PAPER

TEXTURAL, PHYSICAL AND RETROGRADATION PROPERTIES OF MUFFIN PREPARED WITH KAMUT (*TRITICUM TURANICUM* JAKUBZ)

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ABSTRACT

The effects of kamut flour substitution levels (0%, 25%, 50%, 75%, 100%) on muffin properties were investigated. As kamut flour level increased muffins showed lower height and volume and the L value decreased. The crumb hardness increased with increasing kamut level, and the control showed the lowest value in elasticity, chewiness, and brittleness. The increment of kamut flour level resulted the total flavonoid and polyphenol contents, reducing power, and ABTS and DPPH radical scavenging activities. During storage, the avrami exponent decreased between the control to the sample added with 75% kamut. The crumb air cell number decreased, but the area increased with kamut flour level increment. In sensory evaluation, the samples with kamut level 25% and 50% were acceptable. Therefore, muffins with an appropriate level of kamut flour improve the nutritional profile, and quality of baking products.

Keywords: antioxidant, kamut (Khorasan wheat), muffin, physical properties

1. INTRODUCTION

In human nutrition, wheat is an important component of cereal-based foods, and it is one of the most consumed food sources on a global scale (SOFI *et al*., 2013). As the awareness of healthy life increases, healthy food products are more in demand. Following this trend, various wheat varieties such as whole wheat, organic wheat, and ancient wheat, have emerged in the market (ANGIOLONI and COLLAR, 2011; FATMA *et al*., 2017; DINU *et al*., 2018). Among the wheat varieties, kamut (Khorasan wheat, *Triticum turanicum* Jakubz) is one of the noted ancient grains due to the higher content of selenium content and the protein content. Kamut contains 400-1000 ppb of selenium depending on harvesting condition and contains relatively large amounts of 12-18/100 g of protein (WIJNGAARD and ARENDT, 2006; DI LORETO *et al*., 2017). In addition, consuming whole grain and the products from its derivative could be providing antioxidant substances and various cofactors such as Copper, Iron, Zinc and Selenium (BENEDETTI *et al*., 2012).

In recent studies (BORDONI *et al*., 2017; CARNEVALI *et al*., 2014; DI LORETO *et al*., 2017), the whole grain kamut was found to contain higher phytochemical contents than common wheat, and it has been attracting attention due to its nutraceutical properties such as high antioxidant, prebiotic activities, and reduction of irritable bowel syndrome symptoms. In addition, in human intervention studies, a volunteer group that consumed kamut products for 8 weeks demonstrated a significant decrease in total and LDL cholesterol and glucose levels while the control group showed no significant changes.

The popularity of bakery products, especially with health functionalities, is increasing and as the consumption of cereal-based products increase, these products are important for taking essential nutrients in daily life (ALPASLAN and HAYTA, 2006). Among the bakery products, muffins are easy to make into various products depending on the ingredients to be added, so the studies on functional muffins such as legume blended muffin, coffee ground residue water extracts muffin, flaxseed muffin and buckwheat muffin (KIM *et al*., 2016; BAE and JUNG, 2013; KAUR and KAUR, 2018; QIAN *et al*., 2017) are briskly. Also, muffins have high acceptance for the consumer due to sweet taste and soft texture and are characterized by typical pore formation.

Previous studies on kamut have been on antioxidant effects of kamut in the rat liver, sourdough bread, flake and muesli, tortillas and cookies (BENEDETTI *et al*., 2012; CARINI *et al*., 2010; CHANDI *et al*., 2015; CHOI *et al*., 2016; SUMCZYNSKI *et al*., 2015). The present study, therefore, focused on antioxidant, baking, rheological, microstructural, storage and quality characteristics of muffins with whole grain kamut and with the aim to find the optimal addition level of kamut for increasing utilization of kamut and development of functional bakery products.

2. MATERIALS AND METHODS

2.1. Muffin materials

Kamut (KAMUT® International, Ltd., Missoula, USA) cultivated in Canada in 2016 was purchased. Kamut (Khorasan wheat) grains were washed three times and freeze-dried (FD8508, Ilshinbiobase Co., Dongducheon, Korea) at -80℃ for 5 days. Dried kamut grains were ground (RT-04, Wongangbio Co., Taiwan) for 2min, passed through a 40 mesh sieve twice to obtain kamut flour (KF), and stored at -20℃ until use. A soft wheat flour (WF) (Q1, Samyang Co., Asan, Korea), salt (Chungjungwaon, Shinan, Korea), sugar (Beak-seol, Incheon, Korea), egg (Nature egg, Yeo-ju, Korea), butter (Unsalted Pure New Zealand Butter, Fonterra, New Zealand), milk (Seoul milk, Chung-ju, Korea), and baking powder (Baking soda, Gimpo, Korea) were acquired at a local market.

2.2 Preparation of muffin samples

The muffin formulations are presented in Table 1. Muffins were prepared using a modified method as described by QIAN *et al*. (2017). Five different muffin samples with various ratio of KF and WF [100 WF (CON), 75 WF:25 KF (K25), 50 WF:50 KF(K50), 25 WF:75 KF(K75), 100 KF(K100)] were prepared along with a control sample (100 WF). Butter, salt and sugar were whipped with a blender (KMM020, Kenwood, Havant, England) for 3 min at 40 rpm. The egg was added in two portions and mixed for 5 min at 54 rpm. Flour mix and baking powder were added and mixed at 40 rpm for 2 min. Finally, milk was blended at 40 rpm for 2 min. The muffins were cooled for 1 h at 25℃ after baking and were used for analyses.

Table 1. Formula for a muffin with different levels of KF.

