



Effect of mulberry (*Morus alba L.*) leaf enrichment on antioxidant, textural, and sensory properties of cassava pasta

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Mulberry leaf is usually employed for sericulture in various parts of the world; however, its potential utilization for human consumption is not widely recognized. This study aimed to determine the suitability of using mulberry leaf powder (MLP) as a novel ingredient for improving the nutritional and bioactive profile of cassava pasta while maintaining favourable textural and sensory attributes. Three pasta formulations containing 2, 4, and 6 % MLP were developed. Positive relationships between the level of added MLP and its phenolic compound as well as its antioxidant capacity were observed. Results demonstrated that MLP enrichment significantly increased the total phenolic content and antioxidant activity in the enriched pasta. Textural analysis showed that pasta with an increase of MLP showed a lower hardness, springiness, cohesiveness, gumminess, and chewiness. The MLP enrichment determined the lower L, a*, and b* values indicating that the enriched pasta became dark greener. The incorporation of MLP up to 4 % had a sensory score similar to control for appearance, colour, odour, and texture. Above this level, a significant reduction in the sensory attributes was observed caused by the increased bitterness and softer texture. Fortification also increased the nutritional value of fibre and ash contents. The present study showed that mulberry leaf is a promising food ingredient for producing functional pasta with potential health benefits to meet the consumer's acceptance.

1. Introduction

Natural antioxidants extracted from plants and herbs are in high demand by consumers. Mulberry (*Morus alba L.*) has been studied for their functional antioxidant activity and other health benefits due to their high amount of phenolic compounds such as anthocyanins, flavonoids, and chlorogenic acid (Chen *et al.* 2017). Mulberry has been widely cultivated in Asian countries such as China, Japan, and Thailand, where its leaves have been utilized as natural feed for silkworms in sericulture. Traditionally, the mulberry leaf has been used narrowly as human food, such as mulberry leaf tea and spices. The utilization rate of

mulberry leaves is still low, causing waste of raw materials. This biomass is usually used as animal feed or disposed of in landfills, which causes health and environmental hazards (Ma *et al.* 2022). Mulberry leaves have bioactive components, including phenolic acids and flavonoids, which make them a potential ingredient for the production of nutritious foods; however, it is underused due to inadequate application in the food industry (Ren *et al.* 2015). The use of mulberry leaves for healthy food production prevents the waste of mulberry leaves.

A few studies indicated that mulberry leaf powder can improve the nutritional, functional, physical, and sensory characteristics of functional foods. Recent studies reported that MLP could improve the nutritional, textural, and organoleptic qualities of rice cakes (Son and Park 2007; Park *et al.* 2021). The addition of MLP reduced staling by preventing starch retrogradation and decreasing relative crystallinity and moisture loss. In addition, the water-holding ability of fibres in MLP decreased the moisture loss and firmness of steamed rice cakes, leading to a softer texture. Previous research has shown that phenolic extracts from mulberry leaf extract could improve quality and extend the shelf life of fresh-cut cantaloupe during storage (Yu and Shi, 2021). MLP treatments could delay softening, maintain colour, and reduce weight loss of fresh-cut cantaloupe during storage. The antioxidant ability of phenolic compounds in MLP effectively prevented oxidizing and decreased bacterial growth, thus enhancing the shelf life of fresh-cut cantaloupe. Another study by Kim *et al.* (2000) was also conducted on the hypolipidemic effect of regular consumption of rice cakes prepared with MLP. However, there are still limited studies on the utilization of mulberry leaves as natural antioxidants in various food products. Applying mulberry leaves as value-added ingredients in functional foods is a promising option to avoid risks associated with chemical additives and enhance the utilization of agricultural residues.

A certain part of the world population suffers from food allergies directly associated with gluten-containing products. The study to develop foods that are possible alternatives with the potential to fully or partially substitute wheat gluten has aroused interest considerably. Pasta is a traditional food product normally made from wheat flour and is widely consumed due to its convenience, tastiness, and simple cooking (Simonato *et al.*, 2015). Replacement of alternative flours in pasta production could provide interesting quality attributes, contributing to modifications in the nutritional quality of products (Fiorda *et al.* 2013). One suitable alternative for the development of low-gluten pasta could be cassava. Cassava is the world's fifth most important food product, after corn, wheat, rice, and potatoes, and 304 million tons of cassava were produced globally in 2019 (Ramírez *et al.* 2019). Cassava is a gluten-free starch with high availability and relevant properties; however, it is underutilized in the food industry. Previous research has demonstrated

the feasibility of cassava starch as a main ingredient to obtain gluten-free pasta (Fiorda *et al.* 2013; Ramírez *et al.* 2019; Milde *et al.* 2020). Utilizing cassava, a local crop, in pasta formulation will not only reduce the reliance on wheat flour in non-wheat-growing countries but also encourage the suitability of domestic crops as industrial raw materials.

