Research Article

Gluten Free Alternative: Quality and Antioxidant Activity of Mulberry (*Morus alba*) Bread

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ABSTRACT

Innovative food products with health benefits are increasingly becoming popular over the past decade. Therefore, an alternative to produce bread added with composite flour to increase its nutritional value has been developed. This research was conducted to determine the quality of bread added with mulberry powder. Bread quality was assessed through sensory evaluation, bulk density, texture profile analysis (TPA), colour, nutritional value, and antioxidant activity. Five bread formulations were produced with the addition of mulberry powder at 0% (control), 1% (F1), 2% (F2), 3% (F3), and 4% (F4). Through sensory evaluation, the addition of mulberry powder at 1% (F1) obtained the highest mean score value for most attributes and therefore was the best formulation. The bulk density of the mulberry bread increased with the addition of mulberry powder from 0.14±0.00 in F1 to 0.16±0.00 in F4, indicating a denser texture that hindered proper expansion during baking. Texture profile analysis showed that F1 possesses the lowest hardness of 3.16±0.02 and stickiness of 2.24±0.03 compared to F2, F3 and F4 but still showed no significant difference for all attributes compared with the control bread which is a good indicator of bread texture. The values of hardness and stickiness increased with increase amount of mulberry powder, meanwhile, cohesiveness and springiness decreased. Nutritional value analysis showed the bread contained a moisture content of 32.11±0.35%, ash (0.08±0.00%), protein (11.60±0.01%), fat (2.72±0.01%), crude fibre (0.70±0.08%) and carbohydrate (52.81±0.40%). The DPPH scavenging activity increased with the increased amount of mulberry powder from 13% to 26% in F1 to F4, respectively. In conclusion, the addition of mulberry powder in bread formulation could produce bread with high content of nutrient and fibre and increase antioxidant activity in bread. However, the use of mulberry powder should be limited as it affected the texture and organoleptic properties of bread.

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1. Introduction

Recently, there has been a significant increase in the popularity of functional food products, driven by an increasing societal emphasis on healthier dietary choices. Various experts and organisations have defined the term functional food to reflect the changing knowledge of its importance (Baker *et al.*, 2022). Generally, functional food is food that goes beyond basic nutrition to provide additional health benefits by including certain substances known for their physiological benefits (Schmidt, 2000). Addressing the challenges posed by refined flour production aligns with broader concerns about future food security. By exploring alternative raw materials rich in essential nutrients, such as unconventional grains or supplements, the quality of refined wheat flour products can be enhanced, ensuring that staple foods like bread continue to have a favourable impact on overall nutrition and health (Weegels, 2019).

Mulberry fruit is rich in volatile compounds with higher phenolic contents than other berries and mulberry varieties (Butkhup *et al.*, 2013; Chen *et al.*, 2017). Mulberry also has a high nutraceutical value due to its low lipid value and high levels of protein, carbohydrates, fibre, organic acids, vitamins, and minerals that are comparable to other berries (Jiang and Nie, 2014; Sanchez-Salcedo *et al.*, 2015). In addition, mulberry is proven to exhibit excellent pharmacological properties such as antioxidative, diuretic, anti-obesity, hypo-glycaemic, hypo-tensive, anti-cholesterol, anti-diabetic, and antimicrobial (Chen *et al.*, 2017). Recently, mulberry fruit has been widely used in cooking due to its sweet taste and attractive bright colour (Sanchez-Salcedo *et al.*, 2015). The antioxidative properties of *M. alba* leave incorporated into paratha as a functional food showed an increase in the dough's DPPH, ABTS, and Fe^{3+}-reducing and chelating capabilities in a dose-dependent manner. However, the values decreased upon frying. High total phenolic acids, flavonols and antiradical activities were observed in bread added with mulberry leaf and fruit extract, both after baking, and after 30 days of frozen storage (Piechocka *et al.*, 2020).

