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Effect of Drying and Broccoli Leaves Incorporation on the Nutritional Quality of Durum Wheat Pasta

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Pasta is a great vehicle for the incorporation of vegetable-derived ingredients to increase the consumption of the health-beneficial components originating from vegetables. Notably, by-products of vegetable processing can also serve as a rich source of phytochemicals. An important step in pasta processing is drying which can affect the content of bioactive compounds in pasta. This study aimed to evaluate the effect of drying on the nutritional quality and cooking properties of durum wheat pasta fortified with broccoli leaves. Pasta enriched with broccoli leaf powder (BLP) at 2.5% (B2.5) and 5% (B5), and control pasta without BLP (C), which differed in drying conditions: fresh pasta without drying (F), pasta dried at 50°C for 8 h (L), and pasta dried at 80°C for 3 h (H) were formulated. The obtained pasta products were analysed for the cooking properties (optimal cooking time, cooking loss, water absorption and swelling capacity); colour parameters; proximate composition; and contents of free amino acids (FAA), fatty acids and sugars. BLP significantly improved the contents of ash by up to 35 g/100 g, FAA and fatty acids to up to 1298 nmol/g dry matter (DM) and 16741 μg/g DM, respectively, without compromising the cooking quality of pasta. Drying had a significant effect on fatty acids, which content in pasta processed at the highest temperature tested decreased. From the nutritional point of view, the low-temperature drying seems to be an interesting method for pasta preparation, with the highest content of FAA, fatty acids, especially unsaturated ones, and the lowest content of sugar. However, at the same time, the dried pasta products were characterised by greater cooking loss approximating 10%.

INTRODUCTION

The growing world's population demands an increase in the production of foodstuffs. The food industry generates a huge amount of waste and by-products during processing, which adversely impact the natural environment and whose utilization boosts production costs. At the same time, very often, the by-products from plant-derived food have a similar chemical composition to the edible parts and can still be a valuable source of phytochemicals. Therefore, a growing interest has been observed over the past few years in the utilization and valorisation of by-products [Gómez-García *et al.*, 2021]. Recent studies have shown that many of the so-called by-products of vegetable processing can be successfully deployed to develop novel, functional food products due to the high content of bioactive compounds [Dominguez-Perles *et al.*, 2011; Liu *et al.*, 2018; Michalak-Majewska *et al.*, 2020; O'Shea *et al.*, 2012; Reguengo *et al.*, 2022; Sedlar *et al.*, 2020]. An interesting example of a by-product, which showed very good nutraceutical potential in baked, gluten-free products, is the leaves of broccoli [Drabińska, 2022; Drabińska *et al.*, 2018; Krupa-Kozak *et al.*, 2021]. The gluten-free sponge cake and bread obtained in the aforementioned studies had improved nutritional quality, antioxidant capacity, and a more rich profile of bioactive compounds with their texture and sensory properties remained uncompromised. However, it is worth mentioning that these products were baked at a high temperature, which could lead to thermal degradation of phytochemicals.

In turn, pasta is not only willingly consumed worldwide by consumers of all age categories across all social groups [Nilusha *et al.*, 2019], but is also an example of a food product requiring milder temperatures during processing. Studies have shown that pasta is a good vehicle for incorporating potentially bioactive ingredients due to its capacity of maintaining acceptable physical and sensory properties despite the modification of its formula [Bruno *et al.*, 2019; Nilusha *et al.*, 2019]. Moreover, considering the low consumption of fruits and vegetables, especially in children, the incorporation of a vegetable-derived ingredient into the pasta could

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increase the intake of health-beneficial components originating from vegetables [Oliviero & Fogliano, 2016].

Durum wheat semolina is the most suitable material for the preparation of top-quality pasta [Fuad & Prabhasankar, 2010], characterised by high firmness and chewiness, low surface stickiness, and a bright and intense yellow colour. The most desirable cooking properties of top-quality pasta include a moderate water uptake at the optimal cooking time (OCT) and a low cooking loss [Bustos *et al.*, 2015; Cole, 1991]. Moreover, the nutritional properties of pasta have recently spurred a growing interest mainly due to the development of new, fortified products [Hoehnel *et al.*, 2020].

A pivotal stage in pasta production is drying which prolongs its shelf-life. Importantly, drying conditions significantly affect the quality, cooking behaviour and sensory properties of the final product [Piwińska *et al.*, 2016]. Different technologies of drying are applied and three main categories can be distinguished: drying at low temperature (<60°C), drying at high temperature (70–80°C) and drying at very high temperature (>110°C) [Giannetti *et al.*, 2021]. Higher temperatures are in general applied as they ensure an improvement in pasta colour and firmness, lower cooking loss, higher cooked weight and decreased stickiness [Anese *et al.*, 1999]. On the other hand, a higher temperature may result in the degradation of bioactive compounds and consequently reduction of the bioactive potential.

The effect of drying on the physical and aroma properties of gluten-free [D'Amico *et al.*, 2015] and durum wheat pasta was well established [Giannetti *et al.*, 2021; Piwińska *et al.*, 2016]. However, to the best of our knowledge, no studies have been performed to evaluate the effect of drying on the nutritional quality of the fortified pasta products. Therefore, the aim of this study was to evaluate the effect of drying on the nutritional quality and cooking properties of durum wheat pasta fortified with broccoli leaves. Its results were expected to help answer the arising question of whether the potential improvement in the nutritional quality caused by phytochemical-rich ingredients can be lost by drying.

MATERIALS AND METHODS

Broccoli leaf powder

Broccoli leaves were generously donated by GEMIX (Olsztyn, Poland). Broccoli leaf powder (BLP) was prepared from fresh, undamaged leaves of broccoli (*Brassica oleracea* L. var. *italica* cv. Sebastian), which were blanched in hot water for 1 min to inactivate enzymes, freeze-dried and ground into powder. Details of BLP preparation can be found in our previous work [Drabińska *et al.*, 2018].

Pasta preparation

Pasta formulation consisted of durum semolina flour, olive oil, salt (all purchased in local stores in Olsztyn, Poland) and tap water. The formulations were enriched with 2.5% (B2.5), and 5% (B5) of BLP as an additional ingredient to the optimal, control pasta formulation without BLP (C). The compositions of all formulations are presented in Table 1. To prepare the pasta, all ingredients were mixed for 5 min and then extruded through a penne-forming die in an electric pasta maker (Pastamatic 1581, Ariete, Florence, Italy).

TABLE 1. Composition of control and fortified pasta products (g/100 g).

	C	B2.5	B5
Semolina	71.8	70.5	69.3
Water	25.1	24.7	24.3
Olive oil	2.7	2.6	2.6
Salt	0.4	0.4	0.3
BLP	0.0	1.8	3.5

C – control pasta; B2.5 – pasta fortified with 2.5% of broccoli leaf powder (BLP); B5 – pasta fortified with 5% of BLP.