Considering this background, the feasibility of using MLP as a natural antioxidant for cassava-wheat pasta was evaluated. The purpose of the research was to investigate the fortification of MLP as a functional ingredient in the quality characteristics of pasta. The total phenolic compound and antioxidant activities of MLP-enriched pasta during the formulation were examined. Moreover, colour, texture properties (hardness, springiness, cohesiveness, gumminess, and chewiness), and sensory characteristics of the MLP-fortified pasta were determined. The best-known formulation of pasta enriched with MLP was then selected to evaluate its nutritional value.

2. Materials and methods

2.1 Mulberry leaves preparation

Mulberry leaves cv. Sakonnakorn was obtained from the Department of Home Economics, Faculty of Science and Technology, Suan Sunandha Rajabhat University (Bangkok, Thailand). Mulberry leaf powder (MLP) was prepared by the method described by Khanprom (2005). Fresh mulberry leaves (1,000 g) were cleaned and cut into pieces approximately 0.5 x 4.0 centimetres, blanched in hot water for about 1 min, and soaked in cold water for 30 sec. Leaves were dried in a hot air oven at 50 °C for 6 hours until their remaining weight was approximately 600 g (60 % yield). Then, dried leaves were ground into a fine powder with a blender, sieved through 80 mesh, and packed in a vacuum aluminium foil bag.

2.2 Pasta formulation and preparation

The pasta mixture was formulated with cassava flour (Bangkok Natural, Bangkok, Thailand) 50 %, wheat flour (Divella, Rutigliano, Italy) 16 %, eggs 14 %, egg yolk 5 %, olive oil 1 % (w/w), and MLP prepared as described above (2, 4, and 6% (w/w) substituted for wheat flour) before placing in the mixing chamber (Weenuttranon, 2021). Dry ingredients were mixed

together at minimum speed for 1 min (Kitchen-Aid® Professional Mixer, Model KPM50, USA). Egg and egg yolk were mixed at minimum speed for 3 min, and olive oil was then added and mixed at speed 2 for 2 min. Cold water 14 % (w/w) was slowly added and the dough was kneaded until homogenous for 10 min. Pasta-making machine (Perfetta-28400, Sauerland, Germany) was used to make the wide noodle into a length of 250 mm, a width of 5 mm, and a thickness of 1.3 mm.

2.3 Pasta colour measurement

The colour analysis was carried out by the colorimeter equipment (Hunter Lab, Colour Quest XE, Reston, Virginia, USA). The colorimetric method was used with the following conditions: reflectance mode; illuminant D65; and 10 observers. The L* value indicates brightness (brightness to darkness), defines black at 0 and white at 100; The a* value indicates greenness (green to red), and -a* direction for green increase; The b* value indicates yellowness (blue to yellow), and the higher the +b* value, the more yellowness. Measurements were performed in triplicate at three random locations on the surface of each sample before and after cooking (Charles *et al.* 2007).

2.4 Pasta texture measurement

The texture profile of pasta was determined using a texture analyser (TA.XT, plusC, Stable Micro Systems, Surrey, UK). Measurements were performed using a cylinder probe (diameter of 25 mm) with the following conditions: sample weight 15 g; test speed 0.3 mm/s; post-test speed 5 mm/s; and pressing distance of 25 mm of the original height. The hardness (force required to cause a deformation), gumminess (energy required to break a semi-solid food to a state ready for swallowing), chewiness (time required to chew a food sample to a state suitable for swallowing), springiness (the rate at which a deformed material goes back to its intact state after deformation), and cohesiveness (extent to which a sample can be deformed before rupturing) were calculated by the instrument software (Charles *et al.* 2007).

2.5 Antioxidant activity

The MLP-fortified pasta was cut into a size of 0.5 x 0.5 cm and weighed approximately 2 g. The sample was

suspended in 3 mL of 70 % ethanol and underwent sonication under the following conditions: temperature 40 °C; 10 min; working frequency 40 kHz; and ultrasonic power 200 W (LT-200-PRO, TielTaTech®, Guarnizo, Spain). The whole mixture was centrifuged at 5,000 rpm for 15 min to separate the aqueous extract. The sample residues were then extracted twice with 3 mL of 70 % ethanol using the same extraction procedure. Afterward, the total volume of the sample extract was adjusted to 10 mL to obtain the initial concentration of the extract (200 mg/mL).