Mulberry fruits have an interesting colour contributed by their anthocyanins content which has high antioxidant and colour-enhancing properties. Therefore, mulberry can act as a natural, functional food colourant since it is safer to consume than synthetics and can deliver enhanced colour quality with value-added properties to the product (Chen *et al.*, 2017; Chen *et al.*, 2021). These high values show significant mulberry functionality in enhancing health benefits for food consumption. Given the health benefit of mulberry, and the functionality of bread as a convenient and staple food, therefore, it is of interest to study the effect of the addition of mulberry powder at four different percentages in wheat flour for bread making on bread quality related to the physicochemical characteristics, sensory evaluation, and antioxidant activity.

2. Materials and Methods

2.1 Materials

Mulberry was purchased from a local villager in Kg. Tudan, Sabah with the composition of 8.97% protein, 0.35% ash and 14.53% fibre, 0.09% fat and 12.08% moisture content. Refined wheat flour (12.06% protein content, 0.04% ash content, 0.11% fibre, 1.19% fat and 10.89% moisture content) (Gunung Mas brand, Malaysia), sugar (Prai brand, Malaysia), dry instant yeast (Bunga Raya brand, Malaysia) and salt (Bake with Me, Malaysia) were purchased from a local market.

2.2 Mulberry powder preparation

Fresh mulberry was properly rinsed with tap water to eliminate contaminants and then frozen (-20°C) for a maximum of 7 days before the analysis. The mulberry was dried for 24 h at 60°C in a cabinet dryer (Thermoline, Australia) and then crushed to a fine powder with a Waring miller. Subsequently, the mulberry was sieved (US standard testing sieve, A.S.T.M.E-11, W.S Tyler, Mentor, OH, USA) and kept in an airtight container (particle size 250 µm). To make bread, dried mulberry powder (1, 2, 3 and 4%) were combined with refined wheat flour. Before the analysis, the mulberry powder was kept sealed in plastic containers at room temperature (25°C). A pretest was undertaken to establish an acceptable level of mulberry powder in the formulations.

2.3 Bread-making process

The wheat bread added with mulberry powder was made according to Nor *et al.* (2016) with some modifications. All ingredients were adjusted according to the specific formulation as in Table 1 and were mixed in a dough kneading machine (Tefal OW 300101, UK) and set to knead for 20 min. After kneading, 100 g of dough from each batch was transferred to a baking tin and then placed in a fermentation chamber at 37°C for 30 min. The dough was then punched and proofed for the second time in the same conditions. The dough was baked at 180°C in a convection oven (Binder, German) for 15 min to an internal temperature of 99°C at the centre of the bread. After baking, the loaves were cooled at room temperature for 15 min, then removed from the baking tin, and further cooled on a grate at the same temperature for another hour before storage at -18°C for further analysis.

Table 1. Bread formulation

	Materials						
Formulation	**Wheat flour (g)**	**Mulberry powder (g)**	**Salt (g)**	**Sugar (g)**	**Instant yeast (g)**	**Shortening (g)**	**Water (mL)**
F0	56	0					
F1	55	1					
F2	54	2	1	6	1	2	34[1]
F3	53	3					
F4	52	4					

[1]The amount of water was adjusted to get a 100% total weight for each formulation

2.4 Analysis of nutritional composition

The determination of moisture, lipids, ash, proteins, and crude fibre were carried out in triplicate, according to AOAC (2005) method. Carbohydrates were estimated by difference.

2.5 Analysis of physical properties of bread

2.5.1. Volume, specific volume and density measurement

The weight of bread samples was determined using a digital balance (Precisa, Switzerland) and the bread volume was determined using the rapeseed displacement method and the specific loaf volume (mL/g) was calculated, based on the average of 3 slices of bread. The specific volume of each bread was calculated as in equation (1) shown below:

$$\text{Specific volume (cm}^3\text{/g)} = \frac{\text{Loaf volume of bread (cm}^3\text{)}}{\text{Loaf weight (g)}} \quad (1)$$

The bread density was calculated as shown in equation (2) below:

$$\text{Density (g/ cm}^3\text{)} = \frac{\text{Weight of bread (g)}}{\text{Loaf volume of bread (cm}^3\text{)}} \quad (2)$$

2.5.2. Bread colour

After baking, bread samples were cooled and stored at room temperature for colour measurement on the same day. The colour of the bread crumb was determined by measuring lightness (L*), redness (a*), and yellowness (b*) utilizing a Minolta Chroma Meter (CR-310, Konica Minolta Sensing, Inc., Osaka, Japan) in triplicate.

