



Effect of Adding Freeze-dried Kale Powder on Physicochemical, Functional, and Sensory Properties of Fresh Pasta

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Nutritionally enhanced pasta was prepared using wheat flour substituted with freeze-dried kale powder at ratios of 1%, 3%, and 5%. Pasta incorporated with 5% kale powder significantly exhibited the highest fiber and total ash content values. Replacing wheat flour with freeze-dried kale powder resulted in higher cooking time and cooking loss but, decreased water absorption due to lesser wheat flour. Similarly, higher kale powder incorporated reduced tensile strength and elasticity. Increased kale content decreased brightness, increased greenness, but no significant difference in yellowness of the pasta, and increased antioxidant and antimicrobial capacity. At 3% of kale powder, the pasta showed a good level of all attributes except for odor, which had the highest negative responses when the kale increased to 5%. Hence, the increased concentration of freeze-dried kale powder on physicochemical properties could enhance the functional potential of the pasta required for maintaining healthy health.

1. Introduction

Growing demands for developing food products with a lower glycemic index, low lipid, high protein, and high fiber contents, are good approaches to modifying a health-promoting nutritional added foods (Di Pede et al., 2021; Moolwong, Srilasak, & Chuacharoen, 2024; Ringuette, Finley, Prinyawiwatkul, & King, 2018; Vignola, Bustos, & Pérez, 2018). Pasta made from wheat flour (*Triticum aestivum* L.) is a famous globally consumed food due to its easiness in preparation, good sensory attributes and longer shelf life. However, it contains a low number of vitamins, and minerals, including bioactive constituents. Several studies have focused on increasing the nutritional quality of pasta by fortifying different types of non-gluten flour or protein isolate (El-Sohaimy, Brennan, Darwish, &

Brennan, 2020; Pinel et al., 2024; Reddy Surasani, Singh, Gupta, & Sharma, 2019; Sissons, 2022).

One alternative approach to enhance the functional properties of pasta in terms of nutrition is to add phytochemicals and fibers obtained from vegetables, which could be a promising way to develop pasta products with interesting health benefits. Leafy green kale, belonging to the Brassicaceae family is intense with carotenoids, especially lutein and zeaxanthin (approximately 14.7-39.6 mg/100 g fresh kale), including chlorophylls, flavonoids, hydroxycinnamic acids, and glucosinolates, which are considered high antioxidant potential against inflammation and cancer also known as a superfood (Ferioli et al., 2013). Developing pastas by incorporating kale could be a strategy to increase the intake of these health-promoting



phytochemicals which is a challenging way to diminish the risk of many diseases.

However, beneficial compounds in fresh vegetables like kale are heat sensitive and could be easily degraded by pasta making process and cooking (Wang, Brennan, Serventi, & Brennan, 2022). Drying technology could be addressed to stabilize bioactive compounds leading advantages for value-added kale pastas. The study of Hennig, Verkerk, Bonnema, and Dekker (2012) indicated thermal degradation as the major mechanism leading to losses of chlorophylls, flavonoids, hydroxycinnamic acids, and glucosinolates in kale. The freeze-drying technique operates under low temperatures and pressure to allow water to sublime directly from solid to vapor inside the material, which could preserve the targeted heat-sensitive phytochemicals of the materials better compared to other processes such as spray-drying, hot-air ovens, etc. Vargas, Kapoor, Nemzer, and Feng (2022) examined the effect of different drying methods on physical and phytochemical properties of kale. The result confirmed that freeze-dried kale had the highest glucosinolate retention and remained green color due to less chlorophyll degradation compared to hot-air sample. Moreover, the SEM images of hot-air kale powder noticed rough surface, shrinkage and physical changes while freeze-dried sample showed relatively fewer changes.

Thus, the aim of this study was to develop functional pasta by adding freeze-dried kale powder as enriching phytonutrients containing fiber and phytochemical compounds. The incorporating freeze-dried kale powder into pasta at various ratios ranging from 0% to 5% was studied and pasta samples were characterized in terms of physical, color, textural, and functional properties. The microbial, and cooking quality including sensorial

acceptance of the pasta were also evaluated.

2. Materials and Methods

Curly kale, commercial wheat flour, and other pasta ingredients were purchased from a local market in Bangkok, Thailand. Folin-Ciocalteu reagent was obtained from Fisher Scientific (Pittsburg, PA). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 3,4,5-trihydroxy benzoic acid (gallic acid) all were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical-reagent grade.

2.1. Freeze-dried Kale Powder Preparation

Cleaned and selected curly kale leaves were cut and left to dry at room temperature. The leaves were subjected to a two-step freeze-drying method (Labconco Corporation Freeze Dryer 6L, Kansa, MO, USA). First, the sample was kept under -50 °C for 24 h and later frozen at 5 °C with a pressure of 13.3 Pa. The sample was further dried at a shelf temperature of 25 °C for 48 h. The freeze-dried sample was ground, and the obtained powder was kept at -20 °C for further use.