Samples	CON	K25	K50	K75	K100
Wheat flour (g)	200	50	100	150	0
Kamut flour (g)	0	150	100	50	200
Salt (g)	2	2	2	2	2
Sugar (g)	120	120	120	120	120
Egg (g)	70	70	70	70	70
Butter (g)	80	80	80	80	80
Milk (g)	100	100	100	100	100
Baking powder (g)	6	6	6	6	6

CON: Control. Without added KF. K25: 75% WF, 25% KF. K50: 50% WF, 50%KF. K75: 25% WF, 75% KF. K100: 100% KF.

2.3. Physicochemical measurement

2.3.1 Moisture and Brix degree

The moisture content was measured at 105℃ in 5.0 g of crumb parts of muffins with a moisture analyzer (MB, OHAUS, Zurich, Switzerland). The sugar content was measured with a digital refractometer (PR-201 α, Tokyo, Japan) having a range of 0-60% by stirring 5.0 g of sample and 50 g of distilled water for 5 min (SONG *et al*., 2017).

2.3.2 Batter measurement

The batter specific gravity was measured at 25℃ by standard methods of analysis (AACC, 2000). The baking loss and baking yield were calculated according to the following formulas using the batter weigh.

Baking loss (%) = 〔(batter weight – muffin weight)/batter weight〕 × 100
Baking yield (%) = (muffin weight/batter weight) × 100

2.3.3 Physical properties

Muffin weight was measured with a digital scale (EB-2200HU, Dong-il Shimadzu Corp., Seoul, Korea). Muffin volume was measured by the method of seed displacement (Pyler, 1979). Specific volume (mL/g) was determined by dividing the muffin volume (mL) by muffin weight (g). Muffin height was measured as the vertical distance from the bottom to the top of the muffin center using vernier calipers.

2.3.4 Appearance

The color values of both crumb and crust were measured by a spectrophotometer (CR-400, Konica Minolta Co., Ltd, Tokyo, Japan). The color values were shown as lightness (L), redness (a), yellowness (b) and total color difference (‣E). The ‣E was calculated by the following equation. The appearance and cross section of muffins were captured by a digital camera (X-T20, Fujifilm, Tokyo, Japan).

$$\Delta E = \sqrt{(L_{\text{sample}} - L_{\text{standard}})^2 + (a_{\text{sample}} - a_{\text{standard}})^2 + (b_{\text{sample}} - b_{\text{standard}})^2}$$

2.4. Textural analysis of muffin crumb

Texture Profile Analysis (TPA) was performed on muffins at a 25℃. The samples (20 mm × 20 mm × 20 mm) were measured by a two-bite compression test using rheometer (Compac-100Ⅱ, Sun Scientific, Tokyo, Japan). In this measurement, the cylindrical probe (20 mm diameter) was mounted and operated at 1.0 mm/s. Hardness (N), springiness (%), cohesiveness (%), chewiness (g) and brittleness (g) were determined. Hardness refers to the maximum force with the maximum peak of the first compression. Springiness is the deformation rate between the first compression and the second compression, defined as the ratio of distances (d1: the maximum distance of the first bite; d2: the distance to the deformed sample surface in the second bite). Cohesiveness is the strength of internal bonds and defined as the ratio of area. Chewiness is calculated by multiplying the hardness value by the cohesiveness value. Brittleness, also called fracturability, is a measure of force at the first peak.

$$\text{Springiness (\%)} = d_2/d_1 \times 100$$
$$\text{Cohesiveness (\%)} = A_2/A_1 \times 100$$

2.5. Antioxidant activity properties

2.5.1 Antioxidant compound extraction

The muffins were freeze-dried at -80℃ for 48 h and ground for 1 min (40 mesh). The muffins were defatted with hexane at a ratio of 1:5 w/v (3 min, 3 times), dried at 45℃ for 5 h, and extracted in a water bath (BS-20, Jeio Tech, Seoul, Korea) at 185 rpm for 1 h at 40℃

with 70% methanol at a ratio of 1:10 w/v. The sample extracts were filtered using Whatman filter (No. 4) and kept at 4℃ for subsequent experiments.

2.5.2 Total polyphenols content

Total polyphenols content was determined by the Folin-Ciocalteu method. Briefly, 50 μL of 0.9 N Folin-ciocalteu's reagent (Merk KGaA, Darmstadt, Germany) and 150 μL of 20% sodium carbonate solution (Merck KGaA, Darmstadt, Germany) were added sequentially to the extracted samples (20 μL) mixed with distilled water (790 μL). After incubating for 2 h incubation at 25℃, the absorbance of the mixed sample was read at 700 nm using an ELISA microplate reader (Apollo11LB913, Berthhold Thechnolosies Co., Ltd., Bad Wildbad, Germany). The total polyphenol content (㎍ GAE/g) was converted to gallic acid equivalents.

2.5.3 Total flavonoids content

Total flavonoids content was examined by the method of ZHANG *et al*. (2017). Briefly, 150 μL of 5% sodium nitrie (Junsei Chemistry) was added to 1 mL of samples and incubated in the darkroom at 25℃ for 6 min. Subsequently, 0of 10% $AlCl_3$ was added to the mixed samples and incubated in the darkroom at 25℃ for 5 min. Finally, 1 mL of 1 N NaOH was mixed, and the absorbance of the sample mixture was read at 520 nm using an ELISA microplate reader (Apollo11LB913, Berthhold Thechnolosies Co., Ltd., Bad Wildbad, Germany). The total flavonoids content (㎍ QE/mg) was converted to quercetin equivalents.