2.5.3. Bread texture

Bread samples were measured using a texture analyser TA-XT2PLUS (Stable Microsystem) (AACC, 1988). Before analysis, the bread crumb was cut into small pieces (15 × 15 × 15 mm). A cylindrical aluminium probe with a 21 mm diameter was used in the TPA model with twice compression which penetrated 40% of the crumb depth. A pre-test speed of 2 mm/s, a test speed of 1.7 mm/s, and a post-test speed of 10 mm/s were used. The delay between the first and second compression was set as 5 s. The testing was performed in triplicate for each bread sample, and the force was recorded in N.

2.6 Sensory evaluation

The sensory attributes of the bread were examined in a sensory laboratory at the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah (UMS), Malaysia. Panellists were chosen from the Faculty of Food Science and Nutrition, UMS. All panellists provided informed consent to participate in this study. Bread samples were cut into slices (2 x 2 cm), coded with three-digit numbers, and rearranged before being served on a tray at random to panellists. Only 1 slice of bread was given to each panellist for each formulation. The hedonic tests used a total of 40 semi-trained panellists to rate bread samples based on colour, aroma, taste, texture, and general acceptability on a seven-point hedonic scale ranging from 1 (extremely dislike) to 7 (extremely like) (AACC, 1988). The Friedman test was used to examine data.

2.7 Determination of 2.2-Diphenyl-1-Picrylhydrazyl Radical Scavenging (DPPH) Assay

The assay was conducted according to Chen *et al.* (2016) with minor alteration. First, 2.4 mL of 0.1 mM DPPH (Merck, EMD Millipore Corporation, Darmstadt, Germany) solution was added to 1.6 g of the sample, vortexed (Heathrow Scientific, Vernon Hills, IL, USA), and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV–VIS spectrophotometer (Lambda 35 UV–VIS Spectrometer, PerkinElmer, Waltham, MA, USA). Trolox (Merck, EMD Millipore Corporation, Darmstadt, Germany) was used as a reference compound, and the percentages of DPPH radical scavenging activity were calculated by using the equation (3) shown below.

$$\text{DPPH } (\%) = [(A0 - A1)/A0] \times 100, \quad (3)$$

where A_0 = absorbance of control (2.4 mL of 0.1 mM DPPH + 1.6 mL of 70% (v/v) methanol) and A_1 = absorbance of the sample (2.4 mL of 0.1 mM DPPH + 1.6 mL of the sample in respective solvents).

2.8 Statistical analysis

All studies were done in triplicate. One-way ANOVA was used to analyse the data. The Tukey's test was used to estimate the statistical significance at $p < 0.05$ for the least significant differences. SPSS Version 16 was used for the analysis.

3. Results and Discussion

3.1 Nutritional values of bread

The nutritional content of all bread formulations is shown in Table 2. The moisture content (29.91±0.13 - 35.69±0.38), ash (0.08±0.01 - 0.11±0.01), and crude fibre (0.52±0.06 - 1.31±0.05) increased with the increasing percentage of mulberry powder in the formulation. Meanwhile, the content of protein (11.71±0.05 - 11.35±0.06), fat (2.73±0.00 - 2.57±0.01), and carbohydrate (55.07±0.02 - 48.99±0.48) was the highest in control bread and the values significantly decreased as the amount of mulberry powder increased from F1 to F4. This result was in line with the content of raw or dried mulberry and wheat flour (Table 2). Mulberry is high in minerals and fibre, which is reflected in the formulation. In contrast to dried mulberry, wheat contains more protein, carbohydrates, and fat. The high content of crude fibre in F4 shows that it has the potential to be applied as a high fibre source in bakery products; bran fractions are removed during the milling of refined wheat flour rendering it a poor source of dietary fibre. These results also demonstrated that, while some nutrients were lost throughout the bread-making process due to thermal processing, the nutritional composition remained high as the amount of mulberry powder increased. However, a high moisture content in all formulations is not advantageous because it reduces the shelf life of the final products due to the high-water content in the bread. As a result, some formulation tweaks must be made to limit the quantity of moisture in the bread, so preserving the product.

