wheat flour (5% and 10%), while cold-pressed broccoli and cherry seed oils served as alternative lipid sources. This combined use of fiber- and polyphenol-rich powders with alternative seed oils represents a novel, circular approach to functional bakery design. Four experimental formulations (C1-C4) were prepared alongside a control, replacing wheat flour with 5% or 10% *Brassica oleracea* var. *sabauda* by-products plus broccoli seed oil (C1, C2) or with 5% or 10% *Annurca* apple peel powder plus cherry seed oil (C3, C4). Enrichment increased total polyphenol content and DPPH radical-scavenging activity compared with the control, with the highest values observed in the 10% *Annurca* peel formulation (C4; 267.7 μg GAE/g and 43.9%, respectively). The enriched crackers also showed lower IC50 values in the in vitro assays against α-amylase, α-glucosidase and pancreatic lipase, particularly C4, which exhibited the strongest inhibitory activity. In vitro starch digestion resulted in significantly lower predicted glycemic index (pGI) values in all enriched formulations compared with the control, with reductions ranging from approximately 17.5 to 23.8%. These findings indicate that incorporating plant-based by-products can modify the DPPH radical-scavenging activity, in vitro digestive enzyme inhibitory potential, and predicted glycemic response of crackers. Overall, the results support the potential of these by-products for the development of more resource-efficient bakery products, while further studies are needed to characterize individual and combined contributions of these ingredients.

Keywords: enriched crackers; DPPH radical-scavenging activity; α-amylase inhibition; lipase inhibition; predicted glycemic index-pGI

1. Introduction

Crackers rank among the most popularly consumed baked snack foods because they are practical, have a long storage stability, and pleasant sensory characteristics. Conventional formulations are based on soft wheat flour, water, and fats, producing a low-moisture product with a crisp texture [1]. However, the growing demand for foods that are both healthy and sustainable has prompted research into the reformulation of conventional bakery items with functional and upcycled ingredients. In this context, crackers represent an ideal vehicle for nutritional improvement and the valorization of food processing residues [2]. Food by-products, particularly those rich in phytochemicals and dietary fiber, have attracted increasing interest as natural functional ingredients capable of enhancing the nutritional profile of cereal-based foods. Phenolic compounds are of particular interest due to their antioxidant activity and their reported contribution to the prevention of non-communicable diseases, including diabetes, cardiovascular disorders, and obesity [3]. Among the various plant sources, by-products from *Brassica oleracea* var. *sabauda* (Savoy cabbage) and *Malus domestica* var. *Annurca* (apple peels) are well recognized for their richness in bioactive substances, such as flavonoids, phenolic acids, and glucosinolates, as demonstrated in previous analytical and functional studies [4,5]. Despite this well-documented potential, their application in bakery formulations remains limited, particularly in products with a strong regional identity.

A complementary and underexplored strategy for functional enrichment involves the use of seed-derived oils from fruit and vegetable processing residues. Fruit seeds constitute a substantial proportion of food processing residues and are recognized as a precious resource of essential fatty acids, dietary fiber, amino acids, micronutrients, and bioactive lipids. Oils extracted from grape, pomegranate, and rosehip seeds are appreciated for their rich content of linoleic and oleic acids and their associated health-promoting properties [6,7]. In line with these findings, previous studies conducted by our research group demonstrated that cold-pressed broccoli and cherry seed oils are rich in unsaturated fatty acids, particularly erucic, linolenic, and linoleic acids, and exhibit antioxidant and anti-inflammatory activities [8,9]. In addition to their health-promoting properties, these oils respond to consumer demand for sustainable products and enable the food industry to valorize by-products that would otherwise be discarded. Cold-press extraction further preserves their nutritional quality, reinforcing their suitability as functional ingredients. When incorporated into baked goods such as biscuits, snacks, or creams, seed oils can replace less healthy fats, offering consumers affordable and healthy products while also supporting more sustainable and economically advantageous production chains [10,11]. Nevertheless, their technological and nutritional potential in baked goods has received little scientific attention.

The present study proposes an integrated upcycling approach that simultaneously exploits two classes of agri-food by-products: solid plant residues, converted into powders, and seed oils used as alternative lipid sources for the reformulation of crackers with enhanced nutritional and functional properties. This dual valorization strategy addresses multiple waste streams while combining phenolic- and lipid-derived bioactives in a single product matrix. Specifically, wheat flour was partially replaced (5% and 10%) with powders from *Brassica oleracea* var. *sabauda* or *Malus domestica* var. *Annurca*,

while cold-pressed broccoli or cherry seed oils were incorporated at 10%. The resulting formulations were evaluated for total polyphenol content and DPPH radical-scavenging activity, as well as in vitro enzymatic inhibitory activities related to glycemic control. The study provides a basis for future investigations into the individual and potential synergistic contributions of these ingredients.