2.6 Determination of Total Phenolic Content

The total phenolic content (TPC) of pasta was determined using a modified Folin-Ciocalteu colorimetric method according to Wolfe *et al.* (2003). The diluted extract (125 µl) and deionized water (500 µl) were added to a test tube. A 125 µl of Folin-Ciocalteu reagent was added to the solution and allowed to react for 6 min. Afterward, 7 % sodium carbonate solution (1.25 mL) and deionized water (1 mL) were added to the mixture and left for colour development for 90 min. The absorbance was read at 760 nm using a UV-vis Evolution 201 spectrophotometer (Thermo Fisher Scientific Inc., Madison, USA). The results were reported as mg gallic acid equivalents (GAE)/g of pasta. The TPC was calculated independently in triplicates.

2.7 Determination of DPPH Radical Scavenging Activity

Determination of DPPH radical scavenging activity was measured using the modified method described by Maduwanthi and Marapana (2021). DPPH solution was prepared by weighing 2 mg of DPPH and dissolved in 100 ml of ethyl alcohol. 150 µl of the extract was mixed with 2850 µl of DPPH solution and incubated in the dark for 30 min. Afterward, the absorbance was measured at 517 nm using a UV-vis Evolution 201 spectrophotometer. Trolox standard was used to prepare an external standard curve. The activity was expressed as a mg Trolox/g sample. The radical scavenging activity was calculated using the following equation.

$$\% \text{ radical scavenging activity} = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100 \quad (1)$$

2.8 Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was performed according to the method described by Kubola and Siriamornpun (2008). The FRAP reagent was newly prepared by mixing 100 ml of acetate buffer (300 mM, pH 3.6), 10 ml TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution dissolved in 40 mM/HCl, 10 ml FeCl₃·6H₂O (20 nM) and 12 ml deionized water. Then, 1.8 ml of FRAP reagent, 60 µl of a sample, and 180 µl of deionized water were added to the test tubes and incubated at 37 °C for 4 min. The absorbance was measured at 595 nm using a UV-vis Evolution 201 spectrophotometer. The FRAP assay was evaluated from a standard curve plotted using the FeSO₄·7H₂O linear regression equation to calculate the FRAP values and reported as mmol of FeSO₄/kg.

2.9 Sensory evaluation

Sensory analysis was carried out using a 9-point hedonic scale with 50 panellists aged from 18 to 60 who were not allergic to all ingredients used in this experiment. The panellists were requested to first evaluate the acceptability of product appearance and colour, followed by taste, odour, texture, and overall preference of each sample based on a scale ranging from 1 (dislike extremely) to 9 (like extremely) (Sriprapai *et al.* 2018). This sensory study was reviewed and approved by the Ethics Committee of Suan Sunandha Rajabhat University (certificate number: COE. 1-043/2021). Afterward, fortified pasta with an optimized formulation was chosen to evaluate proximate composition (AOAC, 2019) including moisture (Oven-drying method), ash (Gravimetric Method), fat (Soxhlet method), protein (Kjeldahl method), and crude fibre (Enzymatic-gravimetric method).

2.10 Statistical analysis

All Data were statistically tested by analysis of variance using one-way ANOVA with a Completely Randomized Design (CRD). The differences between the mean values were carried out by Duncan's multiple range test (DMRT) at a 95 % confidence interval ($P < 0.05$). Statistical processing was performed by SPSS 25.0 software (IBM, Somers NY, USA) for the Windows statistical package. All data described in figures and tables were the mean values of triplicate determi-

nations.

3. Results and discussion

3.1 Colour quality of cassava pasta supplemented with MLP

Colour is a crucial characteristic of food as it influences consumers' choices and preferences. The colour attributes of the MLP-fortified pasta and control are demonstrated in Table 1. Results showed that the addition of MLP significantly affected the colour of the fortified pasta. The value of lightness (L^*), greenness (a^*), and yellowness (b^*) of the enriched pasta decreased with an increase in MLP enrichment. The pasta with 6% MLP showed the lowest L^* , a^* , and b^* values indicating that the pasta became darker and greener while less yellow.

The green colour in the cassava pasta could be attributed to the addition of MLP, which has an intrinsic green colour due to the presence of chlorophyll. The dark green of the cassava pasta with MLP became more profound compared to the original colour of cassava pasta. More green colours of healthy food products could appeal to consumer's acceptance (Schuldt, 2013). According to the result reported by Park *et al.* (2021), rice cake became darker and more greenish when the number of mulberry leaves increased, which might indicate an increase in colour changes during thermal processing. The colour development during heat processing might be because pigments and polyphenol compounds in MLP underwent oxidation. Considering that chlorophylls are the major pigments in mulberry leaves, the lighter green of cooked pasta during thermal processing is mainly because of the loss of chlorophyll pigments (Von and Schwartz, 1996). The loss of chlorophyll pigments resulting in the pale green colour of the food during cooking is a consequence of heat-induced structural disruption, the conversion of chlorophyll to pheophytin, and the release of intracellular enzymes (Suman *et al.* 2008). Control and enriched pasta samples with different concentrations of MLP are presented in Figure 1.