Table 2. Nutritional composition (%) of mulberry bread per 100 g dry weight basis

Composition (%)	Control	F1	F2	F3	F4
Moisture	29.91±0.13[a]	32.11±0.33[b]	33.31±0.01[c]	34.31±0.25[c]	35.69±0.38[d]
Ash	0.08±0.01[a]	0.08±0.00[ab]	0.09±0.01[ab]	0.10±0.01[ab]	0.11±0.01[b]
Protein	11.71±0.05[c]	11.60±0.01[bc]	11.55±0.02[bc]	11.46±0.04[ab]	11.35±0.06[a]
Fat	2.73±0.00[d]	2.72±0.01[cd]	2.69±0.01[bc]	2.67±0.00[b]	2.57±0.01[a]
Crude fibre	0.52±0.06[a]	0.70±0.08[a]	0.98±0.05[b]	1.14±0.08[bc]	1.31±0.05[c]
Carbohydrate	55.07±0.02d	52.81±0.40[c]	51.40±0.01b	50.34±0.20[a]	48.99±0.48[a]

[a, b] Means with different letters in each column are significantly different ($p < 0.05$)

3.2 Physicochemical properties of bread

3.2.1 Volume, specific volume, and density

The volume, specific volume, and density of wheat bread with mulberry powder are shown in Table 3. The volume and specific volume of bread added with mulberry powder were observed to decrease (1180 ± 3.54 to 1043 ± 3.54 cm^3) with the increasing amount of mulberry powder. This was in line with the value of the bulk density of bread ranging from 0.1 g/ cm^3 to 0.16 g/cm^3. The bulk density (g/cm^3) of flour is the density measured without the influence of any compression. The highest bulk density was observed in F4 and F3 bread (0.16 g/cm^3) followed by F2 (0.15 g/cm^3), and the lowest for F1 bread which has the same value as wheat bread as a control (0.14 g/cm^3). The present study revealed that bulk density of flours is determined by particle size and initial moisture content. Figure 1 shows that the bread with F1 formulation is virtually the same size as the control bread. The particle size decreased, and the structure became denser as the amount of mulberry powder added to the formulation increased from F1 (1% mulberry) to F4 (4% mulberry). The bulk density of composite bread increased when more mulberry powder was included with wheat flour. Decreasing the proportion of wheat flour raises the bulk density of composite bread. Substituting wheat flour with various grains and other raw materials such as barley, oat, roselle, jackfruit and seaweed resulted in a reduction in the volume of loaves of bread (Nor Qhairul Izzreen *et al.*, 2023; Feili *et al.*, 2013). This is due to the substitution of bread with mulberry, which dilutes gluten and hence affects proper gluten matrix formation throughout the mixing, fermentation and baking stages. In contrast, low bulk density might be advantageous in the formulation of complementary foods, as demonstrated by the F2 mulberry bread, which had a bulk density comparable to wheat bread.

Table 3. Volume, specific volume and bulk density of mulberry bread

Formulation	Volume (cm^3)	Specific volume (cm^3/g)	Bulk density (gcm^3)
Control	1180 ± 3.54[a]	7.24 ± 0.01[a]	0.14 ± 0.00[a]
F1	1173 ± 3.54[a]	7.18 ± 0.01[a]	0.14 ± 0.00[a]
F2	1071 ± 3.54[b]	6.51 ± 0.00[b]	0.15 ± 0.00[b]
F3	1056 ± 3.54[c]	6.35 ± 0.03[c]	0.16 ± 0.00[c]
F4	1043 ± 3.54[c]	6.24 ± 0.05[c]	0.16 ± 0.00[c]

[a, b] Means with different letters in each column are significantly different ($p < 0.05$)

