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Pasta Fortification with Leaves of Edible Wild Plants to Lower the P Glycaemic Index of Handmade Fresh Noodles

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Abstract

Edible wild plants are a largely available food at no cost and an emblem of sustainability. Among the numerous varieties of edible wild plants, purslane (*Portulaca oleracea L.*) and common mallow (*Malva sylvestris L.*) are good sources of healthful bioactive compounds. Therefore, there is a growing interest in their consumption for health-related nutritional and sustainable perspectives. Fresh durum wheat tagliatelle fortified with dried and pulverized leaves of mallow or purslane at two distinct percentages of integration (3%, 6%) were handmade. Polyphenols, pigments, and carotenoids were extracted and quantified. The *in vitro* inhibitory effect against digestive enzymes and the predicted glycaemic response were assessed. All samples exhibited appreciable quantities of polyphenols, pigments, and enzymatic inhibition of α-amylase and α glucosidase *in vitro*. The estimated glycaemic index for pasta fortified with 3% or 6% purslane powder was reduced by 10.8% or 28.3%, respectively, compared to pasta with durum wheat semolina alone. For mallow-enriched pasta at 3 and 6%, the reductions were 24.3% and 21.6%, in the order. The lowest expected glycaemic index was obtained with pasta 6% purslane powder enriched (P6c = 53 +/- 2.2). In this study, mallow and purslane were tested to be used as natural sources for producing handmade enriched pasta. All the fortified samples presented a lower pGI concerning control

pasta, thus indicating that plant powder addition could be suitably adopted. Their valuable and functional compounds reduced the pGI and imparted a pleasant natural color to the pasta.

Keywords
Enriched pasta; plant powders; expected glycaemic index

1. Introduction

Wild edible herbs grow easily in nature and can be harvested and included in human nutrition. Today they are acquiring importance as useful food sources for their nutritional value, displaying healthier characteristics and suggesting innovative usages in gourmet cuisine. Significantly these native species arise naturally in the environment; they can more easily adapt to different climatic conditions, also requiring less expense for their eventual cultivation than other commercially cultivated plants [1]. In addition, consumers are more sensitive to making conscious, healthy, and sustainable food choices, demanding new natural-origin products with these requirements. Moreover, they are resources of important compounds including carbohydrates, proteins, lipids, and other molecules such as vitamins, polyphenols, and carotenoids. The latter possess beneficial properties such as antioxidant, anti-inflammatory, or anticancer activity. Therefore, their utilization could be advantageous from a well-being perspective. Since ancient times, these plants have been applied in traditional medicine and food; more recently particular attention has been paid to their bioactive components, especially secondary metabolites mainly responsible for their biological activity and functionality [2].

Common mallow (*Malva sylvestris L.*) and purslane (*Portulaca oleracea L.)* are annual plants belonging to two different plant families widespread in an area rich in biodiversity, namely Southern Italy in the Mediterranean basin. These plants are rich in fatty acids, carotenoids, fibers, polyphenols, and other non-nutritive bioactive phytochemicals. These compounds could have industrial applications by including them in some foods to realize innovative functional foods with healthful implications on common illnesses, including cardiovascular disorders, diabetes, and chronic inflammation. In addition, numerous studies have shown that mallow and purslane possess antioxidant, anti-inflammatory, hypoglycaemic, and cholesterol-reducing properties [3, 4].

Pasta is among the most popular and frequently consumed grain products, for its nutritional quality, somewhat long shelf life, ease of preparation, and relatively low price [5]. Moreover, it can be easily enriched with vegetable raw materials containing phytochemicals with antioxidant, blood sugar-lowering, or cholesterol-lowering effects [6]. An enriched pasta formulation that can induce a low glycaemic response could be a strategy to comply with glucose control diets. This study aimed to evaluate the effects of a leaf powder addition (3%, 6%) of the aforesaid wild species on starch digestibility and *in vitro* GI of the enriched pasta to obtain functional durum wheat products with enhanced health benefits.