2.5.4 Reducing power

Reducing power of the extracted samples was determined by a modified OYAIZU (1986) method. Briefly, 250 μL of 0.2 M phosphate buffer, the mixture of sodium phosphate monobasic solution and sodium phosphate dibasic solution (1:2), and 250 μL of 1% potassium ferricyanide solution were added to 250 μL of samples and incubated for 30 min at 50℃. Subsequently, 250 μL of 10% trichloroacetic acid solution was mixed. Finally, 500 μL of distilled water and 100 μL of 0.1% $FeCl_3$ were added to the sample supernatant (500 μL) and the absorbance of the sample mixtures was read at 700 nm using an ELISA microplate reader (Apollo11LB913, Berthhold Thechnolosies Co., Ltd., Bad Wildbad, Germany).

2.5.5 DPPH radical scavenging assay

DPPH assay refers to the method of JOUNG *et al*. (2017). The 200 µmol DPPH reagent was added to the diluted samples extracts (10, 12.5, 20, 25, 50, 100 mg/mL) and reacted in the darkroom at room temperature for 30 min. The absorbance of the sample mixtures was read at 520 nm using an ELISA microplate reader (Apollo11LB913, Berthhold Thechnolosies Co., Ltd., Bad Wildbad, Germany).

2.5.6 ABTS radical scavenging assay

The ABTS assay was measured with reference to the method of ZHANG *et al*. (2017). The ABTS reagent with the absorbance of 1.5 at 405 nm was added to the diluted samples (10, 12.5, 20, 25, 50, 100 mg/mL) and reacted in the darkroom at room temperature for 60 min. The absorbance of the sample mixtures was read at 405 nm using an ELISA microplate reader (Apollo11LB913, Berthhold Thechnolosies Co., Ltd., Bad Wildbad, Germany).

2.6. Air cells determination

Muffins were cut at the height of muffin mold (2 cm), and images of the bottom half were obtained using a digital camera (X-T20, Fujifilm, Tokyo, Japan). By pore size, air cell number and area were calculated using the ImageJ software (MARCET *et al*., 2015).

2.7. Microstructure of batter and muffins

2.7.1 Batter microstructure

A drop of batter was placed on a microscope glass slide and covered with a cover glass. The cover glass was used to apply constant force (1 kg) to equalize and thinly spread the batter layer. The batter samples were observed using a microscope (TS100, Nikon, Tokyo, Japan). The Infinity Capture V6.5.6 for Windows software was utilized with the Infinity lite camera (Lumenera, Ottawa, Canada).

2.7.2 Crumb microstructure

Scanning electron microscopic (SEM) studies were examined using the JSM-6701F (JEOL Ltd., Tokyo, Japan). Sample preparation for SEM was according to the modified method of SHIN *et al*. (2018). The crumb samples were freeze-dried (FD8508, Ilshinbiobase Co.), and pieces of samples (size 2 × 4 mm) were placed separately on aluminum specimen mount using Nem tape and conductive graphite (Ted Pella Inc., California, USA). Mounted samples were coated with Au using the JSM 670-1F (JEOL Ltd., Tokyo, Japan) at 10 mA for 2 min. Each sample was observed at 10 kV and 1.16×10^{-4} torr vacuum.

2.8. Retrogradation kinetics of Avrami model

Retrogradation of the muffin was analyzed with reference to BERSKI *et al*. (2018). Muffin samples were stored at 25℃ for 35 days, and hardness was measured on a rheometer (Compac-100Ⅱ, Sun Scientific, Tokyo, Japan) at the date of production and every week after preparation. According to the Avrami equation, the retrogradation status of muffins was measured by analyzing the alteration of hardness with the storage period. Avrami equation was as follows:

$$\theta = e^{-kt^n} \qquad (1)$$

Where θ is the amorphous part remaining after a certain time (t), k is the rate constant, n is the Avrami exponent, and t is the storage period.

The operating conditions for the rheometer were a TPA test using the cylinder probe No.1 (Φ 20 mm), 2 × 2 × 2 cm sample size, 120 mm/min table speed, 66.67% distance, and 10 kg max weight.

$$(E_L\text{-}E_t) / (E_L\text{-}E_0) = e^{-kt^n} \quad (2)$$

Where E_0 is the hardness of the initial period (t = 0), E_t is the hardness after a certain time (t) and E_L is the greatest hardness value to be reached theoretically (the hardness value of a muffin stored at 25℃ for 35 days).
The following equation was obtained by taking a common logarithm of equation (2) as:

$$\log〔- \ln (E_L\text{-}E_t) / (E_L\text{-}E_0)〕 = \log k + n \log t \quad (3)$$

Where n is the Avrami exponent (1 ~ 4 values, depending on the crystallization status), and k is rate constant.

2.9. Sensorial evaluation

The muffin samples were baked 3 h before sensory evaluation. The samples were divided into four parts and supplied with water to each panelist at once in a white plastic plate. The sensory evaluation was completed by 51 panelists from Korea University (age range 20-60). The panels evaluated the muffins based on their appearance, flavor, texture, taste and overall acceptability using a hedonic scale of 9 points (9 score is "I like very much" and 1 score is "I dislike very much").

2.10. Statistical analysis

The statistical analysis of results was completed using the SPSS (IBM SPSS Statistics 23, International Business Machines Corporation, New York, USA) program. Significant differences were assessed by one-way ANOVA (analysis of variance) followed by Duncan's multiple range test at a 95% significance level (P<0.05).