The colour of foods is the first sensory perception of people, which can strongly influence consumer preference. The level of greenness is critical in evaluating

Table 1. Color attributes of pasta supplemented with MLP in the ratio of 2, 4 and 6 % and control. Numbers with different superscripts in the same column are statistically different ($p \leq 0.05$).

Fortified pasta	Color of pasta			Color of boiled pasta		
	L^*	a^*	b^*	L^*	a^*	b^*
Control	62.43 ± 0.37^a	9.10 ± 0.04^a	43.55 ± 0.28^a	72.58 ± 0.12^a	4.76 ± 0.08^a	34.22 ± 0.26^a
2% MLP	39.45 ± 0.31^b	-0.33 ± 0.01^b	19.61 ± 0.13^b	53.26 ± 0.27^b	-1.84 ± 0.02^b	18.57 ± 0.22^b
4% MLP	37.12 ± 0.22^c	-1.08 ± 0.07^c	19.81 ± 0.44^b	48.12 ± 0.23^c	-2.28 ± 0.07^c	17.83 ± 0.11^c
6% MLP	35.55 ± 0.12^d	-1.35 ± 0.18^d	16.72 ± 0.03^c	48.32 ± 0.58^c	-2.52 ± 0.11^d	17.77 ± 0.10^c

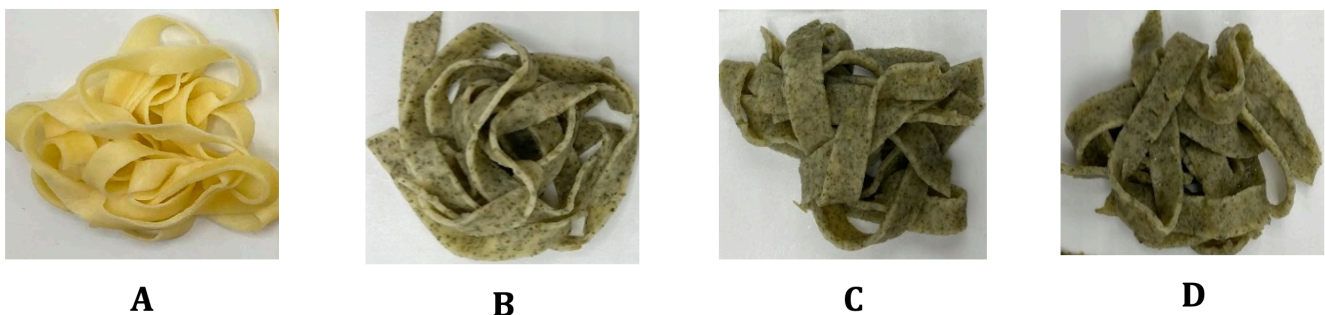


Figure 1. Pasta with different concentrations of MLP, control(A), pasta with 2% MLP(B), pasta with 4% MLP(C), and pasta with 6% MLP(D).

the quality characteristics and consumer appeal, and thermally processed food exhibits low colour quality compared to uncooked food. (Li *et al.* 2017). According to Zhang *et al.* (2023), the addition of chlorophyll microcapsules (0.1%–0.5%) gave the noodles an attractive bright green, which can be considered as healthy and nutritious products by consumers. In addition, the colour value (L^* and b^*) of fresh noodles was lower than cooked noodles, which may be due to the degradation of chlorophyll and gelatinization of starch during the boiling process, resulting in the change of the noodle’s colour.

3.2 Texture profile of the cassava pasta fortified with MLP

The texture is a significant parameter for consumer acceptance of pasta (Bhattacharya *et al.* 1999). The texture attributes of pasta with different percentages of MLP are displayed in Table 2. The addition of mulberry leaves to pasta mixtures resulted in a decrease in

the hardness, springiness, cohesiveness, gumminess, and chewiness of the supplemented pasta. An increase of MLP fortified with pasta was expected to give a lower springiness value (0.23–0.19 g), the tendency of a compressed pasta to return to its original shape after the first bite. The MLP fortification in pasta decreased the cohesiveness (0.19–0.13) resulting in the lower strength of the internal bonds of pasta samples. The highest hardness value (4,331.48 N) observed in the unfortified pasta expressed the high bite forces of pasta. Pasta’s hardness is related to the hydration of starch granules during boiling and the embedding of gelatinizing granules in a network of denatured proteins (Baigts-Allende *et al.* 2022). This change in texture could be due to the high fibre content of mulberry leaves associated with a reduction of gluten in pasta (Sethi *et al.* 2020).