2.2. Pasta Preparation

A composition of pasta was prepared in different proportions, and the amount of freeze-dried kale powder was added at different ratios (Table 1). An optimum of approximately 22% tap water was mixed into flour blends in an extruder mixing chamber with a thin ribbon-shaped die for 7 min. The extruded samples were stored in vacuum seal bags until further analyses.

Table 1: Pasta Composition Incorporated at Various Ratios of Freeze-dried Kale Powder.

Ingredient	Pasta Incorporated with Freeze-dried Kale (grams)			
	A0 (control)	A1	A3	A5
Flour	250	247.5	242.5	237.5
Egg	50	50	50	50
Egg yolk	17	17	17	17
Olive oil	6	6	6	6
Salt	1	1	1	1
Water	55	55	55	55
Freeze-dried kale powder (%)	0 (0%)	2.5 (1%)	7.5 (3%)	12.5 (5%)

Results are presented as weight (grams). Formulations referred to freeze-dried kale powder at ratios of 0% as A0 (control), 1% as A1, 3% as A3, and 5% as A5, respectively.

2.3. Proximate Composition

Proximate analyses of pasta were conducted following the

official methods; the Association of Official Analytical Chemists (AOAC) 1995; American Association of Cereal Chemists (AACC) 2000 with slight modifications proposed by Ohmura et al. (2023) and Romero & Zhang (2019). Briefly, a sample in a Petri plate was placed in an oven at 110 °C for 2 h and then kept in a desiccator for 30 min to previously weigh and dry. Moisture content was evaluated

as weight loss per fresh weight of the sample in grams multiplied by 100 to get a percentage. Fat content was evaluated using the soxhlet extraction method and the fat extract was determined as a percent of the equal amount of fat extract per weight sample in grams multiplied by 100. For ash content, the sample was placed in a muffle furnace set at 550 °C for 4 h and then was kept in a desiccator. The ash content was presented as a percent of ash equal to ash weight per weight sample in grams multiplied by 100. The amount of total protein in pasta was determined using the Kjeldahl method with a conversion factor of 6.25 to convert total nitrogen to protein concentration. Crude fiber was analyzed by digesting fat-removed samples using sequential acid and alkali extraction methods. The total carbohydrate content in the pasta was established by the phenol-sulphuric method described by Masuko et al. (2005). Water activity (a_w) was checked using an AquaLab 4TE (Meter Group Inc., USA). All sample analyses were carried out in triplicate.

2.4. Physical Characteristics and Color

Cooked pasta was prepared by boiling 25-30 cm (10-12 in) lengths of raw pasta in deionized water for 1 min, draining and rinsing it in tap water for 10 s, and then covered with a wet paper towel before further analysis. The cooked pasta strand was subjected to analyze tensile strength and maximum distance using a texture analyzer (TA.XTplus, Lloyd Instruments, Hants, UK) equipped with spaghetti tensile grip (A/SPR).

The cooked pasta color was also evaluated using a HunterLab ColorQuest XE (Hunterlab, Reston, VA, USA). Ten grams of pasta strands were arranged against a black background. The components were shown as lightness ranging from darkest to lightest (L^* value), as greenish to reddish (a^* value), and as bluish to yellowish (b^* value).

2.5. Cooking Quality

Pasta cooking quality was evaluated following the official method; American Association of Cereal Chemists (AACC) 2000 with slight modifications (Romero & Zhang, 2019). During the cooking step, three basic parameters including time taken, amount of loss, and yield were used to indicate the quality of pasta samples. Briefly, the sample was weighed approximately 25 g and cooked in 250 ml of boiling deionized water. The cooking time was recorded when the central opaque core in the pasta strand disappeared. Subsequently, the cooking loss was evaluated as the amount of loss occurring by transferring cooking water to a pre-weight beaker, evaporating the water overnight at 100 °C, and weighing the solid residues, presented as the percentage of solid loss. Later, the cooking

yield is defined as the amount of water absorbed by the pasta during cooking and measured as the weight ratio of cooked to uncooked pasta.

2.6. Microscopy

The cross sections of cooked pasta samples were imaged at microstructures using a scanning electron microscope (SEM) (SU3500, Hitachi High-Technologies Co., Tokyo, Japan) described by (Aravind, Sissons, Egan, & Fellows, 2012). All cooked pasta samples were freeze-dried for 48 h to obtain dried cooked pasta. The dried sample was fixed onto a circular metal stub using double-sided conductive carbon adhesive tape and then vacuum coated with a thin gold layer. The thinly coated samples were observed at 1000x magnification with an acceleration voltage of 10keV.