3. RESULTS AND DISCUSSIONS

3.1. Muffin physicochemical characteristics

The physicochemical features of muffins are shown in Table 2 (Fig. 1.) The results suggest that the addition of KF significantly affected the moisture content and brix degree (P<0.05). Muffin crumb moisture content was the highest in K100 (28.18%) and the lowest in CON (24.78%). Similar to the description by GURPREE *et al*. (2015), this is due to the water absorption of KF. With increasing KF replacement level, muffin moisture content tended to increase. As the result of comparing the water binding capacity of KF and WF, the water binding capacity of KF (225.09%) showed a higher value than that of WF (198.13%) (data not shown). As the water binding capacity results showed, the moisture content of muffins was significantly increased with the addition of KF. The sugar content of muffins was expressed in brix degree, which is the basic criterion to evaluate sugar contents. Muffin sugar contents diminished significantly (P<0.05) as the KF substitution increased. Due to the Maillard reaction, which is the reaction between the reducing sugar and the amino acids. The Brix degree represents the content of soluble solids, and the reducing sugar involved in the Maillard reaction is also included in the soluble solids. As the KF replacement level increased, the muffin lightness value decreased and became significantly darker, suggesting that the number of soluble solids was reduced by increasing the nonenzymatic browning reaction. According to a study by ZHANG *et al.* (2016), the brownish or yellowish color of bakery products with high sugar content were caused by the Maillard reaction, which is mainly found in the baking process. The baking loss significantly decreased with increasing KF substitutions (P<0.05). The lowest baking loss was found in K100 (10.67%). The baking yield was the lowest in CON (83.43%) and the highest in K100 (89.33%), and it significantly increased as the KF substitution ratio increased. The results are similar to the study of KOTOKI and DAKA (2010), suggesting that the water binding capacity affects the water content of muffin crumb, which may affect baking yield and baking loss. The specific gravity of the batter was the lowest in CON (0.94), and it significantly increases with the increased in KF substitution levels. The muffin height significantly decreased as the KF substitution rate rose in the batter (P<0.05). The CON muffin had the highest volume (121.83 mL) and specific volume (2.89 mL/g), and these were significantly decreased as the KF substitution ratio decreased. While the CON muffin showed the lowest weight (42.11 g), it significantly increased with the increasing KF substitution percentage.

The lightness (L), yellowness (b), redness (a), and color difference (‣E) values of both crust and crumb are shown in Table 3. Crust L, a and b were the lowest in K100, and the L and b values of crust were the highest in CON. The crust color was affected by the Maillard or caramelization reaction between proteins and sugar. Crumb L and b values decreased significantly, while the a value increased significantly as the KF substitution increased (P<0.05). The crumb color value was affected by the color of the raw material, which was not heated sufficiently to drive the Maillard or caramelization reaction (MARCHETTI *et al.*, 2018). The crust ‣E values of CON and K25 were lower than other samples. As the KF proportion increased, the crumb ‣E values increased significantly (P<0.05).

Figure 1. Photograph of muffins and cross section of muffin crumbs with different levels of KF.

Table 2. Some physicochemical properties of muffin with different levels of KF.

Samples	Moisture (%)	Brix degree	Baking loss (%)	Batter yield (%)	Specific gravity	Height (mm)	Volume (mL)	Specific volume (mL/g)	Weight (g)
CON	24.78±0.29$^{1)d}$	3.03±0.20^{a}	16.57±0.20^{a}	83.43±0.20^{d}	0.94±0.00^{e}	48.56±0.43^{a}	121.83±2.02^{a}	2.89±0.05^{a}	42.11±0.07^{d}
K25	25.95±0.17^{c}	2.63±0.06^{b}	14.22±0.28^{b}	85.78±0.28^{c}	0.95±0.01^{d}	47.24±0.42^{b}	117.5±1.50^{b}	2.72±0.03^{b}	43.16±0.09^{c}
K50	26.92±0.43^{b}	2.50±0.00^{bc}	11.85±0.03^{c}	88.15±0.03^{b}	0.96±0.00^{c}	47.26±0.47^{b}	114.23±0.76^{c}	2.58±0.02^{c}	44.45±0.02^{b}
K75	27.30±0.45^{b}	2.40±0.00^{c}	11.66±0.13^{c}	88.34±0.13^{b}	1.04±0.00^{b}	46.64±0.21^{b}	107.83±1.26^{d}	2.43±0.03^{d}	44.33±0.10^{b}
K100	28.18±0.22^{a}	2.33±0.06^{c}	10.67±0.09^{d}	89.33±0.09^{a}	1.04±0.00^{a}	45.3±0.33^{c}	90.33±1.26^{e}	2.01±0.03^{e}	45.04±0.05^{a}
F-value	46.567***	23.100***	591.725***	591.725***	616.542***	28.328***	226.868***	317.512***	782.063***

1)The data are mean±SD in triplicates.

a-eDifferent superscripts indicate there are significant differences between values in the same row according to Duncan's multiple range test at $p<0.05$.

*$p<0.05$, ***$p<0.001$.

Table 3. Color measurement of muffin crust and crumb with different levels of KF.

