Physical properties and cellular structure of bread enriched with pumpkin flour

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Abstract. Production of a variety of bread is driven by the consumer interest in bringing a unique and healthier bread to the table. This study was aimed to evaluate the effect of partial substitution of pumpkin flour on physical properties and cellular structure of pumpkin bread. Pumpkin breads were made by partially substituting wheat flour with 5% to 20% of pumpkin flour. The physical properties and cellular structures of breads were evaluated. Breads specific volume decreased significantly at 10% and at higher level of pumpkin flour enrichment. Pumpkin flour did not affect the texture of enriched bread compared to that of control breads. The lightness of crumb decreased significantly with 10% of pumpkin flour enrichment. Inversely, the redness and yellowness increased significantly at the level of enrichment of 5% and 10%, respectively. Pumpkin flour enrichment affected significantly the cellular structured of enriched breads except of cell density. Mean cell area of enriched breads was smaller than that of control breads. At the level of 20%, pumpkin flour enrichment increased significantly the cell area of enriched bread. We suggest the use of pumpkin flour less than 10% in manufacturing pumpkin enriched bread without compromising its physical properties. At higher level, the pumpkin flour enrichment can be done to improve the cellular structures of bread.

1. Introduction
Pumpkin is a food that is rich in dietary fiber, especially pectin, functional compounds, vitamins (A, B6, K, C, E) and minerals (K, Mg, P, Se and Fe). High food fiber content in the form of pectin can control serum insulin levels, lower blood sugar levels, increase glucose tolerance and combating of various diseases such as heart attack, diabetes, high-blood pressure, constipation and colon cancer [1;2;3].

Public awareness to consume healthy bread continues to increase along with the high incidence of diseases caused by advanced civilization such as coronary heart disease, obesity, and diabetes. This encourages the development of the bread industry for certain purposes such as high nutritious bread and for diets [4]. Some studies were reported on the use of ingredients other than wheat flour for certain purposes include; the influence of fungal mycelial supplementation on the quality of bread [5], the effect of the use of inulin soluble fiber and beta-glucan oats on the quality of bread [6], the effect of adding Jerusalem artichoke flour to the quality of bread [7], and the effect of adding pumpkin flour to the volume and quality of organoleptic bread [8].

Previously Ptitchkina et al. [8] reported that adding pumpkin flour gave a very large increase in the volume and organoleptic characteristics of the bread produced. Based on this, the addition of pumpkin flour in bread making is very promising to improve its quality. Bread making with the addition of pumpkin flour which has been optimized for the functional component content has never been done to date. Therefore, in this study, we made breads partially...
substituted with pumpkin flour at different concentrations. The objective of this study was to evaluate the effect of partial substitution of pumpkin flour on the physical properties and cellular structure of bread.

2. Materials and Methods

2.1 Materials
The yellow pumpkin used was *bokor* or *cerme* cultivar with flat rounded shape having orange yellowish color and does not rotten, obtained from Jember area. Other ingredients were wheat flour (high protein contents), sugar, salt, butter, yeast (Saf-Instant), water, and xanthan gum.

2.2 Methods

2.2.1 Bread making procedure

Bread formula is presented in Table 1. Bread making was performed by substituting wheat flour with pumpkin flour (PF). PF substitution was done at concentration of 0, 5, 10, 15 and 20%, based on the weight of wheat flour. Bread formulations are shown in Table 1. A bread maker (National SD-BT102, Panasonic, Osaka, Japan) was used to bake breads using 4-h bread making setting. Baking was performed three times (triplicate). Before analysis, the bread loaves were placed at room temperature (28–30 °C) for tempering.

Table 1. Bread formulations

| Ingredients        | Sample  |
|--------------------|---------|
| Wheat flour (g)    | C% 280  |
|                    | PF5% 266|
|                    | PF10% 252|
|                    | PF15% 238|
|                    | PF20% 224|
| Pumpkin flour (g)  | 0      |
|                    | 14     |
|                    | 28     |
|                    | 42     |
|                    | 56     |
| Xanthan gum (g)    | 1.4    |
|                    | 1.4    |
|                    | 1.4    |
|                    | 1.4    |
|                    | 1.4    |
| Sugar (g)          | 16.8   |
|                    | 16.8   |
|                    | 16.8   |
|                    | 16.8   |
| Salt (g)           | 5.6    |
|                    | 5.6    |
|                    | 5.6    |
|                    | 5.6    |
| Water (mL)         | 200    |
|                    | 200    |
|                    | 200    |
|                    | 200    |
| Yeast (g)          | 2.8    |
|                    | 2.8    |
|                    | 2.8    |
|                    | 2.8    |

2.2.2 Physical properties analysis

Specific volume of bread was measured using the seed displacement method [9]. The crust and crumb colors were determined by using color reader (CR-400, Konica-Minolta, Tokyo, Japan); Hunter’s values were then analyzed (lightness, redness, yellowness) from six regions of bread crust and crumb (Wahyono et al., 2015). The bread texture was analyzed using texture analyzer. The flat probe of Warner-Bratzler with a 3.2 mm thickness aluminum blade was used to test the level of hardness of bread.