2.7. Phenolic Compound Extraction

The dried pasta weighed one gram was ground in a mortar and pestle grinder and then extracted with 10 ml of 70% methanol aqueous solution in darkness at room temperature for 7 h. After shaking for 30 min, the obtained extract was centrifuged at 5000xg using a centrifuge (MPW-352R, Warsaw, Poland) at 4 °C for 30 min, and the supernatant was stored for further analysis. All the samples were prepared in triplicate.

2.8. Total Phenolic Content (TPC)

Polyphenol content in the extracts obtained above was measured using the Folin–Ciocalteu method studied by Lisiecka, Wójtowicz, Dziki, and Gawlik-Dziki (2019) with slight modification. Briefly, 100 µl of diluted extracts was mixed with 500 µl of Folin–Ciocalteu reagent for 5 min, subsequently, 400 µl of sodium carbonate solution (7.5%) was added. The obtained mixture was incubated in darkness at room temperature for 30 min and measured absorbance at 750 nm using a UV-vis spectrometer (Biospectrometer, Eppendorf, Germany). Finally, the TPC in each extract was compared with the standard curve of gallic acid and the results were expressed as mg of gallic acid equivalents (GAE) per gram sample. All samples were done in triplicate.

2.9. Antioxidant Ability

In this study, DPPH and ABTS assays were used to determine the antioxidant ability of prepared pasta according to Chuacharoen, Moolwong, and Chysirichote (2021). For the DPPH assay, 0.1 mM DPPH solution was prepared in ethanol as a stock solution. Briefly, 0.1 ml of obtained extract was mixed with 1.9 ml of DPPH solution and incubated in darkness for 30 min and measured

absorbance at 515 nm. To prepare the ABTS+ radical cation solution, 7 mM ABTS+ was reacted with 2.47 mM potassium persulfate solution at a 1:1 (v/v) ratio. Subsequently, the stock solution was diluted with ultra-pure water at a ratio of 1:80 to obtain a working solution with an absorbance of approximately 0.70 at 734 nm. Then, 60 µl of extract solution was added to 40 µl distilled water and mixed well with 1.9 ml of the working solution. After 15 min of incubation in darkness, the absorbance of the mixtures was read at 734 nm using a UV-vis spectrometer (Biospectrometer, Eppendorf, Germany). Trolox was used as a standard and the results were expressed as µmol Trolox equivalents (TE) per gram sample. All samples were done in triplicate.

2.10. Microbiological Evaluation

Microbiological quality was assessed as described by Milde et al. (2021) with a slight modification. Approximately 100 g of uncooked pasta stored in a refrigerator were analyzed at fresh and 1, 3, and 5 days of storage. Subsequently, 1 gram of packed pasta was mixed and vortexed with 9 ml of normal saline (0.85%) for 2 min. The mixture was diluted to acquire 10⁻¹ to 10⁻⁵ decimal dilutions and each 100 µl of the dilutions was spread on appropriate media. Total bacterial count, total yeast and mold count, and *E. coli* were enumerated on different agars; total bacterial count was employed using plate count agar, yeast, and mold counts were determined using dextrose agar, and *E. coli* was quantified with Levine's eosin methylene blue, all using the serial plate dilution method. The dilution plates containing 30-300 colony-forming units (CFU) were used for counting. The plates for total bacterial count and *E. coli* were incubated at 37 °C for 24 to 48 hours, whereas at 28 ± 2 °C for 24 to 48 hours for total yeast and mold count. The obtained results were expressed as log CFU per g of pasta (log CFU/g). All procedures were conducted in triplicate.

2.11. Sensory Evaluation

Sensory evaluation on attributes: appearance, color, odor, taste, texture, and overall acceptability of cooked pasta was carried out with the approval of the SSRU Ethics Committee, Certificate number COE.1-038/2022, Study Code: 65-041-1-1. Sixty-two untrained evaluators (both sexes and ages between 20-27 years), who belong to the academic community were participated using the 9-point hedonic scale ranging from dislike extremely (1) to like extremely (9) (Sharma, Dar, Sharma, & Singh, 2021). They were notified of the implications of participating and gave their consent to participate in the test. Distilled water was used to prepare pasta samples to optimum

cooking time and approximately 20 g of the samples were served with salt and pepper in randomized codes to avoid biases. The evaluators were instructed to clear the palate by rinsing their mouths and drinking water before and between tastings. The test was conducted under the required environmental conditions.

2.12. Statistical Analysis

All experiments were performed in triplicates and the results were reported as mean value ± standard deviation. All data were tested statistically by one-way analysis of variance (ANOVA) with a completely randomized design (CRD) followed by Duncan's multiple range test at $p < 0.05$ to determine the differences among various variables using SPSS version 26 (IBM Corp., Armonk, NY, USA). Analysis of sensory descriptive data was done by means of the attributes.