2. Materials and methods

2.1. Materials

Pancreatin from porcine pancreas (P7545), pepsin (77161), pancreatic α-Amylase (A 6255), yeast α-glucosidase (G5003), dinitro salicylic acid (DNSA) color reagent (D0550), pancreatic lipase (L3126), acarbose (A8980), orlistat (O4139), pNPB (p-nitrophenyl butyrate), p-nitrophenyl-α-D-glucopyranoside (N1377), and gallic acid (8426490025) were bought from Merck KGaA, Darmstadt, Germany. Acetone, hydrochloric acid, sodium hydroxide, ethanol, and sodium carbonate were purchased from Carlo Erba Reagents, Italy. Wheat flour, Mela *Annurca* PGI (Protected Geographical Indication), edible broccoli seed oil, cherry seed oil, and bread wheat flour were acquired in a shop in Avellino (Italy). As specified by the manufacturers, the BioPurus Certified Organic Seed Oils (United Kingdom) were obtained from cultivated plants and mechanically cold-pressed, avoiding the use of solvents. Samples were stored at 4 °C, avoiding exposure to light until use.

2.2. Preparation of vegetable by-product powders

The apples and brassica leaf scraps were cleaned with deionized water. The *Annurca* apple peels were sliced and dried at 50 ± 2 °C in a tray dryer (Melchioni Babele 250 W, 5 shelves) for ten hours. The scrap leaves of the cabbage Brassica oleracea were dried at 50 ± 2 °C for two hours. Then, the dried materials were milled (Kenwood CH580) and sifted through a 500 μm sieve to obtain the powders. These were either sealed in dark bags for further analysis or added to wheat flour at two concentrations (5% or 10%) for the sample preparations.

2.3. Preparation of crackers

Crackers were prepared by combining 250 g of wheat flour or wheat flour partially substituted with different quantities of plant-based by-product powders (designated as C1, C2, C3, C4) in a stainless-steel mixing bowl. The dry ingredients were homogenized using a laboratory mixer (Kenwood CH580) for 2 minutes. Subsequently, 1.5 g of salt and 25 g of seed oil were added and mixed until a uniform blend was achieved. Water (120 mL) was slowly incorporated to obtain the desired dough consistency, ensuring proper hydration and cohesiveness of the matrix. The dough was allowed at room temperature for 10–15 minutes to promote relaxation and facilitate subsequent handling. Following resting, the dough was laminated to a thickness of 3 mm using a dough sheeter (Ampia 150). Circular cracker shapes were obtained using a standardized round cutter. The shaped dough pieces were baked in a hot air oven (Moulinex OX4648) at 160 °C for 15 minutes. The crackers were cooled to ambient temperature and then immediately packed in airtight bags to preserve quality and prevent moisture absorption. The samples were labelled according to their formulation as follows: C: Control (100% wheat flour, 10% sunflower seed oil), C1 (wheat flour crackers with 5% Brassica

oleracea powder, 10% broccoli seed oil), C2 (wheat flour crackers with 10% Brassica oleracea powder, 10% broccoli seed oil), C3 (wheat flour crackers with 5% apple peel powder and 10% cherry seed oil), and C4 (wheat flour crackers with 10% apple peel powder and 10% cherry seed oil). All cracker samples were stored at ambient conditions (22–25 °C) in a dry environment until further analyses were conducted.

2.4. Physicochemical properties of samples

2.4.1. Water and oil absorption capacity

The method described by Wang et al. [12] was applied to measure the water and oil absorption capacities of the various samples, with some modification. In brief, the cracker 1.0 g was mixed with 10 mL of deionized water or seed oil, left at room temperature for 60 min, and centrifuged for 10 min at 2000×g. The water holding capacity (WHC) and oil holding capacity (OHC) were reported as the number of grams of water or oil retained per gram of sample. All analyses were conducted in triplicate (n = 3). The following equation was applied:

$$\text{WHC or OHC} = [\text{Residue weight (g)} - \text{Sample weight (g)}]/\text{Sample weight (g)} \quad (1)$$

2.4.2. Swelling power

Swelling power (SP) was evaluated with the procedure reported by Gani et al. [13], with some modifications. The experiments were performed in triplicate (n = 3), and the SP value was calculated using the equation:

$$\text{SP} = \text{Paste weight (g)}/\text{Dry flour weight (g)} \quad (2)$$

2.5. Total phenol content

The total polyphenol content (TPC) was calculated with the method described by Ombra et al. [14]. Briefly, the Folin–Ciocalteu reagent and sodium carbonate solution were added to the samples and to the gallic acid standard solutions used for calibration. The reaction mixtures were incubated for 2 h in the dark at room temperature, after which the absorbance was measured at 765 nm using a Cary 50 Bio spectrophotometer (Varian-Agilent). The calibration curve was prepared using gallic acid at concentrations ranging from (0.05 to 0.5 mg/mL), and the results were expressed as μg gallic acid equivalents per g of sample (μg GAE/g).

