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Cauliflower by-products as functional ingredient in bakery foods: Fortification of pizza with glucosinolates, carotenoids and phytosterols

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ABSTRACT

Industrial cauliflower by-products still represent a no-value food waste, even though they are rich in bioactive compounds. With the aim of valorizing them, optimized special flours rich in glucobrassicin, lutein, β-carotene, and β-sitosterol obtained from leaves, orange and violet stalks were used at 10 and 30% w/w in the formulation of functional leavened bakery. For the first time, the effect of bioactive compounds enrichment in pizza products as well as the rheological properties were evaluated. As results, pizza making process affected the recovery of the bioactive compounds. The recovery of glucobrassicin and carotenoids in pizza depended on the aerial part of cauliflower. Pizza with violet stalks was the richest in glucobrassicin, providing 8.4 mg per portion (200 g). Pizza with leaves showed the highest carotenoid content with a 90% of recovery rate while pizza with orange stalks provided up to 5.8% of the phytosterols health claim requirement. All 10% enriched pizzas revealed viscoelastic and springiness properties similar to the control, contrary to 30% fortification level. Therefore, the use of 10% special flour in pizza should meet both technological industrial processing and consumer acceptance. Orange stalks are the most promising ingredients for high levels of fortification in pizzas.

1. Introduction

The SDG (Sustainable Development Goal) target 12.3 aims to halve global food waste at the retail and consumer level and reduce food losses along the food production and supply chains by 2030 (United Nations, 2015). Food waste implies not only high social and environmental impacts, but also economic costs estimated at € 143 billion in the EU, including those associated with the collection, management, and treatment of food waste at the producer, processor, retailer, and household level.

More than one-third of total food waste accounted for vegetable by-products, or rather the wastes generated from industrial processing (Bharat Helkar and Sahoo, 2016). Among them, Brassica species have a considerably high waste rate, e.g., 45–60% for cauliflower, 60–75% for broccoli (Khedkar et al., 2017; Petkowicz and Williams, 2020; Castelão-Baptista et al., 2021). These plants are worldwide cultivated, with China and India being the largest global producers, while in Europe are Spain and Italy (FAO, 2022). As their demand is increasing, new varieties started to appear on the market, such as the newly coloured cauliflowers resulting in spontaneous mutation of the white counterpart (*Brassica oleracea* L. var. botrytis). Cauliflowers, as well as other Brassica species, are rich in bioactive compounds like vitamins, phenolics, glucosinolates (GLS), and phytosterols, which exert health-promoting activities (Ahmed and Ali, 2013; Jahangir et al., 2009). For instance, epidemiological studies highlighted the anticarcinogenic effects of isothiocyanates, which are biologically active molecules derived from the enzymatic degradation of GLS (Arumugam and Razis, 2018). Carotenoids, specifically lutein and β-carotene which are the most abundant in Brassicaceae, contribute to reducing oxidative stress and related disorders such as cancer, diabetes, and cardiovascular diseases (Gul et al., 2015; Jahangir et al., 2009). On the other hand, phytosterols with their cholesterol-like structure can lower the LDL cholesterol in the blood, and therefore the onset of coronary heart diseases (Tolve et al., 2020). For this purpose, EFSA authorised a specific health claim for plant sterols/stanol esters, specifying that "the beneficial effect is obtained with a daily intake of 1,5-3 g plant sterols/stanols" (EFSA, 2009).

However, it is important to preserve the vegetable by-products with their bioactive compounds for their reuse as functional ingredients. A

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solution could be their transformation into flours employing blanching, drying, and milling (Salehi, 2020; Santos et al., 2022). This allows for reducing the water activity (a_w) therefore stabilizing the product against enzymatic and microbiological agents, as well as managing vegetable by-products and using the flour for food formulations (Salehi, 2020). By-products from brassica species, especially broccoli, have been already used to develop functional food, mostly bakery products such as bread (Klopsch et al., 2019; Krupa-Kozak et al., 2021; Lafarga et al., 2018), crackers (Lafarga et al., 2019), and cakes (Krupa-Kozak et al., 2019), but also snacks (Stojceska et al., 2008), soups (Alvarez Jubete et al., 2014) and beverages (Dominguez-Perles et al., 2011; Amofa--Diatuo et al., 2017). However, these studies determined mostly antioxidant activities, polyphenols, and proteins, while few focused on GLS, carotenoids, and phytosterols.

Pizza making (yeast fermentation, mechanical and thermal phases) may affect the recovery of bioactive compounds after enrichment with special flours. In fact, it would differ from other bakery products mentioned because of differences in the food matrix, leavening agents, and baking parameters. Therefore, depending on the fortification level and the type of by-product, a different impact on the rheological and textural properties may be obtained. The aim of this study was to evaluate the effect of the enrichment of special flours from leaves and stalks of different varieties of coloured cauliflowers for pizza making, by assessing the bioactive compounds (GLS, phytosterols, and carotenoids) improvement, the rheological and textural properties of the leavened final product.

2. Materials and methods

2.1. Chemicals and reagents

GLS standards (100%, sinigrin, glucobrassicin, 4-methoxyglucobrassicin, neoglubrassicin potassium salts) were purchased by PhytoLab GmbH & Co (Vestenbergsgreuth, Germany), carotenoids standards (>95% purity; lutein and β-carotene), 19-hydroxycholesterol standard, Sylon BTZ, solvents HPLC grade (>95% purity, acetone, acetonitrile, dichloromethane, ethanol, methanol, *n*-hexane, diethyl ether, isopropanol, acetic acid, water), ammonium acetate (HPLC grade, >99%) were purchased by Merck (Darmstadt, Germany). Potassium hydroxide (85%) and anhydrous sodium sulphate were purchased by ITW Company (Darmstadt, Germany). MilliQ water was purified with Millipore System (Millford, USA).

2.2. Preparation of flours from cauliflower industrial by-products

By-products from industrial processing of cauliflowers *(Brassica oleracea* L. var. botrytis) were provided from a local company (Agrinovana S.r.l Petritoli, Fermo, Italy). Stalks and whole leaves of *Cheddar* (orange) and *Depurple* (violet) cauliflower varieties were selected. After removing the damaged tissue, each by-product was cut into small pieces and treated in hot water for 5 min to inactivate enzymes hydrolysing biologically active compounds (i.e., myrosinase and polyphenol oxidase). Blanched by-products were freeze-dried and ground to produce fine powder flours. In detail, three special flours were obtained: flour from orange cauliflower stalk (OF), flour from violet cauliflower stalk (VF), flour from combined leaves of both varieties (LF). The flours were stored under vacuum at −18 °C until their use for pizza preparation.

2.3. Preparation of pizza

Three pizza dough formulations were developed: i) the control formulation and ii) addition of 10% and iii) 30% by-products flours (OF, VF and LF) in replacement of refined wheat flour. A total of six fortified dough formulations and a control dough were obtained (Table 1). Such relatively high levels of incorporation (10 and 30%) were chosen to have a significant impact on the valorisation of cauliflower by-products.

Samples of pizza were prepared (Fig. 1) in a professional bakery laboratory (Pomorilla.it, Ascoli Piceno, Italy) to standardize the production till a half-processed product (pre-baked and packed pizzas, stored at −18 °C) to be sold to catering services. The preparation of pizza included the preparation of biga dough by mixing the ingredients with a professional spiral mixer with a fixed bowl (SPI 60 F E, Esmach Ali Group, Vicenza, Italy) as starting and once the final dough was obtained, it was sized and round in dough portions of 70 g, allowed to leaven in a leavening chamber (Cella, 1900 × 2300, 2021/085, Atrepan, Verona, Italy) at 35 °C for 60 min, pizzas were draft, allowed to leaven a second time. Pizzas were pre-baked (300 °C, 2.30 min) in an electric rotary rack oven (Esmach-Bongard, Esmach Ali Group, Vicenza, Italy) and then stored at −18 °C for 3 months, then samples were baked setting a pizza specific program (220 °C, 8 min) in a modern oven for consumer use (Bosch, HSG636ES1) purchased from a local distributor (MediaWorld Italy). After baking, pizzas were freeze-dried and milled to a fine powder, submitted to the analytical determinations.

2.4. Glucosinolates determination

GLS were extracted in accordance with Baenas et al. (2019). Special flours (200 mg) and baked pizza powders (1 g) were extracted (30 min, 70 °C) by a vortex mixer for 5 min using 2 mL of methanol/water (70:30, v/v). Samples were cooled in ice and centrifuged (6000 rpm, 2 min). The supernatant was taken to dryness with a rotary evaporator (35 °C), reconstituted in water (0.5 mL), centrifuged (13,500 rpm, 12 min) filtered through a 0.2 μm (Sartorius Regenerated Cellulose Membrane) and injected (5 μL) in an Acquity Ultra Pressure Liquid Chromatographic H-class system (Waters Corporation, Milford, US), equipped with Photodiode Array Detector (PDA). The separation was carried out following Lin et al. (2014) on an analytical column UPLC CSH C18 (2.1 mm × 100 mm, 1.7 μm) with some modifications. The mobile phase was

Table 1
Ingredients in control and fortified (PLF10, POF10, PVF10, PLF30, POF30, PVF30) pizza dough (g/100 g of fresh dough).

g/100 g of dough (fresh weight)									
Pizza Dough formulation	Flour Manitaly	Flour Beta	Flour "0"	Special flour (Powder from by-products)	Malt	Sugar	Salt	Sunflower oil	Water
Control	28.9	14.6	14.6	–	0.6	1.1	1.5	3.9	34.9
Fortification level 10 [a]									
PLF10	26.0	13.1	13.1	5.8	0.6	1.1	1.5	3.9	34.9
POF10	26.0	13.0	13.0	5.8	0.6	1.1	1.5	3.9	34.9
PVF10	26.0	13.0	13.0	5.8	0.6	1.1	1.5	3.9	34.9
Fortification level 30 [b]									
PLF30	19.6	9.8	9.8	16.8	0.5	1.1	1.4	3.7	37.3
POF30	14.8	7.4	7.4	12.7	0.4	0.8	1.1	2.8	52.5
PVF30	17.2	8.6	8.6	14.7	0.5	0.9	1.2	3.3	45.1

(P = pizza; F = flour; L = leaf; O = orange cauliflower; V = violet cauliflower). Water was added in biga and final dough preparation.
[a] Fortification level 10 means that the dough was formulated using special flour and wheat flour in the ratio 1:10.
[b] Fortification level 30 means that the dough was formulated using special flour and wheat flour in the ratio 3:10.