Samples	Crust				Crumb			
	L$^{2)}$	a	b	ΔE	L	a	b	ΔE
CON	48.51±0.44$^{1)a}$	8.99±0.05^{b}	15.21±0.06^{a}	50.90±0.54^{b}	78.51±0.29^{a}	-3.74±0.32^{d}	24.26±0.42^{a}	28.68±0.35^{e}
K25	47.78±0.10^{b}	8.75±0.16^{bc}	15.57±0.54^{a}	51.74±0.28^{b}	67.68±0.34^{b}	-1.02±0.12^{c}	23.05±0.27^{b}	36.42±0.50^{d}
K50	42.87±0.59^{d}	9.64±0.07^{a}	14.17±0.57^{b}	56.11±0.56^{a}	61.45±0.40^{c}	-0.79±0.09^{c}	22.02±0.82^{c}	41.63±0.31^{c}
K75	44.02±0.30^{c}	7.80±0.18^{d}	13.13±0.32^{c}	55.08±0.99^{a}	55.77±0.16^{d}	0.97±0.11^{b}	20.98±0.12^{d}	45.68±0.20^{b}
K100	43.67±0.40^{c}	8.53±0.21^{c}	12.85±0.75^{c}	56.03±0.23^{a}	52.04±0.32^{e}	1.57±0.07^{a}	19.89±0.22^{e}	49.45±0.36^{a}
F-value	125.283***	60.174***	17.172***	53.359***	3370.949***	455.629***	44.380***	1557.270***

1)The data are mean±SD in triplicates.

2)L: lightness, a: redness, b: yellowness, ΔE: total color difference.

a-e Different superscripts indicate there are significant differences between values in the same row according to Duncan's multiple range test at $p<0.05$.

*$p<0.05$, ***$p<0.001$.

3.2. Textural properties of muffin crumb

The texture of food products is a close factor to the body, such as the feeling of the mouth or fingers. The results of the muffin textural analysis prepared from the various ratio of WF and KF are presented in Table 4. The results showed that KF significantly affected the muffin crumb texture ($P<0.05$). Hardness was 1.15 N in CON and as the KF ratio increased, the hardness increased significantly ($P<0.05$), reaching K100 at 3.09 N. The increment in hardness is related to fewer and smaller air cells inside the muffin crumb, and it corresponds to the muffin volume, height, and weight. TESS *et al*. (2015) also mentioned that volume and hardness have an inverse relationship. Springiness is related to aeration and freshness of products, and especially in bakery products, high springiness has close relevance to high quality (MATOS *et al*., 2014). The K100 muffin showed the highest value in springiness, and an appropriate addition of Kamut flour seemed to have a good effect on the muffin textural quality. Cohesiveness is the parameter that signifies the perception linked to the energy required to bite the piece of food and sensory brittleness (SANZ *et al*., 2009). In comparison to CON, the other samples were significantly ($P<0.05$) decreased in cohesiveness, which suggests that energy was required for the second compression. The chewiness reflects the parameter associated with the energy required for biting activity from a solid form to a swallowable state (TESS *et al*., 2015). CON chewiness showed the lowest value at 0.54 N and it increased significantly ($P<0.05$) with the increasing KF ratio. K100 chewiness was approximately twice as high as that of CON. Brittleness is related to the muscle motion of biting food, and it has a correlation with hardness (PENG *et al*., 2002). Brittleness in CON was 56.57 g, and it significantly ($P<0.05$) increased, reaching 214.27 g in K100 and following a similar trend as hardness and chewiness. The previous study carried out by PASQUALONE *et al*. (2011) demonstrated that the crumb firmness of durum wheat bread was slightly lower than Kamut bread, but there was no significant difference.

Table 4. Textural profile analysis of muffin crumbs with different levels of KF.

Samples	Hardness (N)	Springiness (%)	Cohesiveness (%)	Chewiness (N)	Brittleness (g)
CON	1.15±0.12[1)c]	69.65±3.27[c]	67.03±2.63[a]	0.54±0.06[b]	56.57±9.74[e]
K25	1.40±0.15[c]	70.25±1.75[c]	61.56±0.24[b]	0.61±0.05[b]	84.04±3.08[d]
K50	1.73±0.25[c]	75.29±2.11[b]	59.92±0.92[b]	0.78±0.10[b]	110.62±2.52[c]
K75	2.39±0.30[b]	79.86±1.37[a]	54.46±1.48[c]	1.04±0.16[a]	150.87±3.54[b]
K100	3.09±0.60[a]	82.32±0.96[a]	49.17±0.55[d]	1.25±0.24[a]	214.27±3.24[a]
F-value	16.882[***]	21.252[***]	68.269[***]	13.309[**]	426.197[***]

[1)]The data are mean±SD in triplicates.

[a-e]Different superscripts indicate there are significant differences between values in the same row according to Duncan's multiple range test at p<0.05.

[**]P<0.01, [***]p<0.001.

3.3. Antioxidant properties of muffin crumb

The Kamut flour showed significantly higher antioxidant activities than wheat flour. The total polyphenol content was 23.44 μgGAE/mg in kamut flour and 17.74 μgGAE/mg in wheat flour. The total flavonoids content was 27.75 μgQE/mg in kamut flour and 8.44 μg QE/mg in wheat flour. In the results of reducing power, kamut flour (0.84) was about four times higher than wheat flour (0.21). DPPH and ABTS results also showed that kamut flour (129.91 μg/mL and 130.91 μg/mL respectively) had lower IC_{50} values than wheat flour (162.21 μg/mL and 325.79 μg/mL respectively) so that kamut flour had higher radical scavenging activities. SOFI *et al*. (2013) reported a comparison of antioxidant activities between Kamut flour and wheat flour, and Kamut was determined as superior in DPPH and Fe^{2+} chelation. In addition, polyphenols and flavonoids were higher in Kamut. The antioxidant properties of muffins are presented in Table 5. The total polyphenol content and total flavonoid content were determined in terms of gallic acid equivalent and quercetin, respectively. K100 (17.33 μgGAE/mg) was the richest in polyphenols, which was 2.62 times higher than CON. The total flavonoids content was also the highest in K100 (18.54 μgQE/mg), and it increased significantly as KF level increased (P<0.05). The replacement of WF with KF showed an increment in reducing power. The reducing power of extracts is regarded as an indicator of antioxidant activities (KAUR and KAUR, 2018). A significant increment in ABTS and DPPH radical scavenging activities of muffin crumbs was observed as the KF level increased (P<0.05). K100 showed a relatively lower IC_{50} in comparison to CON.