3.2.2 Bread colour

Table 4. summarises the impact of mulberry powder on bread colour. The addition of mulberry powder had a significant effect on Lightness (L*,), greenness/redness (a *,), and yellowness (b*, 5). The control bread with no mulberry powder added had the greatest L* and b* values (L*: 47.19 ± 0.98; b*: 12.64 ± 0.43), whereas the colour declined as the powder level increased. Based on colour variations with the control colour as a reference, the addition of mulberry changed the hue of the bread. Mulberry powder increased bread's greenness/redness, as evidenced by the greatest a* value of 11.55 ± 0.72 in the F4 formulation. As the amount of mulberry powder in the formulation increased from F1 to F4, the values of ΔE* fell from 34.36 ± 0.18 to 20.81 ± 0.50, corresponding to L* and b* values. Martínez *et al.* (2001) found that the human eye perceives colour variations for values over 3. And the values were significantly higher than those in the present study. The colour of the bread is supplied by Maillard and caramelization processes during baking; however, with the addition of mulberry powder, the red colour became darker with the increased percentage of mulberry powder. This could be attributable to the amount of fermentable sugar provided by mulberries, which stimulates the Maillard reaction in bread. Mulberry powder, which is reddish purple causes the changes of yellowish white bread in control bread to turn purple. According to Nayak *et al.* (2013), powdered mulberry fruit is an effective colouring agent. Furthermore, colour is a significant aspect in determining a food product's acceptance.

Table 4. Colour characteristics of mulberry bread crumb

Formulation	*L**	*a**	*b**	ΔE*
Control	47.19 ± 0.98d	-2.26 ± 0.03a	12.64 ± 0.43c	48.81± 0.48
F1	32.49 ± 0.21c	5.58 ± 0.08b	10.78 ± 0.49b	34.36±0.18
F2	22.85 ± 0.14b	8.74 ± 0.31c	9.40 ± 0.02a	26.29±0.16
F3	21.42 ± 0.50b	10.67 ± 0.14d	9.25 ± 0.10a	25.36±0.37
F4	15.48 ± 0.50a	11.55 ± 0.72d	8.26 ± 0.22a	20.81±0.50

[a, b] Means with different letters in each column are significantly different ($p < 0.05$)

3.2.3 Bread texture

Table 5 shows the findings of the texture profile analysis. As can be observed, the addition of mulberry to bread samples had a substantial effect on the product's textural features. The hardness and stickiness characteristics rose as the percentage of mulberry powder increased. However, as the amount of mulberry powder increased, the values of springiness and cohesiveness decreased.

Adding mulberry powder to bread formulas increased hardness significantly ($p<0.05$) (3.14 ± 0.04 in control bread; 3.16 ± 0.02 in F1; 3.60 ± 0.01 in F2; 3.70 ± 0.03 in F3 and 4.09 ± 0.18 in F4). The amylose and amylopectin matrix, which contribute to the overall bread texture, are primarily responsible for its hardness (Schiraldi and Fessas, 2001). Bread hardness was also caused by gluten-fibre interactions (Feili *et al.*, 2013). Tolve *et al.* (2021) reported a similar observation, stating that adding grape pomace powder causes the resulting bread to become hard due to its high fibre content. Gluten formation and gas retention capacity were poor, therefore the dough could not develop properly. As a result, the baked bread gets denser and has a firmer texture. This finding was consistent with Kowalczewski *et al.* (2019), who discovered that the enhanced bread hardness was due to the replacement of wheat flour with fibre-rich components. The inclusion of mulberry powder enhanced the stickiness of the bread samples significantly ($p<0.05$); compared to all other formulations, F4 had the highest value of 3.85 ± 0.04. The stickiness of control bread (2.14 ± 0.03) and F1 (2.24 ± 0.04) did not differ significantly from each other. Adhesiveness describes how sticky the gels are when they come into touch with a solid. The addition of mulberry powder did not significantly ($p>0.05$) impact springiness, although the values reduced from 1.03 ± 0.05 in control to 0.93 ± 0.02 in F4 mulberry bread. According to Hoseney *et al.*, (1994), the interaction between gelatinized starch and gluten dough causes the dough to be more elastic and can form a continuous sponge structure of bread after heating. As a result of the gluten structure in control bread, it is projected to have the maximum springiness among the formulations. The present study's results were consistent with those of Chen *et al.* (2019), who discovered that adding mango peel powder to wheat flour had no significant effect on the springiness of their bread.

