


Effects of Mulberry Pomace Addition and Transglutaminase Treatment on the Quality of Pasta Enriched with Antioxidants and Dietary Fiber

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Mulberry pomace powder, a by-product of mulberry juice processing, was added to pasta recipe to make pasta with high dietary fiber and antioxidant contents. The effects of mulberry pomace ratio on the nutritional, textural and cooking properties as well as the sensory overall acceptance of the product were investigated. A significant increment in dietary fiber and total anthocyanin contents as well as decrement in cooking quality, texture and color change were observed. The cooking loss increased with the substitution level of mulberry pomace while the optimal cooking time, swelling index and water absorption index decreased. The quality improvement of 10% mulberry pomace fortified pasta was investigated by adding a transglutaminase preparation with enzyme dosage from 0.25 to 1.00 U/g protein. The fortified pasta treated with transglutaminase at 0.50 U/g protein showed a significant improvement in chewiness, tensile strength and elongation rate but was not significantly affected in terms of the swelling index and water absorption. The use of transglutaminase also improved the overall acceptability of the fortified pasta. Mulberry pomace powder may, therefore, be considered a potential antioxidant-rich and dietary fiber-rich material for incorporation into pasta products.

Key words: antioxidant, dietary fiber, mulberry pomace, pasta, transglutaminase

INTRODUCTION

Pasta is a common staple food over the world with a production rate of 16.9 million tons in 2021 [IPO, 2021]. Durum wheat semolina is a key ingredient in making high-quality pasta. Despite being rich in calories, durum wheat semolina contains very little dietary fiber and bioactive compounds, such as antioxidants. In this regard, pasta made of durum wheat semolina could be supplemented with functional ingredients to satisfy the growing demand of consumers for healthy food products [Bresciani *et al.*, 2022].

Fruit processing by-products are reported to contain many functional components such as dietary fiber, protein, minerals, vitamins, and antioxidants [Martins *et al.*, 2017]. As a sustainable approach, the utilization of fruit by-products to improve the nutritional value of pasta has been attracting attention from researchers [Bianchi *et al.*, 2021]. Using cashew apple pomace from 5 to 20% of the semolina weight increased total dietary fiber of the pasta up to 4.1 times [Nguyen *et al.*, 2023b]. For pasta supplemented with 10% grape pomace, the total phenolic content and antioxidant capacity were 6-fold and 38-fold, respectively,

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higher than those of the control sample [Tolve *et al.*, 2020]. Fruit peels from mango and banana were also reported as mineral and ascorbic acid-fortified ingredients for pasta preparation [Jalgaonkar *et al.*, 2018; Puraikalan, 2018]. However, a significant consequence of adding fruit by-products to pasta formulation is significant deterioration of the cooking and textural attributes as well as the sensory quality of the fortified pasta [Carpentieri *et al.*, 2022; Padalino *et al.*, 2017, 2018]. In order to improve textural and cooking properties of high-fiber pasta, use of auxiliary ingredients or additional treatment of pasta dough are essential [Padalino *et al.*, 2016].

Transglutaminase (TG, EC 2.3.2.13) has been reported to improve the protein network due to its cross-linking reaction between the protein molecules in food materials [Zhu *et al.*, 2019]. In biochemistry, TG catalyzes the formation of a covalent bond between the γ -carboxamide group of protein or peptide-bound glutamine (acyl donors) and the free amino group of protein or peptide-bound lysine (acyl acceptors). In pasta making, this covalent bond formed by protein intermolecular crosslink could help to considerably decrease the quantities of soluble protein fraction, thereby strengthening the structural integrity of pasta [Gharibzadeh *et al.*, 2019]. The effects of TG on pasta texture have been demonstrated in different studies to improve the overall quality of pasta made with low-protein durum wheat semolina [Aalami & Leelavathi, 2008], semolina-pollard enriched pasta [Sissons *et al.*, 2010], cellulase-treated wheat bran-fortified pasta [Nguyen *et al.*, 2020] or corncob powder-supplemented pasta [Nguyen *et al.*, 2023a]. However, the use of TG in the making of pasta with fruit pomace added has not been reported.

Fruits of mulberry, a tropical *Morus* genus of the Moraceae family, are widely used in the processing of juice, jam, vinegar, and alcoholic beverage [Jan *et al.*, 2021]. The by-product of mulberry juice processing, mulberry pomace, is rich in dietary fiber and phenolic compounds with high antioxidant activity [Zhang *et al.*, 2011]. Mulberry pomace is supplemented to recipes of various food products including chocolate [Hwang *et al.*, 2012], bread [Kim *et al.*, 2013] and cookies [Jeon *et al.*, 2013] to improve their antioxidant capacity. Nevertheless, the addition of mulberry pomace to pasta formulation has not been considered in the literature.

In this study, mulberry pomace powder and transglutaminase preparation were used in pasta recipe to make the product with high-fiber and antioxidant contents. The objective of the study was to investigate the effects of the mulberry pomace ratio on the nutritional, textural and cooking properties as well as the overall acceptability of the product. The effects of transglutaminase dosage in the treatment of pasta dough supplemented with mulberry pomace were also evaluated.

MATERIALS AND METHODS

Materials

Fresh mulberry (*Morus alba* L.) fruits were harvested from a farm in Da Lat City (Lam Dong province, Vietnam) in July 2022, packed in perforated carton boxes and transported to the laboratory within 8 h after harvesting. At the laboratory, the fruits without

physical damage and decay were manually selected and washed with tap water. After 1 h draining, the fruits were pressed in a screw press (AC-130, Kheo Sung World Inc., Seoul, South Korea). The obtained pomace was subsequently dried in a convection oven (CD-20-08, Tung Viet Ltd., Dong Nai, Vietnam) at 60°C for about 6 h. The dried mulberry pomace with moisture content of 10–12 g/100 g was powdered in a hammer mill (HM-20-10, Tung Viet Ltd., Dong Nai, Vietnam), sieved through a 40-mesh sieve and stored in polyethylene bags at –18°C for experimentation.