2.6. Polyphenols extraction

Polyphenols were extracted from crackers according to the method described by Bustos et al. [15], with some modifications. Briefly, 1 g of each cracker sample was extracted with 5 mL of 70 % acetone (in H_2O) and stirred at 26 °C for 2 hours. Following centrifugation at 10,000 g for 12 minutes, the supernatants were recovered. The extraction process was repeated, and the collected supernatants were combined. Solvent removal was achieved using a rotary evaporator, and the resulting dried residue was dissolved in water before subsequent assays. The extracts were used for the determination of total

polyphenol content, DPPH radical-scavenging activity, and for the in vitro enzyme inhibition assays. The extraction procedure was intended to obtain a polyphenol-rich fraction for comparative evaluation among formulations and did not provide a qualitative or quantitative characterization of individual phenolic compounds.

2.7. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) test

The free-radical scavenging activity of the extracts was evaluated using the DPPH assay according to Brand-Williams et al. [16], with modifications. The test was carried out in microplates by mixing 15 µL of extract with 300 µL of 6×10^{-5} M DPPH methanolic solution. The absorbance was measured at 517 nm using a Cary 50 MPR spectrophotometer (Varian-Agilent). The absorbance of the DPPH solution without extract was measured as a control. The percentage of DPPH free radical scavenging activity (I%) was calculated using the formula:

$$I\% = [(A\ blank - A\ sample)/A\ blank] \times 100 \quad (3)$$

where A blank represents the absorbance of the control (containing all reagents except the test sample) and A sample corresponds to the absorbance of the tested extract read at 517 nm after 60 min. The results were expressed as the percentage of DPPH radical-scavenging activity. This assay was used as an in vitro indicator of antioxidant capacity and does not directly reflect antioxidant effects under physiological conditions.

2.8. Alpha-amylase inhibition assay

The α-amylase assay was evaluated according to a modified Sigma-Aldrich [17], including the negative controls to ensure accuracy. The reaction reagent was obtained by mixing 20 mL of 96 mM DSN solution (3,5-dinitro salicylic acid), 8 mL of sodium potassium tartrate (5.31 M) dissolved in 2 M NaOH, and 12 mL of deionized water. Porcine pancreatic α-amylase was prepared in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl. Briefly, 90 µL of the sample were added to 10 µL of amylase solution (575 U/mL) and incubated at 25 °C for 20 min. Subsequently, 100 µL of 1.0% starch solution was added and the mixture was incubated at 25 °C for 3 min. The reaction was stopped with 100 µL of DNS reagent, the incubation at 85 °C for 10 minutes, and the dilution with 1.0 mL of deionized water. Negative controls were prepared by substituting the extract with 90 µL of distilled water, while the blank samples contained the extract without the α-amylase enzyme. Acarbose (1.0 mM) was used as the positive control at a fixed concentration to provide a reference inhibitory response under the experimental conditions. Absorbance was recorded at 540 nm, and the blank values were subtracted from the corresponding sample readings. The IC50 value, defined as the concentration of the extract required to inhibit 50% of enzyme activity, was calculated from three independent experiments using dose–response curves obtained at three extract concentrations, corresponding to 0.5×, 1× and 1.5× the intermediate concentration reported above (90 µL). The IC50 values were subsequently expressed as mg/g dry weight (DW), based on the initial amount of dry sample (mg) in 1 mL of extract.

2.9. Alpha-glucosidase inhibition assay

The assay was performed following the method of Sharp et al. [18]: 10 µL of extract was added to a 5 mg/mL solution of α-glucosidase from Saccharomyces cerevisiae (10 µL), 1 mM solution of p-nitrophenyl-α-D-glucopyranoside (25 µL) in 20 mM phosphate buffer (pH 6.0), and incubated at 37 °C for 10 minutes. Subsequently, 80 µL of 0.1 M Na_2CO_3 solution was added, and the absorbance was read at 400 nm. Acarbose (1.0 mM) was the positive control. The IC50 value was calculated for the cracker samples from three independent preparations, using three extract concentrations, corresponding to 5, 10, and 15µL of extract.