Bread cohesiveness dropped from 0.85 ± 0.02 to 0.66 ± 0.02 when mulberry powder was added to the formulation (from control to F4). The values, however, were not significantly different ($p>0.05$) between control bread and F1 indicating that the texture of F1 remained cohesive, exactly like the control bread, and that adding 1% mulberry powder had no effect on the bread's cohesiveness. Control bread and F1 show significant difference ($p<0.05$) from F2, F3, and F4. This demonstrates that adding mulberry powder will impact the stickiness of bread at the lowest amount of 2%. Cohesiveness refers to the strength of the breadcrumb's intrinsic linkages. This reduction suggests that the bread formulated with mulberry powder has a limited ability to resist before the bread structure is distorted by teeth, whereas the matrix integrity of control bread was unaffected when compared to the bread combined with mulberry powder. The same results were reported by Mironeasa *et al.* (2019) and Chen *et al.* (2019), who found that adding grape skin powder and mango peel powder decreased the cohesive value of bread.

Table 5. Texture analysis of mulberry bread with different formulations

Formulation	Hardness	Springiness	Cohesiveness	Stickiness
Control	3.14 ± 0.04a	1.03 ± 0.05a	0.85 ± 0.02c	2.14 ± 0.03a
F1	3.16 ± 0.02a	1.04 ± 0.04a	0.85 ± 0.02c	2.24 ± 0.04a
F2	3.60 ± 0.01b	1.00 ± 0.002a	0.72 ± 0.02b	2.85 ± 0.04*b*
F3	3.70 ± 0.03b	0.94 ± 0.03a	0.63 ± 0.02a	3.07 ± 0.04b
F4	4.09 ± 0.18c	0.93 ± 0.02a	0.66 ± 0.02ab	3.85 ± 0.04c

[a, b] Means with different letters in each column are significantly different ($p < 0.05$)

3.3 Sensory evaluation

Table 6 summarises the mean scores of hedonic sensory evaluations for colour, aroma, taste, texture, and overall acceptability of bread samples. Table 6 shows that replacing control bread with 1%, 2% and 3% and 4% mulberry bread significantly ($p < 0.05$) affected all sensory measures. The colour of the bread scored the best in F4, which had the most mulberry power, but the lowest score for the remaining attributes. This was owing to the attractive colour of mulberry itself which is reddish and purplish (Figure 1). This aligned with the findings for whole wheat bread with added roselle powder, where the colour changes as the amount of roselle powder increases due to the Maillard and caramelization processes during baking (Nor Qhairul Izzreen *et al.*, 2023). Control bread had the highest scores for aroma, taste, texture, and overall acceptability; nevertheless, the scores for attribute aroma, taste, texture, and overall acceptance were not statistically different between control bread and F1. Generally, the inclusion of mulberry powder had significant effects on sensory properties and acceptance of the bread sample. The addition of mulberry powder caused darker colour and denser texture, which at 4% seems acceptable for consumers (Table 6). Consumers consider the colour of the bread to be an essential feature in sensory evaluation (Matos and Rosell, 2012), depending on their perception of bread type. In this present study, F4 has a more attractive colour, shown by the greatest approval rating across all the other formulations.

Table 6. Sensorial attributes of mulberry bread

Formulation/ percentage of mulberry powder (%)	Colour	Aroma	Taste	Texture	Overall acceptance
Control	5.82 ± 1.10^{b}	6.24 ± 1.04^{c}	6.38 ± 1.35^{b}	6.38 ± 1.37^{b}	6.66 ± 1.11^{d}
F1 (1%)	4.96 ± 1.40^{a}	5.94 ± 1.20^{c}	6.32 ± 1.27^{b}	6.28 ± 1.05^{b}	6.32 ± 1.06^{d}
F2 (2%)	6.50 ± 0.91^{c}	5.58 ± 1.62^{bc}	5.72 ± 1.63^{ab}	5.28 ± 1.68^{a}	5.54 ± 1.54^{bc}
F3 (3%)	7.32 ± 0.62^{d}	4.96 ± 1.68^{ab}	5.06 ± 1.86^{ab}	5.62 ± 1.32^{ab}	5.32 ± 1.98^{ab}
F4 (4%)	7.76 ± 1.19^{d}	4.20 ± 1.92^{a}	4.60 ± 2.09^{a}	5.40 ± 1.81^{a}	4.52 ± 2.28^{a}