Table 5. Antioxidant activities of muffin crumbs with different levels of KF.

	CON	K25	K50	K75	K100	F-value
Polyphenols (μgGAE/mg)	6.61±0.06$^{1)e}$	8.07±0.12^{d}	10.45±0.13^{c}	15.30±0.20^{b}	17.33±0.08^{a}	3953.926***
Flavonoids (μgQE/mg)	9.61±0.25^{e}	11.77±0.00^{d}	12.80±0.11^{c}	16.75±0.33^{b}	18.54±0.14^{a}	987.048***
Reducing Power	0.23±0.00^{d}	0.22±0.00^{c}	0.32±0.00^{b}	0.36±0.00^{a}	0.36±0.00^{a}	9770.3***
DPPH (IC_{50}, μg/mL)	243.09±3.79^{d}	234.95±1.55^{c}	223.95±.6.67^{c}	224.90±0.87^{b}	213.47±3.48^{a}	25.945***
ABTS (IC_{50}, μg/mL)	263.08±2.90^{d}	237.89±3.58^{c}	223.68±1.91^{c}	219.92±6.32^{b}	207.34±0.22^{a}	104.716***

1) The data are mean±SD in triplicates.

a-e Different superscripts indicate there are significant differences between values in the same row according to Duncan's multiple range test at p<0.05.

*p<0.05, ***p<0.001.

3.4. Crumb air cells

Table 6 presents the number and area of air cells categorized by a particular size range. Cross section images of muffin crumbs are shown in Fig. 2. During the mixing process, pores are generated in the batter and they grow while baking as CO_2 production results.

Table 6. Air cell number and area in muffin crumbs with different levels of KF.

Samples	Air cells number						Air cells area				
	<1 pixel^2	1-10 pixel^2	10-100 pixel^2	100-1000 pixel^2	1000 pixel^2 <	Total	1-10 pixel^2	10-100 pixel^2	100-1000 pixel^2	1000 pixel^2 <	Total
CON	61	90	87	81	13	332	4.80±2.71	40.80±27.79	331.22±210.10	2336.69±1194.21	2713.51
K25	52	88	123	66	12	341	4.53±2.55	39.36±23.97	265.05±158.10	2230.58±1445.59	2539.52
K50	98	177	187	59	5	526	4.37±2.28	38.63±24.47	222.32±152.39	1723.6±907.82	1988.92
K75	132	214	257	55	2	660	4.29±2.26	33.77±22.74	224.58±113.84	1412.50±433.46	1675.14
K100	129	218	251	53	3	654	4.28±2.32	34.53±22.09	211.47±110.25	1171.33±152.32	1421.61

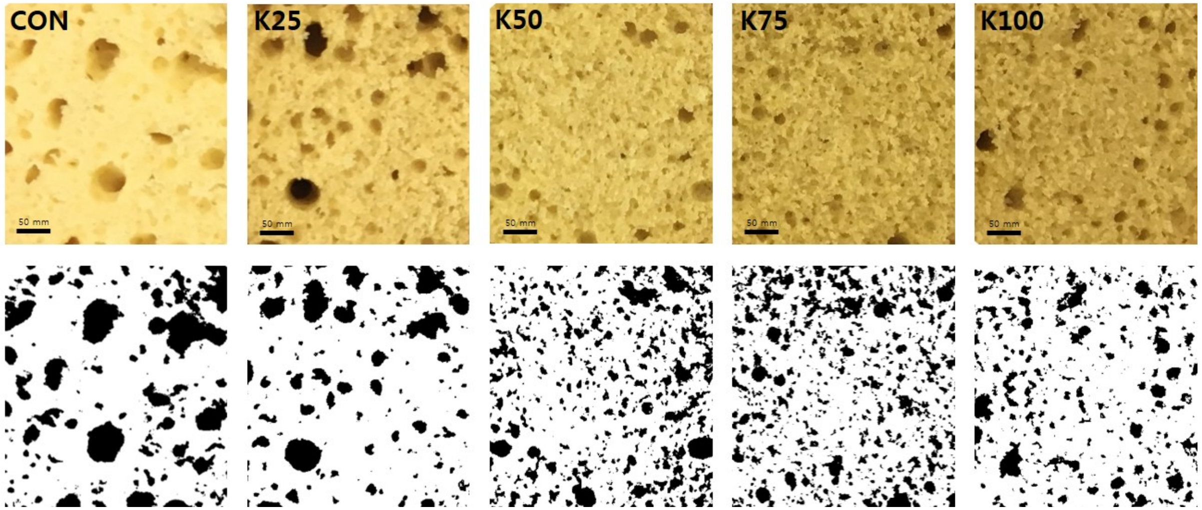

Figure 2. Cellular structure of Muffin crumb with different levels of KF. Top line: scanned images of the cross section of muffin crumb, bottom line: modified images using ImageJ.

As the substitution ratio of WF to KF increases, the number of pores with the size of 100 $pixel^2$ or less increased, whereas the number of pores decreased over a size of 100 $pixel^2$. The air cells area result showed the opposite tendency to the air cells number. As the KF substitution rate increased, average air cell size increased in all pore size classes. Therefore, substitution of WF by KF resulted in a tiny air cell and densely structured crumb. The size of air cells is an important factor affecting the texture of final bakery products (GIACOMOZZI *et al*., 2018). The air cells growing during baking affects the tender quality, which is related to the crumb hardness. CON showed a greater number of large air cells than other samples, which had weak crumb hardness. As mentioned earlier, the hardness increased as the pore size decreased and became dense.