3. Results

3.1. Chemical Composition and Physical Analyses

The nutritional compositions of uncooked pasta supplemented with freeze-dried kale powder at ratios of 1%, 3%, and 5% are demonstrated in Table 2. Pasta without any supplement (A0) was used as a reference sample. A significant reduction ($p < 0.05$) in moisture content from 52.33% to 49.66% was noticed in pasta samples when the additional level of freeze-dried kale powder increased. This decreasing trend related to the interaction among proteins of wheat flour holding water molecules but then was interrupted by adding the fiber of kale powder. The result agrees with the study of Desai, Brennan, and Brennan (2018).

There were no significant differences ($p < 0.05$) found in protein, fat, and carbohydrate contents when the level of supplementation of freeze-dried kale powder in pasta increased. Even though the amount of wheat flour was reduced, and then replaced by freeze-dried kale powder 1%, 3%, and 5% w/w. Kale is classified as a protein-rich vegetable (Thavarajah et al., 2016). Replacing wheat flour with freeze-dried kale powder slightly increased the total protein contained in the pasta, whereas total fat and carbohydrate contents of pasta samples showed decreased trends from 0.17% to 0.13% and 38.41% to 37.59%, respectively with no significant ($p > 0.05$) by increasing freeze-dried kale powder. This is because the amount of wheat flour decreased as well.

Significantly increased trends were found for crude ash and crude fiber contents increased by 1.42% to 1.96% and 0.65% to 3.27%, respectively. It was due to fresh kale

is recognized as a highly nutritious vegetable providing significant mineral micronutrients consisting of K (188-873 mg), Ca (35-300 mg), and Mg (20-100 mg) per 100 g. It also provides a great source of dietary fiber and contains non-digestible carbohydrates with low molecular weight (Pathirana et al., 2017; Thavarajah et al., 2016). It agreed with the study on the nutritional values of fresh kale by

Thavarajah et al. (2016) mentioned that kale is considered a low-calorie food with 36–98 kcal of energy and has a moderate level of protein (up to 5.9 g) per 100 g serving. Thus, fresh kale is recommended for consumption and the replacement of wheat flour by freeze-dried kale powder should be a promising way to enhance nutrition for pasta products.

Table 2: Proximate Compositions of Pasta Incorporated with Freeze-dried Kale Powder.

Parameter	Pasta Incorporated with Freeze-dried Kale			
	A0 (control)	A1	A3	A5
Moisture (%)	52.33±0.08 ^a	51.36±0.01 ^b	50.21±0.01 ^{bc}	49.66±0.01 ^c
Protein (%) ^{ns}	7.35±0.11	7.37±0.14	7.38±0.11	7.39±0.14
Fat (%) ^{ns}	0.17±0.03	0.17±0.04	0.14±0.03	0.13±0.03
Carbohydrate (%) ^{ns}	38.41±0.39	38.08±1.83	37.02±1.50	37.59±0.55
Ash (%)	1.42±0.01 ^a	1.60±0.06 ^{ab}	1.80±0.02 ^b	1.96±0.02 ^c
Fiber (%)	0.65±0.37 ^a	1.12±0.27 ^b	2.33±0.20 ^c	3.27±0.60 ^d

Results are presented as mean values (mean ± SD, n=3). Data superscripted with ^{a-d} and ^{ns} are the significant difference ($p < 0.05$) and not a significant difference ($p > 0.05$) within the same row, respectively according to Duncan's multiple range test. Formulations referred to freeze-dried kale powder at ratios of 0% as A0 (control), 1% as A1, 3% as A3, and 5% as A5, respectively.

Deionized water was used to cook pasta because it has a significant impact on the texture of cooked pasta as mentioned by Sozer and Kaya (2008). All cooked pasta samples were examined for tensile strength and elasticity (Table 3.), which implies the stress that is applied before a pasta strand breaks down. Increasing the level of freeze-dried kale powder substitution from 1% to 5% significantly ($p < 0.05$) decreased both the tensile strength (0.64 to 0.24 MPa) and elastic distance (24.97 to 13.45 mm) of the pasta compared to the control.

These decreased trends might be explained by increased kale powder acting as a non-gluten flour (Pathirana et al., 2017), which reduces the proportion of gluten in wheat flour. Additionally, a higher level of substitution resulting in increased insoluble-dietary fiber in kale powder hindered gluten development by blocking the proper contact among the particles of wheat flour, leading to a weak internal structure and less elasticity. Similar results were reported by Shiau, Wu, and Liu (2012) that fresh noodles supplemented with rigid natural fiber also showed an adverse effect on the tensile property.

The weakened gluten network interrupted by kale fiber significantly resulted in a reduction in water absorption parameters as similarly reported by Park and Baik (2002). These phenomena affect the overall texture property regarding less tensile strength and elasticity of the pasta as well.

Table 3: Physical Properties, Cooking Properties, Total Phenolic Content (TPC), and Antioxidant Ability of Fresh Pasta Incorporated with Freeze-dried Kale Powder.