2.10. Pancreatic lipase inhibition assay

The inhibitory capacity of the extracts on pancreatic lipase was assessed in 96-well microplates, following the method described by Ombra et al. [14]. Porcine pancreas type 2 lipase was prepared in ultrapure water at a concentration of 2.5 mg/mL and centrifuged at 2,000 g for 6 minutes. The resulting supernatant was collected and used immediately. For each reaction, 40 µL of enzyme solution was mixed with 40 µL of extract solution and 20 µL of 10 mM p-nitrophenyl butyrate (NPB) substrate solution. The reaction mixture was incubated at 37 °C for 10 minutes, and the absorbance was read at 405 nm. A solution of 1 mg/mL of Orlistat was employed as the positive control at a fixed concentration (20µg/mL). IC_{50} values were determined for the cracker samples from three independent experiments and using dose–response curves obtained at three extract concentrations, corresponding to 0.5×, 1× and 1.5× the intermediate value reported above.

2.11. In vitro starch digestion and pGI

In vitro starch digestion was evaluated using the method reported by Brennan and Tudorica [19], with changes as described in Ombra et al. [20]. Crackers (4 g) were suspended in 20 mL of sodium phosphate buffer (pH = 6.9). The pH was adjusted to 1.5 using hydrochloric acid to provide proper conditions for porcine pepsin activity. Then, 4 mL of porcine pepsin solution (115 U/mL) was added, and the mixture was incubated at a temperature of 37 °C for 30 minutes. The pH was then adjusted to 6.9 using 2 M NaOH. Porcine pancreatic α-amylase (1 mL, 110 U/mL) was then added, and the final reaction volume was adjusted to 50 mL. The mixture was incubated at 37 °C in a stirred water bath throughout the digestion period. At predetermined time points (5, 10, 15, 20, 30, 60, 90, 120, 180, 240 min), 100 µL aliquots were collected and 400µL of stop solution (0.3 M Na_2CO_3) was added to stop enzyme activity. The collected aliquots were centrifuged at 2000 g for 5 minutes, and the concentration of reducing sugars released during starch hydrolysis was determined using the 3,5-dinitrosalicylic acid method, reading the absorbance at 546 nm [21]. White wheat bread, prepared without inhibitors, was used as the reference for the starch digestion rate. The method reported by Brennan and Tudorica [22] was adopted to calculate the amount of reducing sugar (maltose) released (%RSR eq.4), hydrolysis index (HI eq.5), and pGI (pGI eq.6).

$$\%RSR = (A_{sample} \times 500 \times 0.95)/(A_{maltose} \times Carbohydrate) \times 100 \quad (4)$$

where A sample was the absorbance at 546 nm, A maltose was the absorbance of maltose solution (1 mg/mL), and carbohydrate was the total amount of starch and reducing sugar present in 4 g of

sample. The starch and sugar contents used in the calculation were determined for each cracker formulation. Sugar content was experimentally determined using the 3,5 Dinitrosalicylic-Acid colorimetric method [21], with maltose as the reference standard and absorbance measured at 546 nm. Since starch was not directly determined analytically, its content was estimated from the compositional data reported in the INRAN food composition tables for the individual ingredients used in each formulation.

The hydrolysis index was determined as:

$$HI = AUC\ (0–240min)\ sample/AUC\ (0–240min)\ bread \times 100 \quad (5)$$

where AUC represents the area under the sample curve from 0 to 240 minutes, normalized to wheat bread.

The predicted GI was estimated according to the equation:

$$Predicted\ GI = 0.862HI + 8.189 \quad (6)$$

The pGI values were used as the final comparative parameter to evaluate differences in starch hydrolysis among the cracker formulations.

2.12. Statistical analysis

Results were expressed as means ± standard deviation from three independent sample preparations. For each sample, three crackers were prepared independently, and a separate extract was obtained from each preparation. Results were subjected to a statistical analysis to evaluate differences among samples. For enzyme inhibition assays, a concentration-response curve was generated for each independent extract, and IC_{50} values were calculated accordingly. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used to assess differences among more than two sample groups whereas comparisons between two means were assessed using Student's t-test. In all cases, a significance level of 95% was applied ($p < 0.05$). The IC50 values and statistical analyses were determined using the GraphPad Prism software.

3. Results and discussion

Apple (*Malus domestica)* cv. *Annurca* and *Brassica oleracea var. sabauda* formed the basis of our previous investigations [23,24]. As described previously, *Annurca* peel powder or Brassica waste powder was incorporated into wheat flour at 5% or 10%, together with broccoli seed oil or cherry seed oil, to produce enriched crackers (C1–C4). Figure 1 also shows the control sample (C), prepared with wheat flour and sunflower oil only. The 5% and 10% substitution levels were selected based on a preliminary internal evaluation indicating no evident negative impact on the overall quality of crackers enriched with up to 10% of either powder.