Likewise, polyphenols from a mulberry leaf can interact with starch, disturbing starch hydration, and gelatinization, which changed the properties of the



starch matrix, and subsequently resulted in hardness reduction. Although there were no studies of pasta supplemented with mulberry leaf, the incorporation of MLP in rice cake that weakened the dough network resulting in softer textured cakes was reported by Park *et al.* (2021). A similar result was also reported by Ungureanu-Iuga and Mironeasa (2021), and Baigts-Allende *et al.* (2022) that pasta supplemented with plant materials (grape seed, mango peel, and moringa leaf), the plant substitution of more than 1% of wheat flour showed a reduction of hardness and cohesiveness, similar to the results observed in the current study.

Changes in the texture of pasta can have significant implications for its cooking properties and consumer's sensory perception. Cohesiveness is a reliable index to how products hold together upon cooking and contributes to the density and energy required to chew a piece of food. Springiness is related to an elastic texture and physical ability to spring back after deformation during the first bite (Ji *et al.* 2007).

The strong correlation between springiness value and sensory attributes is related to elasticity. As shown in this study, the cohesiveness and springiness of cooked pasta decreased significantly with an increase of MLP substitution from 2 to 6%, which was consistent with the results of pasta fortified with black rice bran reported by Sethi *et al.* (2020). Zhang *et al.* (2010) also demonstrated that the elastic evaluation score of fortified noodles decreased significantly with higher addition of sweet potato flour, probably resulting from the dilution of gluten in noodle dough. Hardness is the maximum force to compress a sample between molar teeth, which affects the formation of pasta structure

during boiling processes. Gumminess is a measure of the material's resistance to deformation, which could be related to the elastic force of the protein matrix in pasta. Chewiness, a textural parameter dependent on hardness, is the energy required to chew a solid food (Epstein *et al.* 2002). The gluten content and starch properties of pasta might play an important role in the hardness, gumminess, and chewiness. It has been found that the instrumental hardness and chewiness of pasta enriched with black rice bran were correlated directly with the sensory hardness, and both attributes increased with higher flour additions to the formulation (Sethi *et al.* 2020). The pasta fortified with the MLP addition of more than 2% was less preferred by consumers due to a lower texture-sensory evolution score. These results could be associated with the decreased gluten protein content pronounced in the formulation resulting in lower cooking quality and soft texture attributes; thus negatively affecting the sensory properties and consumer acceptance (Zhang *et al.* 2010). The utilization of various hydrocolloids and tropical starches for improving the texture quality of non-gluten products seems to be a promising technique that can be applied to gain higher acceptance in sensory evaluation.

3.3 Total phenolic compound and antioxidant activity of MLP-enriched cassava pasta

The antioxidant properties of the mulberry leaf are generally attributed to their phenolic content, as well as to their active ingredients, such as rutin, isoquercitrin, quercetrin, and chlorogenic acid, all of which have been reported to exhibit antioxidant capacity (Iqbal *et al.* 2012; Ma *et al.* 2022). Total phenolic content was

Table 2. Physical characteristics of cassava flour in pasta supplemented with MLP in the ratio of 2, 4 and 6 % and control. Numbers with different superscripts in the same column are statistically different ($p \leq 0.05$).

Fortified pasta	Textural properties				
	Hardness (g)	Springiness (g)	Cohesiveness	Gumminess (g)	Chewiness (g x mm)
Control	4,331.48 ± 363.44 ^a	0.23 ± 0.02 ^a	0.19 ± 0.01 ^a	871.26 ± 96.67 ^a	214.33 ± 44.30 ^a
2% MLP	3,472.29 ± 207.65 ^b	0.19 ± 0.02 ^b	0.16 ± 0.02 ^b	558.25 ± 53.29 ^b	112.82 ± 15.00 ^b
4% MLP	2,922.74 ± 179.36 ^c	0.20 ± 0.02 ^b	0.14 ± 0.01 ^c	420.98 ± 31.34 ^c	86.38 ± 11.51 ^c
6% MLP	2,521.54 ± 255.17 ^d	0.20 ± 0.01 ^b	0.13 ± 0.02 ^c	357.51 ± 77.29 ^c	73.19 ± 15.19 ^c

measured by the Folin-Ciocalteu method and the antioxidant capacities of the pasta were determined by the DPPH radical scavenging activity and FRAP assays. All the tests showed that pasta with MLP led to a significant improvement in phenolic content (from 0.01 to 0.16 mg GAE/g) and antioxidant capacity (DPPH: from 0.18 to 2.04 mg Trolox/g; FRAP: from 0.13 to 0.30 mmol Fe/kg) and that the increases were proportional to the fortification level as presented in Table 3. The obtained results are consistent with those previously reported by Charunuch *et al.* (2008) who found that the antioxidant activity was correlated with the amount of total phenolic present in mulberry leaves. The previous study by Armellini *et al.* (2018) also found that the antioxidant activity of pasta containing saffron powder was higher in comparison to the control pasta.