2. Materials and Methods

2.1 Materials

Acarbose (A8980), porcine pancreatic pepsin (P7545), pancreatic α-Amylase (A 6255), yeast α-glucosidase (G5003), dinitro salicylic acid (DNSA) color reagent (D0550), and p-nitrophenyl-α-D-glucopyranoside (N1377) were acquired from Merck KGaA, Darmstadt, Germany. Sodium hydroxide, hydrochloric acid, ethanol, acetone, and sodium carbonate, were bought from Carlo Erba Reagents, Italy. Durum wheat semolina (Cappelli), and bread wheat flour (Selex, Trezzano sul Naviglio, Milan, Italy) were acquired in a commercial store in Avellino (Italy).

2.2 Preparation of Vegetable Powders and Plant Extracts

The plants were harvested on private land in Meridional Italy. Plant recognition application PlantNet was used for botanical identification [7]. The leaves were cleaned with distilled water and dried at 50 ± 1°C in a tray dryer (Melchioni Babele) for 6 h. The dried leaves were milled (Kenwood CH580), and sifted with a 500 µm sieve, obtaining plant powders. The powders were stored in sealed dark boxes until analyzed or mixed with durum wheat flour for the two pasta formulations (3% or 6%).

The powders were extracted with 50% aqueous ethanol (1.0 g:10 mL; w:v). The samples were stirred for 24 h at 20°C and centrifuged at 13000 g for 5 min. Supernatants were utilized for the *in vitro* assays.

2.3 Fresh Eggless Pasta Preparation

Four different samples of noodles enriched with mallow or purslane powders and one control sample (Ctrl) were handmade according to the traditional Italian fresh eggless pasta recipe. In brief, 7.5 g or 15 g plant powder and 242.5 g or 235 g of durum wheat semolina, for 3%, and 6% formulation, respectively, were mixed with tap water, until a dough was obtained. Thus, the dough was extruded in the shape of a noodle with 2.0 mm in thickness and 20 cm in length and named P3, P6 (purslane powder), and M3, M6 (mallow powder). After the cooking step, the enriched tagliatelle were called P3c, P6c, and M3c, M6c. The reference pasta was prepared utilizing 100% durum wheat semolina. After one day at 20°C, the samples were stored in sealed bags at 4°C until used within 2-3 days.

2.4 Cooking Time Calculation

The cooking time was established using the AACC-approved method 66-50, as reported by Samaan et al., 2006 resulting in 5 min [8]. Tagliatelle (10 g) were cooked in 100 mL of distilled water. Every 60 s, a small sample was cut in half widthwise and compressed between two transparent glass slides. The point at which the white starch core completely vanished, was accounted for the adequate cooking time.

2.5 Total Phenol Content

Total polyphenol content (TPC) was calculated according to the method explained in Ombra et

al., 2018 [9]. TPC value was reported as gallic acid equivalent (GAE) in μg per g of the specimen.

2.6 Chlorophylls and Carotenoids Content

Chlorophylls (chl a and b) and carotenoids (car) contents were calculated using the method described by Złotek et al., 2014 [10]. Chl and car extractions from plant powders (1 g) or pasta samples (2 g) were performed with 80% (v/v) acetone at 4°C overnight. The extracts were centrifuged at 13 000 g for 5 min. Absorbance for supernatants at 663, 645, and 470 nm by Varian Cary 50 Bio spectrophotometer. The chl a, chl b, and car contents were determined from the following equations:

$$chl\ a = 12.72 \times A663 - 2.59 \times A645$$

$$chl\ b = 22.88 \times A645 - 4.67 \times A663$$

$$car = (1000 \times A470 - 3.27 \times chla - 104 \times chlb)/229$$

and reported in μg/g dm.

2.7 Polyphenols Extraction from Pasta Samples

Polyphenolic extraction from pasta samples was performed with the method explained by Bustos et al. 2020, with modifications [11]. First, 5 mL of acetone: water (70:30) was added to each g of pasta and mixed for 2 hours at 26°C, followed by centrifugation at 10,000 g, for 10 minutes and collection of the supernatants. The extraction was repeated and the supernatants were combined. A rotary evaporator eliminated the solvent, and the dried residue was then dissolved in water for subsequent analyses.