Three types of pasta were prepared for all formulations, differing in drying method: (F) fresh pasta without drying, (L) pasta dried at 50°C for 8 h, and (H) pasta dried at 80°C for 3 h. The images of the obtained pasta products are shown in Figure 1. Pasta products were cooked for the optimal cooking time (OCT) and then freeze-dried, ground into a fine powder (particle size <0.60 mm) and stored in tightly closed containers at –20°C until analyses.

Determination of cooking properties

The analysis of cooking loss (grams of solid material in cooking water per 100 g of pasta as is), OCT, water absorption and swelling capacity were performed according to the AACC approved method 66–50 [AACC, 2000].

Colour parameter analysis

The colour parameters of freeze-dried powders of BLP and pasta samples were evaluated using a HunterLab ColorFlex

FIGURE 1. The images of experimental pasta products. F – fresh pasta; L – pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h); C – control pasta; B2.5 – pasta fortified with 2.5% of broccoli leaf powder (BLP); B5 – pasta fortified with 5% of BLP.

spectrophotometer (Hunter Associates Laboratory, Inc, Reston, VA, USA). The colour was expressed in accordance with the CIELab system and the parameters determined were: lightness: L^* (0 (black) – 100 (white)) and chromatic components: a^* ($-a^*$ (greenness) – $+a^*$ (redness)) and b^* ($-b^*$ (blueness) – $+b^*$ (yellowness)). Each sample was measured in nine replicates. Moreover, a total colour difference (ΔE) in response to the F-C pasta was calculated using the following formula:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Proximate composition analysis

The proximate composition was established according to the AOAC approved methods: moisture content was analysed using the drying method (AOAC 925.10), protein content was determined with the Kjeldahl method (AOAC 979.09), and lipid content using Soxhlet extraction with hexane (AOAC 923.03); ash was determined using the gravimetric method (AOAC 923.03). [AOAC, 2005].

Amino acid analysis

Free amino acid (FAA) composition was analysed using the method described by Drabińska [2022]. Briefly, FAA were extracted from approx. 100 mg of freeze-dried powder with 3 mL of a 50% (*v/v*) methanol solution. The mixture was incubated for 20 min at 50°C with shaking at 500 rpm using a MultiTherm shaker (Benchmark Scientific, Edison, NJ, USA). Next, the mixture was centrifuged for 15 min at 11,200×*g*, and the supernatant was collected for analysis. A portion of 100 μL of the extract was analysed using the EZ: Faast™ kit for free amino acids (Phenomenex, Torrance, CA, USA) according to the protocol provided by the manufacturer. FAA were analysed in an Agilent 7890A gas chromatograph (GC) coupled with a 5975C mass selective detector (MSD) and 7683B autoinjector (Agilent Technologies, Santa Clara, CA, USA). The compounds were separated in a ZB-AAA EZ: Faast™ capillary column provided by the manufacturer (10 m × 0.25 mm, Phenomenex). The temperature gradient and GC and MS settings were according to the producer's protocol. Identification and calculation of individual FAA were performed using external standards provided by the manufacturer and normalised to the internal standard (norvaline). The results were expressed as nmol/g dry matter (DM).

Fatty acid analysis

Fatty acid methyl esters (FAME) were obtained and determined using a standard procedure with transesterification with sodium methylate (0.4 N methanolic solution). First, lipids were isolated from BLP and pasta samples. To 1 g of the sample, 3 mL of *n*-hexane were added and the mixture was vortexed vigorously and centrifuged for 15 min at 2268×*g*. The supernatant was collected and the extraction was performed three times. The collected *n*-hexane layer was dried in the flow of nitrogen. The remaining oil was then dissolved in 2 mL of *n*-hexane containing C19:0 internal standards (1 mg/mL), and 2 mL of derivatization agent were added. The mixture was vortexed and left at room temperature for 20 min. Afterwards, water was added and the upper *n*-hexane layer was collected to the GC vial. FAME were analysed using Agilent Technologies 6890 GC with a flame ionisation detector (FID) (Agilent Technologies). Separation of FAME was conducted in a Supelcowax-10 column (30 m × 0.32 mm × 0.5 μm, Supelco, Bellefonte, PA, USA) with hydrogen as a carrier gas with the flow rate of 1.5 mL/min. The injection volume was 1 μL at a split ratio of 50:1. The operating conditions were as follows: initial oven temperature 170°C (2 min) followed by 2°C/min increase to 230°C (held for 10 min). FAME were identified by comparison with the commercial standard, FAME Mix C4-C24 (Supelco, Bellefonte, PA), and quantified in response to the internal standard. Results were expressed in μg/g DM.

Sugar analysis

The extraction of sugars was performed following the method described by Wieczorek *et al.* [2022]. Approx. 1 g of BLP and pasta samples was extracted with water for 20 min at 60°C. Afterwards, the samples were centrifuged at 2268×*g* for 15 min. Supernatants were filtered through 0.45 μm cellulose acetate syringe filters (Lab Logistics Group GmbH, Meckenheim, Germany) and the extracts were directly analysed by high-performance liquid chromatography (HPLC).

Analysis of individual sugars was performed using an Agilent Technologies 1100 series HPLC system with a refractive index detector (Agilent Technologies), isocratically with water as a mobile phase at a flow rate of 0.6 mL/min at 80°C on the Rezex RPM-Monosaccharide Pb^{+2} 300×7.8 mm column (Phenomenex). Commercial standards of sugars purchased in Sigma Aldrich (Steinheim, Switzerland) were used for the identification and quantification of individual sugars. The results were expressed in mg/g DM.

Statistical analysis

Pasta was prepared with one batch of 700 g for each pasta type. All analyses were performed in triplicate unless differently stated, and the data are presented as mean ± standard deviation (SD). Differences between pasta samples were assessed using one-way analysis of variance (ANOVA) with the Fisher's least significant difference (LSD) post-hoc test. Moreover, two-way ANOVA was performed for contents of FAA, fatty acids and sugars to assess the effect of BLP addition and drying temperature (D), and interactions thereof (BLP×D) on the chemical composition of the experimental pasta samples. The differences were considered significant at $p<0.05$. All the statistical analyses were performed using STATISTICA version 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) software.

