Turnitin_2_The Application of Pandan Leaves Powder (Pandanus amaryllifolius Roxb.) as a Natural Coloring on the Pandan Steamed Bun Production

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The Application of Pandan Leaves Powder (*Pandanus amaryllifolius* Roxb.) as a Natural Coloring on the Pandan Steamed Bun Production

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Abstract Pandan leaves (*Pandanus amaryllifolius*) have been popularly used as a natural colorant in food and beverage production. In this research, we applied the zero-waste concept. Following the zero-waste concept, this research was carried out to evaluate the changes in the phsyco-characteristics of steamed bun with the addition of pandan leaves powder (PLP) as a natural coloring. The study was done by Randomized Block Design ($\alpha = 0.05$) with pandan leaves age (young and old), PLP concentration (0%, 1%, 3%, and 5%), and the storage time (1, 3, and 5 days) as the factors. The results showed that the pandan leaves age and PLP concentrations significantly affected the water content and color of the dough. They also significantly affected the physical characteristics (weight, volume, diameter, & height) and texture profile (hardness, springiness, cohesiveness, gumminess, chewiness, & resilience) of the steamed bun. The old pandan leaves and higher concentration of PLP significantly increase the green color of the dough and bun, weight, and diameter but reduce the height and volume of the bun. It also impacts the increase in the hardness of the steamed bun texture. The longer storage time of the steamed bun significantly reduces its size but will increase its hardness.

Keywords Bun, Color, Leaves, Pandan, Powder, Steamed, Storage, Texture

1. Introduction

Pandanus amaryllifolius, also known as pandan, is a local plant that is widely used as a natural colorant in traditional food and beverage processing in Southeast Asia, mainly in Indonesia [1]. Based on Mataliana et al. [2], in 2013, in Subak Tegenungan, Gianyar, Indonesia, pandan leaves have high availability, reaching 9.87 hectares of the area in that village. The pandan leaves can give green color because they contain an amount of Chlorophyll [1] and some volatile compounds named 2-Acetyl-1-pyrroline [3]. In addition, pandanus leaves are also known to contain lots of polyphenols like steroids, isoflavones, phenols, lignans, tannins, glycosides, alkaloids, terpenoids, saponins, flavonoids, amino acids, and vitamins that have functional effects on the body [4]. Pandan leaves also have a high dietary fibre content, which is good for the digestive tract [5].

As a natural source of color and aromatics in food, chlorophyll content is a consideration in using pandan leaves to produce products with attractive colors. Therefore, the selection of pandan leaves based on the maturity levels needs to be done to make the products according to the desired characteristics. The pandan leaf maturity or pandan leaf age was grouped into (1) young with leaf numbers 1 to 6, (2) medium with leaf numbers 7 to 12, (3) mature with leaf numbers 13 to 18, and the last is (4) over mature with leaf numbers 19 to 24. The higher level of leaf maturity will contain more chlorophyll content than younger maturity [6]. Bleszynski et al. [7] stated that in older leaves, there is an increase in metabolism and genetics so that chlorophyll content and other metabolites, such as 2-Acetyl-1-pyroline in the leaf number 12-14 from the apex [3]. As a result of their metabolism, the older leaves had increased fibers and phenolic compounds [7]. These changes occur during the growth and development period of the leaf [7,8].

The application of pandan leaves in food and beverage processing in Indonesia has been done by the extraction method. But this kind of use lacks stability during storage. In this research, we offer the zero waste concepts to utilize all parts of the pandan leaves, which are converted into powder as a natural colorant and aroma. As a natural ingredient, the whole of pandan leaves powder has been incorporated into sponge cake [9] but has never been used in a steamed bun. This research aimed to observe the effects of PLP with different ages of leaf and 0.00 %, 1.00 %, 2.00 %, and 3.00 % levels on the characteristics of the steamed bun quality. We also evaluate the characteristics of steamed bun during the storage period.