2.8 Alpha-Amylase Inhibition Assay

The α-amylase bioassay was derived from the Sigma-Aldrich protocol, with some modifications [9]. Briefly, a 96 mM solution of 3,5-dinitro salicylic acid (20 mL) was diluted with 8 mL of sodium potassium tartrate (5.31 M) in 2 M NaOH and 12 mL of deionized water. Porcine pancreatic α-amylase was dissolved in 20 mM phosphate buffer (pH 6.9) and NaCl (6.7 mM). Each sample (90 μL) was mixed with 10 μL of amylase solution (575 U/mL) and incubated at 25°C for 20 min. A 1.0% starch solution (100 μL) was added, followed by incubation at 25°C for 3 min. DNS reagent (100 μL) was included, at a temperature of 85°C for 10 min, finally diluting with distilled water (1.0 mL). Negative controls were performed by replacing the extracts with 90 μL of deionized water. The extract was added to the reaction mix excluding the α-amylase enzyme to obtain a blank. Absorbance at 540 nm was recorded and the blank value was detracted from that of the sample. Acarbose (1.0 mM) was used as a positive control. The IC50 value, defined as the concentration of the extract where the percent inhibition equals 50, was calculated from three independent experiments.

2.9 Alpha-Glucosidase Inhibition Assay

A 5 mg/mL solution of α-glucosidase from *Saccharomyces cerevisiae* (10 μL) and a 1 mM solution

of p-nitrophenyl-α-D-glucopyranoside (25 µL) in 20 mM phosphate buffer (pH 6.0) [12] were incubated with 10 µL of extract at 37°C for 7 min. Then a solution of 0.1 M Na_2CO_3 (80 µL) was added, measuring the absorption at 400 nm. Acarbose (1.0 mM) was used as a control. The test was repeated three times and the IC50 value was calculated.

2.10 In Vitro Starch Digestibility and pGI Calculation

The method described by Brennan & Tudorica, 2008 [13] was applied for the simulated *in vitro* digestion of starch and modified as described previously [14]. First, the samples were cooked at adequate cooking time, as described above. Cooked noodles (4 g) were mixed with 20 mL of sodium phosphate buffer (pH = 6.9). Next, the pH was adjusted to 1.5 by hydrochloric acid to obtain an appropriate environment for porcine pancreatic pepsin activity. A 4 mL of porcine pancreatic pepsin (115 U/mL) was added to the reaction mixture and incubated at 37°C for 30 min. Then, pH was adjusted to 6.9 by sodium hydroxide (2 M) followed by adding 1 mL of porcine pancreatic α-amylase (110 U/mL) to the final reaction mixture of 50 mL. The resulting mixture was incubated in the stirred water bath at 37°C. At each time interval (5, 10, 15, 20, 30, 60, 90, 120, 180, 240) µL of Stop Solution (0.3 M Na_2CO_3) was added to 100 µL aliquot to block ulterior enzymes activity. Aliquots were centrifuged at 2000 g for 5 min and the 3,5 dinitro salicylic acid method was applied to calculate the reducing sugar quantity (546 nm) [15]. White wheat bread was considered as a reference, without inhibitors, and used for starch digestion rate. Brennan and Tudorica's 2007 method was applied to calculate the amount of Reducing Sugar (maltose) Released (%RSR eq.1), Hydrolysis Index (HI eq.2), and predicted GI (pGI eq.3) [16]:

$$\%RSR = (Asample \times 500 \times 0.95)/(Amaltose \times carbohydrate) \times 100 \quad (1)$$

A sample was the absorbance at 546 nm; A maltose represented the absorbance of a solution comprising released maltose (1 mg/mL) by enzyme starch hydrolyzing; carbohydrate represents mg starch and sugars contained in 4 g sample.

The Hydrolysis Index was measured with the equation:

$$HI = AUC(0 - 240min)sample/AUC(0 - 240min)bread \times 100 \quad (2)$$

AUC represents the area under the sample curve from 0 to 240 min as a percentage of the corresponding area of the reference wheat bread.

The predicted GI was calculated with the equation:

$$predictedGI = 0.862HI + 8.189 \quad (3)$$

2.11 Statistical Analysis

The results represent the means ± standard deviation of three tests. Statistical analysis by ANOVA (one-way analysis of variance) with a high confidence level (95%, $p < 0.05$) was used to evidence differences between samples. Student's t-test analyzed the means and significant differences were accepted at $p < 0.05$. "Statistics-Excel" was applied for calculations. For IC50 calculations the online software "ED50plus v1.0" was utilized [17].