RESULTS AND DISCUSSION

Cooking properties

Cooking properties are important drivers of the final quality and acceptance of pasta. The ideal pasta should feature high water absorption, low cooking loss and good texture [Bruneel *et al.*, 2010]. The results of determinations of the cooking properties of pasta with BLP dried under different conditions and control pasta (without BLP) are presented in Figure 2. Drying significantly prolonged the cooking time required to obtain *al dente* pasta. While in the F pasta,

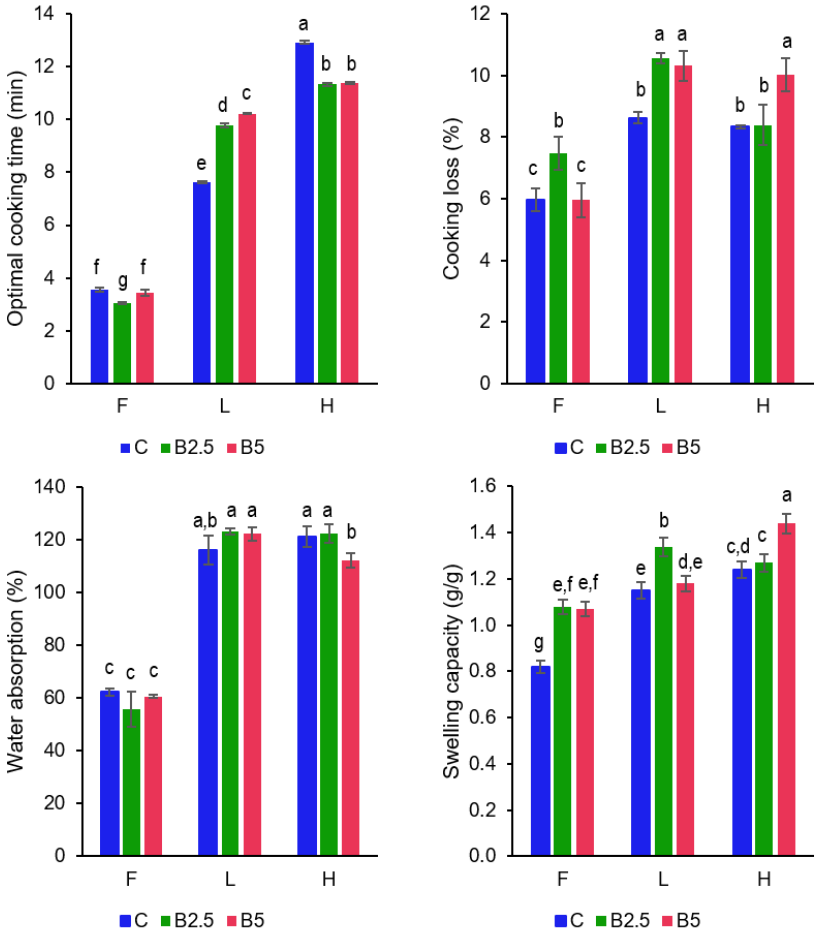

FIGURE 2. Cooking properties of the experimental pasta products. B2.5 – pasta fortified with 2.5% of broccoli leaf powder (BLP); B5 – pasta fortified with 5% of BLP; C – control pasta without BLP; F – fresh pasta; L –pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h). Different letters above bars indicate a significant difference ($p<0.05$).

the addition of BLP did not affect much the OCT, it caused differences in dried pasta samples. Interestingly, for L pasta, the OCT was significantly longer with a higher percentage of BLP, while an opposite phenomenon was observed for H pasta. The OCT of F pasta was definitely shorter due to a higher moisture content and faster rehydration compared to the dried pasta [Piwińska *et al.*, 2016].

Cooking loss should not exceed 8% for good-quality pasta [Piwińska *et al*., 2016; Desai *et al.*, 2018a]. All F pasta products were within this range (Figure 2). The cooking losses were greater after drying. The C and B2.5 pasta samples dried at the highest temperature tested were on the edge of the acceptance; whereas the cooking loss determined for H-B5 pasta approximated 10%. The greatest cooking loss was noted for L-B2.5 (10.55%) and L-B5 (10.31%). Similarly, Piwińska *et al.* [2016] reported that pasta dried for a longer time at a lower temperature was characterised by the greatest cooking loss.

Irrespectively of the drying method applied and BLP addition, the water absorption determined for the L and H samples was similar and much higher compared to F pasta (Figure 2). The high-temperature drying is believed to result in the best cooking quality of pasta, mainly due to the polymerisation of the protein network which entraps the starch granules and consequently reduces water absorption and prevents starch leaching [Lamacchia *et al.*, 2007]. Contrary, Bruneel *et al.* [2010] reported the highest cooking quality for pasta dried under mild conditions. The application of protein-rich ingredients was reported to increase cooking loss [Kaur *et al.*, 2013]. Raczyk *et al.* [2022] observed a high increase in the cooking loss after pasta fortification with spirulina, which was explained by solubilization and washing out of the protein fraction and gluten matrix weakening, which decreases the capacity for entrapping swollen starch granules during boiling.

The swelling capacity was significantly affected by BLP addition (Figure 2). For F pasta, it was higher in the BLP-enriched products, irrespective of BLP percentage. For L pasta, the highest swelling capacity was established for B2.5, which was the opposite for H pasta. In turn, F pasta had a lower swelling capacity compared to dried pasta products, likely due to shorter OCT. After BLP addition and drying, a stronger protein network might have been formed, which could have resulted in hampered starch swelling and lower water uptake [Piwińska *et al.*, 2016].

Colour parameters

The colour of pasta is affected by the quality of semolina and the addition of colour-containing ingredients [Piwińska *et al.*, 2016]. The results of the colour parameters analysis

TABLE 2. Colour parameters of experimental pasta products.

Pasta	L^*	a^*	b^*	ΔE
F-C	87.51±0.01^{a}	-0.12±0.01^{c}	14.05±0.02^{g}	-
F-B2.5	76.05±0.03^{d}	-6.25±0.02^{h}	28.14±0.03^{d}	19.17
F-B5	67.61±0.02^{g}	-6.26±0.02^{h}	31.48±0.02^{a}	27.15
L-C	84.98±0.01^{b}	-0.28±0.01^{a}	14.56±0.01^{f}	3.90
L-B2.5	68.88±0.03^{f}	-3.67±0.01^{d}	27.32±0.02^{e}	12.92
L-B5	66.99±0.04^{i}	-5.66±0.01^{g}	28.69±0.03^{c}	21.57
H-C	84.78±0.02^{c}	-0.25±0.01^{b}	12.63±0.03^{h}	1.88
H-B2.5	72.10±0.01^{e}	-4.23±0.02^{f}	28.16±0.03^{d}	15.38
H-B5	67.10±0.05^{h}	-4.33±0.01^{e}	29.52±0.03^{b}	19.14

B2.5 – pasta fortified with 2.5% of broccoli leaf powder (BLP); B5 – pasta fortified with 5% of BLP; C – control pasta without BLP; F – fresh pasta; L – pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h). Different letters in superscript in the same column indicate a significant difference ($p<0.05$).