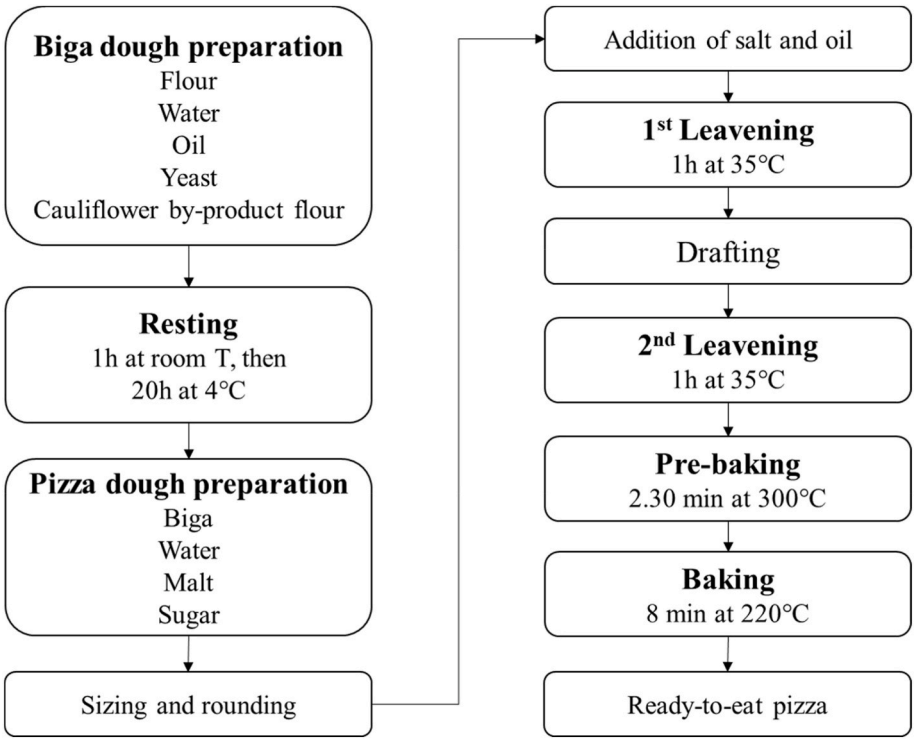

Fig. 1. Flow chart of professional pizza preparation.

composed of A (0.01% formic acid in water, v/v) and B (0.01% formic acid in acetonitrile, v/v). The linear gradient was from 4 to 15% B (v/v) at 10 min, from 15 to 70% at 20 min, isocratic for 5 min, and decreased to 4% B (v/v) at 30 min. The flow rate was 0.3 mL/min, column oven was set at 35 °C and sample loading was carried out at 20 °C. PDA analysis was performed at 225 nm wavelength upon a spectrum scanning in the 210–500 nm range. GLS were identified by comparison of retention time and absorbance spectrum with pure standards. The quantification was performed by external calibration using sinigrin and glucobrassicin as standards of aliphatic and indole GLS, respectively. Stock and working standards of each glucosinolate were prepared by dissolving the salts in deionized water. Good correlation coefficients (R^2) of 0.999 were obtained in all cases. The instrumental limit of detection (LOD) and quantification (LOQ), calculated at a signal-to-noise ratio of 3:1 and 10:1 respectively, were 23 and 75 (indole GLS), 18 and 59 ng/mL (aliphatic GLS).

2.5. Carotenoids determination

Carotenoids were extracted and analyzed according to Nartea et al. (2021a). Briefly, to special flours (100 mg) and baked pizza powder, acetone (5 mL, 4 °C) was added, and the mixtures kept at 4 ± 1 °C (15 min), vortexed (5 min), and centrifuged (1370 rpm, 10 min, 4 °C), repeating the acetone extraction a second time. The supernatant was filtered (0.45 μm, Sartorius Regenerated Cellulose Membrane), dried, resuspended in 0.5 mL acetone for pizza samples and 0.25 mL for tissue vegetable and injected in an Acquity Ultra Pressure Liquid Chromatographic H-class system (Waters Corporation, Milford, US), equipped with PDA and an Acquity column UPLC BEH C18 (2.1 mm × 100 mm, 1.7 μm). The mobile phase was composed of phase A consisting of acetonitrile (75%), dichloromethane (10%), and methanol (15%), and phase B consisting of acetate ammonium in water (0.05 M). Gradient started at 75% A, held for 10 min, up to 98% in 1 min and held in isocratic mode till 20 min. The flow rate was 0.4 mL/min, column oven was set at 35 °C and sample loading was carried out at 20 °C. PDA analysis was performed at 450 nm wavelength upon a spectrum scanning in the 210–500 nm range. Carotenoids were identified by comparison of retention time and absorbance spectrum with pure standards. Their quantification was performed by external calibration. Good correlation coefficients (R^2) of 0.999 were obtained in all cases Good correlation coefficients (R2) of 0.999 were obtained in the range of 1–100 μg/mL for lutein and 0.05–100 μg/mL for β-carotene. The instrumental LOD and LOQ, calculated at a signal-to-noise ratio of 3:1 and 10:1 respectively, were as followed: lutein, 5 and 16, and β-carotene, 5 and 18 ng/mL. Retinol activity equivalent (RAE) was calculated considering 1 μg retinol for every 6 μg of β-carotene or 12 μg or another provitamin A carotenoids (EFSA NDA Panel, 2015).

2.6. Phytosterols determination

Acid hydrolysis and alkaline saponification were performed to extract the total amount of phytosterols (esterified and acylated) in special flours and baked pizzas as suggested by Toivo et al. (2001) for cereal-based matrices and adapted with the new modification. Special flours (0.5 g) and baked pizza powder (2.0 g) were added to internal standard solution (19-hydroxycholesterol, 50 μg in 2.5 mL ethanol) absolute ethanol (1 mL), shaken, added again with hydrochloric acid (6 M, 5 mL), shaken and samples were placed in a water bath (80 °C, 60 min) and shaken every 10 min. Then, samples were cooled down, added of absolute ethanol (5 mL) and *n*-hexane:diethyl ether (20 mL, 1:1, v/v), vortexed again (10 min), centrifuged (4500 rpm, 10 min). The organic phase was collected and taken to dryness with a rotary evaporator (40 °C). The saponification (80 °C, 10 min) was performed by adding pyrogallol/ethanol solution (8 mL 3%, w/v), aqueous KOH [(0.5 mL, 1.3% (w/v)] to each dried sample, placed in the water bath, and shaken every 2 min. The samples were cooled down and extracted with *n*-hexane (20 mL) and deionized water (12 mL), vortexed (10 min), centrifuged (4500 rpm for 10 min). The upper organic phase (15 mL) was evaporated to dryness at 40 °C and added with Sylon BTZ allowing the derivation reaction (1 h at room temperature). The sample was dried and dissolved in 250 μL of *n*-hexane. All samples were injected (1 μL) in a GC/EI-MS (ThermoScientific, Waltham, MA, USA) system, equipped

with a split/splitless injector, and a single quadrupole analytical column MDN-5 (30 m length, 0.25 mm internal diameter, 0.1 μm film thickness). The oven temperature was set at 90 °C, held for 0.5 min, increased to 290 °C (30 °C/min), then to 300 (1 °C/min) and held for 15 min, using helium flow at 1.0 mL/min. The injector in split mode (ratio 10) was set at 300 °C, the ionization source (70 eV) was set at 250 °C, and the auxiliary line at 300 °C. The acquisition was performed in total ion current (TIC) in a mass range of 70–650 m/z and with a detector gain of 1.0. Trimethylsilane-phytosterols were identified by comparison with pure standards of each phytosterol (retention time and mass spectra). Quantification was performed by internal calibration. The instrumental LOD and LOQ were 0.6 and 1.8 ng/mL.