Durum wheat semolina and table salt were purchased from Vietnam Flour Mills Ltd. (Ba Ria – Vung Tau, Vietnam) and Southern Salt and Trade Joint Stock Company (Ho Chi Minh City, Vietnam), respectively.

Protiact TG-RA, a clean-labelling preparation used to improve the texture of protein-rich food products, was supplied by Rama Production Co. (Bangkok, Thailand). The transglutaminase (TG) activity of Protiact TG-RA was 100 U/g. One unit (U) of TG activity was defined as the quantity of enzyme that catalyzes the formation of 1 μ mol of hydroxamate from *N*-carbobenzoxy-L-glutaminyglycine within 1 min under the assay conditions [Ando *et al.*, 1989].

Protease (Alcalase 2.5 L), amyloglucosidase (Dextrozyme GA) and α -amylase (Termamyl SC) used for determination of dietary fiber content were from Novozyme Inc. (Bagsvaerd, Denmark). All chemicals of analytical grade quality used in this study were purchased from Sigma Aldrich Co. (Saint Louis, MO, USA) and Merck Co. (Darmstadt, Germany).

Pasta preparation

Pasta was prepared with 200 g of a blend of durum wheat semolina and mulberry pomace, 1 g of table salt and 95 g of water. The ratio of mulberry pomace was 0, 5, 10, 15, 20% of the blend weight. Semolina, mulberry pomace and table salt were premixed in a KitchenAid 5K5SSWH flour mixer (Whirlpool Co., Guangzhou, China) at about 80 rpm for 5 min. Water was heated to 42°C, added to the flour mixer and mixed at 80 rpm for 2 min. The dough was kneaded for 18 min at 60 rpm. The pasta was prepared using a Philips HR2355 extruder (Philips Co., Guangdong, China) with extrusion force of 7,117 N. The fresh pasta was dried at 50°C until the moisture content achieved 10–12 g/100 g. The product was then stored in 18x26 cm polyethylene bags at –18°C for further analysis. Pasta fortified with 10% mulberry pomace was used for enzymatic treatment. TG was mixed with water before the wet mixing. The enzyme was added with the dosage of 0.25, 0.50, 0.75, and 1.00 U/g protein of the blend weight.

Proximate composition analysis

Moisture content was determined at 105°C using a moisture analyzer (ML-50, A&D Co., Tokyo, Japan). Crude protein content was evaluated by Kjeldahl method with a nitrogen-to-protein conversion factor of 5.8 for wheat. Lipid content was measured using Soxhlet method with diethyl ether. Ash was quantified by incineration at 600°C in a furnace (EF11/8, Lenton Co., Hope Valley, UK). Insoluble dietary fiber (IDF) and soluble dietary fiber

(SDF) contents were estimated according to AOAC International methods no. 991.42 and 993.19, respectively [AOAC, 2000].

■ Determination of total phenolic and anthocyanin contents and antioxidant capacity

Total phenolic content (TPC) of dried mulberry pomace, durum wheat semolina and dried pasta samples was determined according to the procedure reported by Biney & Beta [2014] with some modifications. About 1 g of dried mulberry pomace, durum wheat semolina or dried pasta was extracted with 10 mL of 60% (v/v) acetone for 30 min. The extract was recovered by centrifugation at 1,600×g for 20 min (3K30, Sigma Zentrifugen Ltd., Osterodeam Harz, Germany). The obtained supernatant (0.2 mL) was added to 1 mL of the Folin-Ciocalteu reagent followed by vortexing for 30 s. The tubes with the reaction mixture were left at room temperature in dark for 2 h, and the absorbance was measured at 760 nm (UV 2600i spectrophotometer, Shimadzu Co., Kyoto, Japan). TPC was calculated as mg gallic acid equivalent *per* 100 g dry weight of the dried mulberry pomace, durum wheat semolina or dried pasta (mg GAE/100 g dw).

For total anthocyanin content determination, 1 g of dried mulberry pomace, durum wheat semolina or dried pasta was extracted with 10 mL of acidified ethanol (the volume ratio of ethanol to 2% HCl was 1:1, v/v) for 2 h [Peng *et al.*, 2021]. The centrifugation was performed at 1,600×g for 20 min. The resulting supernatant was then diluted in acetate buffer with pH 1.0 and pH 4.5. Total anthocyanin content was calculated as follows:

$$A = (A_{520}^{\text{pH}1.0} - A_{700}^{\text{pH}1.0}) - (A_{520}^{\text{pH}4.5} - A_{700}^{\text{pH}4.5}) \quad (1)$$

$$a = \frac{A \times M_w \times D_f \times 1000}{\epsilon \times l} \quad (2)$$

in which, a is the total anthocyanin content (mg cyanidin 3-O-glucoside equivalent/100 g dw); M_w is molecular weight of cyanidin 3-O-glucoside, equal to 449.2 g/mol; D_f is dilution factor; ϵ is molecular extinction coefficient, equal to 26,900 L/(mol×cm); l is length of the cuvette (cm), $A_{520}^{\text{pH}1.0}$ and $A_{700}^{\text{pH}1.0}$ are absorbances of the reaction mixture with extract diluted in the buffer with pH 1.0 at 520 and 700 nm, respectively, $A_{520}^{\text{pH}4.5}$ and $A_{700}^{\text{pH}4.5}$ are absorbances of the reaction mixture with extract diluted in the buffer with pH 4.5 at 520 and 700 nm, respectively.