[a, b] Means with different letters in each column are significantly different ($p < 0.05$)

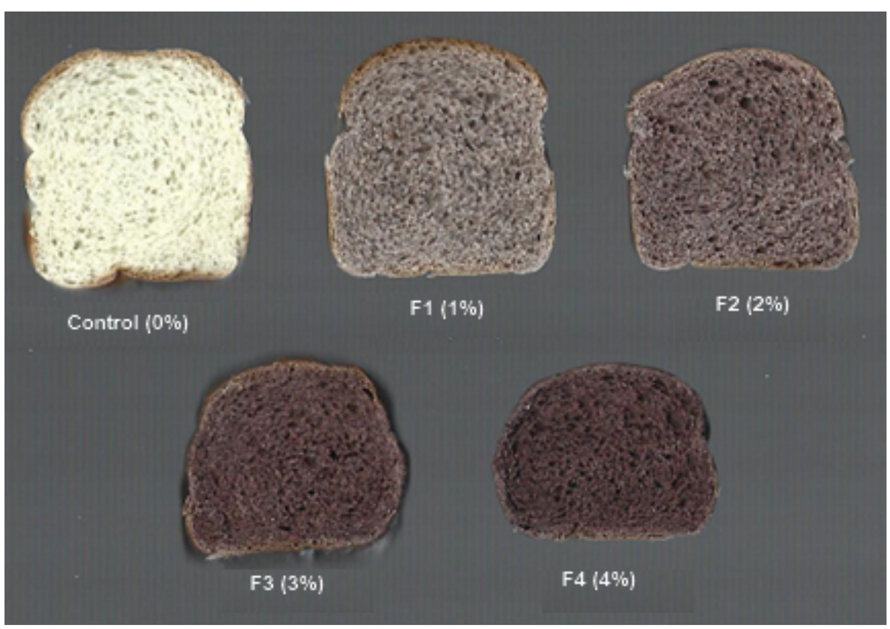

Figure 1. Bread added with different percentages of mulberry powder

Average bread aroma scores, as evaluated by the sense of smell, were considerably lower as the amount of mulberry powder increased from F1 to F4, indicating that adding more than 3% mulberry powder had a negative effect on the final product's aroma. This could be attributed to the loss of wheat smell which is a common perception of bread and the increase in sour aroma supplied by mulberry (Lasekan and Dabaj, 2020). Furthermore, the use of mulberry might impact the fermentation process since it can release volatile compounds that can contribute to the aroma of the finished bread (Cho and Peterson, 2010). Table 6 shows a similar pattern. The texture attribute scores were consistent with the results of texture analysis, which revealed that increasing the amount of mulberry powder in the bread formula can result in harder bread. Wheat flour has a high gluten content that leads to the formation of good bread texture and the usage of mulberry powder that was high in fibre causes gluten development to be interrupted (Schiraldi and Fessas, 2001; Feili *et al.*, 2013). Bread samples with scores more than 4 (neither liked nor disliked) were deemed acceptable. All formulations, F1 through F4 obtained acceptable scores as their total acceptability was greater than 4.50. The control bread had the highest score (6.66 ± 1.11), which did not differ significantly from F1 (6.32 ± 1.06).

3.4 2.2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) assay

Figure 2 shows that the antioxidant activity of bread extract was significantly increased from 13 % DPPH radical scavenging activity to 26 % with the addition of mulberry powder from F1 to F4, indicating that a higher-level of mulberry powder addition resulted in a higher antioxidant activity.