3.5. Microstructure of batter and muffins

Batter microstructure is shown in Fig. 3. The micrograph of CON batter shows a uniformly spread air bubble and the air bubble size is relatively large and not concentrated in a particular area. In the former study by RAJIV *et al*. (2011), the control muffin batter showed an even and constant size of air bubble distribution. Micrograph of K25 batter shows relatively small-sized air bubbles appeared. The air bubbles of K25 batter showed closer formation and density compared to those of the CON batter. Micrograph of K50 batter exhibits both large-sized and small-sized air bubbles, and it does not form the uniform distribution. Compare to CON and K25 batter, the smaller air bubbles can be observed to stick together slightly in the K50 batter. The K75 batter micrograph shows unevenly distributed air bubbles, and the small-sized air bubbles are gathered around medium-sized air bubbles. Micrograph of the K100 batter shows an air bubble distribution similar to that of the K75 batter is observed, and the air bubbles are denser.
Fig. 4 presents the scanning electron micrographs. In Figs. 4A-1 (CON), 4B-1 (K25), 4C-1 (K50), 4D-1 (K75) and 4E-1(K100), the muffin crumbs are magnified 100 times, and the change of the pore size, distribution, and matrix surface can be observed. As the KF level increased, the small-sized pores gradually formed and the granular structure on the matrix surface became larger, such that the matrix appeared to be disconnected. Fig. 4A-2 shows the micrograph of the CON muffin crumb prepared entirely with WF, which shows gelatinized starch granules buried under the denatured protein matrix. The microstructure of starch granules embedded in a protein matrix is also described by GAO *et al*. (2018). LEE *et al*. (2001) reported that starch granules can be divided into two types, spherical and lenticular-shaped, which were also observed in the blended wheat flour dough. Fig. 4B-2 displays the micrograph of the K25 muffin crumb, and it shows fewer starch granules and smoother continuous matrix than that of CON. In Fig. 4C-2, which is the micrograph of the K50 muffin crumb, a rather rough and ruptured protein matrix is observed. RAJIV *et al*. (2011) reported that disruption of the protein matrix became greater when wheat was replaced by 60% of finger millet. In Fig. 4D-2, which exhibits the micrograph of the K75 muffin crumb, the gelatinized starch granules are large, and it appears to be coated. Fig. 4E-2 is the micrograph of the K100 muffin crumb, which shows greater and thin starch granules that seem to be entangled in a discontinuous protein matrix. Therefore, when the KF level is increased, the starch granules become greater, and continuity of the protein matrix is lost.

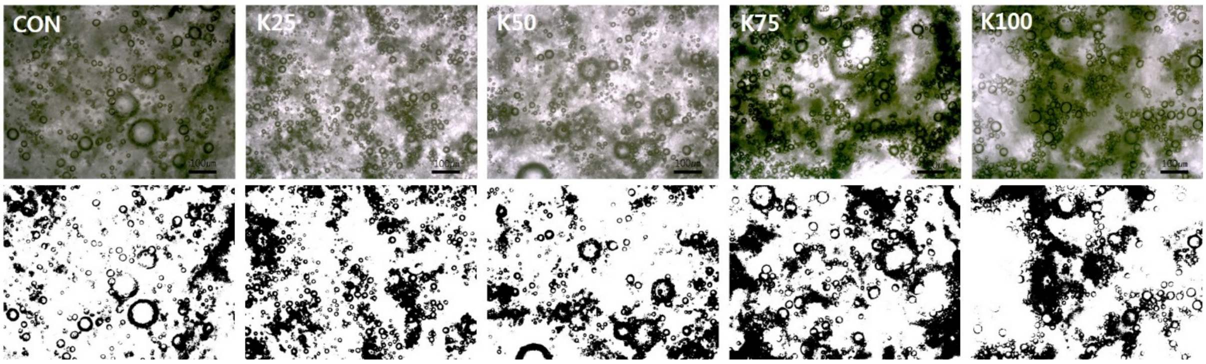

Figure 3. Batter microstructure (×100). Top line: micrograph of muffin batters CON, K25, K50, K75, K100, bottom line: modified images of batter CON, K25, K50, K75, K100 using ImageJ.

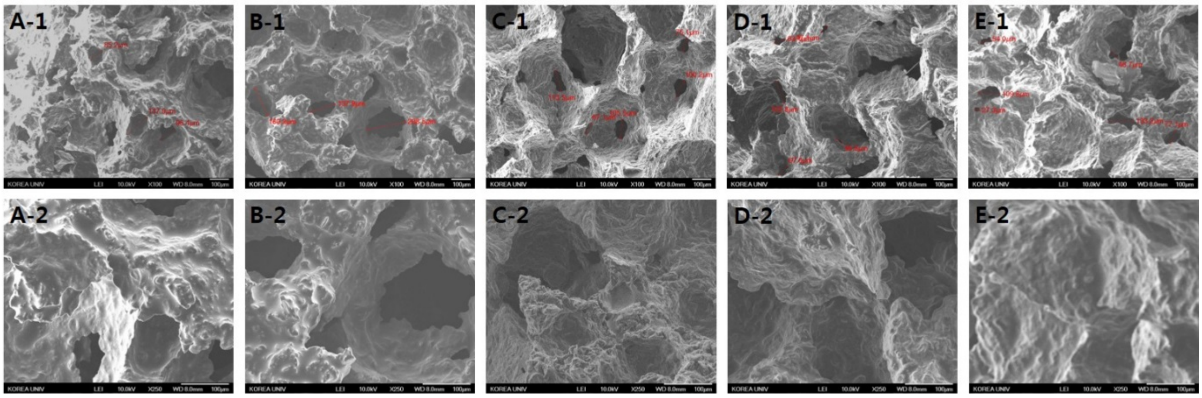

Figure 4. Scanning electron micrograph of muffin crumbs. Top line: SEM micrograph of magnification 100×. Bottom line: SEM micrograph of magnification 250×.