Antioxidant capacity of dried mulberry pomace, durum wheat semolina and dried pasta was evaluated as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) as described by Nguyen *et al.* [2020]. Briefly, about 1 g of dried mulberry pomace, durum wheat semolina or dried pasta was mixed with 10 mL of a 60% (v/v) aqueous acetone solution for 30 min. The supernatant recovered by centrifugation at 1,600×g for 20 min (3K30 centrifuge, Sigma Zentrifugen Ltd.) was diluted and used for antioxidant capacity determination. For DPPH assay, 0.1 mL of diluted extract was combined with 3.9 mL of a 60 μM DPPH radical solution in methanol. The reaction mixture was incubated at room temperature and in the dark. Absorbance at 515 nm was measured at zero

and after 30 min of incubation using methanol as the blank. The results were given as μmol of Trolox equivalent (TE) *per* 100 g dw of dried mulberry pomace, durum wheat semolina or dried pasta. For FRAP assay, 3.8 mL of the FRAP working solution (25 mL of 0.3 M acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine solution in 40 mM HCl, and 2.5 mL of 20 mM FeCl₃×6H₂O) was mixed with 0.2 mL of the diluted extract. The reaction mixture was incubated in the dark at 37°C for 5 min, and the absorbance at 593 nm was measured with an acidified methanol as blank. The FRAP was expressed as μmol TE/100 g dw of dried mulberry pomace, durum wheat semolina or dried pasta.

■ Determination of water holding capacity

Water holding capacity (WHC) of mulberry pomace and semolina was determined as described by Caprez *et al.* [1986] with slight modification. About 1 g of the sample was soaked in 10 mL of distilled water for 2 h and then centrifuged at 1,200×g for 10 min at room temperature. The supernatant was discarded, and the residue was weighted. WHC (g water/g) was calculated by the following equation:

$$\text{WHC} = (M_1 - M_0)/M_0 \quad (3)$$

in which, M_0 is the initial weight of the sample and M_1 is the weight of the obtained residue.

■ Determination of cooking properties

Cooking quality of pasta was evaluated through optimal cooking time (OCT), cooking loss (CL), swelling index (SI) and water absorption index (WAI) according to the method described by Nguyen *et al.* [2020]. About 10 g of the pasta sample (~5 cm in length) were cooked in 100 mL of boiling distilled water until the white inner core of pasta strand disappeared for its OCT. The cooked pasta strands were drained for 2 min, then dried at 105°C to constant weight. The total dry matter of cooking water was determined by drying at 105°C. The CL, SI and WAI were calculated as follows:

$$\text{CL} = P_1/P_0 \times 100 (\%) \quad (4)$$

$$\text{SI} = (P_2 - P_3)/P_3 \quad (5)$$

$$\text{WAI} = (P_2 - P_0)/P_0 \quad (6)$$

in which, P_1 is the total dry matter of cooking water, P_2 is the weight of cooked and drained pasta, P_3 is the weight of cooked pasta after drying at 105°C, and P_0 is the weight of raw pasta.

■ Instrumental color analysis

Color of uncooked pasta, mulberry pomace powder and semolina was determined using a chromameter CR400 (Konica Minolta Co., Osaka, Japan) with CIE Lab color space. The total color difference (ΔE) was determined through L^* (lightness), a^* (redness) and b^* (yellowness) values as follows:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (7)$$

in which, L_0^* , a_0^* and b_0^* are the color values of the pasta without mulberry pomace supplementation; L^* , a^* , and b^* are the color values of the mulberry pomace-enriched pasta.

■ Determination of textural properties

Texture profile analysis (TPA) was employed to assess the textural quality of pasta samples using a TA-XT plusC (Stable Micro Systems Co., Godalming, UK) with a Windows version of Exponent Connect Lite 7.0 software (Texture Technologies Co., Hamilton, MA, USA). The measurements were done on 5 sections from 5 different cooked pasta strands, using a 40 mm diameter acrylic probe with 70% axial compression and compression speed of 1 mm/s. The second compression cycle was set after 1 s. The hardness and chewiness of cooked pasta samples were calculated from the force-time curve. In the tensile strength test, two ends of a 15 cm cooked pasta strand were fixed to a pair of parallel rollers (Pasta Tensile Rig, Stable Micro Systems). The extension speed was 1 mm/s. The tensile strength (TS) and elongation rate of cooked pasta samples were determined by the formulas reported by Nguyen *et al.* [2020].

■ Estimation of overall acceptability

The overall acceptability of pasta was evaluated using a consumer test with 60 untrained panelists, including 32 men and 28 women, aged from 18 to 25, recruited from the students and staff at the Ho Chi Minh City University of Technology (Ho Chi Minh City, Vietnam). Pre-screening for potential wheat allergies and pasta consumption (at least once a week) was done on the panelists. Pasta samples were prepared by boiling 100 g of pasta in 1 L of water at OCT. Three-digit codes were used to label samples of pasta. Each panelist received approximately 30 g of cooked pasta at 40°C, one serving at a time and in a random order. Water was provided between samples for mouth cleansing. The overall acceptability was evaluated using a 9-point hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like).

■ Statistical analysis

Each type of pasta was prepared in triplicate. The data were subjected to one-way analysis of variance (ANOVA) following a Tukey post-hoc test with $p \leq 0.05$, and correlations between the overall acceptance and the pasta hardness and elasticity were evaluated based on the Pearson correlation coefficient (Statgraphics ver. 18.1.12, Statgraphics Technologies, Inc., The Plains, VA, USA).