2.7. Rheological properties of pizza samples

Both dough (pre-baked) and baked pizza samples (90 mm^2 area, 15 mm thickness) were investigated for their viscoelastic properties. Creep and recovery tests were conducted under small-deformation conditions using a Universal Testing Machine (Zwick 1 kN, GmbH and Co, Ulm, Germany). A creep load target (5 N) was reached during the loading step by imposing high crosshead speed (600 N/min) and kept constant for 300 s (15 min) under load-controlled mode. Then the target load was removed, and the displacement (mm) was recorded for 300 s (5 min) during the unloading step, allowing the equilibrium recovery in the residual structure. A preload of 0.05 N was used to keep in touch with the samples before loading and during unloading steps. Six experimental replicates were performed for each pizza formulation. Creep behavior was modeled by a tailored Burger's function using Robust algorithm and Profit software ver. 7 (QuantumSoft, Zurich, Switzerland), as reported by Nartea et al. (2021b). A square relative mean error (Equation (1)) inferior to 2, tested the correctness of the model in describing the creep behavior in the small-scale deformation range.

$$\overline{E}\% = \frac{100}{N-8} \bullet \sqrt{\frac{\sum \left[J(t) - J(t)_{esp}\right]^2}{J(t)}} \quad \text{(Equation 1)}$$

Monte Carlo simulations (500) were performed simultaneously to estimate the model parameters with 95% of confidence.

2.8. Texture profile analysis of baked pizza

Texture properties were investigated under large-deformation conditions using Universal Testing Machine for uniaxial compression tests. Baked pizza samples of 90 mm^2 area and 15 mm thickness were analyzed after a preload of 5 N (reached with a crosshead speed of 5 mm/s) and 10 s of resting time to relax preload stress. Two consecutive compression cycles each with 50% of deformation as final target were performed under position-controlled mode with a crosshead speed of 10 mm/s. Load *vs* time data were analyzed to obtain some conventional textural parameters, i.e., hardness, cohesiveness, resilience, springiness, gumminess, chewiness, and adhesiveness. Six replicates were performed for each pizza formulation.

2.9. Statistical analysis

Chemical data are reported as mean values ± standard deviation (SD) of three replicates. Data were analyzed by one-way ANOVA and Tukey's mean comparison test at a significance level of $p < 0.05$ using R software version 3.5.0. Principal component analysis (PCA) was performed using Statistica 10.0 (StatSoft 2011; Tulsa, OK, USA) to highlight the pattern of changes as induced by wheat flour substitution by cauliflower flours in terms of technological quality of the functional doughs as well as of microstructural-related properties that can affect sensory perception of the obtained functional pizzas.

3. Results and discussion

3.1. Bioactive compounds in special flours

The bioactive compounds investigated are GLS (glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin), carotenoids (β-carotene and lutein) and phytosterols (campesterol, stigmasterol and β-sitosterol). Their contents (mg/kg powder) in flours obtained from cauliflower by-products are reported in Table 2 and an example of the obtained chromatograms is reported in the Supplementary material.

It is noteworthy to notice that glucobrassicin, carotenoids (lutein and β-carotene), and phytosterols (campesterol, stigmasterol, and β-sitosterol) are differently accumulated in the aerial parts of cauliflower. Moreover, the levels of these compounds in leaves and stalks were markedly affected by the genetic factor (cauliflower variety). As a result, the investigated by-product flours presented different chemical features.

In detail, glucobrassicin resulted the most plentiful glucosinolate (GLS) in flour from stalks (OF, VF), while neoglucobrassicin was dominant in leaves (LF). The highest content of glucobrassicin was revealed in flour from violet stalks (VF). Powder from leaves showed the highest content of total indole GLS (around 830 mg/kg powder), followed by VF (750 mg/kg powder) and OF (290 mg/kg powder). The findings are consistent with previous reports where the glucobrassicin was the predominant GLS in white, green, and purple cauliflower florets (Volden et al., 2009; Picchi et al., 2012; Dong et al., 2021). Accordingly, Drabińska et al. (2021) evidenced that the dominant GLS in white cauliflower florets was glucobrassicin, while in leaves the amounts of glucobrassicin and neoglucobrassicin were comparable, and in stems, the major GLS was neoglucobrassicin. Aliphatic GLS were not detected differently from Drabińska et al. (2021) who quantified sinigrin and progoitrin in leaves (boiled 1 min) and stems (2 min) of white cauliflower, in lower amount than indole GLS. These differences could be related to the different cauliflower cultivars examined (white *vs* coloured), the GLS extraction (desulphation *vs* no desulphation) and to the lasting of blanching pre-treatment (1–2 min *vs* 5 min). In a targeted semi-quantitative study based on LC-HRMS, GLS of florets of the same varieties, here tested, were lost at the increasing time of boiling (10, 25, 40 min) (Nartea et al., 2022).

Considering the carotenoid matter, the most abundant was β-carotene, followed by lutein, in all the flours. The contents of β-carotene and lutein were markedly higher in flour from leaves (LF) than flour from stalks of both varieties (OF, VF). Regarding the influence of the variety on stalk composition, OF and VF presented comparable amounts of lutein whereas OF was 10-fold richer in β-carotene than VF. These results reflected the evidence reported by Nartea et al. (2021a) highlighting that the total carotenoid content was 10-fold higher in orange florets (*Cheddar* variety) than in *Depurple* variety. Moreover, the most abundant carotenoid in orange was β-carotene, followed by lutein, whereas in *Depurple* was lutein the most plentiful followed by β-carotene. It is possible to suppose that the spontaneous mutation in *Cheddar* cauliflower alter the carotenoid accumulation in floret but also in stalks (Li et al., 2006; Nartea et al., 2021b).

As concern the phytosterol fraction, its profile did not vary among the special flours. In all cases, the predominant compounds were β-sitosterol, followed by campesterol and stigmasterol. This finding agrees with the phytosterol profile of *Cheddar* and *Depurple* cauliflower florets reported in previous studies (Nartea et al., 2021b).

The flours from stalks (OF = 2530,26 and VF = 1567,41 mg/kg) contained more phytosterols than flour from leaves (LF = 801,6 mg/kg). Considering the varieties, the total phytosterol content was 1.5-fold higher in OF than in VF. The cultivar affects the plant sterols levels (Gajewski et al., 2009). In florets, violet cauliflower has been reported to be richer than orange one (Nartea et al., 2021b). This difference could be related to the fact that the distribution of phytosterols along the aerial part of cauliflowers changes according to the cauliflower varieties. Broccoli stems were found to contain lower concentration of free sterols

Table 2
Bioactive compounds (mg/kg powder) in special flours from orange stalks (OF), violet stalks (VF) and leaves (LF). Data are reported as mean of three replicates ± standard deviation.

	OF	VF	LF
Glucobrassicin	161.21 ± 3.39	476.62 ± 4.36	283.97 ± 7.61
4-Metoxyglucobrassicin	39.46 ± 5.48	15.35 ± 2.03	207.15 ± 19.20
Neoglucobrassicin	84.92 ± 3.51	261.90 ± 16.50	334.99 ± 16.34
β-carotene	68.37 ± 1.14	6.81 ± 0.26	357.26 ± 6.15
Lutein	0.43 ± 0.02	0.45 ± 0.01	103.47 ± 5.09
Campesterol	508.13 ± 38.17	323.64 ± 14.68	131.78 ± 11.17
Stigmasterol	16.71 ± 0.99	19.09 ± 1.09	4.61 ± 0.33
β-sitosterol	2005.42 ± 58.87	1224.68 ± 31.66	665.21 ± 21.67

than florets (Gajewski et al., 2009).

The stabilization of the raw stalks and leaves was performed with a prolonged blanching step, 5 min instead of 1–2 min, to enhance the content of selected bioactive compounds. From our previous studies on cauliflowers and broccoli, boiling (10 min) enhanced the β-carotene, lutein (Nartea et al., 2021a; Orlando et al., 2022) and phytosterols from raw material, mainly because of tissue softening (Nartea et al., 2021b). While GLS, expressed as isothiocyanates equivalents, decreased with respect to raw broccoli (Orlando et al., 2022). The compromise to obtain special flour rich in both carotenoids, phytosterols and GLS was 5 min of blanching, based on our previous experiments.

3.2. Bioactive compounds in fortified pizzas

The content of indole GLS, carotenoids and phytosterols in baked pizza (control and fortified) are reported in Table 3.

Generally, the qualitative profile of bioactive compounds in fortified pizzas reflected the bioactive profile of by-product flours. However, the extent of bioactive compounds enrichment in pizza resulted from the combination of three factors: the content of bioactive compounds in by-products flours, the level of replacement of wheat with by-products flour, and the stability of each compound to the thermal treatment applied to bake the pizza. The thermal treatment has involved a pre-baking (300 °C, 2.3 min) and then a baking step (220 °C, 8 min).

In detail, glucobrassicin ranged from 5.7 to 13.8 mg/kg (wet weight) in fortified pizzas with 10% of by-products flour, while from 19.2 to 42.0 in pizza with 30% of special flour incorporation. The glucobrassicin enrichment of pizza is proportionally enhanced with the amount of by-products flours used for pizza formulation (fortification level). In fact, for all by-product flours incorporated, the sample resulting from the replacement of 30% of wheat with by-product flours, had a 3-fold higher amount of glucobrassicin than the respective sample obtained with 10% of replacement. Differently, the glucobrassicin content in pizza samples (PVF > POF > PLF) did not follow the same order of the glucobrassicin level in by-product flour (VF > LF > OF). As a result, pizza made with 30% of violet cauliflower stalks (PVF30) resulted to be the significantly richest ($p < 0.05$) sample in glucobrassicin. The PLF10 (made with leaf flour) resulted the poorest ($p < 0.05$). Despite the lower concentration of glucobrassicin in OF flour (161.2 ± 3.4 mg/kg powder) than in LF (283.9 ± 7.6 mg/kg powder), all pizza samples that contained OF flour were significantly richer in glucobrassicin (2-fold more) than the respective made with LF flour. This outcome can be explained by

Table 3
Level of bioactive compounds in control and fortified baked pizza (P) with OF, VF and LF at 10 and 30% of incorporation. Data are reported as mean of three replicates ± standard deviation. Different letters in the same row mean statistical difference (ANOVA, $p < 0.05$).