3.6. Retrogradation kinetics

The Avrami equation describes the retrogradation process kinetics and the result is shown in Table 4. Starch retrogradation is an important quality determinant in the staling of bakery products. Avrami exponent (n) indicates the value of nucleation in crystallization, and it depends on the growth rate of crystallites in short storage periods (COLLAR *et al*., 1999). The lower the Avrami exponent, the slower the crystallization rate, which is more effective for delaying retrogradation (KIM *et al*., 2006; S.-S. KIM and CHUNG, 2010; ZHANG *et al*., 2017). Crystallization is an important factor as it is related to the texture and shelf-life of a bakery product. The Avrami exponent showed the highest value in K100, but the value tended to decrease from CON (1.5540) to K75 (0.7669). The rate constant (k) refers to the retrogradation time. The reduction in the rate constant describes delays in retrogradation in the presence of carbohydrates. The rate constant tended to decrease with Kamut replacement from CON (1.8095) to K75 (1.2904), but the K100 (2.1541) showed a sharp increase. According to Avrami kinetic results, muffins prepared only with Kamut had similar values to the CON. CARINI *et al*. (2010) reported that whole Kamut tortillas were very similar to the control group in textural changes during the storage period.

Table 7. Avrami parameters for muffin crumbs with different levels of KF.

AVRAMI	Avrami exponent (n)	Rate constant (k)	R^2
CON	1.554	1.8095	0.9988
K25	1.3208	1.4036	0.9998
K50	1.1402	1.3113	0.9996
K75	0.7669	1.2904	0.9995
K100	1.5774	2.1541	1.0000

3.7. Sensorial evaluation

The sensory evaluation scores for appearance, flavor, texture, sweetness, and overall acceptability of muffins are presented in Table 8. The data showed that the sensory score for appearance, flavor, texture, sweetness, and overall acceptability decreased as the Kamut flour replacement level was over 50%. In the case of appearance and sweetness, except for CON, K25 and K50 scored higher than other samples and became not preferred at levels above 75% KF addition. The lowest appearance score of K100 could be explained due to the dark crumb color by the Maillard reaction between sugar and amino acids, increased brittleness, and small muffin volume. The decreased score in sweetness could be related to a decrement in brix degree. Regarding flavor and overall acceptability, both K25 and K50 were as high as CON. According to statistically analyze result, the CON and both K25 and K50 showed no significant difference in flavor and overall acceptability (P>0.05). It showed that the Kamut replacement had no significant negative effect on product preference at levels below 50%. The texture is one of the important parameters of sensory evaluation of processed foods (ALPASLAN and HAYTA, 2006). The texture was not significantly affected by Kamut flour substitution (P>0.05), unlike the mechanical texture results. In previous studies, the partial or complete replacement of wheat flour with KF provided equal or better sensory characteristics (BORDONI *et al*., 2017), and cooked Kamut grain ranked highly for sweetness among the various wheat varieties (STARR *et al*., 2015). Based on the sensory evaluation results, 25-50% of the Kamut added to muffins is considered as a desirable substitute addition level.

Table 8. Sensory evaluation of muffins with different levels of KF.

	CON	K25	K50	K75	K100	F-value
Appearance	7.18 ± 1.80^{a}	6.76 ± 1.45^{ab}	6.27 ± 1.54^{bc}	5.72 ± 1.39^{cd}	5.49 ± 1.55^{d}	10.435^{***}
Flavor	6.84 ± 1.49^{a}	6.54 ± 1.43^{a}	6.27 ± 1.61^{ab}	5.84 ± 1.54^{bc}	5.53 ± 1.65^{c}	5.948^{***}
Texture	6.10 ± 1.71^{NS}	6.25±1.56	6.11±2.00	5.75±1.62	5.37±1.77	2.161
Sweetness	6.94 ± 1.22^{a}	6.25 ± 1.44^{bc}	6.55 ± 1.63^{ab}	5.86 ± 1.46^{c}	6.00 ± 1.93^{bc}	3.985^{**}
Overall acceptability	6.69 ± 1.69^{a}	6.37 ± 1.57^{a}	6.45 ± 1.80^{a}	5.73 ± 1.42^{b}	5.47 ± 1.74^{b}	5.009^{**}

[1)]The data are mean±SD in triplicates.

[a-e] Different superscripts indicate there are significant differences between values in the same row according to Duncan's multiple range test at p<0.05.

**p<0.01, ***p<0.001.

4. CONCLUSIONS

Replacement of wheat flour by Kamut flour in muffins affected the air bubble distribution in batter and this affected on the muffin volume, weight and height. The air cell distribution became denser as KF level increased so that caused increased harness. In addition, with increasing KF level, the greater starch granules and the less protein matrix continuity could be descripted through the microscopic observation. Textural properties of crumb changed significantly. Addition of KF improved antioxidant capacity. Study of muffin firming kinetics revealed Avrami exponent value of K100 significantly high, but K75 showed the lowest value. All muffins were considered acceptable and the muffin containing less than 50% of Kamut flour level showed a preference score in the sensory evaluation

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Paper Received February 19, 2019 Accepted September 5, 2019