RESULTS AND DISCUSSION

■ Characterization of mulberry pomace powder

The results of nutritional quality and physical characteristics of mulberry pomace powder and durum wheat semolina used in the present study are presented in Table 1. The mulberry pomace powder contained slightly less protein than the durum wheat semolina but had a greater content of lipid (9.8-fold) and ash (2.8-fold) than the semolina. In addition, starch was

also present at low level in the mulberry pomace while it was the main compound in the durum wheat semolina. It can be noted that the total dietary fiber (TDF) content of mulberry pomace powder was 13.9-fold higher than that of the semolina. Nevertheless, the mulberry pomace powder used in this study contained less TDF than the pomace powder of blueberry (62.3 g/100 g dw), cranberry (62.2 g/100 g dw) [Wang *et al.*, 2019] and strawberry (67.6 g/100 g dw) [Juśkiewicz *et al.*, 2015]. The IDF:SDF ratio of mulberry pomace powder (6.8:1.0, w/w) was also greater than that of durum wheat semolina (1.4:1.0, w/w) (Table 1). Both soluble and insoluble dietary fibers provide positive effects to human health; SDF is mainly associated with lowering blood cholesterol and reducing the absorption of glucose in the small intestine while IDF is in charge of improving fecal bulk, and water absorption that improves laxative effects [Esteban *et al.*, 2017; Yangilar, 2013].

The mulberry pomace contained a considerable amount of anthocyanins (Table 1). This group of mulberry pomace phenolics is known for its high antioxidant activity [Du *et al.*, 2021; Zhang *et al.*, 2011]. The total phenolic content of mulberry pomace powder was 7.7-fold greater than that of the semolina (Table 1). Different non-anthocyanin monomeric phenolics, including resveratrol, catechin, rutin, quercitrin and quercetin, were identified in mulberry pomace and these compounds were also reported to exhibit high antioxidant activity [Du *et al.*, 2022]. As a result, the ferric reducing antioxidant power and DPPH radical scavenging activity of mulberry pomace powder were 38.9-fold and 15.1-fold, respectively, greater than those of durum wheat semolina (Table 1). Mulberry pomace can be considered as an antioxidant source for incorporation into different food products.

In terms of water holding capacity, the mulberry pomace exhibited a threefold increase relative to the semolina (Table 1). Fiber is reported to have good water holding capacity due to a high number of hydroxyl groups in the chemical structure of its components [Tejada-Ortigoza *et al.*, 2017]. Supplementation of mulberry pomace to pasta recipe could change cooking quality of the product since fiber can compete for water with starch granules during pasta cooking, affecting starch gelatinization [Qiu *et al.*, 2016].

The mulberry pomace had different color compared to the wheat semolina. The lightness (L^*) of mulberry pomace was 2.3-fold lower than that of the semolina counterpart (Table 1), meaning it was much darker. The redness (a^*) of mulberry pomace was also 5.9-fold greater while its yellowness (b^*) was 7.7-fold lower than those of the semolina. The color of pasta supplemented with mulberry pomace could be changed as compared to that of the conventional pasta.

■ Effects of mulberry pomace ratio in the pasta formulation on nutritional quality and antioxidant capacity of the product

The nutritional quality and antioxidant capacity of pasta samples incorporated with different mulberry pomace powder ratios are shown in Table 2. The use of mulberry pomace was found to have no impact on the protein content of pasta probably due

Table 1. Nutritional quality, antioxidant capacity and physical characteristics of mulberry pomace and durum wheat semolina.

Component/characteristic		Mulberry pomace	Durum wheat semolina
Proximate composition	Crude protein (g/100 g dw)	12.88±0.41 ^b	13.89±0.56 ^a
	Lipid (g/100 g dw)	10.82±0.42 ^a	1.05±0.18 ^b
	Ash (g/100 g dw)	2.81±0.05 ^a	0.95±0.03 ^b
	Starch (g/100 g dw)	3.33±0.20 ^b	81.72±1.10 ^a
Fiber composition	TDF (g/100 g dw)	45.80±0.89 ^a	3.32±0.47 ^b
	IDF (g/100 g dw)	39.89±1.54 ^a	1.92±0.18 ^b
	SDF (g/100 g dw)	5.91±0.69 ^a	1.39±0.30 ^b
	IDF:SDF ratio (w/w)	6.84±1.09 ^a	1.40±0.18 ^b
Phenolic content	Total anthocyanin content (mg C3GE/100 g dw)	508.9 ± 1.9	ND
	Total phenolic content (mg GAE/100 g dw)	1,095±43 ^a	141.6±4.7 ^b
Antioxidant capacity	Ferric reducing antioxidant power (µmol TE/100 g dw)	8,558±429 ^a	220±23 ^b
	DPPH radical scavenging activity (µmol TE/100 g dw)	2,529±82 ^a	167±21 ^b
Physical characteristics	Water holding capacity (g water/g dw)	2.35±0.13 ^a	1.02±0.08 ^b
	<i>L</i> *	39.58±0.03 ^b	91.19±0.01 ^a
	<i>a</i> *	5.25±0.05 ^a	0.89±0.01 ^b
	<i>b</i> *	1.30±0.02 ^b	9.97±0.01 ^a

Data are expressed as mean ± standard deviation ($n=3$). Values that do not share a lowercase letter within a row are significantly different ($p \leq 0.05$). TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; C3GE, cyanidin 3-O-glucoside equivalent; GAE, gallic acid equivalent; TE, Trolox equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; dw, dry weight; *L**, lightness; *a**, redness; *b**, yellowness; ΔE , total color difference; ND, not determined.

to the little difference in protein content between mulberry pomace powder and wheat semolina. However, the mulberry pomace addition to the pasta recipe increased its lipid and ash contents but reduced the starch content. Mulberry pomace was reported to be rich in essential fatty acids, including omega-3 and omega-6 fatty acids [Yilmaz & Durmaz, 2015]. In addition, various minerals including macro-minerals (K, Ca, Na, Mg) and micro-minerals (Fe, Zn, Ni) were identified in mulberry [Imran *et al.*, 2010]. As a result, the partial replacement of semolina with mulberry pomace in pasta formulation significantly improved nutritional value of the product.