C	C1	C2	C3	C4
250 g wheat flour	237.5 g wheat flour	225 g wheat flour	237.5 g wheat flour	225 g wheat flour
1.5 g salt	1.5 g salt	1.5 g salt	1.5 g salt	1.5 g salt
25 g sunflower oil	25 g broccoli seed oil	25 g broccoli seed oil	25 g cherry seed oil	25 g cherry seed oil
120 ml water	120 ml water	120 ml water	120 ml water	120 ml water
-	12.5 g Brassica ol. powder	25 g Brassica ol. powder	12.5 g Apple peel powder	25 g Apple peel powder

Figure 1. Appearance and formulation of control C and experimental cracker samples (C1–C4).

3.1. WHC, OHC, and SP

The control sample (CTRL) showed slightly higher WHC values than all enriched formulations (C1–C4). However, the observed differences were negligible and did not reach statistical significance ($p > 0.05$; Figure 2). Overall, the treatments did not enhance water retention capacity, with values comparable to or slightly lower than the control. A similar trend was observed for OHC. The control sample showed marginally higher values compared with the treated formulations, suggesting a limited reduction in the availability of hydrophobic sites involved in lipid binding. Regarding SP, values across all formulations were largely similar to the control. C1 presented a slightly higher value, whereas C2–C4 were marginally lower, but none of these differences was statistically significant. This indicates that the swelling capacity of the samples was generally preserved after enrichment. These findings suggest that the applied treatments did not substantially modify the physical properties examined. In the case of crackers, lower WHC has been associated with the maintenance of crispness in previous studies [25]. In the literature, higher OHC has also been associated with flavor retention and crispness, although greater lipid retention may increase the susceptibility of products to rancidity [26]. In comparison, crackers enriched with legume flours typically show higher WHC and OHC values, attributable to the greater fiber and protein content of legumes, which enhances water and lipid retention. While this can improve satiety and internal hydration, it may also reduce crispness and increase the risk of lipid oxidation during storage [27]. In the present work, the goal was to achieve a balanced formulation enriched with vegetable by-products, providing fiber and polyphenols while maintaining the overall physical properties examined.

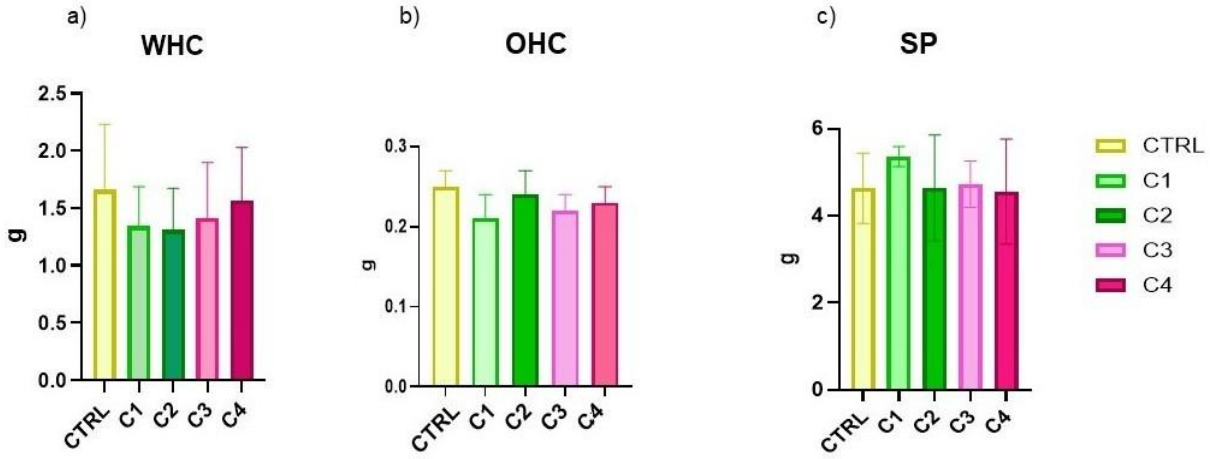


Figure 2. a) WHC = Water-holding capacity, b) OHC = Oil-holding capacity, c) SP = Swelling Power of enriched crackers (C1–C4) and control (CTRL). Data are presented as means ± standard deviation (n = 3). No significant differences were observed among the samples ($p > 0.05$).