of pasta fortified with BLP and dried under different conditions are presented in Table 2. The highest lightness (L^*) value was determined for F-C pasta, which was observed to decrease in C pasta with an increasing drying temperature. In the case of pasta fortified with BLP, the L pasta was the darkest. As expected, the BLP-fortified pasta products were characterised by the highest greenness (a^*). Interestingly, for F and H pasta, the difference between the 2.5% and 5% BLP was very small, while for L pasta, the B5 pasta was almost twice more green compared to B2.5. The yellowness (b^*) value was observed to increase with an increasing BLP content in the formulation, irrespective of the drying conditions. A change in colour parameters of pasta was also reported by other authors who fortified pasta products [Duda *et al.*, 2019; Raczyk *et al.*, 2022]. Notably, the difference in sample colour (ΔE) differed between the drying methods (Table 2). As stated by Duda *et al.* [2019], the difference in ΔE of more than 3 is noticed by the eye without additional tools. The differences between F-C and H-C were lower than 3, while the difference between F-C and L-C which accounted for 3.90 could be perceived as noticeable. Similarly, for B2.5 pasta, the difference between the F and H samples was smaller as compared to L. The tendency was opposite at the highest BLP content, and compared to F-B5, the H pasta differed the most; however at the same time, the difference to F-C was the smallest. The green colour of BLP is due to chlorophylls, detected in Brassica leaves in high amounts [Drabińska *et al.*, 2021]. Notably, the loss of greenness was observed in B5 pasta products with the increasing drying temperature, which can be explained by the thermal degradation of chlorophylls. As reported by Van Loey *et al.* [1998] and Weemaes *et al.* [1999], degradation of chlorophylls in broccoli occurs when the temperature exceeds 50°C.

Proximate composition

The nutritional quality of pasta depends on the quality of ingredients used for its production. Semolina is the main component of pasta and is considered a relatively good source of protein with a low amount of minerals and lipids [Fuad & Prabhasankar, 2012; Pérez & Pérez, 2009]. Various ingredients, such as fish products, vegetables and crickets, were incorporated to improve the nutritional quality of pasta [Desai *et al.*, 2018a; Duda *et al.*, 2019; Michalak-Majewska *et al.*, 2020]. BLP was previously characterised as a rich source of protein and minerals [Drabińska *et al.*, 2018]. The proximate composition of cooked experimental pasta obtained in this study is shown in Figure 3. Except for F pasta, the addition of BLP resulted in the increased moisture content of cooked pasta compared to C, which can be associated with the high hygroscopic properties of freeze-dried BLP. The content of ash increased with an increasing percentage of BLP in all pasta products. However, as can be seen in Figure 3, the ash content decreased with increasing drying temperature. Similarly, Manthey & Hall [2007] reported that H pasta had a lower ash content compared to L. In our study, less pronounced changes were found for protein, the highest content of which was detected in H-B5. The insignificant changes in protein content can be explained by a relatively high protein content in semolina itself. No clear tendency was observed for lipid content in the analysed pasta samples. The lowest lipid content was noted for F-C pasta and the highest one for L-B5 pasta. For F and H pasta, the trend was similar (with the highest content of lipids in B2.5), whereas an opposite tendency was observed for L pasta. As summarised in the meta-analysis, the changes in the proximate composition of fortified pasta products vary depending on the type of additive and enrichment level [Mercier *et al.*, 2016]. And although the effect of drying on starch and protein digestibility and cooking properties of pasta was extensively studied [Bresciani *et al.*, 2022], the effect of drying on its proximate composition did not gain much scientific attention to date.

Free amino acid profile

Wheat flours used for pasta preparation are a source of protein in our diet. However, it has to be kept in mind that wheat proteins are of low quality with unbalanced amino acid composition, especially with lacking lysine and threonine [Filip & Vidrih, 2015; Hoehnel *et al.*, 2020]. Therefore, there have been many scientific attempts to incorporate the protein-rich ingredient into pasta formulations [Duda *et al.*, 2019; Hoehnel *et al.*, 2020; Raczyk *et al.*, 2022].

The contents of individual FAA in BLP and experimental pasta are presented in Table 3. BLP was found as a rich source of FAA with 15 individual FAA. The major FAA in BLP were aspartic and glutamic acids, alanine and glutamine followed by serine, valine and threonine. A similar profile of FAA in BLP was reported previously [Drabińska, 2022]. In control pasta, the presence of 11 FAA was detected, with dominant asparagine, aspartic acid and alanine, which is in agreement with the previous study with semolina-based pasta [Fois *et al.*, 2019]. The incorporation of BLP significantly affected the content of FAA in experimental pasta products, which increased upon all drying methods applied. Notably, threonine, tyrosine and glutamic acid were detected only in the BLP-enriched pasta and surprisingly also in the L-C pasta. The statistical analysis showed that BLP percentage influenced the total FAA content and the content of almost all individual FAA, except leucine. A similar effect of BLP was reported

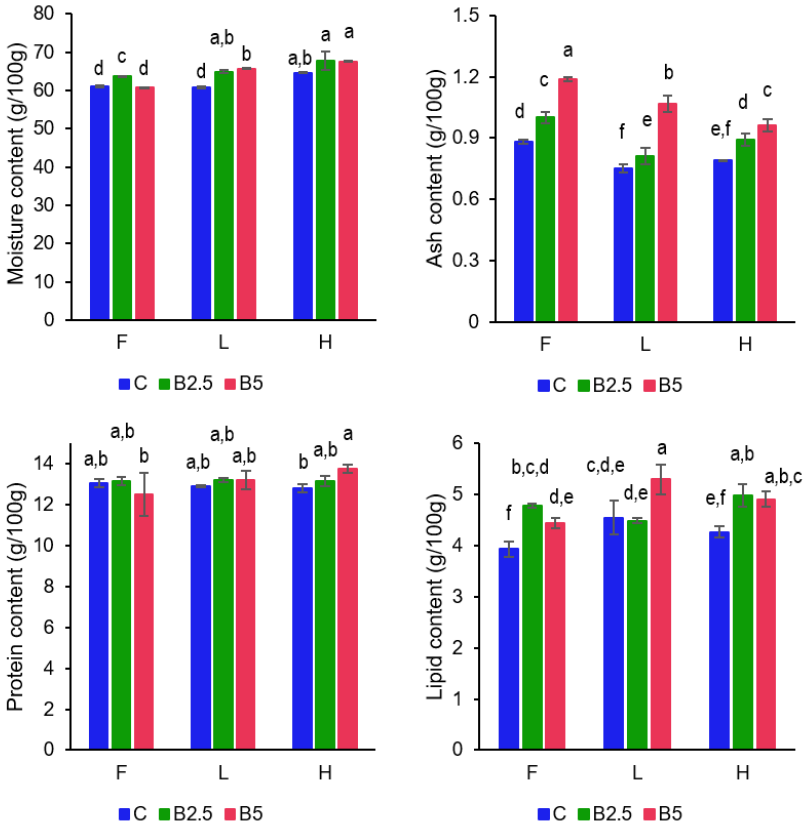

FIGURE 3. Proximate composition of the experimental pasta products. B2.5 – pasta fortified with 2.5% of broccoli leaf powder (BLP); B5 – pasta fortified with 5% of BLP; C – control pasta without BLP; F – fresh pasta; L – pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h). Different letters above bars indicate a significant difference ($p<0.05$).

by Drabińska [2022] in BLP-fortified gluten-free sponge cake muffins. Importantly, the semolina was previously reported to be lacking in lysine [Raczyk *et al.*, 2022]. In our study, lysine was not detected in experimental pasta although the BLP contained this amino acid. Maybe the incorporation of a higher level of BLP could result in products with excellent nutritional properties. Notably, the content of the second limiting amino acid in wheat, threonine, increased with increasing BLP fortification. After pasta enrichment with BLP, the content of polar FAA (aspartic and glutamic acids) increased with an increasing BLP content, which was suggested to increase protein solubility [Arise *et al.*, 2022].