3. Results

3.1 Polyphenols and Pigments Contents

The total polyphenol content (TPC) of mallow and purslane powders was first calculated. As reported in Table 1, the purslane extract presented no significantly lower TPC than the mallow extract, ($p > 0.05$). Furthermore, the contents of chlorophyll a, chlorophyll b, and carotenoids are indicated in Table 1. Appreciable chlorophyll a and b quantities were measured in mallow and purslane extracts, with a higher content of chlorophyll a in mallow extract than in purslane extract, but not at a significant level ($p > 0.05$).

Table 1 Total Polyphenol content and pigments of dried leaves extracts.

SAMPLE	TPC mg GAE/g ± sd	chl a mg/g ± sd	chl b mg/g ± sd	car µg/g ± sd
***Portulaca oleracea* extract**	5.99 ± 0.5 (a)	1.7 ± 0.3 (a)	1.3 ± 0.2 (a)	118.3 ± 3.2 (b)
***Malva Sylvestris* extract**	6.58 ± 0.7 (a)	1.9 ± 0.2 (a)	1.6 ± 0.1 (a)	104.1 ± 2.1 (a)

Results are means of three experiments ± standard deviation. Abbreviations: TPC = total phenolic content; chl a = chlorophyll a; chl b = chlorophyll b; car = carotenoids; values within a column with the same lowercase letters are not significantly different ($p > 0.05$).

Carotenoids are typical plant pigments and, due to intrinsic beneficial properties, are arousing growing attention for potential application in industry as natural pigments [18, 19]. In Table 1, a significantly higher content of carotenoids in the *Portulaca oleracea* extract was observed compared to the *Malva sylvestris* extract, ($p < 0.05$), in agreement with results obtained in the literature where statistically significant differences in total carotenoids were observed between spinach, kale leaves, and purslane with the latter having the highest total carotenoid content [20].

The quantities found with *Portulaca oleracea* and *Malva sylvestris* extracts strengthen the utilization of both vegetable powders for pasta functionalization. On the whole, following the replacement of semolina with vegetable flours, the polyphenol content of raw and cooked pasta samples augmented significantly ($p < 0.05$) in comparison to the control pasta (Ctrl-P), up to a maximum of 192.9 ± 3.5 µg GAE/g in M6 raw pasta (Table 2). The phenolic content of M6-enriched pasta was almost 5.6 times higher than that of pasta without any addition.

Table 2 Total Polyphenol content and pigments of pasta extracts.

SAMPLE	TPC µg GAE/g ± sd	chl a µg/g ± sd	chl b µg/g ± sd	car µg/g ± sd
Ctrl-P	34.0 ± 4.1	nd	nd	nd
P3	101.7 ± 2.6	2.10 ± 0.7	2.70 ± 0.7	0.60 ± 0.2

P6	185.8 ± 3.9	4.60 ± 1.1	6.00 ± 1.3	1.50 ± 0.6
M3	127.0 ± 5.3	1.90 ± 0.3	2.10 ± 0.8	0.54 ± 0.2
M6	192.9 ± 3.5	3.70 ± 0.9	3.40 ± 1.1	0.82 ± 0.3
P3c	96.7 ± 7,1	0.24 ± 0.1	0.18 ± 0.0	0.36 ± 0.1
P6c	135.5 ± 3,5	0.84 ± 0.3	0.54 ± 0.2	0.84 ± 0.3
M3c	99.2 ± 5.5	0.70 ± 0.2	0.36 ± 0.2	0.18 ± 0.0
M6c	105.2 ± 4.6	1.70 ± 0.3	0.66 ± 0.3	0.48 ± 0.2

Ctrl-P = control pasta; P3 = 3% of *Portulaca oleracea* powder enrichment; P6 = 6% of *Portulaca oleracea* enrichment; M3 = 3% of *Malva sylvestris* addition; M6 = 6% of *Malva sylvestris* addition; P3c; P6c; P3c; P6c = cooked enriched pasta; TPC = total phenolic content; chl a = chlorophyll a; chl b = chlorophyll b; car = carotenoids; nd = not detectable; all mean values resulted significantly different respect to Ctrl-P, $p < 0.05$ by t-test.