Parameter	Pasta Incorporated with Freeze-dried kale			
	A0 (control)	A1	A3	A5
Water activity (a_w)	0.87±0.02 ^a	0.65±0.03 ^b	0.57±0.01 ^c	0.50±0.04 ^c
Tensile strength (MPa)	0.64±0.06 ^a	0.57±0.01 ^b	0.44±0.01 ^c	0.24±0.03 ^d
Elasticity (mm)	24.97±1.23 ^a	23.93±2.88 ^a	19.45±2.18 ^b	13.45±2.57 ^c
Cooking time (min)	5.30±0.01 ^a	6.15±0.01 ^b	6.10±0.01 ^b	6.05±0.02 ^b
Cooking loss (%)	6.40±0.15 ^a	7.00±0.10 ^a	8.40±0.12 ^b	8.60±0.14 ^b
Cooking yield (%)	61.08±1.74 ^a	57.04±8.75 ^{ab}	47.57±3.75 ^c	43.44±2.74 ^d
Color				
L^*	76.35±2.47 ^a	71.14±1.41 ^b	67.50±0.70 ^c	60.81±1.78 ^d
a^*	5.52±0.55 ^a	1.07±0.19 ^b	-1.59±0.39 ^c	-0.80±0.75 ^d
b^* ^{ns}	29.89±0.98	31.52±0.69	30.70±1.57	30.87±0.43
TPC (mg GAE/g sample)	2.10±0.24 ^c	2.62±0.23 ^c	7.08±0.82 ^b	12.06±1.39 ^a
DPPH (µmol TE/g sample)	21.66±1.52 ^d	25.40±0.20 ^c	36.51±0.03 ^b	44.72±1.13 ^a
ABTS (µmol TE/g sample)	73.53±5.21 ^d	88.60±17.67 ^c	103.16±11.23 ^b	132.40±6.48 ^a

Results are presented as mean values (mean ± SD, n=3). Data superscripted with ^{a-d} are the significant difference ($p < 0.05$) within the same row according to Duncan's

multiple range test. Formulations referred to freeze-dried kale powder at ratios of 0% as A0 (control), 1% as A1, 3% as A3, and 5% as A5, respectively.

Cooking parameters are related to final product quality. Table 3 shows there were no significant differences ($p < 0.05$) in cooking time among pasta samples (A1 to A5), and all had longer times than the control. The cooking loss increased slightly, ranging from 6.40% (A0) to 8.60% (A5), whereas the cooking yield by measuring water uptake reduced from 61.08% (A0) to 43.44% (A5) when increasing the additional level of freeze-dried kale powder.

Generally, a cooking process involves water absorption and swelling, gelatinization, and protein denaturation of starch granules. The gluten network formation can prevent excessive starch swelling and protein can restrict starch swelling. When wheat flour in pasta was substituted with kale powder acting as a gluten-disrupting agent, this implied the reduced ability to hold water and accelerated the solid loss in pasta during cooking. Kumari and Gupta (2022) observed similar findings on cooking properties that increased cooking loss as well as decreased water absorption in noodles prepared with less gluten-content flour.

3.2. Structural Analysis

SEM images of cross-section pasta samples are depicted in Figure 1 to confirm the results of cooking quality. At high magnification (1000x), the control pasta has many unembedded starch molecules implied to ungelatinized starch mixed with partially gelatinized starch (Figure 1A). When the level of freeze-dried kale powder increased, the pasta samples exhibited denser networks of gelatinized starch suspended or embedded in a smooth surface forming more compact and dense networks of continuous network in the pasta, which showed a very small difference among samples (Figure 1C, 1E, and 1G).

Unlike a close-up look, control pasta (Figure 1B) showed a firm texture without big holes or cracks appearing at low magnification (100x). This suggests a denser protein network where more starch granules were connected to form networks in the texture of the pasta. While there were more holes and cracks that appeared when pasta was incorporated with higher freeze-dried kale powder (Figure 1D and 1F), especially at 5% added noticeable large cracks (Figure 1H). It was consistent with the physical property in terms of tensile strength and water uptake during cooking. Freeze-dried kale powder containing insoluble-dietary fiber might have a role in disrupting the continuity of the gluten-starch network and this phenomenon creates a melting-like gelatinization lacking the development of the network

and losing the ability to uptake water. These are relevant to those reported by Aravind et al. (2012) and la Gatta et al. (2017) indicated that the fortification of insoluble fiber into pasta disrupted the formation of the protein matrix. These findings may be used to explain the higher cooking loss and possibly less ability to uptake water of pasta incorporated with a higher amount of freeze-dried kale powder during cooking.

Similar studies showed the swelling index of pasta is reduced by adding protein to the formula which causes the formation of protein networks stronger resulting in reduced water delivery used for swelling of starch granules and gelatinization (Desai et al., 2018).