2. Materials and Methods

2.1. Materials

The main material of this research is fresh pandan leaves obtained from Materia Medica Batu and it was sorted as young and old leaves based on their maturity level. Young pandan leaves with leaf numbers 1 to 7, and old leaves with leaf numbers up to 7. Young pandan leaves had greener color than older leaves which had a darker color. Older pandan leaves also had more complex and thick tissue. Pandan leaves powder was made from fresh; dried pandan leaves using a vacuum dryer in the Food Processing Technologies Laboratory, Faculty of Agriculture Technology, Brawijaya University. The supporting materials for steamed buns making were obtained in the Prima Rasa Store Malang, including the high protein wheat flour (Cakra), shortening (Blueband), sugar (Rose Brand), salt (Refina), powdered milk (Frisian Flag), instant yeast (Fermipan), and mineral water.

2.2. Preparation of Powdered Pandan Leaves

Pandan leaves were sorted based on maturity level. It was divided into two categories: young leaves with leaves 1 to 7 from the apex and old leaves with up to 7 from the apex. They were cleaned and washed using water flow. They were cut into small size, around 3x0.5 cm. Pandan leaves were dried using a vacuum dryer at 40°C for 6 hours. Then pandan leaves were crushed using a blender for around 10 minutes and ball mill 115-118 rpm for 30 minutes until they became smooth small particles. Powdered pandan leaves were sieved with 100 mesh and stored using a closed food container.

2.3. Physical and Chemical Analysis

Chemical contents, including moisture, ash, and also fiber of PLP, were analyzed with AACC standard methods [10]. The density of powdered pandan leaves was obtained by measuring their weight and volume [11]. The color of PLP and steamed bun dough were obtained using a colorimeter CR-300 (Minolta, Japan). The colorimeter showed color L*, a*, and b* values of the products [12].

2.4. Water and Oil Absorption Capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) were analyzed using Edun et al. [11] methods. WAC was obtained with weighed 1 gram of PLP and poured with 10 ml distilled water in a centrifuge tube. Samples were mixed using a vortex for 1 min. Samples were placed at room temperature (±30C) for 1 hour. Then the centrifuge tubes were put into a centrifuge for the separation session. Set the centrifuge speed at 2000 rpm for 30 min. Then the supernatant was separated, and their volumes were measured. WAC was measured in the percentage of the PLP weight used. OAC was obtained with 1 gram of PLP and 10 ml of corn oil mixed in a centrifuge tube. Tubes were mixed using a vortex for 1 min. The suspensions in the tube were put in centrifuged (*Gemmy*) and set the centrifugal speed at 3000 rpm for 15 min, then the supernatant was separated. The tube content was re-weighed to determine the oil absorbed by PPL. Oil absorption capacity was measured in the percentage of the weight of the PLP used.

2.5. Total Chlorophyll

The total chlorophyll of PLP was determined using the spectrophotometry method with modified [7]. Fresh pandan leaves were mashed first using mortar before maceration. Then 1 gram of PLP was weighed and placed into an Erlenmeyer. Then poured 50 ml of 96% ethanol into the Erlenmeyer and closed the Erlenmeyer to avoid light exposure. The samples were placed in a water bath shaker at 60°C for 45 minutes. The maceration process results were filtered using filter paper to separate the undissolved material of PLP. The extracted filtrates were analyzed using UV-Vis spectrophotometry (*Spectro 20D Plus*) at 645 and 663 nm to calculate the chlorophyll content. Following is the formula to calculate the chlorophyll content of the sample [7]:

$$\text{Total chlorophyll content (mg/L)} = 8.02 \times \text{Abs } 663 + 20.2 \times \text{Abs } 645$$

2.6. Total Phenolic Content

The total phenolic content of PLP was calculated by spectrophotometry method at 756 nm based on Abdulkadir et al. [8] with modification. 10 mg PLP was mixed with 50 ml of ethanol using a vortex until dissolved. Then 0,5 ml of the solution between PLP and ethanol was added to 2,5 ml 10% Folin Ciocalteau and 2 ml 7,5% Na_2CO_3. Then, it was mixed using a vortex. The samples were incubated for 30 minutes. Absorbance was determined using a spectrophotometer at 756 nm. The total phenolic content concentration was obtained from calculations using the equation of gallic acid as standard phenolic content.