3.2. TPC and DPPH radical-scavenging activity of crackers

The TPC and DPPH radical-scavenging activity of the cracker extracts were evaluated to compare the effect of incorporating plant-based by-products into the formulations. Previous studies by our research group showed that *Brassica oleracea* var. *sabauda* by-products and *Malus domestica* var. *Annurca* peel contain phenolic compounds and exhibit antioxidant potential [23,24]. In addition, cold-pressed broccoli and cherry seed oils have previously been reported to contain bioactive lipid fractions and to exhibit antioxidant properties [8,9]. Based on these findings, the present study investigated whether the incorporation of these ingredients was associated with differences in the TPC and DPPH radical-scavenging activity of the resulting crackers. As shown in Table 1, the control sample exhibited the lowest TPC (109.4 μg GAE/g) and DPPH radical-scavenging activity (5.9%). All enriched formulations showed significantly higher TPC values than the control. The highest TPC was observed in C4 (267.7 μg GAE/g), followed by C2 (256.9 μg GAE/g), whereas C1 and C3 showed intermediate values of 208.4 and 186.4 μg GAE/g, respectively. A similar trend was observed for DPPH activity, with C4 showing the highest value (43.9%), followed by C2 (36.6%), C3 (18.0%) and C1 (10.6%). Overall, a positive correlation was observed between phenolic content and DPPH results ($r = 0.882$; $p < 0.05$), based on five formulation means. This correlation should be considered exploratory. The control sample, prepared using only wheat flour with no added powders, exhibited a markedly lower TPC than all the experimental formulations. These findings indicate that the incorporation of the two types of by-products and seed oils was associated with higher measurable polyphenol content and DPPH radical-scavenging activity in the fortified crackers. However, the Folin–Ciocalteu assay provides an estimate of total reducing phenolic compounds, and the observed differences cannot be attributed to specific compounds or to the retention of individual bioactive molecules after baking.

Similarly, the DPPH assay provides an indication of radical-scavenging capacity but does not comprehensively characterize the overall antioxidant potential of the samples. Further studies using targeted compositional analyses and complementary antioxidant assays would be required to determine which compounds contribute to the observed activity and to assess their stability during baking.

3.3. Analysis of the in vitro inhibitory potential of fortified crackers on digestive enzymes

A growing body of evidence highlights the ability of phenolic compounds to interact in vitro with digestive enzymes α-amylase, α-glucosidase, and lipase, principally through non-covalent interactions [28]. For this reason, enzyme inhibition assays are frequently employed as preliminary screening tools to compare the relative bioactivity of food formulations enriched with plant-derived ingredients. However, these assays do not reproduce the complexity of gastrointestinal digestion and the observed inhibitory effects cannot be directly extrapolated to physiological conditions. Several studies have applied this approach to different bakery formulations, although quantitative comparisons between studies are not possible due to differences in formulations, polyphenolic composition, and analytical conditions. For instance, studies have reported measurable in vitro inhibitory trends in biscuits enriched with pumpkin seed powder [29], orange peel powder [30], and *Tenebrio molitor* flour [31]. Similarly, the incorporation of borage powder in biscuit production has been associated with significant inhibitory potential, an effect that may be associated with the bioactive constituents naturally occurring in the powder [32]. In line with these findings, the enriched crackers tested in this study (C1–C4) also showed in vitro distinct inhibitory activities against the three digestive enzymes (Table 2). Among them, C4 exhibited the strongest inhibition, with IC_{50} values of 38 mg/g for α-amylase, 68 mg/g for α-glucosidase, and 33 mg/g for lipase. This was followed by C2, which also performed well, particularly against α-amylase and lipase. By contrast, C3 was consistently the least effective, especially in terms of α-glucosidase inhibition. The control cracker was not included in the enzyme inhibition assay, as its very low polyphenol content resulted in an inhibitory response below the assay's quantifiable range. Taken together, these results indicate that C4, characterized by the highest polyphenol content among the tested samples, exhibits the greatest potential to modulate both carbohydrate- and lipid-digesting enzymes. Similar findings have been documented in other fortified snack formulations. Crackers fortified with Joha rice, bamboo shoot, and green gram powders were found to inhibit α-amylase and α-glucosidase [33]. Similarly, biscuits enriched with spices and white pea powder exhibited significant inhibitory activity [34]. Moreover, a snack formulated with an acha-pigeon pea blend (70:30) not only displayed strong antioxidant properties but also showed marked inhibition of α-amylase and α-glucosidase, the crucial enzymes implicated in carbohydrate digestion [35]. Although differences in formulation and polyphenol profiles, assay conditions, and expression of IC_{50} values limit direct quantitative comparisons, the overall consistency among these studies reinforces the relevance of polyphenol-rich food matrices in modulating digestive processes *in vitro*. The inhibitory effects are commonly attributed to non-covalent interactions between polyphenols and digestive enzymes, such as hydrogen bonding and hydrophobic interactions at or near the active sites [36]. However, the specific molecular interactions responsible for the observed inhibition in complex food matrices, such as C4, remain insufficiently characterized, highlighting the need for further mechanistic studies to better define their relevance and potential health implications. In addition, future studies should investigate the influence of the baking process on the preservation of phenolic compounds and related bioactivities, for instance by incorporating baking loss tests to better assess the retention of

bioactive components after thermal processing [37]. These findings provide preliminary evidence of the functional potential of the formulations. Further work involving detailed characterization of the bioactive compounds, assessment of their stability during processing and digestion, and appropriate *in vivo* studies would be necessary to establish physiological relevance.