	Control	POF10	POF30	PVF10	PVF30	PLF10	PLF30
mg/kg of pizza (dry weight)							
Glucobrassicin	<LOD	11.6 ± 0.1^{b}	41.3 ± 0.9^{e}	15.7 ± 0.1^{c}	53.8 ± 0.7^{f}	6.5 ± 0.2^{a}	24.0 ± 0.8^{d}
4-Methoxyglucobrassicin	<LOD	4.7 ± 0.2^{a}	12.4 ± 1.0^{c}	6.5 ± 0.8^{b}	14.5 ± 1.5^{c}	7.4 ± 0.4^{b}	18.5 ± 0.9^{d}
Neoglucobrassicin	<LOD	7.5 ± 0.2^{a}	25.2 ± 3.1^{c}	11.2 ± 0.6^{ab}	35.8 ± 1.8^{d}	12.4 ± 1.0^{b}	39.0 ± 2.5^{e}
Indole glucosinolates	<LOD	23.9 ± 0.4^{a}	78.8 ± 3.0^{c}	33.4 ± 1.4^{b}	104.2 ± 2.6^{d}	26.2 ± 1.0^{a}	81.6 ± 3.6^{c}
β-Carotene	<LOQ	2.3 ± 0.0^{b}	7.0 ± 0.2^{d}	0.2 ± 0.0^{a}	1.0 ± 0.7^{ab}	3.8 ± 0.2^{c}	40.9 ± 1.2^{e}
Lutein	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}	0.2 ± 0.0^{a}	0.8 ± 0.0^{a}	5.3 ± 0.3^{b}	30.4 ± 1.5^{c}
Ergosterol	4.4 ± 0.3^{bc}	4.0 ± 0.10^{b}	7.4 ± 0.8^{d}	4.3 ± 0.2^{bc}	5.0 ± 0.1^{c}	1.8 ± 0.0^{a}	2.5 ± 0.2^{a}
Campesterol	21.8 ± 1.7^{a}	29.6 ± 2.7^{ab}	76.6 ± 5.6^{e}	40.1 ± 4.0^{c}	56.4 ± 4.6^{d}	38.9 ± 1.9^{bc}	58.7 ± 3.3^{d}
Campestanol	4.6 ± 0.30^{b}	7.5 ± 0.6^{c}	4.3 ± 0.30^{b}	4.4 ± 0.30^{b}	4.9 ± 0.3^{b}	2.4 ± 0.2^{a}	3.3 ± 0.5^{a}
Stigmasterol	6.0 ± 0.5^{a}	5.9 ± 0.4^{a}	9.5 ± 0.5^{c}	7.2 ± 0.4 ^{ab}	8.6 ± 0.6^{bc}	6.8 ± 0.8^{a}	8.9 ± 1.0^{bc}
β-Sitosterol	97.6 ± 4.4^{a}	140.2 ± 5.7^{b}	331.9 ± 9.3^{d}	161.5 ± 4.2 ^{b}	248.3 ± 8.1^{c}	156.5 ± 6.4^{b}	302.6 ± 22.7^{d}
Δ^7-Avenasterol	17.0 ± 1.3^{a}	28.5 ± 2.1^{b}	25.4 ± 1.7^{b}	14.9 ± 0.7^{a}	17.8 ± 0.9^{a}	14.4 ± 0.7^{a}	15.4 ± 1.0^{a}
Stigmastadienol	6.9 ± 0.5^{ab}	6.0 ± 0.4^{a}	9.3 ± 0.8^{c}	8.6 ± 0.5^{c}	7.0 ± 0.4^{ab}	8.5 ± 0.4^{bc}	12.0 ± 0.9^{d}
mg/kg of pizza (wet weight)							
Glucobrassicin	<LOD	9.8 ± 0.1^{b}	32.3 ± 0.6^{e}	13.8 ± 0.1^{c}	42.0 ± 0.5^{f}	5.7 ± 0.1^{a}	19.9 ± 0.5^{d}
4-Methoxyglucobrassicin	<LOD	4.0 ± 0.2^{a}	10.0 ± 0.6^{c}	5.7 ± 0.6^{ab}	11.3 ± 0.9^{c}	6.5 ± 0.3^{b}	15.4 ± 0.6^{d}
Neoglucobrassicin	<LOD	6.3 ± 0.1^{a}	20.3 ± 2.1^{c}	9.8 ± 0.4^{ab}	27.9 ± 1.2^{d}	11.0 ± 0.7^{b}	32.4 ± 1.7^{e}
Indole glucosinolates	<LOD	20.9 ± 1.2^{a}	61.9 ± 1.6^{c}	30.1 ± 1.1^{b}	91.9 ± 1.8^{d}	20.4 ± 1.5^{a}	62.6 ± 2.1^{c}
β-Carotene	<LOQ	1.9 ± 0.0^{b}	5.6 ± 0.2^{d}	0.2 ± 0.0^{a}	0.8 ± 0.0^{ab}	3.4 ± 0.16^{c}	34.0 ± 0.80^{e}
Lutein	0.3 ± 0.0 ^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.2 ± 0.0 ^{a}	0.7 ± 0.0 ^{b}	4.7 ± 0.2 ^{c}	25.3 ± 1.0 ^{d}
Vitamin A *(RAE, μg retinol/200g)**	41.7 ± 0.1^{a}	68.8 ± 0.6^{b}	193.4 ± 5.1^{c}	10.2 ± 0.1^{a}	36.9 ± 0.5^{ab}	191.3 ± 2.4^{c}	1554.3 ± 27.9^{d}
Ergosterol	4.0 ± 0.3^{b}	3.4 ± 0.1^{b}	6.0 ± 0.5^{c}	3.8 ± 0.2^{b}	3.8 ± 0.0^{b}	1.6 ± 0.0^{a}	2.1 ± 0.1^{a}
Campesterol	19.8 ± 1.2^{a}	24.8 ± 1.8^{a}	61.8 ± 3.7^{d}	35.1 ± 2.9^{b}	44.0 ± 2.9^{c}	34.4 ± 1.4^{b}	48.7 ± 2.2^{c}
Campestanol	4.2 ± 0.3^{c}	6.3 ± 0.4^{d}	3.5 ± 0.2^{bc}	3.8 ± 0.2^{c}	3.8 ± 0.2^{c}	2.1 ± 0.2^{a}	2.7 ± 0.3^{ab}
Stigmasterol	5.5 ± 0.3^{ab}	5.0 ± 0.2^{a}	7.6 ± 0.3^{d}	6.3 ± 0.2^{ad}	6.7 ± 0.4^{bd}	6.0 ± 0.6^{abc}	7.4 ± 0.7^{cd}
β-Sitosterol	88.8 ± 3.3^{a}	117.9 ± 3.9^{b}	267.8 ± 6.1^{d}	141.4 ± 3.0^{b}	193.7 ± 5.1^{c}	138.4 ± 4.6^{b}	251.4 ± 15.4^{d}
Δ^7-Avenasterol	15.5 ± 0.9^{a}	24.0 ± 1.5^{c}	20.5 ± 1.1^{b}	13.1 ± 0.5^{a}	13.9 ± 0.6^{a}	12.8 ± 0.5^{a}	12.8 ± 0.1^{a}
Stigmastadienol	6.27 ± 0.4^{ab}	5.1 ± 0.3^{a}	7.5 ± 0.5^{b}	7.6 ± 0.4^{b}	5.4 ± 0.2^{a}	7.5 ± 0.3^{b}	10.0 ± 0.6^{c}

LOQ, Limit of quantification; LOD, limit of detection; *Retinol activity equivalent (RAE) was calculated considering 1 μg retinol for each 6 μg of β-carotene or 12 μg of other provitamin A carotenoids. The recommended serving size of pizza is 200 g.

considering the degradation of GLS during thermal processing. It is well known that GLS can be thermally degraded leading to the formation of volatile compounds such as nitriles and other compounds (Hanschen et al., 2012; Wu et al., 2021).

On the other side, in the pizza samples, neoglucobrassicin reflected the composition of powders as the order was PLF > PVF > POF at 10 and 30% level of incorporation. Globally, the total amount of indole GLS showed intermediate behavior between those found for glucobrassicin and for neoglucobrassicin (PVF > PLF > POF).

In bakery products as crackers, cakes, bread, investigated in previous studies, temperature did not overcome 180 °C (Franco et al., 2016; Drabińska et al., 2018; Krupa-Kozak et al., 2021), while in the present study, in pizza samples baking, temperatures are in the range 220–300 °C. In agreement with our results, fortified sponge mini cakes displayed variations in the content of individual GLS, showing differences probably in relation with the thermal stability, where aliphatic compounds were found more stable than indole ones (Drabińska et al., 2018). In crackers, only 5% of the total GLS have been lost in the process of mixing and baking (Franco et al., 2016).

However, the effect of the thermal treatment on the degradation of glucobrassicin can be related to the presence of other bioactive compounds in the by-product flour.