2.7. Antioxidant IC_{50} Analysis

Antioxidant IC_{50} analysis was done by the free radical-scavenging activity method. It was calculated based on the DPPH radical reduction in ethanol, which causes an absorbance drop at 517 nm. DPPH in ethanol 0.1 mM 2 ml was added to 2 ml of 0, 40, 80, 120, 160, and 200 ppm extract PPL. Then the samples were incubated for 20 min in the dark. The absorbance was measured at 517 nm. The antioxidant activity IC_{50} was obtained using a standard linear equation to find the concentration of samples needed to have 50% antioxidant activity [13].

2.8. Evaluation of Physical Characteristics of Steamed Bun

The physical characteristics of the steamed bun were evaluated after 60 min bread was cooled at room temperature. The evaluation was done using by Edun et al. [11] method. The steamed bun was weighed using an analytical scale. Loaf volume was measured using the chia seed displacement test method, and then the bun specific volume was calculated by dividing the volume by the weight of the bun. The diameter and height of the bun were measured using calipers.

2.9. Evaluation of Pandan Steamed Bun Properties during Storage

The bread was wrapped with polypropylene ziplock plastic and stored at room temperature (30ºC). The steamed bun's physical characteristics were measured using a texture profile analyzer, which was conducted using a TA-XTPlus Texture Analyzer (Stable MicroSystem, UK) according to the method stated by Fan et al. [14]. Hardness, cohesiveness, springiness, gumminess, chewiness, and resilience were measured. The analysis was carried out on days 1, 3, and 5.

2.10. Statistical Analysis

Analysis of all parameters of this research was done with four replicates. The data obtained were analyzed by analysis of variance (ANOVA) using Minitab 17 software. Tukey HSD was used at $\alpha < 0.05$.

3. Result and Discussion

3.1. Characteristics of PPL

Table 1 shows the characteristics of PLP. The different aged levels of PLP had a significant effect ($p>0.05$) on moisture content, soluble and insoluble fiber content, WAC, total chlorophyll and phenolic contents, antioxidants IC_{50}, and the PLP color, including L*, a*, and b*. However, it has no significant effect on the ash content, density, and OAC of the PLP.

The PLP derived from old leaves contains total chlorophyll and phenolic content, but the antioxidant value of IC50 is lower than that of young leaves. Chlorophyll is synthesized during the leaf maturity process, along with the phenolic content [7,8], so its content will be increased in mature leaves [15]. Phenolics and polyphenols are the plant's secondary metabolites with antioxidant activities [16]. Mature leaves contained higher phenolic content, which corresponded to lower antioxidant IC_{50}. [17]. The phenolic from pandan leaves also has the potency of being an antioxidant [18]. The phenolic is composed of free phenolic compounds that are acidic such as gallic acid, protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, and sinapic acid, in old leaves resulting in a decrease in the pH of pandan leaf powder [19]. When mature leaves were older, their total chlorophyll increased, affecting the L*, a*, b* values. The older leaves result in PLP with L*, a*, and b* values smaller than the younger ones [6].

Table 1. Characteristics of pandan leaves powder (PLP)