Cooking the samples resulted in a certain reduction of their polyphenolic content. However, the most remarkable difference was observed in M6c, where about 45.4%, was lost compared to the raw counterpart. In Table 2, as for polyphenols, pigments were more abundant in raw pasta, with the P6 sample displaying the highest contents. Furthermore, M6c and P6c pasta showed higher chl a, chl b, and carotenoid contents due to the higher addition of plant powder in the pasta enrichment compared to M3c and P3c.

Lastly, applying a t-test to compare the mean values, a significantly higher amount ($p < 0.05$) of TPC, and pigments was observed in all enriched samples compared to the control pasta (Table 2).

Moreover, although the phytochemical content was partly missed in the cooking process, it still imparted appropriate and palatable colors to the fortified samples (Figure 1).

Figure 1 Image of 3% and 6% *Portulaca oleracea L.* fortified noodles, after cooking (P3c-P6c) and 3% and 6% cooked *Malva sylvestris L*. powder enriched samples (M3c-M6c). Ctrl = pasta with durum wheat semolina.

3.2 Alpha-Amylase and Alpha-Glucosidase Inhibition

Numerous results presented in literature show that phenolic compounds exhibit inhibitory activities on α-amylase and α-glucosidase digestive enzymes [9, 21]. Therefore, the inhibitory potential of different concentration levels of mallow and purslane powders (3%, 6%) incorporated into pasta against α-amylase and α-glucosidase was evaluated. Extracts of *Portulaca oleracea* and *Malva sylvestris* (P and M) possess hypoglycaemic potential to inhibit a-amylase and a-glucosidase

in vitro (Table 3). Similarly, the inhibitory properties of polyphenols in mallow or purslane were preserved in extracts derived from 3% and 6% fortified pasta (Figure 2). For the purslane pasta, as the amount of powder in P6 increased, lower IC50 results were found, indicating enhanced inhibitory activity, although these differences were statistically significant exclusively for α-glucosidase.

Table 3 α-amylases and α-glucosidase inhibition by leaves powder extracts.

SAMPLE	**IC50 (±sd) mg/g against α-amylase**	**IC50 (±sd) mg/g against α-glucosidase**
***Portulaca oleracea* extract**	19.6 ± 2.2 (a)	24.1 ± 2.3 (a)
***Malva Sylvestris* extract**	12.0 ± 1.9 (b)	26.0 ± 3.1 (a)

IC50: Concentration of sample (mg/g of dry weight) required to inhibit 50% of enzyme activity. Nonidentical lowercase letters within the same column evidence significant differences ($p < 0.05$).