2.2.3 Bread crumb image analyses

The ImageJ software (1.47v, National Institutes of Health, Bethesda, MD, USA) was used to analyze the cellular structures of the bread crumb. Then, mean cell area, cell area relative distribution, and the fraction of cell area to total area were calculated. The crumb images were captured using a scanner (Epson Perfection V370 Photo, Epson, Suwa, Japan) at a resolution of 600 × 600 dpi. To reflect the actual size, crumb’s image was calibrated using a known scale and cropped to 60 × 60 mm. Image processing was done using a bandpass filter. To produce binary images, convert to mask feature was used for differentiating the cell and non-cell area. The threshold was adjusted using a black and white and a dark background. To analyze particles (cells), the particle size was adjusted from 0.01 mm² to infinity and the circularity was set from 0 to 1 [7]. The results were then tabulated and calculated using Microsoft Excel 2007v.

2.2.4 Statistical analysis

Statistical analysis was performed using SPSS for windows (ver. 19, SPSS, Inc., Chicago, IL, USA).
Statistical significance among the data were analyzed using one-way analysis of variance. Then, Duncan’s multiple range test (DMRT) at p < 0.05 was performed to compare means between samples.

3. Results and Discussion

3.1 Physical Properties

Specific volume of bread indicates the development of bread dough after baking. The greater the specific volume value indicates the more inflated bread mixture after baking. The effect of Pumpkin Flour to the specific volume of enriched bread is presented in Table 2. Based on the statistical analysis showed that pumpkin flour enrichment had a significant effect (P < 0.05) on the specific volume of enriched bread.

| Sample | Specific Volume (cm$^3$/g) | Texture (N) |
|--------|-----------------------------|-------------|
| C      | 6.98 ± 2.54$^a$             | 16.34 ± 3.14$^a$ |
| PF5    | 4.92 ± 1.04$^{ab}$          | 13.99 ± 2.35$^a$ |
| PF10   | 2.78 ± 0.51$^b$             | 14.15 ± 1.51$^a$ |
| PF15   | 2.92 ± 0.23$^b$             | 14.74 ± 1.01$^a$ |
| PF20   | 2.17 ± 0.36$^b$             | 15.8 ± 1.02$^a$ |

Values presented in table are the mean of the three replications ± Standard Deviation. Values presented in column with different letters imply statistically differences (p<0.05), as determined by DMRT test.

Increased level of pumpkin flour causes a decreased in specific volume of enriched bread. The highest specific volume (6.98 ± 2.54 cm$^3$/g) was obtained in bread without enrichment of pumpkin flour. Whereas, the lowest specific volume (2.17 ± 0.36 cm$^3$/g) was obtained by adding 20% of pumpkin flour. Enrichment of 5% pumpkin flour resulted in a specific volume of 4.92 ± 1.04 cm$^3$/g which was not significant compared to that of control bread (6.98 ± 2.54 cm$^3$/g). See et al. [10] reported that increased level of pumpkin flour in bread making decreased the specific volume. Adding pumpkin flour as much as 5% significantly increased the specific volume of bread compared to that of the control bread. It was suggested that water content was the main cause of an increase in the volume of bread (loaf volume).

The higher level of pumpkin flour reduced the specific volume of enriched bread. The addition of pumpkin flour weakened the gluten-starch matrix which can further reduce gas retention in the dough. The reduced ability of gas retention caused a decrease in the specific volume of bread [11]. In addition, the presence of pumpkin flour might increase solid-like behavior that can inhibit the expansion of dough during fermentation and in turn decreases the specific volume of bread. Peressini and Sensidoni [12] found a decrease in bread dough added with inulin. Similarly, Wahyono et al. [7] reported a decrease in the specific volume of bread with the addition of higher level of Jerusalem artichoke powder.

Based on the Table 2 shows that the enrichment of pumpkin flour had no significant effect (P> 0.05) on the texture of enriched bread. Thus, there is a chance to enrich the bread with a greater level of pumpkin flour without compromising its texture properties.