Parameter	PPL	
	Young Leaves	Old Leaves
Moisture content (%)	7.87 ± 0.18^a	6.30 ± 0.17^b
Ash (%)	10.81 ± 0.51^a	11.55 ± 0.19^a
Density (g/cm^3)	0.17 ± 0.00^a	0.16 ± 0.00^a
Soluble Fiber (%)	6.62 ± 0.20^b	11.14 ± 0.16^a
Insoluble fiber (%)	46.60 ± 0.41^b	51.13 ± 0.24^a
Water absorption capacity (%)	842.38 ± 10.37^b	898.51 ± 30.01^a
Oil absorption capacity (%)	398.89 ± 10.15^a	421.27 ± 19.63^a
pH	5.71 ± 0.02^a	5.53 ± 0.10^b
Total chlorophyll (mg/g)	10.76 ± 0.55^b	13.82 ± 0.77^a
Total phenolic (mg/g)	87.47 ± 1.89^b	96.34 ± 1.96^a
Antioxidant (ppm)	184.70 ± 3.19^a	142.19 ± 1.32^b
Lightness (L*)	55.73 ± 0.17^a	54.53 ± 0.25^b
Redness (a*)	-8.05 ± 0.12^a	-9.01 ± 0.25^b
Yellowness (b*)	20.66 ± 0.20^a	19.79 ± 0.20^b

*Results are reported as means of three replicates ± SD. P-Values < 0.05 indicates a significant difference between Young and Old.

Moisture content is one of the essential parameters to determine the quality of powder products. The powder is expected to have a low moisture content, so it has a longer shelf life. The PLP from young leaves has more moisture content but lower ash content than mature leaves. Liu et al. [20] reported that during the maturation process, the leaf loses its water content, and plants will absorb more minerals from the soil so that old leaves will have a higher mineral content than young leaves [21]. Ash content indicates the presence of mineral content in the powder, chlorophyll is a dye that contains Mg ions in the center of its structure. In addition, the high amount of ash in PLP might be associated with the high chlorophyll content containing Mg ions, the maturation process and lower moisture content during the maturation process.

Fiber is one type of carbohydrate that is formed in the leaves during growth as a result of the plant's metabolism and its contents increase during the maturation process [22]. Table 3 shows that, as leaf maturity increased, the soluble and insoluble fiber contents tended to increase. The presence of dietary fiber will increase powder’s absorption capacity, increasing WAC and OAC [23]. Based on this research, young pandan leaves powder has relatively low dietary fiber causing low oil binding and water binding by pandan leaves powder.

Based on research conducted by Suryani et al. [18], the young pandan leaves had a higher moisture content, ash, and carbohydrate, including dietary fiber. But it has a lower phenolic content than the matured ones. The moisture content of PLP from this research ranged from 6.30-7.87% by the results obtained in the research done by Murtini et al. [9], which ranged from 7.09-10.65%. Also, this research's range of color values, Lightness, redness, and yellowness, is similar to Murtini et al. [9] studies. According to research done by Adhamatika et al. [13], the characteristics of PLP from the young and matured pandan leaves had similar trends to this research.

3.2. Characteristics of Dough and Steamed Bun Influenced by PLP Addition with Different Maturity Levels

The addition of PLP impacted steamed bun dough characteristics are shown in Table 2. The moisture content of the steamed bun dough ranged from 37.46-39.36%.

The maturity levels of PLP and level of addition significantly affected the dough's moisture content. Increasing the level addition could decrease the moisture content of the dough. The increase in the cellulose content in the dough will also affect the water absorption of the dough so that the availability of water for gluten will decrease, resulting in poor viscoelastic properties of the dough [24]. Based on the Bchir et al. [25], they reported that the addition of powder pomace with a concentration of 2% (w/w flour basis) increased the water absorption of dough. The absorption was increased by the interaction between fiber and water through hydrogen bonding with the hydroxyl groups of the fiber.