The major active compounds in mulberry leaves are phenolic antioxidants, especially phenolic acids, flavonols, and flavones. Mulberry leaves consist of different types of proteins, amino acids, fibres, and minerals, which may be a potential source of nutritional components suitable for healthy and functional food (Ma *et al.* 2022). Mulberry leaves and their derivatives may be a promising alternative treatment for regulating blood sugar, lipid metabolism (anticholesterol), and anticancer effects. The main components, including phenols, flavonoids, polysaccharides, and alkaloids, are responsible for the pharmacological effects of resisting oxidation, anti-inflammation, and anticancer (Joh *et al.* 2015). The mechanism of antioxidant activity of phenolic compounds, including flavonoids, is mainly related to their direct scaveng-

ing of free radicals, which may help to protect the human body against damage by reactive oxygen species (ROS) (Heim *et al.* 2002). According to previous reports, mulberry leaves and their extracts can reduce the activity of multiple oxidases, prevent α -amylase and α -glucosidase, inhibit the synthesis of foam cells containing cholesterol, and exert antioxidative (Ma *et al.* 2022).

The noticeable health benefit of mulberry leaves was its anti-diabetes effect. However, the effect may vary due to the significant differences in mulberry leaves related to species, ecology, solvent extracts, and treatment dosage. Chlorogenic acid, rutin, 1-deoxynojirymycin (1-DNJ), and mulberry leaf polysaccharides play crucial roles in the anti-diabetic properties of mulberry leaves (Hunyadi *et al.* 2012). It has been suggested that 12 mg of 1-DNJ is the minimum suitable quantity to reduce postprandial hyperglycemia (Thaipitakwong *et al.* 2020). Polyphenol compounds in mulberry leaves have excellent antioxidant potential as promising functional food ingredients for consumer health promotion and disease risk reduction; however, further studies should be conducted to effectively utilize and clearly understand the pharmacological effects of mulberry leaves.

3.4 Sensory characteristics of cassava pasta fortified with MLP

The results of sensory characteristics of MLP-enriched pasta are shown in Table 4. There was no significant difference observed in colour scores between control and MLP substitution up to 4 %, while 6 % MLP sub-

Table 3. Phenolic content and antioxidant activities of cassava flour in pasta supplemented with different levels of MLP. Numbers with different superscripts in the same column are statistically different ($p \leq 0.05$).

Fortified pasta	Total phenolic content (mg GAE/g)	Free Radical scavenging activities	
		DPPH assay (mg Trolox/g)	FRAP assay (mmol Fe/kg)
Control	0.01±0.00 ^a	0.18±0.01 ^a	0.13±0.01 ^a
2 % MLP	0.09±0.01 ^b	1.07±0.04 ^b	0.21±0.02 ^b
4 % MLP	0.12±0.01 ^c	1.65±0.12 ^c	0.26±0.02 ^c
6 % MLP	0.16±0.01 ^d	2.04±0.09 ^d	0.30±0.03 ^d

stitution significantly reduced the score. This could be due to the presence of chlorophyll in mulberry leaves which made enriched pasta darker and more greenish (Son and Park, 2007). The MLP-enriched pasta up to 2 % was highly rated in terms of taste, while a significantly lower taste score was obtained for a higher substitution of MLP from 4 to 6 %. This could be attributed to the unique taste with the increased bitterness in pasta containing MLP over 4% relating to the presence of catechin, rutin, tannic acid, and chlorogenic acid in mulberry leaves (Park *et al.* 2021).

The pasta with 2 and 4 % MLP had similar texture scores. Whereas the pasta incorporated with 6 % MLP showed much lower scores, which could be attributed to the softer texture of the pasta. Corresponding to the textural hardness results, a complex formation between amylose and phenolic compounds from mulberry leaves might slow down amylose chain entanglement contributing to a decrease in hardness (Park *et al.* 2021). These results concurred with the findings of Baigts-Allende *et al.* (2022), who reported that the replacement of wheat flour with Hibiscus sabdariffa reduces the starch content and proportion of gluten resulting in a decrease in hardness. The scores of overall acceptance of the fortified pasta decreased with an increase in MLP due to their intense dark colour, unique order, bitter taste, and soft texture. However, no significant differences ($p < 0.05$) between 2 % and 4 % MLP-fortified pasta were found. These results suggest that enriched pasta, with good consumer acceptance, could be successfully prepared using 4 % MLP enrichment.