It can be speculated that the by-product from orange cauliflower stalk contained compounds able to preserve the degradation of glucobrassicin. Drabińska et al. (2018) hypothetically attributed the presence of positive interactions between bioactive compounds and food ingredients in mini gluten-free sponge cakes fortified with GLS. Giambanelli et al. (2015) emphasized that the ratio of GLS to other food components is crucial to determine thermal degradation of GLS and that the interactions with the food matrix in bakery products require further investigation. Moreover, yeast fermentation (*Saccharomyces cerevisiae*) can alter the antioxidants profiles doughs because of enzymatic activities (e.g., β-glucosidase activity) and can be influenced by anti-fermentative agents such as GLS and polyphenols (Lafarga et al., 2018; Vlassa et al., 2022). Eighty-one % of glucobrassicin was recovered in POF30 against 37% in PVF30 and 26% PLF30. VF and LF were flours richer in GLS than OF. We exclude the mirosinase activity to explain the different recoveries of the enriched pizzas, inactivated with the prolonged blanching step we performed.

The sum of GLS and carotenoids in special flours resulted negatively correlated with the % of glucobrassisin recovery in pizza at 30% level of enrichment.

Considering the carotenoids, the pizza prepared as control sample solely displayed a low amount of lutein. The β-carotene and the lutein levels in the samples proportionally increased with the amount of by-products flours used for pizza formulation. In all cases, the samples made with 30% of by-product flour were richer than those made with 10%. Moreover, the use of the richest flour in carotenoids, such as LF, at the fortification levels of 30% led to the obtainment of pizza with the highest content of lutein (25.3 mg/kg baked pizza) and β-carotene (34.0 mg/kg baked pizza). The use of flour from violet stalks, having the lowest carotenoid content, provided the samples PVF10 and PVF30 with the lowest amount of carotenoids. In PLF10, only 12% of β-carotene was recovered from the special flour vs 50% in PLF30. For lutein, the recovery rate was higher than β-carotene with a value of 57% and 90% in PLF10 and PLF30, respectively. Carotenoids during thermal treatments can undergo not only degradation but also isomerization and oxidation (Schieber and Carle, 2005). In pak choi and kale leaf bread, only 22% of the initial lutein was recovered during breadmaking process. While β-carotene in pak choi leaf bread was at 48% and at 26% for kale choi leaf (Klopsch et al., 2019). Pizza and bread differed for the time of fermentation (22 h *vs* 40 min) and baking parameters (40 min at 230 °C *vs* 2 min at 300 °C plus 8 min at 220 °C). The combination of yeast fermentation, thermal degradation and food matrix effect could explain the different recovered carotenoids. Concerning phytosterols, the incorporation of all by-product flours at both levels led to a significant enhancement of β-sitosterol and campesterol with respect to the control sample. The stigmasterol content did not significantly change among the pizza samples. As we discussed for glucobrassicin, the phytosterols enrichment of pizza did not reflect the phytosterol content of the by-product's flours. Although the OF flour presented a higher level (3-fold more) of β-sitosterol and campesterol than LF, POF and PLF samples had comparable amounts of β-sitosterol and campesterol. Furthermore, the phytosterols enrichment of pizza proportionally increased with the amount of by-products flour used for pizza formulation (fortification level) when flour orange stalks and leaves were used. On the other hand, no difference in β-sitosterol and campesterol levels were revealed between PVF10 and PVF30 samples. The phytosterols enriched foods are recommended to be consumed with higher content of carotenoids, because of their interaction. Phytosterols compete not only with cholesterol but also with carotenoids for the digestive absorption (Fardet et al., 2017). Thus, the use of and LF as functional food ingredients rich in both phytosterol and carotenoids requires accurate evaluation on their levels to optimize the bioavailability of carotenoids. Thermal degradation of phytosterols in pizza could have been occurred but in low quantities, less than 1.38% per portion of goods cooked with phytosterol enriched margarine (Lin et al., 2016). Better, our data variability could regard the food matrix. Disassembling and assembling mechanisms depending on the cell wall and membrane may occurred during heat processing of pizza making affecting the extractability of phytosterols of pizzas differently from that of flours (Nartea et al., 2021b).

Moving to the health benefits issue, the EU Commission has not yet included any health claim about GLS in the Regulation 432/2012, although many epidemiological studies highlighted the inverse association between the intake of GLS-rich foods and the risk of different types of cancer or other disorders (Marino et al., 2021). It has been reported that 121 mg of GLS per day helps to attenuate chronic inflammation in overweight subjects (López-Chillón et al., 2019). Along with EFSA authorisation, "plant sterols/stanols contribute to the maintenance of normal blood cholesterol levels" can be claimed for food providing an intake of at least 0,8 g of plant sterols/stanols per day (EFSA, 2009).

Within this frame, the nutritional potential of fortified pizza is interesting as the fortification with different cauliflower by-products produced pizzas with 1.44–12.00 μmol of glucobrassicin, 4–670 μg vitamin A, and 22.1–46.4 mg of total phytosterols per 100 g of dry weight pizza. Similar values in total GLS (9.03–15.05 μmol/100 g fresh weight) were reported by Drabińska et al. (2018) in cakes enriched with 2.5–7.5% of broccoli leaf powder. 75 μmol/100 g of crackers added with 1% of *E. sativa* defatted seed meal (DSM), ensured a release of about 20 μmol/100 g isothiocyanates during chewing and 50 μmol GLS in the gastrointestinal tract for the slow hydrolysis by gut microbiota in isothiocyanates, the bioactive metabolites of GLS displaying health effect (Franco et al., 2016). The recipe for the production of crackers enriched with *E. sativa* DSM was defined to maximize the yield of intact GLS in the dough characterized by the absence of activity of the enzyme myrosinase.

Hydrolysis of glucobrassicin produces predominantly indole-3-carbinol and its major in vivo product, 3,3′-diindolylmethane, is a chemopreventive agent in pre-clinical models (Williams, 2021). Thus, intact glucobrassicin in pizza samples represents the potential indole-3-carbinol as the principal compound resulting after the myrosinase hydrolysis of the intestinal microbiome.

A portion of 200 g of fortified baked pizza provides up to 95.7% of the reference intakes of vitamin A, set at 700 μg retinol equivalent per day (EFSA NDA Panel, 2015) and up to 5.8% of the phytosterols health claim requirement (0.8 g of plant sterols/stanols intake per day (EFSA, 2009), obtained with 30% fortification level with orange stalks flour.

3.3. Rheological and textural properties

The quantitative effects of functional flours on the technological

properties of enriched doughs (pre-baked pizzas) as well as on texture properties of derived pizzas were investigated by analyzing their load-bearing ability in a wide range of scale size deformation, as these characteristics may be important both for industries and consumers.

In Fig. 2, the time dependent creep compliance curve, J(t), of the control dough (D-control) showed a typical viscoelastic behavior. J(t), expressed as the ratio between the strain (dimensionless) and the applied constant stress (Pa), registered three distinguishable rheological regimes during the loading step: i) an instantaneous elastic deformation (J_0), phenomenon associated to the stretching of covalent bonds between structural units such as gluten, amylopectin-lipid complexes and/or cellulose microfibril network (Nartea et al., 2021b); ii) a retarded reversible deformation (J_i), during which the dough deformed less rapidly due to the break and the reformation of bonds (Wang and Sun, 2002); iii) a steady-state regime (J_N), which increased until the maximum level (J_{max}), or rather when the force was removed. Similarly, three regimes with an opposite trend can be identified during the unloading stage, i.e., instantaneous, retarded, and steady recovery. However, at the end of the test, the dough recovered only partially the original structure, and therefore J_{rel} represented the extent of irreversible damage caused by the viscous components. The overall creep compliance profile of functional doughs (Fig. 3a) showed a clear distinction between the two fortification levels (10 *vs* 30%). The doughs at 10% displayed a higher J_{max} value (ranging from about 5.0×10^{-3} of LF to 7.5×10^{-3} of OF) than those at 30%, while they were similar to the control dough ($\sim 6.8 \times 10^{-3}$). On the other hand, the doughs containing 30% special flours had J_{max} values between 1.5×10^{-3} of LF and 3.0×10^{-3} of OF, thus indicating a solid-like structure and a greater resistance to flow (Dogan and Kokini, 2006). Since the functional doughs enriched with cauliflower by-products are supposed to have a higher number of fibers (Hussain et al., 2020) and lower gluten compared to the control dough, our findings agree with previous studies, according to which the higher the percentage of dietary fibers in the doughs, the harder the texture (Ma et al., 2020; Abul-Fadl, 2012; Wang and Sun 2002). As regards pizzas (Fig. 3b), similar observations can be made, specifically pizzas containing 30% special flour resulted less prone to deform compared to those at 10%.