Table 2. Water content and color of dough

Sample		Moisture Content (%)	L*	a*	b*
Control		41.14 ± 0.11	79.94 ± 0.83	4.75 ± 0.47	17.50 ± 0.18
Young	1%	39.36 ± 0.24^{b}	72.18 ± 0.33^{a}	-0.51 ± 0.21^{a}	24.51 ± 0.20^{e}
	3%	38.31 ± 0.09^{c}	60.66 ± 0.11^{c}	-0.73 ± 0.03^{ab}	26.54 ± 0.10^{c}
	5%	37.46 ± 0.18^{d}	57.06 ± 1.39^{d}	-1.40 ± 0.14^{c}	27.47 ± 0.25^{b}
Old	1%	40.68 ± 0.21^{a}	68.55 ± 1.42^{b}	-0.81 ± 0.04^{b}	26.08 ± 0.28^{d}
	3%	39.08 ± 0.07^{b}	57.13 ± 0.34^{d}	-1.42 ± 0.12^{c}	27.08 ± 0.18^{b}
	5%	38.16 ± 0.12^{c}	51.49 ± 0.13^{e}	-1.54 ± 0.09^{c}	28.25 ± 0.14^{a}

Notes: Means of 4 repetitions ± standard error. The same symbol shows the insignificant difference on Tukey's HSD test α=0.05.

Table 3. Physical characteristics of pandan steamed bun

Sample		Weight (g)	Volume (mL)	Diameter (mm)	Height (mm)
Control		28.78 ± 0.13	108.75 ± 3.77	73.60 ± 0.68	38.83 ± 0.44
Young	1%	29.19 ± 0.06^{de}	98.75 ± 1.50^{a}	66.81 ± 2.18^{c}	38.55 ± 0.69^{a}
	3%	29.49 ± 0.06^{c}	96.50 ± 0.58^{b}	69.22 ± 1.04^{b}	38.31 ± 1.47^{ab}
	5%	29.76 ± 0.14^{b}	93.50 ± 0.58^{c}	70.21 ± 1.23^{a}	37.39 ± 1.08^{c}
Old	1%	29.35 ± 0.11^{cd}	95.75 ± 0.96^{b}	68.82 ± 1.52^{bc}	38.52 ± 0.37^{a}
	3%	29.82 ± 0.09^{b}	93.25 ± 1.26^{c}	69.36 ± 0.45^{ab}	37.44 ± 0.85^{c}
	5%	30.22 ± 0.06^{a}	92.25 ± 2.06^{d}	70.74 ± 2.04^{a}	37.15 ± 1.49^{d}

Notes: Means of 4 repetitions ± standard error. The same symbol shows the insignificant difference on Tukey's HSD test α=0.05.

The color value (L*, a*, b*) of dough with different maturity and addition levels ranged from 51.49 to 72.18, from -1.54 to -0.51, and from 24.51 to 28.25, respectively. Table 2 shows that there is a significant difference ($P \leq 0.05$) in the color value including L* (brightness), a* (greenness-redness), and b* (blueness-yellowness). The greenest Pandan steamed bun dough was obtained from the addition of mature PLP and was greener with a higher addition level (5%). The chlorophyll in PLP can give the steamed bun dough a green color. In addition, the powder produced from old leaves has a higher chlorophyll content than the younger ones, so the color of the dough that will be greener.

When PLP's concentrations were increased from 1–5% of the flour, all the color values of the dough were significantly different from the control dough (without PLP addition). The increase of PLP's concentration up to 5% reduced the L* (brightness) and also a* (redness) values significantly, otherwise increased the b* value significantly. The results were similar to Ning et al. [26] comparative study about the effect of powdered green tea on the quality of Chinese steamed bun. This research shows that the steamed bread's brightness value decreased after adding green tea powder from 1 to 4% according to control with whole wheat flour.