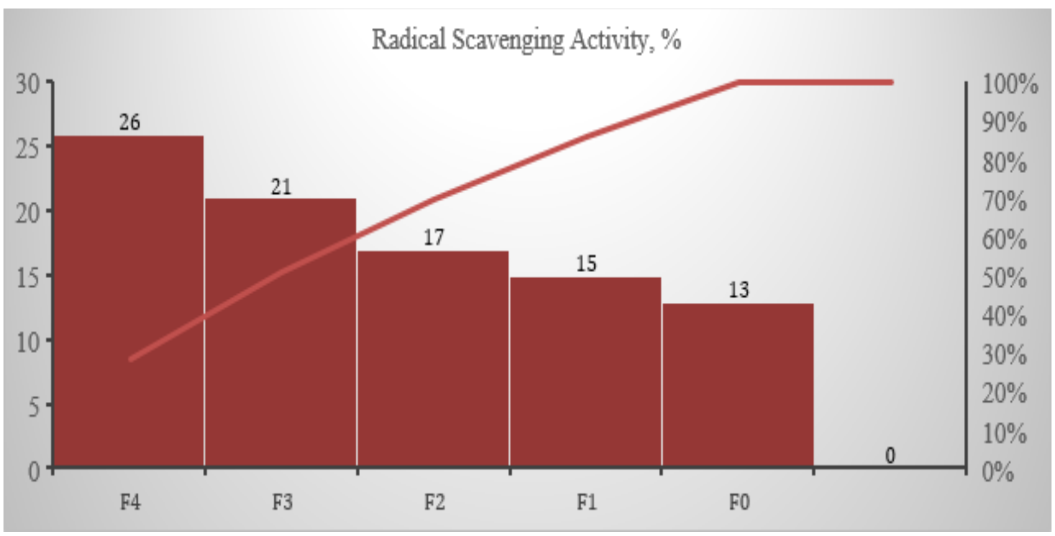

Figure 2. Radical scavenging activity of mulberry bread

Mulberry powder's ability to suppress free radicals suggests that it may work as an antioxidant. According to Chen *et al.* (2021; 2022), this species of mulberry (*Morus alba*) has high flavonoid, phenolic and anthocyanin content in its fruits. However, the antioxidant activity in the present bread samples with various formulations was shown to be low when compared to the antioxidant activity in extracted fruit samples. The high antioxidant activity in F4 was in line with the intense red colour it possesses, which is contributed by the anthocyanin compound (Table 4, Table 6, and Figure 1). Tamaroh and Sudrajat (2021) also indicated that anthocyanin content contributes to antioxidant activity. Mulberry fruit has a high anthocyanin content, so adding mulberry powder to bread helps to improve antioxidant activity in bread. Similarly, Wahyono *et al.* (2020) found that adding pumpkin powder to bread increased antioxidant activity due to its high β-carotene concentration. Furthermore, D'urso *et al.* (2020) found that phenylpropanoids and flavanols were the primary chemicals responsible for the fruit's antioxidant activity with a significant DPPH activity (IC_{50} = 0.518 mg/mL). Meanwhile, Chen *et al.* (2022) discovered that DPPH activity increases by 44.88% as fruit maturity increases, from the lowest in black fruits (IC_{50} = 0.073–0.152 mg/mL) to red fruits (IC_{50} = 0.16–0.77 mg/mL). Because the raw material utilised in this study was black fruit, the value was expected to be lower than if we had used red fruit. However, red fruit tastes sour when compared to black fruit, which is sweet and unsuitable for bakery products. Moreover, low DPPH activity in the bread compared to the mulberry fruits extract could be caused by heat-induced reactions of antioxidant compounds with food components, such as proteins or starch to produce large molecules, that could not be extracted by the solvents that were not used in this experiment. Also, thermal processing may cause the compounds to degrade, resulting in lower antioxidant content (Tamaroh and Sudrajat, 2021).

Conclusion

F1 was the best bread formulation that included 1% mulberry powder. F1 was the most acceptable formulations by the panellists and the only attributes that differed significantly from the control bread were texture and overall acceptance, making it the panellists' favourite bread. Furthermore, F1 bread may retain bread's organoleptic quality as compared to other formulations. The proximate analysis of F1 revealed that adding 1% mulberry powder to the formulation increased the moisture content, ash and crude fibre of bread while decreasing the fat and carbohydrate content. Also, the antioxidant activity in mulberry-added bread increased as the amount of mulberry powder increased, indicating that it could minimise lipid oxidation in bread.

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