3.4. pGI

To investigate the capacity of the enriched crackers to inhibit starch-degrading enzymes more thoroughly, an *in vitro* digestion trial was conducted for the different formulations. This experimental model is a valuable tool for examining the functional features of new food products [38]. The inclusion of supplementary ingredients can, in fact, affect the stability of the food matrix, which is sometimes characterized by structural pores; therefore, it is necessary to confirm whether the functional integrity of enriched products is preserved. The digestion of starch, however, is a multifaceted process and is not determined solely by the protein–starch network or by the granular structure of starch itself [39]. As summarized in Table 3, adding 5% or 10% of *Annurca* apple powder or *Brassica oleracea* powder lowered starch digestibility, resulting in reduced pGI values compared with the control crackers. However, pGI values obtained from *in vitro* assays should not be considered equivalent to glycemic responses measured in humans.

As shown in Table 3, pGI of the control cracker was (80 ± 1.1), while pGI values were 66.0 ± 0.4 for C1, 65.0 ± 2.0 for C2, 61.5 ± 1.3 for C3, and 61.0 ± 3.1 for C4. Therefore, the reduction relative to the control ranged from approximately 17.5% in C1 to 23.8% in C4. For the *Annurca* peel formulations, increasing the level of enrichment from 5 to 10% resulted in only a small additional reduction in pGI (61.5 to 61.0), with no statistically significant difference between the two formulations. A similar trend was observed for the Savoy cabbage formulations, for which pGI values of 66 and 65 were obtained at 5% and 10% enrichment, respectively. This suggests that, under the conditions investigated, increasing the amount of by-product powder from 5% to 10% did not proportionally increase the reduction in starch hydrolysis. Such a response may indicate a plateau effect at the higher enrichment level rather than a linear dose-response relationship.

Previous research conducted by our group had already shown that *Annurca* powder influenced the pGI of pasta [23]. Interestingly, a strong negative correlation was found between total polyphenol content and pGI ($r = –0.9738$). Although this association supports the possibility that polyphenol-rich ingredients contribute to the observed differences in starch hydrolysis, correlation alone does not establish a causal relationship. The reduction in pGI may also be related to other compositional and structural characteristics of the enriched crackers. In particular, dietary fiber may influence water availability, matrix viscosity and enzyme accessibility, while interactions among starch, proteins, fibers and phenolic compounds may modify starch hydrolysis [36,39]. The relative contribution of these mechanisms cannot be distinguished using the present experimental design. The lower pGI values observed for all enriched formulations therefore indicate that incorporation of *Annurca* apple peel or Savoy cabbage by-products was associated with reduced starch hydrolysis under the in vitro conditions used. This result is consistent with previous observations for *Annurca*-enriched pasta [23] and with the reported effects of dietary fiber and plant-derived components on starch digestion [19,39]. Nevertheless, the present findings should be interpreted as an in vitro indication of reduced predicted glycemic response rather than evidence of a corresponding reduction in postprandial glycemia in humans. One potential drawback of using *Annurca* and *Brassica oleracea* powders lies in the visual

appearance of the final products. The incorporation of plant-based powders modified the color of the products compared with the control (Figure 1). Although this change may be perceived positively as an indication of the presence of plant-derived ingredients, consumer acceptance was not formally assessed in the present study. Although some consumers may favor the traditional yellow appearance associated with conventional crackers, others may perceive the darker brown color of the enriched products as an indicator of added functional ingredients and enhanced nutritional value. This duality highlights how consumer perception plays a central role in product acceptance. Therefore, textural and sensory evaluation should be included in future work to determine the acceptability of the formulations.

Overall, the results indicate that the incorporation of 5 or 10% *Annurca* apple peel or Savoy cabbage by-products can reduce the pGI of crackers while simultaneously increasing their measured polyphenol content and DPPH radical-scavenging activity. However, the mechanisms underlying these effects are likely multifactorial, and further studies combining compositional, structural and digestive analyses would be required to establish the relative contribution of the different components. Our work also underscores the substantial but often overlooked value of apple peel in food innovation. Whereas apple pulp is commonly exploited for its nutritional benefits [40], the peel contains an even richer reservoir of phytochemicals with noteworthy bioactive properties. Unfortunately, vast amounts of peel are routinely eliminated during the production of juices, applesauce, or preserved foods.