The effect of drying on FAA content in pasta was much less prominent. The significant effect of drying was detected for alanine, valine, leucine, isoleucine, threonine, proline, phenylalanine, tyrosine and glutamic acid as well as total FAA (Table 3). A previous study showed that higher temperatures of drying negatively affected the content of lysine [Dexter *et al.*, 1984]. Although this amino acid was not reported in this study; similarly to the results reported by Dexter *et al.* [1984], a higher content of total FAA was found in L pasta compared to H. In another study [Manthey & Hall, 2007], drying conditions were considered as not influencing the content of individual amino acids. However, similarly to our study, significant changes were reported for valine and threonine.

The results of the two-way ANOVA showed that there was a clear interaction between the BLP percentage and drying temperature for individual compounds (leucine, isoleucine, threonine, phenylalanine, tyrosine and glutamic acid) and total FAA (Table 3).

Fatty acid profile

Brassica vegetables are one of the most important crops for the oil industry, mainly due to the high oil yield of rapeseed [Sharafi *et al.*, 2015]. Broccoli does not belong to oil crops; however, also contains fatty acids. Leaves of broccoli were found to have the highest compositional ratio of polyunsaturated fatty acid (PUFAs) among all its aerial parts, with linolenic (C18:3 *n*3), palmitic (C16:0) and linoleic (C18:2) acids being the most dominant, accounting for around 80% of total fatty acids [Bhandari *et al.*, 2013]. In our study, the content of fatty acids in BLP and experimental pasta products was evaluated and respective results are presented in Table 4. Eleven fatty acids were identified in BLP with γ-linoleic acid (C18:3 *n*6) and C16:0 being the major acids, followed by *trans*-heptadecenoic acid (C17:1 *trans*), C18:2 and C18:3 *n*3, which is in agreement with previous reports [Bhandari *et al.*, 2013; Joon Lee *et al.*, 2009]. Similarly to other authors, PUFA were found to dominate in the analysed BLP. The vast majority of fatty acids detected in experimental pasta products derived from olive oil used for pasta preparation. Nevertheless, also wheat contains a small amount of lipids (around 3%) with a profile of fatty acids consisting mainly of C18:2, C16:0, C18:1, C18:3, stearic acid (C18:0) and palmitoleic acid (C16:1) [Narducci

TABLE 3. Content of free amino acids (FAA) in broccoli leaf powder (BLP) and experimental pasta products (nmol/g dry matter).

Amino acid	BLP	F-C	F-B2.5	F-B5	L-C	L-B2.5	L-B5	H-C	H-B2.5	H-B5	*p*-Value#		
											BLP addition effect	Drying (D) effect	BLP×D effect
Alanine	1369.02±87.7	70.3±6.7^{d}	104±3.3^{c}	179.7±2.9^{b}	74.6±2.9^{d}	176.4±13.3^{b}	228.9±10.6^{a}	81.4±5.8^{d}	109.4±5.3^{c}	178.3±1.3^{b}	<0.001*	0.049*	0.432
Glycine	/	20.3±1.0^{c}	14.03±0.0^{c}	34.4±1.3^{a}	16.9±5.5^{c}	32.6±0.2^{a}	26.9±1.2^{a,b}	26.0±1.8^{b}	16.9±2.4^{c}	21.6±2.4^{b,c}	/	0.492	/
Valine	342.2±129.4	17.2±1.5^{b}	31.0±2.9^{b}	57.8±0.3^{a}	34.2±6.1^{b}	67.6±6.9^{a}	60.8±3.2^{a}	26.1±6.1^{b}	32.8±6.0^{b}	58.6±1.2^{a}	<0.001*	0.015*	0.158
Leucine	82.3±29.2	13.1±1.1^{c}	13.1±1.1^{c}	27.3±1.7^{b}	28.3±2.6^{b}	66.8±3.7^{a}	29.2±4.4^{b}	22.6±2.1^{b}	26.2±2.8^{b}	29.1±1.3^{b}	0.112	<0.001*	0.002*
Isoleucine	102.3±32.1	9.8±2.8^{d}	13.6±2.5^{d}	24.2±1.3^{b}	14.6±1.9^{d}	34.5±0.2^{a}	22.7±1.9^{b}	/	16.8±1.1^{c}	21.9±1.3^{b}	<0.001*	0.002*	0.002*
Threonine	325.3±111.3	/	/	26.5±2.2^{a}	/	33.5±1.4^{a}	35.8±1.1^{a}	/	22.6±0.7^{b}	34.7±2.7^{a}	<0.001*	0.002*	0.015*
Serine	569.0±192.4	16.8±0.0^{f}	32.1±6.3^{e}	67.3±8.4^{a,b,c}	/	56.9±3.9^{b,c}	73.6±1.4^{a,b}	27.8±4.6^{e}	41.3±7.6^{d}	76.9±5.4^{a}	<0.001*	0.055	0.233
Proline	243.3±82.9	19.6±3.0^{e}	23.9±1.9^{e}	49.9±0.1^{a}	24.0±2.1^{e}	55.0±4.1^{a}	52.0±1.1^{a}	24.7±2.9^{e}	35.5±3.9^{b}	48.7±0.9^{a}	<0.001*	0.046*	0.171
Aspatric acid	2055.7±633.8	87.9±13.3^{c}	121.0±12.2^{b}	301.9±0.1^{a}	79.7±7.2^{c}	168.4±3.9^{b}	341.8±12.8^{a}	78.5±26.5^{c}	213.8±10.2^{b}	296.0±20.2^{a}	<0.001*	0.164	0.628
Asparagine	/	103.7±7.2^{c}	76.4±3.5^{d}	233.1±5.0^{a}	63.6±3.0^{d}	128.3±19.5^{b,c}	186.8±19.9^{b}	124.7±15.3^{b,c}	139.3±9.9^{b,c}	156.8±2.6^{b}	/	0.126	/
Glutamine	1213.7±479.4	/	/	/	/	/	/	/	/	/	/	/	/
Phenylalanine	78.9±23.6	9.4±0.8^{c}	8.7±1.3^{c}	22.5±1.1^{b}	13.1±1.8^{c}	31.9±2.2^{a}	22.2±1.5^{b}	12.3±1.9^{c}	17.4±1.0^{b}	20.0±0.0^{b}	0.007*	0.037*	0.017*
Tyrosine	39.6±10.7	/	/	13.7±0.6^{a}	12.9±3.8^{a}	18.4±1.9^{a}	11.6±2.6^{a}	/	12.0±4.8^{a}	15.7±2.2^{a}	0.001*	0.003*	0.007*
Tryptophan	33.5±11.0	38.7±1.4^{b}	23.5±5.3^{c}	114.4±5.0^{a}	34.9±1.7^{b}	70.3±14.0^{a,b}	97.9±23.3^{a,b}	58.3±22.1^{b}	75.4±3.8^{a,b}	79.9±1.9^{a,b}	0.002*	0.215	0.224
Glutamic acid	1581.4±609.1	/	39.6±2.2^{c,d}	119.9±12.7^{a,b}	32.9±3.0^{d}	48.5±8.0^{c}	119.2±15.6^{a,b}	/	96.9±10.3^{b}	125.8±2.2^{a}	<0.001*	0.025*	0.040*
Lysine	52.0±9.4	/	/	/	/	/	/	/	/	/	/	/	/
Histidine	72.8±25.4	/	/	/	/	/	/	/	/	/	/	/	/
∑ FAA	8161.0±113.4	406.8±37.4^{d}	501.53±42.5^{d}	1262.4±42.8^{a}	429.6±41.5^{d}	989.0±83.1^{b,c}	1298.0±100.4^{a}	482.3±89.1^{d}	856.4±69.8^{c}	1164.0±45.0^{a,b}	<0.001*	<0.001*	<0.001*