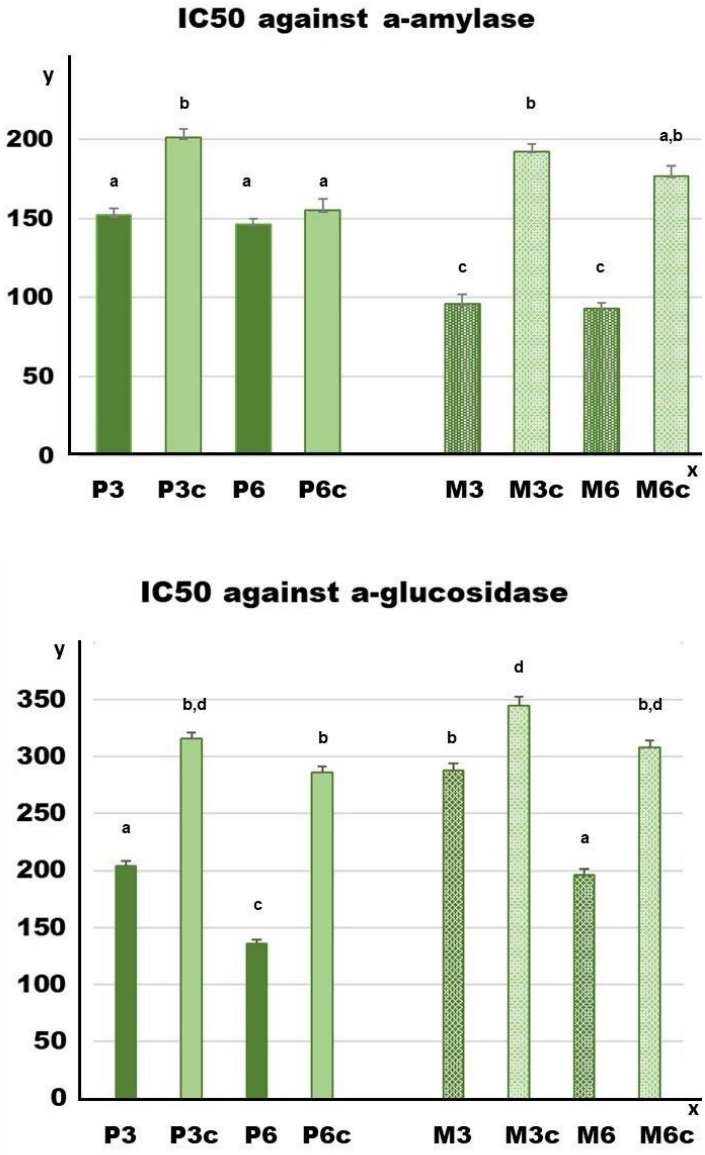

Figure 2 IC50 values of enriched samples to inhibit α-amylase and α-glucosidase. IC50: Sample concentration (mg/g of dry weight) to obtain a percent inhibition equal to 50%. Extract from control pasta (Ctrl-P) was inactive (not shown). P3 - P3c = extracts from raw and cooked samples with 3% *Portulaca oleracea* powder; P6 - P6c = extracts from raw and cooked samples with 6% *Portulaca oleracea* powder; M3 - M3c = extracts from raw and cooked pasta with 3% *Malva sylvestris* powder; M6 - M6c = extracts from raw and cooked pasta with 6% *Malva sylvestris* powder. Data were mean values of triplicate measurements ± standard deviation (SD). Different letters over the columns indicate statistically significant differences ($p < 0.05$).

The observed trend was also found for mallow, with M6 extract showing lower IC50 values for α-amylase and α-glucosidase than M3. Comparing the noodles extracts with 3% supplementation (P3, M3), for a-amylase inhibition, the IC50 values were significantly different ($p < 0.05$). The same result was obtained for the higher P6 and M6 powder contents, ($p < 0.05$), confirming a higher inhibitory

property for mallow powder. Conversely, the values for glucosidase, indicated that purslane powder had higher inhibitory abilities, and the lowest IC50 value was obtained with the P6 extract (Figure 2).

Since noodles are not consumed raw, testing whether the inhibitory potential was retained after cooking was essential. As shown in Figure 2, for α-amylase assay the lowest IC50 value was measured for 6% enriched pasta with flour-reduced purslane while the highest result was obtained for P3c extract. All the extracts also inhibited the α-glucosidase enzyme. As supposed, the sample fortified with 6% mallow or purslane powder showed the lowest values compared with the 3% corresponding samples. The vegetable powder-free extract (Ctrl-P) had no such inhibitory activities (data not shown). Thus, such activities were largely due to the vegetable powders as described in the literature on the two analyzed plant species, significant sources of polyphenols with inhibitory capacities [3, 22].

3.3 In Vitro Starch Digestion and pGI Values

Applying an optimized protocol, the *in vitro* starch digestibility of the noodles P3c, P6c, M3c, and M6c, was evaluated [13]. A pGi value of 74 (+/-1.1) was measured for the control pasta with only durum wheat. In contrast, pGI values for the four supplemented samples were significantly lower than the semolina-only control, as reported in Table 4. In particular, the mallow samples M3c and M6c exhibited 56 (+/-3.0) and 58 (+/-1.3), respectively. Thus, including the mallow powder reduced the fortified pasta's predicted glycaemic index by 24.3% and 21.6% in M3 and M6, respectively. The pGI values of 3% and 6% formulations were similar, and the incorporation of mallow powder, containing amylase and glucosidase inhibitors, into durum wheat semolina significantly lowered the pGI of fresh pasta, under the value of 74. Accordingly, the bioactive components of mallow flour in the final noodle structure of M3 and M6 samples affect the rate of starch degradation. However, the increase to 6% in the formulation did not return a lower value than M3% pGI. This trend was also described for other enriched pasta [23]. Nevertheless, for purslane samples, a reduction to a value pGI = 66 +/- 0.3 was obtained for P3c, while with the increment to 6% powder, the pGI further decreased (pGI = 53 +/- 2.2). Indeed, the expected glycaemic index was reduced by 10.8% and 28.3% in P3 and P6, respectively.