However, to understand deeply the interactions between rheological and texture properties of pizzas, a principal component analysis (PCA) was performed using the latter as independent input variables and the former as supplementary variables (Fig. 4). The first two principal components revealed more than 89% of total variance, the first factor explaining the highest proportion (72.67%). The pizzas containing 30% of VF and LF were higher in hardness, chewiness, cohesiveness, and gumminess compared to control and 10 %-enriched pizzas. These findings are in line with our creep experiments, proving their suitability to

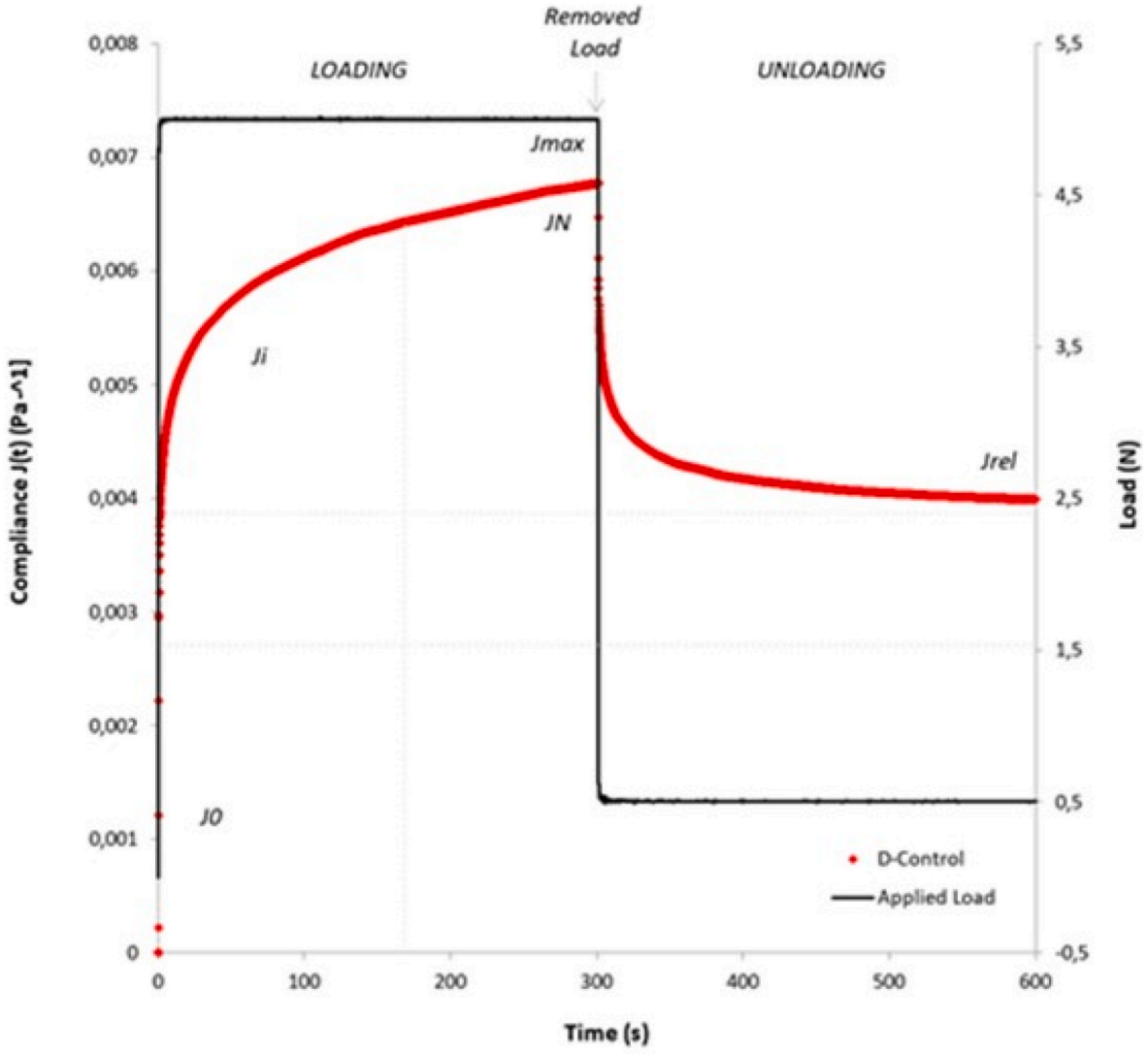

Fig. 2. Time dependent creep compliance of conventional dough (D-Control).

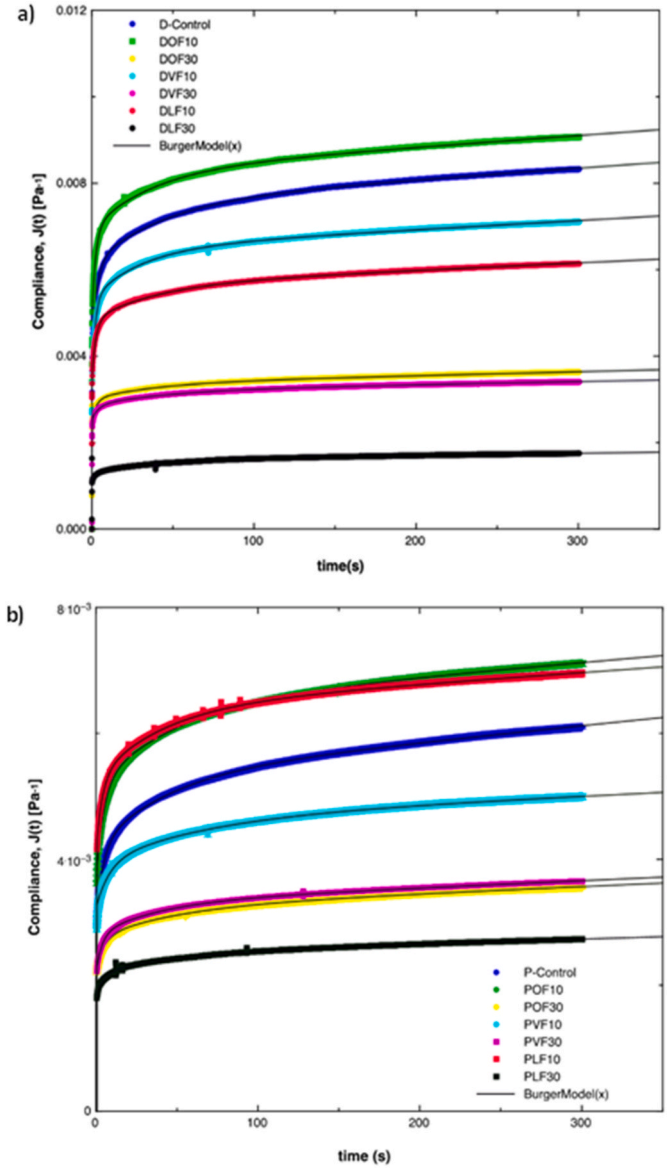

Fig. 3. Elastic, viscoelastic, and plastic behavior of a) control and functional doughs; b) control and functional pizzas.

predict texture properties. In addition, they agree with the outcomes reported by previous authors according to which the addition of fiber-rich vegetable and fruit powders to bakery products such as cakes, biscuits, and bread affects texture properties of the final product especially by increasing hardness, gumminess, and chewiness (Gómez et al., 2003; Salehi, 2020; Salehi and Aghajanzadeh, 2020). By contrast, pizzas with the lowest fortification level (10%) showed characteristic similar to the control pizza, thus having high elastic (J_0), viscoelastic (J_i), and plastic (J_N) properties, as well as springiness. Although sensory tests were not conducted, this outcome led to think that these types of pizza may gain a good consumer acceptance. The pizza with 30% OF displayed intermediate features between the two groups, having a higher springiness compared to PVF30 and PLF30. This difference could be attributed to the impact of the fermentation process, that may have altered the food structure differently from the other two pizzas (Lafarga et al., 2018). To conclude, the enrichment of pizza with 10% of special flour did not strongly affect neither the rheological properties, nor the texture, suggesting that their production is feasible on an industrial scale maintaining the same conditions used for conventional pizzas, and that there are high probabilities to meet consumers' acceptance.

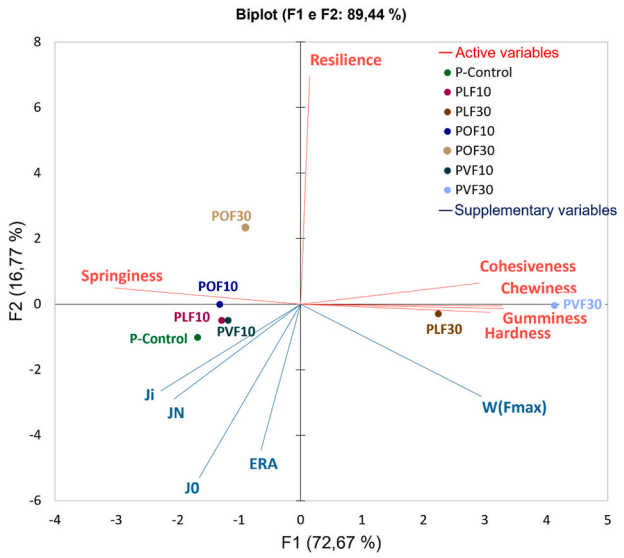

Fig. 4. Principal component analysis (PCA) performed using texture parameters as independent input variables and creep compliance parameters as supplementary variables for conventional and functional pizzas.