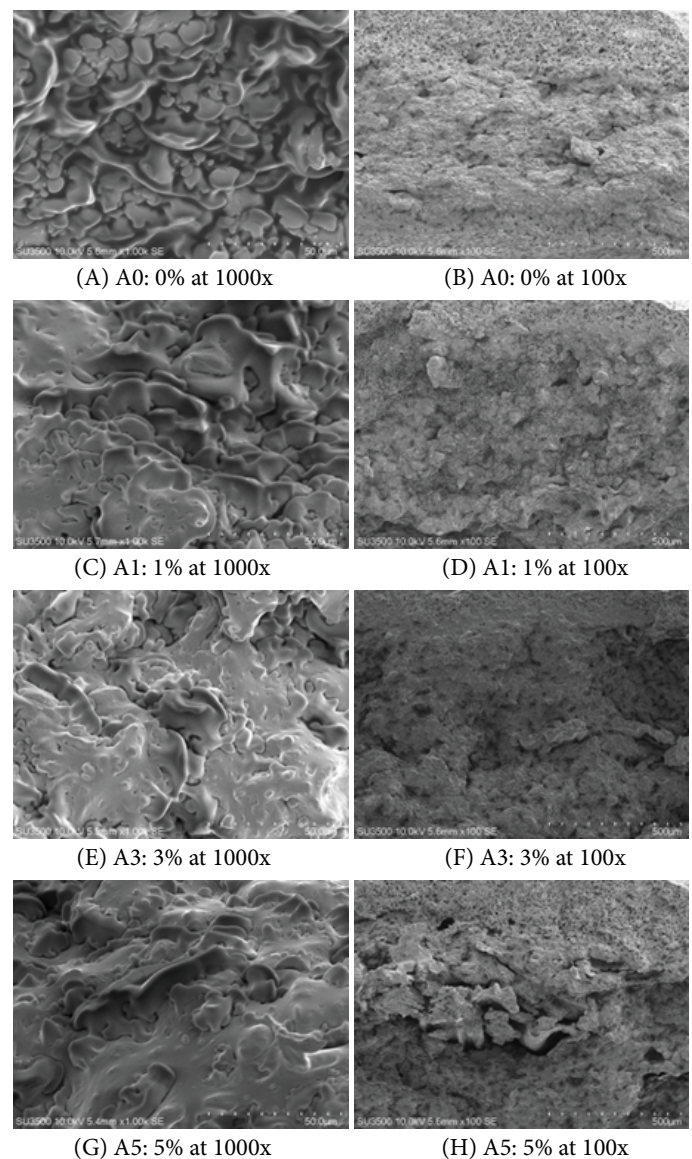


Figure 1: SEM Micrographs of Transverse Cross Sections of Cooked Pasta Samples with Various Ratios of Freeze-dried Kale Powder Incorporation at 1000x Magnification: the Control (A); 1% (C); 3% (E); 5% (G) and at 100x Magnification: the Control (B); 1% (D); 3% (F); 5% (H).

Therefore, the use of hydrocolloids such as guar gum, xanthan gum, carboxymethyl cellulose etc. along with substituted wheat flour pasta formulations should be a promising way to improve physical property and cooking quality of pasta capable to reach higher additional ratio which match consumer's acceptance.

3.3. Color

Color characteristics are an important quality parameter reflecting a certain aspect as the visual attractiveness to consumer acceptability for the pasta product. The physical appearance and color parameters of pasta after supplementing freeze-dried kale powder

with wheat flour pasta at different ratios are presented in Figure 2 and Table 3. The increased level of freeze-dried kale powder substitution caused fresh pasta darker and greener observed by the naked eye. It was consistent with the color values measured by the colorimeter. A significant ($p < 0.05$) decrease in L^* values (76.35 to 60.81) was noticed, similar to a^* values (5.52 to -0.80) indicating deviation to greener. Nonetheless, there was no significant difference was noticed in b^* values. Since kale was powdered using a freeze-drying technique operating under low temperatures, which showed less color changes due to the prevention of chlorophyll degradation (Vargas et al., 2022), thus higher amount of kale powder presented darker green in the pasta.

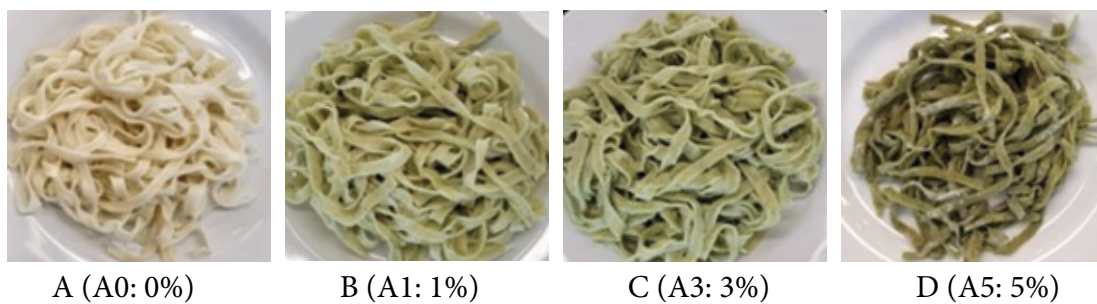


Figure 2: Fresh Pasta Incorporated with Freeze-dried Kale Powder At Ratios of 0% as A0 (A), 1% as A1 (B), 3% as A3 (C), and 5% as A5 (D), Respectively.