Table 4 Predicted Glycaemic Index (pGI) of pasta samples.

SAMPLE	pGI	+/-sd
Ctrl pasta	74^{a}	+/-1.1
P3c	66^{b}	+/-0.3
P6c	53^{b}	+/-2.2
M3c	56^{b}	+/-3.0
M6c	58^{b}	+/-1.3

Results are means of three experiments ± standard deviation. Predicted Glycaemic Index (pGI) of P3c = Pasta with 3% of purslane powder; P6c = Pasta with 6% of purslane powder; M3c = Pasta with 3% of mallow powder; M6c = Pasta with 6% of mallow powder. Nonidentical lowercase letters in the same column evidence significant differences ($p < 0.05$).

4. Discussion

Among the wild plants of the Mediterranean region, mallow and purslane were selected for inclusion in this study according to the following criteria: a wide distribution in the Mediterranean basin, the desirability, and presence of the plants in popular tradition, the non-inclusion in the lists of protected flora, ease of recognition of the species, the harmlessness of the plant i.e., absence of potentially toxic substances, appreciation for their health properties. Common mallow (*Malva Sylvestris* L.) is a member of the Malvaceae family, widespread in many countries. Its leaves contain substances with antioxidant, antiproliferative, anti-inflammatory, antihepatotoxic, and glucose-lowering properties [3]. Pursley (*Portulaca oleracea* L.) belongs to the Portulacaceae family. It includes phytochemicals with antioxidant, hypoglycaemic, and anti-inflammatory capacities. Purslane is a rich source of omega-3 fatty acids, especially alpha-linolenic acid (ALA) more than any leafy green vegetable, and contains vitamins A and C [22, 24]. Given their phytochemical contents, these wild plants should be valued as sustainable foods to provide new functional foods and nutraceuticals. The two plant flours and enriched noodles were examined for biochemical and nutritional properties. In particular, the contents of the two plant powders' total polyphenols, chlorophyll a, b pigments, and carotenoids were measured. Then their quantity in the fortified pasta was verified before and after cooking. A higher content of carotenoids was measured in purslane extract than in mallow extract, while the content of polyphenols and chlorophylls was very similar in the two extracts. Studies have demonstrated that the solvent concentration, incubation time, and temperature affect the polyphenol or other bioactive substances extraction, making complex comparisons with other studies. However, the measured TPC values were not dissimilar to those reported by other authors [24, 25]. The environment and climatic conditions influence pigment content. For example, greater amounts of pigment are measured in plants growing in shady conditions [26]. Pigments are sensitive to temperature and light, nevertheless, consistent results have been recorded in other studies [27]. In addition to their antioxidant activity, polyphenols have many other health benefits, offering protection against the development of diseases. Similarly, chlorophylls are natural pigments of green vegetables possessing antioxidant properties capable of protecting against cellular damage, and a cellular detoxifying capacity. For such properties, chlorophyll and its derivatives could function as chemopreventive agents [28, 29]. Appreciable polyphenols, chlorophyll a, b, and carotenoids quantities were also measured in all enriched pasta, and similar to the results reported by other authors the reduction of polyphenols in cooked noodles could be in some measure due to their degradation while boiling or to the solubilization, especially of the free fraction, in the cooking water [30]. The next passage was to check that the pulverized leaves and the enriched pasta had an inhibitory activity for the enzymes deleting starch, a main component of this food. Starch is a good energy source, and once ingested, first digested by salivary and pancreatic α-amylase, into smaller molecules such as maltose, maltooligosaccharides, and others. Then, these sugars are further hydrolyzed by intestinal brush border α-glucosidase to glucose [31]. Thus, α-amylase and α-glucosidase are essential for starch digestion, and delaying their activity may be useful for controlling postprandial blood glucose levels [32]. As reported in Figure 2 all the enriched samples exhibited inhibitory capacities on amylase and glucosidase enzymes, and the cooking step did not eliminate the inhibitory activities. The lower inhibitory abilities of cooked noodles as the reduced TPC could be due to the loss of polyphenols in the cooking water, particularly free phenolic acids, and the thermal degradation of phenolic compounds [33]; yet, further research