When the amount of mulberry pomace in the recipe increased from 0% to 20%, the TDF, IDF, and SDF contents of pasta increased by 232%, 406%, and 46%, respectively. As the IDF:SDF ratio of mulberry pomace was 4.9-fold greater than that of durum wheat semolina, the enhanced amount of mulberry pomace in the recipe led to an augmented IDF:SDF ratio of the pasta ranging from 1.23 to 4.16. The total fiber content of pasta sample with 10% mulberry pomace powder was greater than 6 g/100 g pasta, and this product could be considered as food "high" in fiber [Bröring & Khedkar, 2018]. It was reported that the IDF:SDF ratio of pasta fortified with 10% orange pomace was 1.9 while that of pasta supplemented with 10% cucumber pomace was 5.2 [Kaur *et al.*, 2021]. Thus, the IDF:SDF ratio of pasta added with 10% mulberry pomace met the recommended value of 3:1 (w/w) suggested by the Dietetic Association to enhance the nutraceutical functionality of dietary fiber [Borderías *et al.*, 2005]. Foods with

proper ratio of IDF:SDF improve physiological effects of both dietary fiber fractions on human health [He *et al.*, 2022].

The higher the mulberry pomace level in pasta formulation, the greater the total phenolic and total anthocyanin contents, as well as the antioxidant capacity of pasta. Results collated in [Table 2](#) reveal that the pasta sample with 20% of mulberry pomace powder (M20) showed the greatest TPC being 2.6-fold higher than in the control sample (M0); in addition, its antioxidant capacity assessed by FRAP and DPPH assays was 11.8-fold and 36.1-fold, respectively, higher than that of the M0 sample. When orange pomace was added to pasta recipe at the level of 20% of the flour blend, a 4.8-fold increase in TPC and a 3-fold increase in DPPH radical scavenging activity were recently recorded [Kaur *et al.*, 2021]. Increment in TPC and antioxidant capacity in the final product may vary depending on the fruit by-product composition and pasta preparation conditions. It can be confirmed that the incorporation of mulberry pomace into pasta recipe highly improved antioxidant capacity of the product.

■ Effects of mulberry pomace ratio in the pasta formulation on textural and cooking quality of the product

■ Textural properties

The textural properties of pasta samples incorporated with different mulberry pomace powder ratios are shown in [Table 3](#). Increase in mulberry pomace ratio in the pasta recipe resulted in an increased hardness of the product. At 20% mulberry pomace level (M20), the hardness of pasta was about 22% higher than

Table 2. Nutritional quality and antioxidant capacity of pasta incorporated with mulberry pomace at different levels.

Component/characteristic		M0	M5	M10	M15	M20
Proximate composition	Crude protein (g/100 g dw)	13.92±0.50 ^a	13.75±0.77 ^a	13.54±0.93 ^a	13.43±0.75 ^a	13.08±0.47 ^a
	Lipid (g/100 g dw)	1.65±0.14 ^b	1.53±0.04 ^{ab}	1.99±0.15 ^{ab}	2.07±0.08 ^{ab}	2.57±0.19 ^a
	Ash (g/100 g dw)	1.00±0.03 ^e	1.09±0.03 ^d	1.22±0.08 ^c	1.52±0.03 ^b	1.65±0.02 ^a
	Starch (g/100 g dw)	81.72±1.10 ^a	77.80±1.05 ^b	73.88±1.01 ^c	69.96±0.96 ^d	66.04±0.92 ^e
Fiber composition	TDF (g/100 g dw)	3.35±0.08 ^e	5.35±0.65 ^d	7.27±0.77 ^c	9.32±0.99 ^b	11.33±0.97 ^a
	IDF (g/100 g dw)	1.85±0.05 ^d	3.73±0.70 ^{cd}	5.56±0.89 ^{bc}	7.32±1.03 ^b	9.14±0.90 ^a
	SDF (g/100 g dw)	1.51±0.03 ^c	1.62±0.14 ^b	1.71±0.14 ^b	2.00±0.10 ^a	2.19±0.08 ^a
	IDF:SDF ratio (w/w)	1.23±0.02 ^c	2.33±0.57 ^{bc}	3.29±0.76 ^{ab}	3.68±0.64 ^a	4.16±0.28 ^a
Phenolic content	Total anthocyanin content (mg C3GE/100 g dw)	0.0±0.0 ^e	22.1±3.0 ^d	33.5±1.9 ^c	62.4±1.5 ^b	83.4±2.8 ^a
	Total phenolic content (mg GAE/100 g dw)	134±9 ^e	190±8 ^d	224±10 ^c	297±6 ^b	348±11 ^a
Antioxidant capacity	Ferric reducing antioxidant power (μmol TE/100 g dw)	60±4 ^e	448±14 ^d	869±31 ^c	1,521±92 ^b	2,168±133 ^a
	DPPH radical scavenging activity (μmol TE/100 g dw)	129±15 ^e	491±92 ^d	855±94 ^c	1,259±201 ^b	1,526±249 ^a