4. Conclusions

Leaves and stalks of cauliflower, transformed into optimized bioactive flours, are valuable ingredients for pizza making. The profile of bioactive compounds of the special flours was affected by the aerial part and the variety of cauliflower, orange or violet. Orange stalks flour contained the highest level of total bioactive compounds, the sum of GLS, carotenoids and phytosterols. The profile of carotenoids and neoglucobrassicin of flours was reflected in the enriched pizzas at different level of fortification, differently for glucobrassicin and phytosterols. However, the higher the enrichment level in pizza formulation, the higher the content of bioactive compounds in the final product. Pizza making process affected the recovery of the bioactive compounds. The recovery of glucobrassicin in pizza was higher for stalks-based flours rather than leaves, vice versa for carotenoids. Moreover, for lutein, the recovery rate was higher than β-carotene in leaves-based pizzas. The level of enrichment with the stalks and leaves-based flours affected the rheological and thus, the texture properties of final leavened products. No matter stalks or leaves, 10% of fortification maintained the elastic, viscoelastic, and plastic properties, as well as springiness characteristics of the control pizza. Differently, 30% of leaves and violet stalks-based flours negatively impacted on the properties of pizza, resulting less keen to deform and more cohesive, chewier, gummier, and harder than control and all 10% enriched pizzas. The most promising formulation was found in POF30 where 30% of orange stalk flour was used, for an optimal combination of good functional (intake of glucobrassicin, vitamin A and phytosterols), technological (keen to deformation), and textural properties (springiness).

Hereafter, cruciferous stalks could be valorized also through the investigation of new eco-friendly and mild technologies such as fermentation to improve their bioavailability and to enhance their sustainability.

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CRediT authorship contribution statement

Ancuta Nartea: Methodology, Software, Formal analysis, Data curation, Writing – original draft, preparation. **Benedetta Fanesi:** Formal analysis. **Deborah Pacetti:** Conceptualization, Investigation, Supervision, Project administration, Funding acquisition. **Lucia Lenti:** Data curation. **Dennis Fiorini:** Conceptualization, Writing – original draft, preparation. **Paolo Lucci:** Conceptualization, Data curation, Writing – review & editing. **Natale G. Frega:** Project administration. **Pasquale M. Falcone:** Software, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100437.

References

Abul-Fadl, M.M., 2012. Nutritional and chemical evaluation of white cauliflower by-products flour and the effect of its addition on beef sausage quality. J. Appl. Sci. Res. 8, 693–704.

Ahmed, F.A., Ali, R.F.M., 2013. Bioactive compounds and antioxidant activity of fresh and processed white cauliflower. BioMed Res. Int. 1–9. https://doi.org/10.1155/2013/367819, 2013.

Alvarez Jubete, L., Valverde, J., Kehoe, K., Reilly, K., Rai, D.K., Barry-Ryan, C., 2014. Development of a novel functional soup rich in bioactive sulforaphane using broccoli (Brassica oleracea L. ssp. italica) florets and byproducts. Food Bioprocess Technol. 7, 1310–1321. https://doi.org/10.1007/s11947-013-1113-9.

Amofa-Diatuo, T., Anang, D.M., Barba, F.J., Tiwari, B.K., 2017. Development of new apple beverages rich in isothiocyanates by using extracts obtained from ultrasound-treated cauliflower by-products: evaluation of physical properties and consumer acceptance. J. Food Compos. Anal. 61, 73–81. https://doi.org/10.1016/j.jfca.2016.10.001.

Arumugam, A., Razis, A.F.A., 2018. Apoptosis as a mechanism of the cancer chemopreventive activity of glucosinolates: a review. Asian Pac. J. Cancer Prev. APJCP 19 (6), 1439–1448. https://doi.org/10.22034/APJCP.2018.19.6.1439.

Baenas, N., Marhuenda, J., García-Viguera, C., Zafrilla, P., Moreno, D.A., 2019. Influence of cooking methods on glucosinolates and isothiocyanates content in novel cruciferous foods. Foods 8, 257. https://doi.org/10.3390/foods8070257.

Bharat Helkar, P., Sahoo, A., 2016. Review: food industry by-products used as a functional food ingredients. Int. J. Waste Resour. 6, 3. https://doi.org/10.4172/2252-5211.1000248.

Castelão-Baptista, J.P., Barros, A., Martins, T., Rosa, E., Sardão, V.A., 2021. Three in one: the potential of brassica by-products against economic waste, environmental hazard, and metabolic disruption in obesity. Nutrients 13, 4194. https://doi.org/10.3390/nu13124194.

Dogan, H., Kokini, J.L., 2006. Rheological properties of foods. In: Handbook of Food Engineering, second ed. https://doi.org/10.1201/b18668-4

Dominguez-Perles, R., Moreno, D.A., Carvajal, M., Garcia-Viguera, C., 2011. Composition and antioxidant capacity of a novel beverage produced with green tea and minimally processed byproducts of broccoli. Innovat. Food Sci. Emerg. Technol. 12, 361–368. https://doi.org/10.1016/j.ifset.2011.04.005.

Dong, M., Tian, Z., Ma, Y., Yang, Z., Ma, Z., Wang, X., Li, Y., Jiang, H., 2021. Rapid screening and characterization of glucosinolates in 25 Brassicaceae tissues by UHPLC-Q-exactive orbitrap-MS. Food Chem. 365, 130493 https://doi.org/10.1016/j.foodchem.2021.130493.

Drabińska, N., Ciska, E., Szmatowicz, B., Krupa-Kozak, U., 2018. Broccoli by-products improve the nutraceutical potential of gluten-free mini sponge cakes. Food Chem. 267, 170–177. https://doi.org/10.1016/j.foodchem.2017.08.119.

Drabińska, N., Jeż, M., Nogueira, M., 2021. Variation in the accumulation of phytochemicals and their bioactive properties among the aerial parts of cauliflower. Antioxidants 10, 1597. https://doi.org/10.3390/antiox10101597.

EFSA, 2009. Scientific Opinion of the Panel on Dietetic Products Nutrition and Allergies on a request from the European Commission and a similar request from France in relation to the authorization procedure for health claims on plant sterols/stanols and lowering/red. EFSA J. 7 (7) https://doi.org/10.2903/j.efsa.2009.1175.

EFSA NDA Panel, 2015. Scientific opinion on dietary reference values for vitamin A. EFSA J. 13 (3), 4028. https://doi.org/10.2903/j.efsa.2015.4028.

FAO, 2022. FAOSTAT. Retrieved June 1, 2019, from. https://www.fao.org/faostat/en/#data/QCL/visualize.

Fardet, A., Morise, A., Kalonji, E., Margaritis, I., Mariotti, F., 2017. Influence of phytosterol and phytostanol food supplementation on plasma liposoluble vitamins and provitamin A carotenoid levels in humans: an updated review of the evidence. Crit. Rev. Food Sci. Nutr. 57 (9), 1906–1921. https://doi.org/10.1080/10408398.2015.1033611.

Franco, P., Spinozzi, S., Pagnotta, E., Lazzeri, L., Ugolini, L., Camborata, C., Roda, A., 2016. Development of a liquid chromatography-electrospray ionization-tandem mass spectrometry method for the simultaneous analysis of intact glucosinolates and isothiocyanates in Brassicaceae seeds and functional foods. J. Chromatogr. A 1428, 154–161. https://doi.org/10.1016/j.chroma.2015.09.001.

Gajewski, M., PrzybyŁ, J.L., Kosakowska, O., Szymczak, P., 2009. Some factors influencing free sterols content in broccoli (brassica oleracea l. var. botrytis italica plenck.). J. Food Biochem. 33 (6), 881–894. https://doi.org/10.1111/j.1745-4514.2009.00262.x.

Giambanelli, E., Verkerk, R., Fogliano, V., Capuano, E., D'Antuono, L.F., Oliviero, T., 2015. Broccoli glucosinolate degradation is reduced performing thermal treatment in binary systems with other food ingredients. RSC Adv. 5, 66894–66900. https://doi.org/10.1039/C5RA11409H.

Gómez, M., Ronda, F., Blanco, C.A., Caballero, P.A., Apesteguía, A., 2003. Effect of dietary fibre on dough rheology and bread quality. Eur. Food Res. Technol. 216 (1), 51–56. https://doi.org/10.1007/s00217-002-0632-9.

Gul, K., Tak, A., Singh, A.K., Singh, P., Yousuf, B., Wani, A.A., 2015. Chemistry, encapsulation, and health benefits of β-carotene - a review. Cogent. Food Agric. 1 (1) https://doi.org/10.1080/23311932.2015.1018696.

Hanschen, F.S., Platz, S., Mewis, I., Schreiner, M., Rohn, S., Kroh, L.W., 2012. Thermally induced degradation of sulfur-containing aliphatic glucosinolates in broccoli sprouts (Brassica oleracea var. italica) and model systems. J. Agric. Food Chem. 60 (9), 2231–2241. https://doi.org/10.1021/jf204830p.

Hussain, S., Jõudu, I., Bhat, R., 2020. Dietary fiber from underutilized plant resources-A positive approach for valorization of fruit and vegetable wastes. Sustainability 12 (13), 5401. https://doi.org/10.3390/su12135401.

Jahangir, M., Kim, H.K., Choi, Y.H., Verpoorte, R., 2009. Health-affecting compounds in Brassicaceae. Compr. Rev. Food Sci. Food Saf. 8, 31–43. https://doi.org/10.1111/j.1541-4337.2008.00065.x.

Khedkar, M.A., Nimbalkar, P.R., Chavan, P.V., Chendake, Y.J., Bankar, S.B., 2017. Cauliflower waste utilization for sustainable biobutanol production: revelation of drying kinetics and bioprocess development. Bioproc. Biosyst. Eng. 40 (10), 1493–1506. https://doi.org/10.1007/s00449-017-1806-y.