is needed to ascertain these mechanisms. By adding other ingredients to pasta, it is necessary to analyze the functional healthy capacity of fortified pasta samples, since the stability of the pasta matrix could be worsened by these components because they could generate pores in its structural configuration [16]. Therefore, the *in vitro* digestion assay could provide interesting information and be a proper analytical system for studying the properties of new formulations of pasta.

In this regard, pasta is considered a low GI food, different from other grain-based foods with high GI, such as rice. Fresh pasta, has a higher pGI at about 70, in contrast, to dry pasta range of 43-60 [34, 35]. In this study, adding vegetable powders modified the pGI of pasta samples; in the case of mallow, the increased quantity likely weakened the gluten arrangement, contributing to major starch readiness for digestive enzymes compared with the 3% enrichment. A similar trend was observed for persimmon flour addition as reported by Lucas-Gonzalez et al. [23]. Spaghetti with 3% persimmon flour had a lower glycaemic index than spaghetti with 6% . The lowest GI value (53 +/- 2.2) was achieved for the pasta enriched with 6% purslane powder. Starch digestion mechanisms are intricate and not referred completely to the peculiarities of the protein-starch matrix or the starch structural status. Pasta fortification at 3 or 6 percent mallow returned a decreased digestibility. However, when more plant powder was added, the weakening of the gluten network allowed contact between enzymes and starch, and the inhibition effect became less significant. Finally, 6% purslane powder could be applied to produce pasta, with lower pGI than traditional fresh pasta. Nevertheless, *in vivo* analyses are needed to validate *in vitro* results. Our research represents a preliminary but crucial step to obtaining pasta with enhanced nutraceutical potential. Including wild herbs in pasta may contribute to more sustainable food consumption. Both wild plant powders efficaciously improved the glucose-lowering potential of the enriched fresh pasta, with likely benefits in diets. A low-glycemic index diet is recommended for diabetic patients and healthy subjects [36]. Further investigations are required to estimate consumer acceptability and the *in vivo* glycaemic effect.

5. Conclusions

Currently, the enrichment of foods with functional natural substances has received much attention from nutritionists, and the use of nutraceuticals in food production promotes human health by reducing the risk of disease. Pasta fortification with dried leafy vegetables could promote the development of low-glycaemic index food, resulting in slower increases in blood glucose and insulin level. Our study investigated the effect of mallow or purslane addition on pasta *in vitro* starch hydrolysis. Two distinct quantities of plant powders were included in the dough. It was concluded that all fresh handmade pasta formulated with 3% or 6% mallow or purslane powders had a significantly lower pGI than control pasta, associated with reduced starch digestive enzymes activity. Results suggested that their addition can improve pasta; an adequate amount is required to reach the desired effects, and adding up to 6% wild vegetable powders is a promising approach to valorize fortified pasta. Finally, due to their valuable and functional compounds, mallow and purslane can be used as natural sources to produce fortified pasta, and be appreciated as sustainable foods. The use of spontaneous edible plants protects the environment and in itself is an example of sustainable nutrition; being wild herbs at zero impact, they do not come from intensive crops. This approach is an opportunity for the pasta sector because it responds to consumer demand for more sustainable processes and greener products, and it could be expanded to obtain pasta containing bioactive

substances derived from additional wild edible plants. Enriched pasta could be part of glucose control diets and thus be relevant to human health and well-being.

Author Contributions

Conceptualization, Investigation, Data curation, Methodology, MNO, FF; Writing—original draft preparation, MNO, Supervision and funding acquisition FN.

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Competing Interests

All authors declare no conflicts of interest in this paper.

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