B2.5 – pasta fortified with 2.5% of BLP; B5 – pasta fortified with 5% of BLP; C – control pasta without BLP; F – fresh pasta; L – pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h). Different letters in superscript in the same line indicate a significant difference ($p<0.05$) based on post-hoc Fisher's least significant difference (LSD) test. #Two-way analysis of variance. *Significant effect ($p<0.05$)

TABLE 4. Content of fatty acids in broccoli leaf powder (BLP) and experimental pasta products (μg/g dry matter).

Fatty acid	BLP	F-C	F-B2.5	F-B5	L-C	L-B2.5	L-B5	H-C	H-B2.5	H-B5	*p*-Value#		
											BLP addition effect	Drying (D) effect	BLP×D effect
C16:0	294.7±27.8	1625.2±49.1^{e}	2275.1±33.7^{a,b}	1951.5±101.4^{c}	1779.1±5.7^{d}	2302.3±10.9^{a,b}	2426.5±87.1^{a}	1852.5±40.6^{c,d}	2251.4±87.1^{b}	2240.4±103.1^{b}	<0.001*	<0.001*	0.007*
C16:1 *n*10	/	11.3±0.2^{d}	25.4±3.3^{a}	20.9±0.4^{b,c}	19.9±1.0^{c}	25.7±1.56^{a}	24.8±1.8^{a,b}	23.3±0.2^{a,b,c}	23.8±1.2^{a,b,c}	26.2±3.9^{a}	/	0.003*	/
C16:1 *n*7	/	97.5±2.9^{e}	133.8±1.5^{b}	113.5±5.2^{d}	112.1±0.8^{d}	128.2±3.6^{b,c}	144.8±2.5^{a}	128.0±7.5^{b,c}	120.8±5.3^{c,d}	136.9±0.6^{a,b}	/	<0.001*	/
C16:2	30.3±4.2	/	2.5±0.6^{d}	5.5±0.1^{b}	/	4.1±0.0^{c}	6.5±0.4^{a}	/	3.0±0.5^{d}	6.0±0.0^{a,b}	<0.001*	0.002*	0.037*
C16:3	35.3±4.8	/	1.4±0.0^{c}	2.0±0.2^{b}	/	1.4±0.0^{c}	2.6±0.3^{a}	/	1.3±0.0^{c}	2.9±0.2^{a}	<0.001*	0.016*	0.006*
C17:0	8.5±1.2	10.0±0.3^{d,e}	14.6±1.5^{c,d}	16.4±4.5^{b,c}	9.9±1.5^{e}	13.7±0.4^{c,d,e}	19.7±2.6^{a,b}	11.8±1.9^{c,d,e}	13.3±0.7^{c,d,e}	21.4±1.6^{a}	<0.001*	0.343	0.337
C17:1 *cis*	/	14.4±1.0^{d}	22.0±0.7^{b,c}	24.0±6.0^{b}	14.5±1.4^{d}	24.2±2.7^{b}	29.0±6.0^{a,b}	16.5±1.6^{c,d}	25.5±0.6^{b}	34.6±0.3^{a}	/	0.041*	/
C17:1 *trans*	192.4±20.7	/	4.1±0.6^{c}	6.3±0.6^{b}	/	1.8±0.2^{e}	8.4±0.1^{a}	/	3.1±0.0^{d}	9.3±0.9^{a}	<0.001*	0.019*	<0.001*
C18:0	46.1±3.0	263.3±12.9^{e}	366.8±20.4^{a,b}	321.2±16.2^{c}	275.0±0.2^{d,e}	382.1±0.2^{a,b}	393.2±8.4^{a}	302.2±16.9^{c,d}	387.1±13.4^{a,b}	360.7±18.0^{b}	<0.001*	0.003*	0.044*
C18:1 *cis*	69.3±3.9	7687.9±147.6^{c,d}	8897.5±30.4^{a,b}	7667.0±697.2^{c,d}	7621.1±14.1^{d}	8584.4±205.7^{b,c}	9508.9±468.7^{a}	8548.7±727.9^{b,c}	8134.9±127.0^{b,c,d}	8133.0±420.4^{b,c,d}	0.073	0.169	0.008*
C18:1 *trans*	21.8±3.0	785.7±230.1^{e}	1352.9±122.4^{a,b,c}	1118.0±18.6^{c,d,e}	933.2±204.2^{d,e}	1300.0±127.2^{a,b,c}	1496.4±127.3^{a,b}	1065.3±135.1^{c,d,e}	1211.6±37.1^{b,c,d}	1605.2±230.8^{a}	0.001*	0.103	0.140
C18:2	192.9±14.0	1668.5±4.8^{e,f}	2049.7±23.8^{b,c}	1785.9±196.7^{d,e,f}	1888.3±0.8^{c,d}	1850.7±2.8^{d,e}	2318.8±37.1^{a}	2119.8±149.1^{b}	1624.4±54.1^{f}	1930.7±45.6^{b,c,d}	0.021*	0.014*	<0.001*
C18:3 *n*6	697.5±83.0	113.2±510^{d}	153.4±19.8^{b}	159.4±22.5^{b}	121.5±0.3^{c,d}	146.6±0.6^{b,c}	207.6±1.3^{a}	139.8±8.0^{b,c,d}	119.0±17.2^{d}	201.6±6.6^{a}	<0.001*	0.104	0.009*
C18:3 *n*3	185.0±13.8	/	10.8±1.4^{c}	21.7±1.6^{b}	/	13.9±1.4^{c}	25.8±3.2^{a}	/	13.6±0.7^{c}	28.6±1.7^{a}	<0.001*	0.011*	0.083
C20:0	/	36.2±6.0^{d}	53.6±8.7^{b,c}	52.8±0.2^{c}	35.7±0.9^{d}	62.2±0.6^{a,b}	63.6±0.0^{a}	40.1±2.23^{d}	63.6±0.1^{a,b,c}	54.5±5.21^{a,b,c}	/	0.067	/
C20:1	/	29.0±4.7^{c}	52.7±12.4^{b}	52.6±5.7^{b}	31.8±0.6^{c}	59.0±2.1^{a,b}	64.6±1.6^{a}	35.4±0.6^{c}	56.4±0.6^{a,b}	57.0±5.8^{a,b}	/	0.106	/
∑ Fatty acids	1773.9±173.3	12341.9±291.8^{e}	15416.3±225.5^{b}	13318.7±1043.3^{d,e}	12842.1±193.8^{e}	14899.9±70.4^{b,c}	16741.2±477.9^{a}	14283.3±735.3^{c,d}	14050.1±126.8^{c,d}	14849.0±265.2^{b,c}	<0.001*	0.009*	<0.001*