The combination of functional ingredients from agri-

cultural by-products in pasta without changing their acceptable quality and sensory attributes while retaining the biological functionality of the added bioactive substances represents a considerable technological challenge for researchers and food producers (Carpentieri *et al.* 2022). Several researchers have studied consumer preference for enriched pasta by surveying the utilization of agricultural by-products as functional ingredients and evaluating their purchase attitudes to pasta products. According to Cecchi *et al.* (2019), consumers positively accepted the idea of re-using agri-food waste to enhance the nutritional value of food products. Based on the visible appearance and the evaluation of the cooked pasta, many customers correlated the darkened colour of enriched products with the characteristics of healthy foods. However, Makhoul *et al.* (2019) have revealed that the original colour of the unfortified pasta sample was scored by consumers higher than the apparent darker colour of pasta fortified with bioactive compounds such as oat bran. This result suggested that consumers may be familiar with the original colour of the wheat pasta, which did not tend to be accepted at the first introduction of fortified pasta to consumers. Accordingly, the colour change after adding bioactive substances is associated with an individual's colour preference and consumer perception of health products, which probably affects consumer liking (Jaworska *et al.* 2020). In the perception of fortifying pasta with antioxidant bioactive compounds, Cecchi *et al.* (2019) observed the deterioration of sensory characteristics, and consumer liking could be slightly affected by the darker appearance, earthy odour and flavour, and the increase in bitterness of fortified products. However, Verbeke (2006) indicated in their study that consumers are

Table 4. Sensory characteristics of cassava flour in pasta supplemented with MLP. Numbers with different superscripts in the same column are statistically different ($p \leq 0.05$).

Fortified pasta	Sensory Characteristics					
	Appearance	Color	Odor	Taste	Texture	overall acceptance
Control	7.14 ± 0.62 ^a	7.06 ± 0.60 ^a	6.54 ± 0.49 ^a	6.96 ± 0.55 ^a	6.92 ± 0.34 ^a	6.80 ± 0.48 ^a
2% MLP	7.10 ± 0.22 ^a	6.94 ± 0.30 ^a	6.26 ± 0.56 ^{ab}	6.88 ± 0.54 ^a	6.72 ± 0.53 ^{ab}	6.46 ± 0.39 ^{ab}
4% MLP	6.86 ± 0.38 ^a	6.78 ± 0.43 ^a	6.00 ± 0.52 ^b	6.30 ± 0.38 ^b	6.65 ± 0.47 ^b	6.32 ± 0.55 ^b
6% MLP	5.88 ± 0.57 ^b	6.10 ± 0.47 ^b	5.86 ± 0.58 ^b	5.60 ± 0.49 ^c	6.14 ± 0.37 ^c	5.86 ± 0.41 ^c

willing to compromise the worsening of the taste of functional foods in exchange for health benefits. Consumer expectations and sensory experiences are critical influences for choosing functional foods.

Several strategies could be employed to enhance the overall quality of the fortified products. Adding seasonings or sauces to fortified pasta could improve the low flavour intensity and saltiness, while firmness could be developed by further optimizing the cooking time. Moreover, some researchers suggested that suitable labelling and marketing of fortified pasta products could actively influence consumer acceptance and create positive expectations of these products (Cecchi *et al.* 2019). Owing to consumers' acceptance of fortified pasta, Jaworska *et al.* (2020) reported that the consumers, especially females and elderly persons, were interested in the consumption of fortified food products with nutritional content and could compromise the lower sensory quality of functional foods to obtain health benefits. Additionally, the study on consumer purchasing behaviour of fortified pasta was carried out by Altamore *et al.* (2017), and the findings showed that consumer preferences are mainly motivated by health benefits and concerns, raw material sources, and environmental impact. Therefore, identifying consumer segments that share similar characteristics and needs is crucial for the food industry to position new products on the functional food market for health-conscious consumers and develop effective strategies to break down barriers to acceptance (Carpentieri *et al.* 2022).

3.5 Proximate composition of cassava pasta supplemented with MLP

Mulberry leaf is recognized as a good source of protein, carbohydrates, vitamins, and dietary fibre (Srivastava *et al.* 2006). Although some food products fortified with mulberry leaves were studied, such as rice crackers and cake (Park *et al.* 2021), few studies on pasta were reported. The fortified pasta with 4% MLP was chosen to evaluate proximate composition compared with unfortified pasta. The result showed that the incorporation of MLP in pasta modified its nutritional value since significant changes in carbohydrate, fat, fibre, and ash were observed (Table 5). The fat content of 4 % MLP-fortified pasta decreased from

4.92 to 3.74 % compared to the unfortified pasta. This result indicated that fat content decreased since low-fat MLP was used in the pasta dough. This is in agreement with Ahn and Yuh (2004), who reported that the fat content of enriched wheat muffins decreased with an increase in the amount of MLP. The carbohydrate content of the MLP-enriched pasta also decreased compared with the control pasta. This could be due to the low starch content of mulberry leaves, which might have functionality impacts on the texture and mouthfeel of food products (Aleman *et al.* 2021). Son and Kang (2014) reported a decrease in carbohydrate contents with an increase in the proportion of MLP in a mixture of wheat noodles. The addition of 4 % MLP in pasta led to an increase in fibre and ash (from 2.18% to 3.60% and 1.12% to 1.32%, respectively), which contributes to the health benefit of fibre and mineral consumption. This was seemingly the case in the previous studies where the addition of mulberry leaves in starchy foods presented higher fibre and ash content, as it contains a high amount of dietary fibre and minerals (Iqbal *et al.* 2012; Baigts-Allende *et al.* 2022).