Klopsch, R., Baldermann, S., Hanschen, F.S., Voss, A., Rohn, S., Schreiner, M., Neugart, S., 2019. Brassica-enriched wheat bread: unraveling the impact of ontogeny and breadmaking on bioactive secondary plant metabolites of pak choi and kale. Food Chem. 295, 412–422. https://doi.org/10.1016/j.foodchem.2019.05.113.

Krupa-Kozak, U., Drabińska, N., Baczek, N., Šimková, K., Starowicz, M., Jeliński, T., 2021. Application of broccoli leaf powder in gluten-free bread: an innovative approach to improve its bioactive potential and technological quality. Foods 10 (4), 819. https://doi.org/10.3390/foods10040819.

Krupa-Kozak, U., Drabińska, N., Rosell, C.M., Fadda, C., Anders, A., Jeliński, T., Ostaszyk, A., 2019. Broccoli leaf powder as an attractive by-product ingredient: effect on batter behaviour, technological properties and sensory quality of gluten-free mini sponge cake. Int. J. Food Sci. Technol. 54, 1121–1129. https://doi.org/10.1111/ijfs.13972.

Lafarga, T., Gallagher, E., Bademunt, A., Bobo, G., Echeverria, G., Viñas, I., Aguiló-Aguayo, I., 2019. Physiochemical and nutritional characteristics, bioaccessibility and sensory acceptance of baked crackers containing broccoli co-products. Int. J. Food Sci. Technol. 54 (3), 634–640. https://doi.org/10.1111/ijfs.13908.

Lafarga, T., Gallagher, E., Bademunt, A., Viñas, I., Bobo, G., Villaró, S., Aguiló-Aguayo, I., 2018. Bioaccessibility, physicochemical, sensorial, and nutritional characteristics of bread containing broccoli co-products. J. Food Process. Preserv. 43 (2), 1–11. https://doi.org/10.1111/jfpp.13861.

Li, L., Lu, S., Cosman, K.M., Earle, E.D., Garvin, D.F., O'Neill, J., 2006. β-Carotene accumulation induced by the cauliflower or gene is not due to an increased capacity of biosynthesis. Phytochemistry 67 (12), 1177–1184. https://doi.org/10.1016/j.phytochem.2006.05.013.

Lin, L.Z., Sun, J., Chen, P., Zhang, R.W., Fan, X.E., Li, L.W., Harnly, J.M., 2014. Profiling of glucosinolates and flavonoids in rorippa indica (Linn.) hiern. (Cruciferae) by UHPLC-PDA-ESI/HRMS(n). J. Agric. Food Chem. 62 (26), 6118–6129. https://doi.org/10.1021/jf405538d.

Lin, Y., Knol, D., Menéndez-Carreño, M., Blom, W.A.M., Matthee, J., Janssen, H.G., Trautwein, E.A., 2016. formation of plant sterol oxidation products in foods during baking and cooking using margarine without and with added plant sterol esters. J. Agric. Food Chem. 64 (3), 653–662. https://doi.org/10.1021/acs.jafc.5b04952.

López-Chillón, M.T., Carazo-Díaz, C., Prieto-Merino, D., Zafrilla, P., Moreno, D.A., Villaño, D., 2019. Effects of long-term consumption of broccoli sprouts on inflammatory markers in overweight subjects. Clin. Nutr. 38 (2), 745–752. https://doi.org/10.1016/j.clnu.2018.03.006.

Ma, S., Han, W., Li, L., Wang, X., 2020. Small and large strain rheology of gluten and gluten–starch doughs containing wheat bran dietary fiber. J. Sci. Food Agric. 100 (1), 177–183. https://doi.org/10.1002/jsfa.10012.

Marino, M., Martini, D., Venturi, S., Tucci, M., Porrini, M., Riso, P., Del Bo', C., 2021. An overview of registered clinical trials on glucosinolates and human health: the current situation. Front. Nutr. 8, 730906 https://doi.org/10.3389/fnut.2021.730906.

Nartea, A., Fanesi, B., Falcone, P.M., Pacetti, D., Frega, N.G., Lucci, P., 2021a. Impact of mild oven cooking treatments on carotenoids and tocopherols of Cheddar and Depurple cauliflower (Brassica oleracea L. var. Botrytis). Antioxidants 10 (2), 196. https://doi.org/10.3390/antiox10020196.

Nartea, A., Falcone, M.P., Torri, L., Ghanbarzadeh, B., Frega, N.G., Pacetti, D., Frias, J. M., 2021b. Modeling softening kinetics at cellular scale and phytochemicals extractability in cauliflower under different cooking treatments. Foods 10 (9), 1969. https://doi.org/10.3390/foods10091969.

Nartea, A., Fanesi, B., Giardinieri, A., Campmajó, G., Lucci, P., Saurina, J., Pacetti, D., Fiorini, D., Frega, N.G., Núñez, O., 2022. Glucosinolates and polyphenols of colored cauliflower as chemical discriminants based on cooking procedures. Foods 11 (19). https://doi.org/10.3390/foods11193041.

Orlando, P., Nartea, A., Silvestri, S., Marcheggiani, F., Cirilli, I., Dludla, P.V., Fiorini, R., Pacetti, D., Loizzo, M.R., Lucci, P., Tiano, L., 2022. Bioavailability study of isothiocyanates and other bioactive compounds of Brassica oleracea L. Var. Italica boiled or steamed: functional food or dietary supplement? Antioxidants 11 (2), 209. https://doi.org/10.3390/antiox11020209.

Petkowicz, C.L.O., Williams, P.A., 2020. Pectins from food waste: characterization and functional properties of a pectin extracted from broccoli stalk. Food Hydrocolloids 107, 105930. https://doi.org/10.1016/j.foodhyd.2020.105930.

Picchi, V., Migliori, C., Lo Scalzo, R., Campanelli, G., Ferrari, V., Di Cesare, L.F., 2012. Phytochemical content in organic and conventionally grown Italian cauliflower. Food Chem. 130 (3), 501–509. https://doi.org/10.1016/j.foodchem.2011.07.036.

Salehi, F., 2020. Recent applications of powdered fruits and vegetables as novel ingredients in biscuits: a review. Nutrire 45 (1). https://doi.org/10.1186/s41110-019-0103-8.

Salehi, F., Aghajanzadeh, S., 2020. Effect of dried fruits and vegetables powder on cakes quality: a review. Trends Food Sci. Technol. 95, 162–172. https://doi.org/10.1016/j.tifs.2019.11.011.

Santos, D., Lopes da Silva, J.A., Pintado, M., 2022. Fruit and vegetable by-products' flours as ingredients: a review on production process, health benefits and technological functionalities. LWT–Food Sci. Technol. 154 https://doi.org/10.1016/j.lwt.2021.112707.

Schieber, A., Carle, R., 2005. Occurrence of carotenoid cis-isomers in food: technological, analytical, and nutritional implications. Trends Food Sci. Technol. 16 (9), 416–422. https://doi.org/10.1016/j.tifs.2005.03.018.

Stojceska, V., Ainsworth, P., Plunkett, A., İbanoğlu, E., İbanoğlu, Ş., 2008. Cauliflower by-products as a new source of dietary fibre, antioxidants and proteins in cereal based ready-to-eat expanded snacks. J. Food Eng. 87 (4), 554–563. https://doi.org/10.1016/j.jfoodeng.2008.01.009.

Toivo, J., Phillips, K., Lampi, A.M., Piironen, V., 2001. Determination of sterols in foods: recovery of free, esterified, and glycosidic sterols. J. Food Compos. Anal. 14 (6), 631–643. https://doi.org/10.1006/jfca.2001.1019.

Tolve, R., Cela, N., Condelli, N., Di Cairano, M., Caruso, M.C., Galgano, F., 2020. Microencapsulation as a tool for the formulation of functional foods: the phytosterols' case study. Foods 9, 470. https://doi.org/10.3390/foods9040470.

United Nations, 2015. THE 17 GOALS | Sustainable Development. Retrieved. https://sdgs.un.org/goals. (Accessed 21 December 2021).

Vlassa, M., Filip, M., Țăranu, I., Marin, D., Untea, A.E., Ropotă, M., Dragomir, C., Sărăcilă, M., 2022. The yeast fermentation effect on content of bioactive, nutritional and anti-nutritional factors in rapeseed meal. Foods 11 (19), 1–17. https://doi.org/10.3390/foods11192972.

Volden, J., Borge, G.I.A., Hansen, M., Wicklund, T., Bengtsson, G.B., 2009. Processing (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (Brassica oleracea L. ssp. botrytis). LWT–Food Sci. Technol. 42 (1), 63–73. https://doi.org/10.1016/j.lwt.2008.05.018.

Wang, F.C., Sun, X.S., 2002. Creep-recovery of wheat flour doughs and relationship to other physical dough tests and breadmaking performance. Cereal Chem. 79 (4), 567–571. https://doi.org/10.1094/CCHEM.2002.79.4.567.

Williams, D.E., 2021. Indoles derived from glucobrassicin: cancer chemoprevention by indole-3-carbinol and 3,3'-diindolylmethane. Front. Nutr. 8, 734334 https://doi.org/10.3389/fnut.2021.734334.

Wu, W., Chen, J., Yu, D., Chen, S., Ye, X., Zhang, Z., 2021. Analysis of processing effects on glucosinolate profiles in red cabbage by LC-MS/MS in multiple reaction monitoring mode. Molecules 26 (17), 5171. https://doi.org/10.3390/molecules26175171.