3.4. Total Phenolic Content and Antioxidant Activity

The antioxidant properties vary depending on the contents of total phenolic compounds, vitamins C, vitamin E, carotenoids, and flavonoids added to food products. Table 3 shows total phenolic compounds extracted from the pasta. The lowest amount of TPC (2.10 mg GAE/g sample) was found in the control (A0) and significantly increased ($p < 0.05$) ranging from 2.62 to 12.06 mg GAE/g sample when the level of freeze-dried kale powder increased from 1% to 5%, respectively.

The DPPH and ABTS assays are efficient procedures commonly used to determine the antioxidant activity of powdered natural antioxidants incorporated in several food products (Chuachoen et al., 2021). A similar increase trend was found in the results of the antioxidant capacity of pasta samples. The scavenging ability stated in Trolox equivalence (TE) against DPPH and ABTS assays with increased incorporation of kale powder from 0% to 5% was between 21.66 and 44.72 μmol and 73.53 and 132.40 μmol , respectively. This is similar to the studies that the addition of natural antioxidants to food products enhances their antioxidant capacities (Hussain & Kaul, 2018).

Indeed, fresh kale contains high concentrations of antioxidants including glucosinolates (Casajús et al., 2021). This increment was due to the presence of polyphenolic constituents in freeze-dried kale powder such as tocopherols, ascorbic acid, carotenoids, and plant phenolics, which was less destructive to the original properties than convective drying (Korus, 2022). This tendency was more noticeable with an increase in the level of freeze-dried kale powder substitution. As seen from the results of TPC and antioxidant values, the nutritional potential of pasta products could be enhanced by adding antioxidant and phytochemical compounds. However, apart from the benefit of antioxidative and fibrous healthy ingredients, the incorporation study in pasta should concern on rather delicious as well.

3.5. Microbiological Evaluation

The existence of spoilage microorganisms adversely decreases the cooking qualities and affects shelf life of fresh pasta products, which endangers consumers' health. In addition, the study on shelf life of fresh noodles elaborated on security for 5 days under 4 °C based on mold and total viable count (Guo et al., 2022).

As seen in Table 4, all pasta samples were negative for

E. coli count known as an indicator of unfavorable hygienic conditions including no fecal contamination and good manufacturing practices in pasta processing. There were no yeast and mold found on the initial day and day 1 and the number of yeast and mold was found not more than 1.48 log CFU/g (<30 CFU/g) after 3 and 5 days of storage. At day 3 of storage, the number of total bacterial counts varied from control to A5, ranging from

4.15 to 3.72 log CFU/g, when the pasta samples were kept longer to day 5 the bacterial count significantly ($p<0.05$) increased ranging from 4.94 to 4.57 log CFU/g for control to 5% kale incorporated pasta, respectively. The highest bacterial count was observed in the control while significantly ($p<0.05$) least count was recorded in the pasta samples prepared by supplementing freeze-dried kale powder 1% to 5%, respectively.

Table 4: Microbiological Evaluation of Pasta Incorporated with Freeze-dried Kale Powder at Initial, 1, 3, and 5 Days of Storage (4 °C).

Storage Time (days)	Analysis (log CFU/g)	Pasta Incorporated with Freeze-dried Kale			
		A0 (control)	A1	A3	A5
0	Yeast/Mold	ND	ND	ND	ND
	Total Bacterial count	ND	ND	ND	ND
	<i>E. Coli</i>	ND	ND	ND	ND
1	Yeast/Mold	ND	ND	ND	ND
	Total Bacterial count	ND	ND	ND	ND
	<i>E. Coli</i>	ND	ND	ND	ND
3	Yeast/Mold	<1.48	<1.48	<1.48	<1.48
	Total Bacterial count	4.15 ^a	3.84 ^b	3.80 ^c	3.72 ^d
	<i>E. Coli</i>	ND	ND	ND	ND
5	Yeast/Mold	<1.48	<1.48	<1.48	<1.48
	Total Bacterial count	4.94 ^a	4.70 ^b	4.66 ^c	4.57 ^d
	<i>E. Coli</i>	ND	ND	ND	ND

CFU/g represents colony forming unit/gram, and ND represents not detected. Results are presented as mean values (mean ± SD, n=3). Data superscripted with ^{a-d} are the significant difference ($p<0.05$) within the same row according to Duncan’s multiple range test. Formulations referred to freeze-dried kale powder at ratios of 0% as A0 (control), 1% as A1, 3% as A3, and 5% as A5, respectively.