Data are expressed as mean ± standard deviation (n=3). Values that do not share a lowercase letter within a row are significantly different (p≤0.05). M0, M5, M10, M15, M20, pasta incorporated with 0% (control), 5%, 10%, 15%, and 20% mulberry pomace powder, respectively; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; C3GE, cyanidin 3-O-glucoside equivalent; GAE, gallic acid equivalent; TE, Trolox equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; dw, dry weight.

that of the control sample (M0). Similar observation was reported when tomato peel or gac fruit powders were supplemented to the pasta recipe [Chusak *et al.*, 2020; Padalino *et al.*, 2017]. Fibers were reported to have high water absorption capacity [Dhingra *et al.*, 2012]; the amount of water for starch gelatinization during the cooking of enriched fiber pasta was therefore reduced [Nguyen *et al.*, 2020], resulting in an enhanced hardness of the product [Gallo *et al.*, 2020]. Various interaction types between phenolics and starch are reported. Tannins might interact with amylose through hydrogen bonding and hydrophobic interaction, resulting in microstructure alteration of starch granules [Amoako & Awika, 2016]; in addition, polyphenols might adsorb onto surface of the starch granules and interact with amylose to form an unordered inclusion complex [Liu *et al.*, 2011], which is suggested to interact with free water. This phenomenon might lead to less hydrated starch granules during the pasta cooking and decreased starch gelatinization. Otherwise, anthocyanins might limit the gluten development by forming disulfide linkages with glutenin and gliadin, resulting in increased starch crystallinity as well as deformed and disrupted microstructure of the starch-gluten network [Ou *et al.*, 2022]. Such disruption of the starch-protein matrix and decrement in starch gelatinization may affect the textural properties of the incorporated pasta. The chewiness of pasta samples supplemented with mulberry pomace was always lower than that of the control pasta sample (Table 3). Similar observation was also recorded by Kultys & Moczowska-Wyrwisz [2022] when carrot and beetroot-apple pomace were incorporated into pasta.

When the mulberry pomace ratio increased from 0 to 20%, the tensile strength and elongation rate of pasta decreased

by 45 and 86%, respectively (Table 3), probably mainly due to the decrement in gluten content. Replacement of gluten by components with high water absorption capacity, like fibers from fruit by-products, was reported to reduce firmness of pasta due to a weak gluten network [Gull *et al.*, 2015]. A sub-incorporation of gluten is reported to compensate for these properties of fiber-enriched pasta [Nguyen *et al.*, 2020].

■ Cooking properties

As seen from Table 3, when the replacement level of semolina by mulberry pomace was increased from 0 to 20%, the optimal cooking time (OCT) of pasta reduced by about 36%, while the cooking loss (CL) increased 2.1-fold. This could be explained by the higher total dietary fiber content of sample M20 (11.33 g/100 g dw), in comparison to that of sample M0 (3.35 g/100 g dw) which could interfere with the starch-gluten network and result in “capillary texture” of fiber-enriched pasta [Gallo *et al.*, 2020]. During the pasta cooking, water diffusion into the central core of the pasta strands could be more rapid, facilitating the gelatinization of starch granules [Tolve *et al.*, 2020]. As a result, the cooking time of pasta supplemented with mulberry pomace was shorter. Moreover, the weakening of the gluten matrix of fiber-enriched pasta could cause higher leaching of starch and other components from the pasta strands into the cooking water, significantly increasing the cooking loss of pasta. Increase in CL and reduction in OCT were previously reported for tomato peel-fortified spaghetti [Padalino *et al.*, 2017].

The presence of 20% mulberry pomace in pasta formula also decreased the swelling index (SI), and water absorption index (WAI) compared to those of the control pasta (Table 3), probably due

Table 3. Texture and cooking quality of pasta incorporated with mulberry pomace at different levels.

Characteristic	M0	M5	M10	M15	M20	
Textural properties	Hardness (g)	2,438±13 ^e	2,600±44 ^d	2,715±44 ^e	2,825±21 ^b	2,977±57 ^a
	Chewiness (g)	1,613±26 ^a	1,537±39 ^b	1,520±10 ^b	1,557±26 ^b	1,542±18 ^b
	Tensile strength (kPa)	31.31±0.82 ^a	21.91±0.93 ^b	19.59±1.05 ^c	18.18±0.64 ^{cd}	17.06±0.91 ^d
	Elongation rate (%)	365±35 ^a	309±48 ^b	159±12 ^c	130±22 ^c	50±9 ^d
Cooking quality	Optimal cooking time (min)	14.00±0.50 ^a	13.00±0.50 ^b	12.50±0.17 ^b	10.50±0.20 ^c	8.88±0.33 ^d
	Cooking loss (%)	3.76±0.19 ^e	5.12±0.17 ^d	5.94±0.24 ^c	6.58±0.37 ^b	8.05±0.45 ^a
	Swelling index	1.97±0.11 ^a	1.79±0.01 ^b	1.69±0.07 ^{bc}	1.65±0.06 ^c	1.59±0.07 ^c
	Water absorption index	1.52±0.03 ^a	1.39±0.02 ^b	1.28±0.06 ^c	1.26±0.01 ^c	1.13±0.04 ^d

Data are expressed as mean ± standard deviation ($n=3$). Values that do not share a lowercase letter within a row are significantly different ($p \leq 0.05$). M0, M5, M10, M15, M20, pasta incorporated with 0% (control), 5%, 10%, 15%, and 20% mulberry pomace powder, respectively.

to the reduction in starch content of the fiber-enriched pasta [Rakshesh *et al.*, 2015]. Similar reduction in SI and WAI was recorded for pasta enriched with tomato peel when the addition level increased from 0 to 15% [Padalino *et al.*, 2017]. However, the opposite results were observed by Simonato *et al.* [2019] when the olive pomace was added to durum wheat semolina pasta. The quantity of water absorbed by pasta cooked at OCT is reported to be associated with starch swelling and gelatinization as well as with physical properties of material flours, such as water-binding capacity and particle size distribution [Bustos *et al.*, 2015; Steglich, 2013]. Further study on fiber-protein-starch-phenolic interaction of high-fiber pasta dough is therefore essential to justify the impacts of mulberry pomace on textural and cooking properties of the product.