Enrichment of pumpkin flour had no significant effect (P> 0.05) on lightness (L), redness (a) and yellowness (b) of crust of enriched bread (Table 3). In addition, enrichment of pumpkin flour did not significantly affect the lightness (L) of crumb of enriched bread, but significantly increased the redness (a) and yellowness (b) as presented in Figure 1. Increased of redness compared to the controls bread occurred when higher level (>5%) of pumpkin flour was added. While the yellowness (b) increased significantly when 10% of pumpkin flour was added. See et al. [10] reported that adding pumpkin flour significantly increased (P <0.05) the redness and yellowness of bread crumb. This is presumably due to the addition of pumpkin flour which has a high value of redness and yellowness. Color is an
important attribute for bread products for consumers. Crust color is depending on the physical and chemical characteristics of the mixture such as water content, pH, amino acid content and reducing sugar. Besides that, it is also influenced by regulation during bread baking such as temperature, airspeed, relative humidity, and heat transfer type [13].

![Figure 1. Color of bread’s crumb made with different level of pumpkin flour (PF)](image)

| Sample | Crust color | Crumb color |
|--------|-------------|-------------|
|        | L    | a    | b    | L    | a    | b    |
| C      | 50.01 ± 4.72 | 27.87 ± 22.06 | 33.42 ± 2.86 | 73.35 ± 1.70 | 0.87 ± 0.64 | 21.66 ± 1.48 |
| PF5    | 50.53 ± 6.68 | 17.27 ± 0.55 | 38.41 ± 5.76 | 69.02 ± 3.09 | 5.27 ± 0.32 | 41.81 ± 5.17 |
| PF10   | 55.35 ± 7.84 | 15.47 ± 0.97 | 47.33 ± 7.26 | 62.90 ± 1.64 | 9.68 ± 0.84 | 50.95 ± 9.36 |
| PF15   | 43.31 ± 1.83 | 18.34 ± 0.64 | 33.82 ± 0.84 | 59.96 ± 1.55 | 11.94 ± 1.5 | 53.65 ± 9.74 |
| PF20   | 47.17 ± 2.83 | 18.44 ± 1.19 | 41.43 ± 6.89 | 57.52 ± 1.76 | 14.02 ± 1.06 | 55.45 ± 9.43 |

Values presented in table are the mean of the three replications ± Standard Deviation. Values presented in column with different letters imply statistically differences (p<0.05), as determined by DMRT test.

3.2 Cellular Structures
Bread is manufactured in random physical processed resulting in a heterogeneous cellular structure. That heterogeneous structure is considered to affect the mechanical behavior of bread’s crumbs. The contribution of cellular features to bread quality can be quantified by using image analysis, [14].

Based on the Table 4 it was observed that PF substitution significantly decreased (P<0.05) cell density and cell area fraction of resulting bread but no significant effect was observed in cell density. Cellular structure of bread’s crumb enriched with pumpkin flour is shown in Figure 2. Incorporation of other ingredients into wheat bread tend to decrease mean cell area and cell area fraction. Previously, we found that incorporating Jerusalem artichoke powder into wheat bread significantly decreased mean cell area and cell area fraction of bread’s crumb. It has been reported that physical texture and the cellular structure of bread crumb are strongly correlated. The two parameter are related quality factors that should be considered as a single entity [15]. Pyler [16] suggested that the crumb feels to the mouth is strongly associated with the cell structure of the crumb. The crumb having finer, thin-walled, uniform cells size produced a softer and more elastic texture than that of the coarse and thick-walled cell structures.

![Figure 2. Cellular structure of bread made with various level of pumpkin flour (PF)](image)
Table 4. Crumb cellular structure of bread made with pumpkin flour (PF)

| Perlakuan | Cell Density (1/cm²) | Mean cell area (mm²) | Cell Area Fraction (%) |
|-----------|----------------------|----------------------|------------------------|
| C         | 38.88±6.7           | 0.93±0.1             | 35.75±3.5             |
| PF5       | 43.09±4.3           | 0.75±0.0             | 32.36±1.5ab           |
| PF10      | 42.76±3.8           | 0.74±0.1             | 31.09±1.1ab           |
| PF15      | 43.12±2.2           | 0.72±0.0             | 31.10±3.0ab           |
| PF20      | 48.89±12.0          | 0.63±0.1             | 30.12±3.1b            |

Values presented in table are the mean of the three replications ± Standard Deviation.
Values presented in column with different letters imply statistically differences (p<0.05), as determined by DMRT test.

4. Conclusions

Pumpkin flour was significantly affect the physical properties and cellular structure of enriched bread. Increased level of pumpkin flour decreased the specific volume of enriched bread. The redness and yellowness of bread’s crumb increased in line with the level of pumpkin flour enrichment. But, no effect was observed on the color of bread’s crust. Mean cell area and cell area fraction decreased as the level of pumpkin flour increased. Thus, we suggest the use of pumpkin flour less than 10% in manufacturing pumpkin enriched bread without compromising its physical properties. At higher level, the pumpkin flour enrichment can be done to improve the cellular structures of bread.

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