It was supported by several studies on the total phenolic contents of kale, which exhibited effective antimicrobial activity against selected pathogenic bacteria including *E. Coli* (Ayaz et al., 2008; Shamlan, Yahya, Ismail, & Yahya, 2020). Another explanation is during the storage period in a refrigerator, a slight decrease in the moisture of fresh pasta was observed (Schettino, Pontonio, Gobbetti, & Rizzello, 2020; Tiwari et al., 2009). When freeze-dried kale powder was increasingly added to pasta, the moisture content was also reduced as mentioned above. Thus, adding freeze-dried kale powder can reduce the growth of bacterial count in fresh pasta.

In summary, the obtained results were satisfactory at no more than 2.00 log CFU/g (100 CFU/g) for yeast/mold and less than 5.70 log CFU/g (5x10⁵ CFU/g) for the total bacterial count according to Standards for Pathogenic Microorganisms in Food (FDA Thailand 2017). Hence, bioactive compounds found in kale leaves are useful for successive applications as food preservatives against

bacteria.

3.6. Sensory Evaluation

Apart from the additional health benefits to pasta products, the taste should be considered. Sensory evaluation plays a key role in measuring the impression of eating quality and this test sought to use hedonic scales to assess consumer behavior in sensory acceptance of pasta.

The sensory assessment of cooked pasta is shown in Figure 3. The highest score of overall acceptability was observed in pasta incorporated with 3% freeze-dried kale powder, which was slightly higher than that of the control. This level of kale added also depicted the best appearance, color, taste including texture, whereas the increase of kale powder substitution in pasta adversely affected the odor assessment because of its too-intensive greenish vegetable flavor of kale. The worst score was significantly ($p<0.05$) noticed by adding up to 5% and all sensory attributes had the lowest scores as well. This result was supported by the study on supplementing pasta with high-fiber fractions of la Gatta et al. (2017), which showed that additional fiber causes gluten protein networks to weaken and detriment its cooking and sensory properties in terms of texture. Thus, the highest score of overall acceptability indicated pasta could be enriched with 3% freeze-dried kale powder as this added level shows good sensory properties.

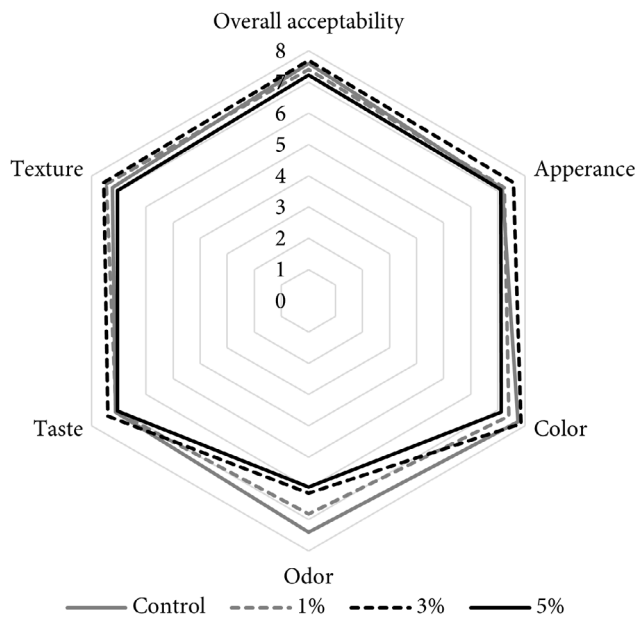


Figure 3: Sensory Evaluations of Pasta Incorporated with Freeze-dried Kale Powder at Different Ratios of 0% (A0), 1% (A1), 3% (A3), and 5% (A5).

4. Conclusion

In this study, supplementation with freeze-dried kale powder enhanced nutritional composition and maintained physicochemical properties including cooking parameters of fresh pasta. The fiber in kale powder added to pasta might have a role in the network structure formation. When protein and starch granules are obstacles to forming networks, these phenomena cause the pasta low strength with easy to break down by stretching and lose the ability to uptake water resulting in losing texture properties and perception in the sensory aspect. Moreover, the addition of freeze-dried kale powder showed effective antimicrobial activity during storage under chilling condition. Based on the obtained results, pasta could be prepared by supplementing up to 3% freeze-dried kale powder as this level added shows the highest acceptability and receives good properties in many certain aspects of physical and functionality. This confirms the addition of kale powder into pasta could affect the textural properties, but the functional aspects of the pasta are increased. Therefore, in the future, it is worth to optimize parameters such as flour types, added vegetables and their ratios, including utilization of hydrocolloids with the aim at maintaining physical property and preventing cooking quality loss. The acquired data on the development of enriched functional pasta could pave the way to design food which is compatible with consumer perceptions and industry demands for sustainable healthy food products.

5. Conflict of Interest

The authors declare no conflict of interest in this research concerning research and authorship.

6. Acknowledgement

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