■ Effects of mulberry pomace ratio in the pasta formulation on instrumental color and overall acceptability of the product

Table 4 reveals that the lightness (L^*) and yellowness (b^*) of pasta samples notably decreased while the redness (a^*) increased with the increasing amounts of mulberry pomace incorporated into the product recipe. It can be explained that color of mulberry pomace had a higher redness value (5.9 folds) but lower yellowness (7.7 folds) and lightness values (2.3 folds) than the durum

wheat semolina. The color of pasta supplemented with mulberry pomace could be changed as compared to that of the conventional pasta. Addition of fruit and vegetable pomace was reported to enhance the pasta darkness [Kultys & Moczowska-Wyrwisz, 2022]. High redness value of berry fruits is due to high anthocyanin content. A similar increment in redness was also observed in berry-enriched pasta [Bustos *et al.*, 2019].

The overall acceptability of the pasta sample fortified with 5% mulberry pomace and the control sample was statistically ($p > 0.05$) similar (Table 4). As the incorporation level of mulberry pomace increased from 5 to 20%, the overall acceptance of pasta decreased by 22% due to changes in its textural properties. Strong correlations between the overall acceptance and the pasta hardness and elasticity were recorded. The Pearson correlation coefficient between the overall acceptance and the hardness was -0.96 ($p = 0.009$) while the coefficient between the overall acceptance and the tensile strength was 0.95 ($p = 0.015$). However, all the pasta samples with mulberry pomace had acceptable scores, ranging from 5.13 to 6.58.

■ Effects of transglutaminase treatment on quality of the mulberry pomace-fortified pasta

Following the recommendation of Dietetic Associations on dietary fiber requirement [Borderías *et al.*, 2005], the fortified pasta

Table 4. Color parameters and overall acceptability of pasta incorporated with mulberry pomace at different levels.

Characteristic	M0	M5	M10	M15	M20
L^*	89.32±0.04 ^a	66.71±0.12 ^b	60.11±0.23 ^c	57.37±0.11 ^d	55.40±0.15 ^e
a^*	1.07±0.01 ^e	4.55±0.07 ^d	6.52±0.03 ^c	8.07±0.06 ^b	8.58±0.06 ^a
b^*	8.74±0.02 ^a	3.63±0.04 ^b	2.68±0.04 ^c	2.18±0.02 ^d	1.52±0.03 ^e
ΔE	0.00±0.00 ^e	23.44±0.08 ^d	30.33±0.22 ^c	33.36±0.13 ^b	35.48±0.13 ^a
Overall acceptability	6.67±1.46 ^a	6.58±1.22 ^a	6.00±1.13 ^b	5.19±1.32 ^c	5.13±1.37 ^c

Data are expressed as mean ± standard deviation ($n=3$). Values that do not share a lowercase letter within a row are significantly different ($p \leq 0.05$). M0, M5, M10, M15, M20, pasta incorporated with 0% (control), 5%, 10%, 15%, and 20% mulberry pomace powder, respectively. L^* , lightness; a^* , redness; b^* , yellowness; ΔE , total color difference.

with 10% mulberry pomace was chosen in the TG treatment for improvement in its textural and cooking quality.

The effects of TG dosage on textural properties, cooking quality, color values as well as overall acceptability of the high-fiber pasta are shown in Table 5. The pasta hardness enhanced with the increased TG dosage from 0.00 to 0.50 U/g protein; however, the chewiness of the fortified pasta increased only 4.2% when increasing the TG dosage from 0.50 to 1.00 U/g protein. Similar observation was recently reported when TG was used to improve the quality of corn cob powder-enriched pasta [Nguyen *et al.*, 2023a]. In addition, increase in TG dosage from 0 to 0.50 U/g protein improved the tensile strength and elongation rate of mulberry pomace-supplemented pasta by 26 and 10%, respectively (Table 5). It is reported that the TG treatment of pasta dough induces the formation of crosslinks between gliadin and glutenin in wheat proteins, strengthening the protein network and enhancing the dough elasticity [Gharibzadeh *et al.*, 2019]. Increase in tensile strength and elongation rate of pasta fortified with 20% wheat bran was recently reported when the pasta dough was treated with TG dosage of 0.75 U/g [Nguyen *et al.*, 2020]. Otherwise, as the TG dosage continued to increase from 0.50 to 1.00 U/g protein, both tensile strength and elongation rate began to decrease (Table 5). High level of TG treatment may lead to high degree of cross-linking and reduce the flexibility of the protein network [Aalami & Leelavathi, 2008]. The tensile strength and elongation rate of corn cob-incorporated pasta were also decreased by 5–8% and 16–28%, respectively when the excessive TG concentration (1.00–1.25 U/g) was used [Nguyen *et al.*, 2023a].