B2.5 – pasta fortified with 2.5% of BLP; B5 – pasta fortified with 5% of BLP; C – control pasta without BLP; F – fresh pasta; L – pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h). Different letters in superscript in the same line indicate a significant difference ($p < 0.05$) based on post-hoc Fisher's least significant difference (LSD) test. #Two-way analysis of variance. *Significant effect ($p<0.05$).

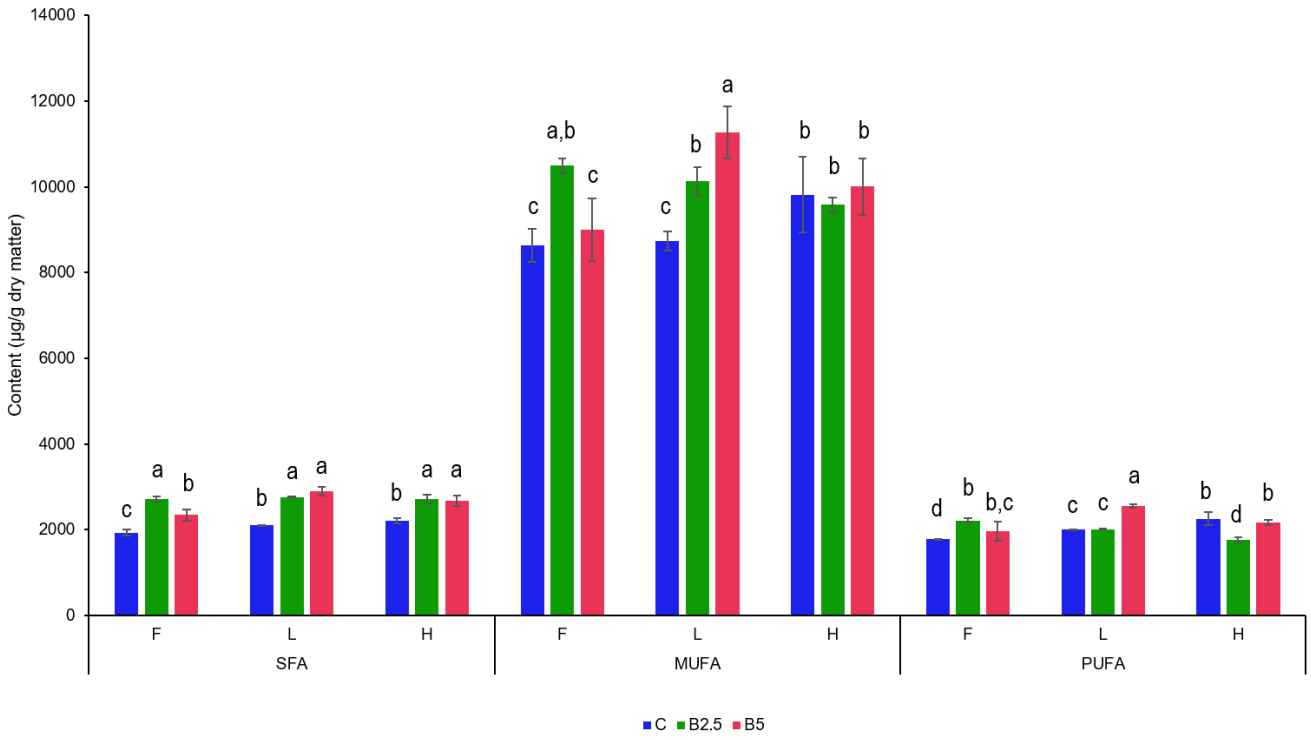

FIGURE 4. The summarized content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in experimental pasta products. B2.5 – pasta fortified with 2.5% of broccoli leaf powder (BLP); B5 – pasta fortified with 5% of BLP; C – control pasta without BLP; F – fresh pasta; L – pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h). Different letters above bars indicate a significant difference ($p<0.05$), separately for SFA, MUFA and PUFA.

et al., 2019]. In control pasta, the presence of 11 fatty acids was detected with oleic acid (C18:1 *cis*), C18:2 and C16:0 as the major ones. The profile of fatty acids in control pasta is in agreement with the fatty acid profile of olive oil [Sánchez-Rodríguez *et al.*, 2019]. Although the percentage of BLP incorporation into pasta formulations was relatively low, changes were noticed in the fatty acid profile. Significant effects were detected for all fatty acids except for C18:1 *cis.* This resulted in a significant effect of BLP percentage on the total fatty acid content. Notably, in the fortified pasta products, hexadecadienoic acid (C16:2), hexadecatrienoic acid (C16:3), C17:1 *trans* and C18:3 *n*3 derived exclusively from BLP as they were not detected in C pasta. Notably, the high consumption of cereal-based products, rich in *n*6 fatty acids is considered to be responsible for the imbalance in the *n*6 to the *n*3 ratio [Fradique *et al.*, 2013]. BLP contained *n*3 fatty acids, which was reflected in the fatty acid profile of experimental pasta. C18:3 *n*3 was detected even in the pasta made with the minimum percentage of BLP (B2.5), which contributed to an important reduction in the *n*6:*n*3 ratio compared to C pasta. The consumption of BLP-enriched pasta may contribute to *n*6:*n*3 ratio normalisation in the diet, which is particularly imbalanced in the Western diet [Asefy *et al.*, 2021]. A summarised content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA is presented in Figure 4. The incorporation of BLP resulted in increased contents of all types of fatty acids. However, noteworthy increases in MUFA and PUFA were noted in L pasta. It is of a particular importance considering the recommendations of the European Food Safety Authority (EFSA), who underlined the importance of increasing the daily intake of PUFA [EFSA Panel on Dietetic Products, 2010]. An increase in fatty acid content in fortified pasta was previously reported by other authors; however, all these studies focused on the fortification of pasta with fish products [Ainsa *et al.*, 2021; Desai *et al.*, 2018b; Monteiro *et al.*, 2016].