Incorporating functional ingredients into pasta has shown promising results in enhancing its nutritional value. Adding mulberry leaves to pasta not only contributes to bioactive ingredients and improves the antioxidant properties but also increases fibre content, with the potential to further deliver health benefits (Wang *et al.* 2022). Dietary fibre plays a role in acting as a prebiotic for promoting the growth of intestinal microflora, decreasing fat absorption, lowering serum Low-density Lipoprotein (LDL) cholesterol, and reducing glycaemic index (Abuajah *et al.* 2015; Theuwissen and Mensink 2008). Previous research has demonstrated the utilization of mulberry leaves as a functional ingredient in food products for lowering glycaemic index (Yazdankhah *et al.* 2019; Thaipitakwong *et al.* 2020; Lange *et al.* 2022). Other studies were also conducted on the feasibility of using mulberry leaves to decrease the level of LDL-cholesterol (Katsube *et al.* 2006; Yang *et al.* 2011).

However, an increment of fibre content could weaken the protein matrix resulting in a disrupted structure of enriched pasta (Marinelli *et al.* 2018). The quality characteristics of pasta depend on the ability of the protein-starch matrix to maintain its integrity and are

Table 5. Chemical compositions of the cassava pasta supplemented with MLP. Numbers with different superscripts in the same column are statistically different ($p \leq 0.05$).

Chemical composition [% , wet basis]	Control	Pasta with 4%MLP
Moisture	23.30 ± 0.59 ^b	25.62 ± 0.40 ^a
Protein	8.78 ± 0.38 ^a	8.51 ± 0.24 ^a
Fat	4.92 ± 0.33 ^a	3.74 ± 0.12 ^b
Ash	1.12 ± 0.04 ^b	1.32 ± 0.08 ^a
Fiber	2.18 ± 0.16 ^b	3.60 ± 0.32 ^a
Carbohydrate	59.70 ± 0.52 ^a	57.21 ± 0.27 ^b

associated with consumer preference (Rakhesh *et al.* 2015). By competing with starch, fibre and its interactions with protein could weaken protein-starch interactions and influence the matrix structure and dough properties (Sissons *et al.* 2010). According to Rakhesh *et al.* (2015), who evaluated the sensory properties of durum wheat spaghetti enriched with different dietary fibers, the increase in fibre content could alter the starch-protein matrix, resulting in an increase in water absorption with a decrease in optimal cooking time. Cooking loss is an important quality characteristic of pasta that was influenced by an increase in fibre content. Aravind *et al.* (2012) reported that the cooking loss of bran-fortified pasta increased from 5.67% to 7.64% when the level of bran substitution increased to 30%. Another study by Tazart *et al.* (2016) was also conducted on the use of broad bean flour (*Vicia faba*) to fortify wheat pasta. The authors described that the increase in fibre content disrupting the gluten-protein network exhibited a decrease in optimal cooking time and an increase in cooking loss resulting in a lower cooking quality. Thus, enriched pasta with functional ingredients from plants is a crucial step in delivering more complete nutrition to consumers. However, further studies on the effects of bioactive ingredient addition need to be conducted to minimize the impact on cooking and nutritional quality and achieve sensory acceptability.

4. Conclusions

The present study provides beneficial information to support the inclusion of mulberry leaf powder in cassava pasta with high nutritional properties and bioactive components. The results showed that with

an increase in MLP amounts, the colour of the enriched pasta became dark greener. At the same time, the texture attributes such as hardness, springiness, cohesiveness, gumminess, and chewiness decreased. The increment of mulberry leaf level also enhanced the phenolic contents and the antioxidant activity of enriched pasta. The sensory evaluation suggested that the addition of low levels of MLP in pasta did not adversely affect its sensory attributes. In addition, the contents of fibre and ash were improved in the pasta with the MLP incorporation. Regarding optimization, pasta containing up to 4 % MLP exhibited good antioxidant, textural, sensory, and nutritional properties. Thus, the results obtained in this study indicate the potential of utilizing MLP as a nutritious and bioactive ingredient for producing high-quality pasta.

Ethics statement

This study was reviewed and approved by the Ethics Committee of Suan Sunandha Rajabhat University (certificate number: COE. 1-043/2021).

Conflict of Interest

The authors declare that there is no conflict of interest concerning the research and authorship of this paper.

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