Table 1. Total polyphenol content and DPPH.

Sample	TPC µg GAE/g ±sd	DPPH (%) ±sd
C	109.4 ± 2.1^{a}	5.9 ± 1.1^{a}
C1	208.4 ± 3.2^{c}	10.6 ± 2.2^{b}
C2	256.9 ± 4.0^{d}	36.6 ± 2.0^{d}
C3	186.4 ± 2.3^{b}	18 ± 1.3^{c}
C4	267.7 ± 3.3^{d}	43.9 ± 2.3^{e}

Values sharing the same superscript letter within a column are not significantly different ($p > 0.05$).

Table 2. IC50 values for enzymatic inhibitions.

Sample	IC50 (±sd) mg/g against α-amylase	IC50 (±sd) mg/g against α-glucosidase	IC50 (±sd) mg/g against lipase
C1	63.0 ± 0.9^{a}	81.1 ± 1.5^{a}	$40 \pm 0.5^{a,b}$
C2	49.0 ± 2.2^{b}	74.0 ± 2.1^{b}	$38 \pm 1.1^{b,c}$
C3	55.1 ± 1.4^{c}	118 ± 2.2^{c}	45 ± 3.5^{a}
C4	38 ± 1.6^{d}	68 ± 1.7^{d}	33 ± 2.1^{c}

IC50 represents the sample concentration (mg/g dry weight) required to inhibit 50% of enzyme activity. Data are expressed as mean ± standard deviation (sd) of three independent determinations. Non-identical lowercase letters in a column indicate significant differences ($p < 0\ 05$).

Table 3. pGI.

Sample	HI	pGI ± sd
C	83.39 ± 1.0[a]	80 ± 1.1[a]
C1	67.06 ± 0.6[b]	66 ± 0.4[b]
C2	65.90 ± 1.8[b,c]	65 ± 2.0[b,c]
C3	61.89 ± 1.4[b,c]	61.5 ± 1.3[b,c]
C4	61.26± 2.1[c]	61 ± 3.1[c]

Results are means of three replicates ± standard deviation. Non-matching lowercase letters in a column indicate significant differences (p < 0 05).

4. Conclusions

Insufficient consumption of fruits and vegetables is widely recognized as a factor contributing to poor dietary quality, highlighting the need for innovative strategies to enhance the intake of plant-derived bioactive compounds through commonly consumed foods [41, 42]. In this context, fortifying staple products with nutrient-rich ingredients recovered from food processing by-products constitutes a sustainable approach to improve the nutritional profile of bakery foods [43].

In this study, the incorporation of *Malus domestica var. Annurca* apple peel powder and *Brassica oleracea var. sabauda* by-product powder into cracker formulations resulted in products with increased DPPH radical-scavenging activity and measurable inhibitory activity against digestive enzymes, including α-amylase, α-glucosidase, and lipase. A lowering in the pGI was also obtained compared to the control formulation. In addition, the study highlights a dual valorization approach that simultaneously exploits solid and liquid by-product fractions, such as plant powders and seed oils, offering a replicable model for circular food system innovation. This integrated use of agro-industrial residues supports the transition toward more sustainable production chains while adding value to food formulations. Nevertheless, the conclusions are based on *in vitro* assays and predicted glycemic responses, which require validation through *in vivo* studies [44]. Furthermore, consumer acceptance remains to be assessed to ensure the technological and sensory feasibility of the proposed formulations. Future work should also explore processing conditions that maximize the stability and bioavailability of phenolic compounds, as well as the synergistic effects between polyphenols and bioactive lipids within complex food matrices. In addition, the seed oils used in this study are relatively unconventional in food applications, and their detailed composition was beyond the scope of this work. Although food-grade, certified cold-pressed oils were used, their full compositional and safety assessment, including fatty acids of nutritional or toxicological relevance, as well as their contribution to the oxidative behavior of crackers and their shelf life, should be addressed in future studies.

Taken together, the present findings provide evidence that regionally distinctive by-products such as *Annurca* apple peel and Brassica residues can be incorporated into bakery formulations and can modify the measured characteristics of the resulting crackers, including polyphenol content, DPPH radical-scavenging activity, in vitro digestive enzyme inhibitory activity and predicted glycemic index. The proposed formulation strategy represents a potential approach for the valorization of underutilized agri-food resources and for the development of resource-efficient bakery products. Further studies are needed to assess their nutritional composition, sensory acceptability, and physiological relevance.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

All authors declare that there are no competing interests.

Author contributions

Experimental design, investigation, data collection, dataset preparation, MNO, FF; Manuscript drafting, MNO, Research supervision and funding acquisition, FN. All authors have read and approved the final version of the manuscript for publication.

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