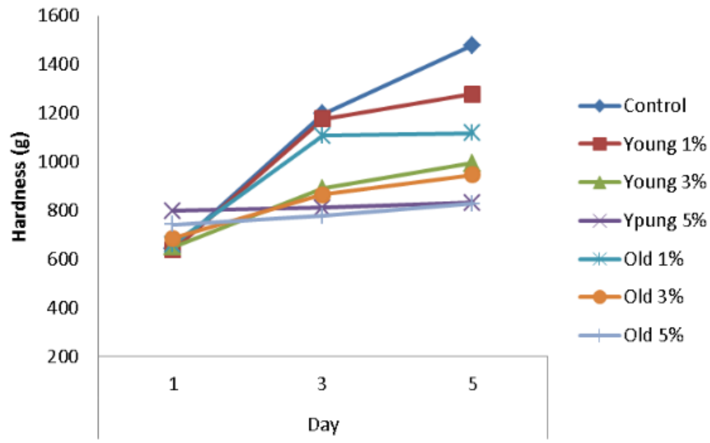
Table 3 shows that the use of PLP from 2 different types of maturity had a significant impact on the steamed bun's weight, volume, diameter, and also height. However, the differences in the level of PLP addition from 1-3% significantly affected volume, diameter, and height but did not significantly affect the weight of the steamed bun. There was a general trend indicating a decline in steamed bun volume and height also increased the diameter of the steamed bun with increasing PLP levels. The reduction of the steamed bun's volume with the higher addition of PLP was caused by the dilution of gluten components and reduced the interactions between fiber, water, fat, and gluten [27].

PLP was known to contain 6.62-11.55% of soluble fiber and 46.60-51.13% of insoluble fiber, which could profoundly affect steamed bun results. According to Anil's [28] studies, this possibly caused a weakening of the structure of the dough and it reduce carbondioxide gas retention during the proofing process. The higher fiber content competes for bonding the water during steamed bun making. Limited water available for the development of the starch-gluten network was affected by the deterioration of the gluten network and it produced bun with lower loaf volume [29]. The reduction of loaf volume also affects the height reduction and increases the diameter of the steamed bun. Ning et al. [26] reported that bread with higher fiber content and antioxidant activity will produce pan bread with a smaller volume. It is caused by the weakening of gluten strength in the dough due to the competition with fiber.

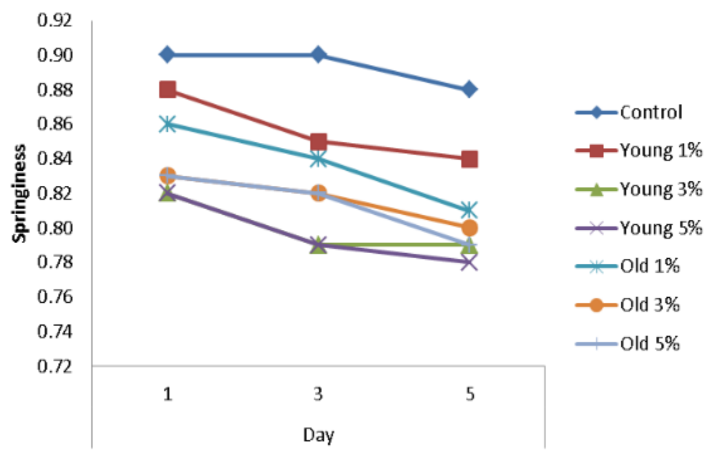
3.3. Steamed Bun Textural Changes during Storage

The steamed bun's textural characteristics are a critical quality affecting consumer preference and acceptability. The textural parameter of the steamed bun including hardness, cohesiveness, springiness, gumminess, chewiness, and resilience was used to determine the bread product quality. The hardness parameter is the key to the representation of bread quality. The higher hardness value of the steamed bun indicates a decreasing in quality.

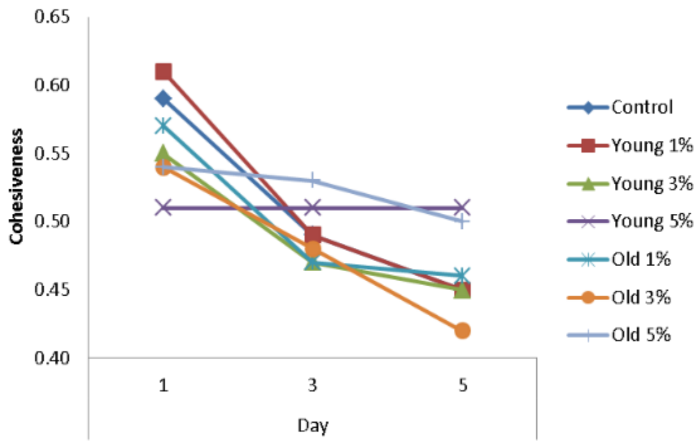
Staling has a negative correlation with bread quality. Increasing crumb hardness, chewiness, and gumminess during storage indicated the bread stalling that had negatively impact on bread's quality [30]. The textural properties of steamed buns with different levels of maturity and concentration during storage showed in Figure 1.