The effect of drying on fatty acid contents could also be noticed, especially in the case of C16:0 and palmitoleic acid (C16:1 *n*7). The clear increasing trend of fatty acid content with increasing BLP percentage was observed only for L pasta products, with L-B5 pasta having the highest content of fatty acids among all analysed pasta products (Table 4). At the same time, this pasta was characterised by the highest proportion of MUFA and PUFA (Figure 4). Unsaturated fatty acids are particularly vulnerable to oxidation [Nawar, 1984], which can explain the lower content of fatty acids in the H pasta. In our study, the most thermally sensitive unsaturated fatty acids were C18:1 *cis*, C18:2 and C18:3 *n*6. Previously, C18:3 *n*3 was found to be particularly thermolabile in pasta fortified with flaxseed [Manthey *et al.*, 2002], which was not noted in our study. We observed an opposite phenomenon, and C18:3 *n*3 content was the highest in H pasta.

The results of the two-way ANOVA showed that there was a clear interaction between the BLP percentage and drying temperature for most individual fatty acids and total fatty acids (Table 4). The most significant interactions were noted for C17:1 *trans* and C18:2.

TABLE 5. Content of sugars in broccoli leaf powder (BLP) and experimental pasta products (mg/g dry matter).

Pasta	Fructose	Glucose	Sucrose	Stachyose	∑ Sugars
BLP	32.44±1.53	17.56±0.16	7.71±5.94	55.38±0.32	113.09±6.99
F-C	0.62±0.28^{e}	0.81±0.21^{d,e}	18.05±1.06^{a,b}	5.36±0.25^{a}	24.83±0.31^{a,b}
F-B2.5	1.04±0.08^{d}	0.89±0.01^{c,d,e}	18.52±0.51^{a,b}	4.51±0.06^{b}	24.96±0.61^{a}
F-B5	1.67±0.23^{b}	1.23±0.07^{b,c}	17.24±0.34^{a,b}	4.60±0.21^{b}	24.74±0.78^{a,b}
L-C	1.11±0.16^{d}	1.32±0.06^{b}	17.46±0.17^{a,b}	3.62±0.16^{e}	23.50±0.37^{a,b}
L-B2.5	2.13±0.51^{a}	2.31±0.19^{a}	17.03±0.66^{a,b}	3.14±0.05^{f}	24.60±1.42^{a,b}
L-B5	1.58±0.11^{b,c}	1.18±0.11^{b,c,d}	16.43±0.88^{a,b}	3.98±0.11^{c}	23.17±0.81^{a,b}
H-C	0.58±0.07^{e}	0.97±0.52^{b,c,d,e}	19.32±7.06^{a}	3.91±0.08^{c,d}	24.78±6.94^{a}
H-B2.5	1.24±0.27^{c,d}	0.76±0.1^{e}	16.26±0.98^{a,b}	3.67±0.18^{d,e}	21.94±1.21^{a,b}
H-B5	1.20±0.10^{d}	0.84±0.07^{d,e}	14.57±0.85^{b}	3.65±0.22^{d,e}	20.26±1.06^{b}
			p-Value$^{\#}$		
BLP addition effect	<0.001*	0.033*	0.252	<0.001*	0.432
Drying (D) effect	<0.001*	<0.001*	0.615	<0.001*	0.169
BLP×D effect	0.006*	<0.001*	0.668	<0.001*	0.559

B2.5 – pasta fortified with 2.5% of BLP; B5 – pasta fortified with 5% of BLP; C – control pasta without BLP; F – fresh pasta; L – pasta dried at low temperature (50°C, 8 h); H – pasta dried at high temperature (80°C, 3 h). Different letters in superscript in the same column indicate a significant difference ($p<0.05$) based on post-hoc Fisher's least significant difference (LSD) test. $^{\#}$Two-way analysis of variance. *Significant effect ($p<0.05$).

Sugar profile

The main sugars detected in broccoli were glucose, fructose and sucrose, which are important contributors to its taste [Rosa *et al.*, 2001; Wieczorek *et al.*, 2022]. The content of individual sugars in BLP and experimental pasta is presented in Table 5. Four sugars were detected in BLP and experimental pasta, including fructose, glucose, sucrose and stachyose. The main sugar in BLP was stachyose. Contrary, sucrose was the main sugar in the experimental pasta products. The incorporation of BLP influenced the content of all sugars except for sucrose, which was purely derived from semolina. The most prominent effect of BLP addition was found for fructose; however, the increasing trend with increasing BLP percentage was observed only for F pasta. In the case of pasta products dried at low temperature, B2.5 pasta had a significantly higher content of fructose than B5. Similar tendencies were observed for glucose in F and L. For H pasta, incorporation of BLP resulted in insignificant changes in glucose content.

The effect of drying was found for fructose, glucose and stachyose. The content of stachyose was significantly lower in dried pasta compared to F. An increasing trend in glucose and fructose content was observed for F pasta with increasing BLP percentage, while no clear trend could be defined for dried pasta products. Notably, irrespective of BLP percentage, the content of glucose and fructose was higher in dried than in fresh pasta. It is in agreement with a previous study, which also reported a small increase in contents of these sugars [Gélinas *et al.*, 2016]. The authors reported also the difference in sucrose levels depending on drying temperature. In L products, sucrose content decreased, while in H products it was observed to increase. A similar tendency was observed also in our study, although statistical analysis did not highlight the effect of drying on sucrose content. In H pasta, the highest content of sucrose corresponded to the lowest content of glucose and fructose, which can suggest that a higher temperature of drying can inhibit sucrose hydrolysis. The lower content of sucrose observed with a higher content of glucose and fructose in L pasta can be explained by the most favourable conditions for possibly the enzymatic reaction.

The results of the two-way ANOVA showed that there was an interaction between the BLP percentage and drying temperature for individual sugars and total sugars (Table 5). The most significant interactions were noted for stachyose and glucose.

CONCLUSIONS

This study presented a successful attempt of incorporating BLP into pasta formulations. BLP significantly improved the contents of ash, FAA and fatty acids, without compromising the cooking quality of pasta. Drying temperature had a significant negative effect on the contents of fatty acids, which were probably partly oxidised at the highest temperature. From the nutritional point of view, the low-temperature drying seems to be an interesting method for pasta preparation, as the highest content of FAA, fatty acids, especially MUFA and PUFA, and the lowest sugar level were found in these pasta products. However, at the same time, dried pasta products were characterised by greater cooking loss approximating 10%, which is higher than the acceptable 8%.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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