Increase in TG dosage from 0 to 0.75 U/g protein slightly enhanced the OCT of high-fiber pasta while reduced its cooking

loss (Table 5) probably due to the strengthened gluten network. Similar results were recently reported when TG treatment was applied to pasta dough supplemented with wheat bran [Nguyen *et al.*, 2020] and corn cob powder [Nguyen *et al.*, 2023a]. However, further increase in TG level from 0.75 to 1.00 U/g protein decreased the OCT while enhanced the CL of mulberry pomace-added pasta (Table 5). According to Aalami & Leelavathi [2008], high degree of gluten crosslinking at high TG dosage might decrease protein-starch interaction, resulting in an increased leaching of starch component into the cooking water. This effect was observed in both non-fortified pasta [Sissons *et al.*, 2010] and pasta enriched with fiber ingredients, such as wheat bran and corn cob powder [Nguyen *et al.*, 2020, 2023a]. In addition, the TG treatment of pasta dough had no effect on SI ($p>0.05$) while the WAI of high-fiber pasta treated with TG at 0.50 U/g protein was slightly greater than that of the counterpart without TG treatment (Table 5).

Table 5 also reveals that the pasta samples treated with TG showed an increased lightness. This could be due to limitation of Maillard reaction during pasta drying as TG crosslink effect could reduce available lysine content [Aalami & Leelavathi, 2008]. Moreover, change in yellowness and redness of the pasta samples was very little; both a^* and b^* values varied in narrow ranges (Table 5).

All pasta samples were considered acceptable since their overall acceptability score was higher than 5 points (Table 5). It should be noted that the high-fiber pasta samples treated with TG at 0.50 and 0.75 U/g protein had similar overall acceptance which was significantly ($p\leq 0.05$) higher than that of the other pasta samples. These results were consistent with the tensile strength and elongation rate of the product. The appropriate

Table 5. Texture profile, cooking quality, color parameters and overall acceptability of pasta supplemented with 10% mulberry pomace and treated with transglutaminase (TG) at different dosages.

Characteristic	TG0	TG25	TG50	TG75	TG100	
Textural properties	Hardness (g)	2,715±44 ^d	2,865±53 ^c	3,044±73 ^b	3,208±114 ^a	3,157±106 ^{ab}
	Chewiness (g)	1,520±10 ^{bc}	1,461±15 ^c	1,506±22 ^{bc}	1,536±52 ^{ab}	1,584±51 ^a
	Tensile strength (kPa)	19.6±1.1 ^c	22.2±1.5 ^b	24.6±0.9 ^a	22.2±0.5 ^b	20.9±0.9 ^{bc}
	Elongation rate (%)	159.3±12.0 ^b	163.8±9.6 ^{ab}	175.1±6.0 ^a	158.4±5.8 ^b	121.7±7.0 ^c
Cooking quality	Optimal cooking time (min)	12.50±0.07 ^c	12.52±0.08 ^{bc}	12.69±0.08 ^{ab}	12.85±0.08 ^a	12.03±0.16 ^d
	Cooking loss (%)	5.94±0.24 ^a	5.46±0.25 ^{bc}	5.49±0.21 ^{bc}	5.37±0.17 ^c	5.82±0.12 ^{ab}
	Swelling index	1.69±0.07 ^a	1.68±0.03 ^a	1.67±0.03 ^a	1.66±0.04 ^a	1.60±0.03 ^a
	Water absorption index	1.28±0.06 ^{bc}	1.32±0.03 ^{ab}	1.33±0.01 ^a	1.30±0.02 ^{abc}	1.25±0.03 ^c
Color and overall acceptability	L^*	60.11±0.23 ^d	62.77±0.18 ^c	63.67±0.38 ^b	64.11±0.71 ^{ab}	64.44±0.50 ^a
	a^*	6.52±0.03 ^b	6.52±0.04 ^b	6.57±0.08 ^b	6.73±0.14 ^a	6.81±0.06 ^a
	b^*	2.68±0.04 ^c	2.77±0.03 ^c	2.77±0.10 ^c	3.12±0.04 ^b	3.27±0.10 ^a
	ΔE	0.00±0.00 ^c	2.66±0.30 ^b	3.56±0.46 ^{ab}	4.02±0.76 ^a	4.37±0.58 ^a
	Overall acceptability	6.04±1.52 ^{cd}	6.25±1.3 ^{bcd}	6.88±1.15 ^a	6.48±1.08 ^{abc}	5.92±1.12 ^d

Data are expressed as mean ± standard deviation ($n=3$). Values that do not share a lowercase letter within a row are significantly different ($p\leq 0.05$). TG0, TG25, TG50, TG75 and TG100, pasta treated with TG at dosages of 0, 0.25, 0.50, 0.75 and 1.00 U/g protein of the pasta dough, respectively. L^* , lightness; a^* , redness; b^* , yellowness; ΔE , total color difference.

dosage of TG for pasta dough enriched with mulberry pomace powder was therefore 0.50 U/g protein.

CONCLUSIONS

The incorporation of mulberry pomace powder into pasta recipe improved dietary fiber content and antioxidant capacity of the product. The pasta supplemented with 10% mulberry pomace was a high-fiber food with a reasonable ratio of IDF:SDF (3.3:1.0, w/w). However, the pasta fortified with mulberry pomace had a decreased optimal cooking time, swelling index, water absorption index and increased cooking loss. The textural properties and overall acceptability of high-fiber pasta were also decreased when the supplementation level of mulberry pomace was 10% or higher. TG treatment slightly reduced the cooking loss of high-fiber pasta while enhanced its tensile strength, elongation rate and sensory score at the enzyme dosage of 0.50 U/g protein. Further studies on fiber-protein-starch-phenolic interaction of pasta dough supplemented with mulberry pomace powder are essential to clarify the effects of this potential material on textural and cooking attributes of high-fiber pasta.

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CONFLICT OF INTERESTS

The authors have declared no conflicts of interest for this article.

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