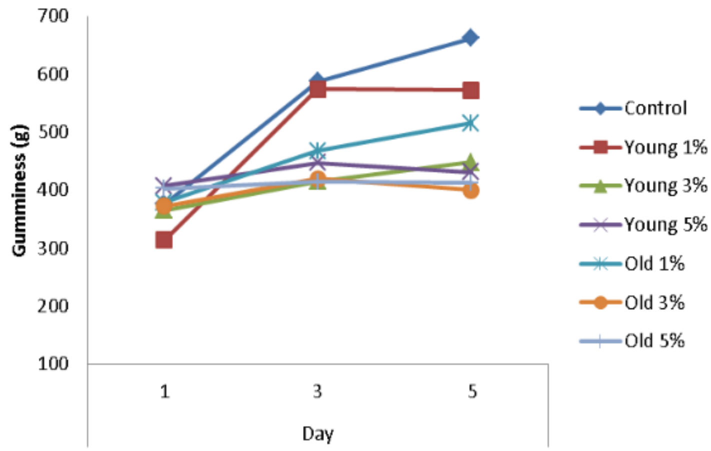
(A)



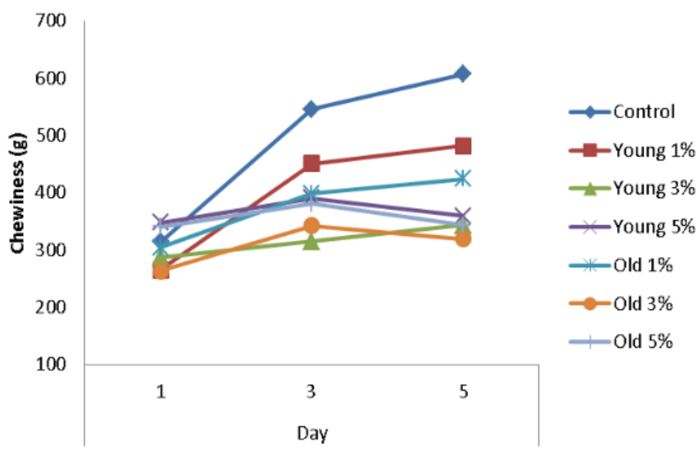
(B)


0.65
0.60
0.55
0.50
0.45
0.40
Cohesiveness
1
3
5
Day
Control
Young 1%
Young 3%
Young 5%
Old 1%
Old 3%
Old 5%

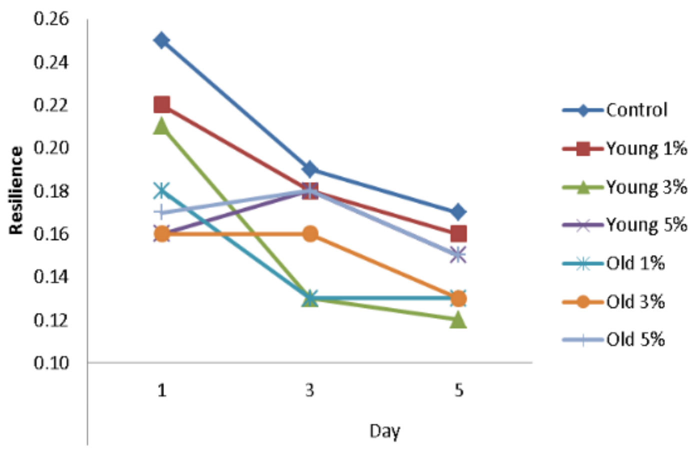
(C)


700
600
500
400
300
200
100
Gumminess (g)
1
3
5
Day
Control
Young 1%
Young 3%
Young 5%
Old 1%
Old 3%
Old 5%

(D)


700
600
500
400
300
200
100
Chewiness (g)
1
3
5
Day
Control
Young 1%
Young 3%
Young 5%
Old 1%
Old 3%
Old 5%

(E)



(F)

Figure 1. The effect of leaf maturity, concentration of PLP on (A) hardness, (B) cohesiveness, (C) springiness, (D) gumminess, (E) chewiness, and (F) Resilience of pandan steamed bun during storage

There was an increase in hardness, gumminess, and chewiness and a decrease in cohesiveness, springiness, and resilience along with the increase in the PLP addition. In general, increased hardness, gumminess and chewiness value, in conjunction with decreased springiness, cohesiveness, and resilience value was observed in both steamed buns made with mature PLP and young PLP during the storage time. This is probably because the addition of PLP with high fiber content replaces part of the gluten in wheat flour, gluten and fiber compete for water, resulting in the prevention of a three-dimensional network structure formation, resulting in an increased the hardness of bakery products [31,32]. The changes during storage were less pronounced in the steamed buns with the addition of 5% PLP and were not too significant from the 1st day to the 5th day compared to the control and steamed bun with the addition of 1% PLP. Majzoobi et al. [33] reported that adding powdered tomato pomace with a concentration 1 to 7% w/w flour basis could reduce the starch retrogradation during storage and further retarding bread staling. Tomato pomace powder contains the hydrocolloid that can avoid the bonding between starches [33]. On the other hand, a decrease in the bread quality in terms of texture profile like an increase in hardness, and a decrease in loaf volume probably occur. Adding another fiber source has also been reported to improve the shelf life of resultant bread. However, according to the control, the increase in steamed bun hardness during storage is slower in a steamed bun with high PLP content. There are two processes that are linked to breadcrumb hardening during storage [34]. First, process redistribution of moisture from proteins and starch to the amylopectin crystals. Second, the recrystallization of amylopectin during bread storage [34].

Cohesiveness, springiness, and resilience are also representations of bread texture quality. After adding powder with high fiber content, bread's cohesiveness, springiness, and resilience tend to decrease with the increased substitution significantly. This result was similar to Ning et al. [26] comparative study about the effect of powdered green tea on the quality of Chinese steamed bun. Adding green tea powder from 1 to 4% tends to decrease the resilience value. Other studies from Shiau et al. [35] show that adding pineapple core fiber into mantou dough could reduce the cohesiveness and springiness value. During the storage period, the steamed bun's cohesiveness, springiness, and resilience tend to decrease for both types of PLP. But the decrease that occurred in adding 5% PLP showed the slowest speed compared to the control and the addition of PLP as much as 1-3 %. As storage time went by, the water content reduced, the gluten network structure was broken as well as denser in the steamed bread. Besides, the hardness, gumminess and chewiness of the steamed bread increased. Due to the breakage of the gluten network structure, the gas holding capacity impaired, the elasticity, cohesiveness and recovery reduced [36].

4. Conclusions

Pandan leaves powder from different maturity levels of fresh pandan leaves had different physicochemical characteristics of the powder. Older leaves produced PLP with higher ash, fiber, WAC, OAC, total chlorophyll, total phenolic, antioxidant, darker green color, and lower moisture content and pH. The results showed that the pandan leaves age and PLP concentrations had a significant effect on the water content and color of the dough, physical characteristics (weight, volume, diameter, & height) and texture profile (hardness, springiness, cohesiveness, gumminess, chewiness, & resilience) of steamed bun. The old pandan leaves and higher concentration of PLP significantly increase the green color of the dough and bun, the weight and diameter of a bun, but reduce the height and volume of a bun. It also impacts the increase in the hardness of the steamed bun texture. The longer storage time of steamed bun significantly reduces the size of steamed bun, but will increase its hardness.

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