Progress in Nutrition 2021; Vol. 23, N. 4: e2021178 DOI 10.23751/pn.v23i4.10651 © Mattioli 1885

ORIGINAL ARTICLE

Effect of Kañiwa (*Chenopodium pallidicaule*) Flour Addition on Textural, Physical, and Sensory Properties of Pound cakes

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Summary. In this study we examined the effect of kañiwa flour as gluten-free material on pound cake quality. The wheat flour to make pound cake (control, designated as CON) gradually replaced with different amounts of kañiwa flour (25, 50, 75, and 100%, designated as KF25, KF50, KF75, and KF100, respectively). The incorporation of kañiwa flour in wheat flour significantly affected the physical properties of the batter and the cakes. As the amount of kañiwa flour increased, baking loss, height, and volume reduced. KF100 showed the best textural properties with respect to hardness, fracturability, and chewiness. Regarding the micrograph of crumbs, with increasing content of kañiwa flour, the pore sizes gradually decreased and starch granules on the matrix surface grew larger. The substitution with kañiwa flour resulted in higher amounts of total polyphenols and flavonoids, and a higher value of the reducing power. The sensory evaluation showed that KF25 scored high with respect to texture, sweetness, bitterness, and overall acceptability in comparison to CON. These results suggest that kañiwa is a potential material for developing gluten-free foods.

Key words: kañiwa flour, pound cake, physicochemical property, sensory evaluation

Introduction

Gluten, the main protein complex of wheat, plays a crucial role in making bread. The interaction between gliadins and glutenins, the gluten components, provides the dough with a unique texture such as viscosity and elasticity. Behind these gluten benefits, however, health-related issues are an increasing concern. Celiac disease and IgE-mediated wheat allergy are typical diseases related to gluten-containing grains. These are adverse reactions to gluten mediated by the immune system (1). In addition, non-celiac gluten sensitivity, a recently described disorder caused by consumption of gluten and/or other cereal components, results in symptoms of intestinal discomfort such as diarrhea or abdominal pain (1,2). In this context, interest in gluten-free diet has increased and related studies are being increasingly carried out to address this problem. According to Juhász et al. (3), the market for gluten-free products currently accounts for about one-third of the global food intolerance market, anticipating an annual growth of about 11.5% by 2021, and expected to be worth $7.4 billion by that time. Most gluten-free products are based on gluten-free cereals, such as rice, maize, millet, oil seeds, nuts, etc. The so-called pseudo-cereals, for example, amaranth, buckwheat, and quinoa, are consumed as non-gluten foods because of their nutritional value, richness in protein contents, essential amino acids, and fatty acids (4).

Among the pseudo-cereals, kañiwa (*Chenopodium pallidicaule*) is one of the indigenous grains which inhabit the Andean regions such as Peru and Bolivia and is used as food for its high nutritional value since 5000 BC in the Inca and preceding cultures (5,6). It is a resistant plant that is well adapted to an extreme environment and can be grown in a high-altitude at 4000 m above sea level, thriving in barren, rocky soil. As such, it has important potential cultivation characteristics because it adapts easily to changing environmental conditions. The chemical composition of kañiwa was reported in a study of Peñarrieta et al. (7), consisting of 63–66% carbohydrate, 15–18% protein, 6–8% lipids, and 3–4% ash. The protein content of kañiwa is 18.8 g per 100 g, which is abundant compared to wheat (10.5 g), barley (11.8 g), oats (11.6 g), and corn (11.1 g). It is noteworthy as a substitute for animal protein because the essential amino acid composition is similar to that of the milk protein, casein. Also, the lysine content, the first limiting amino acid in general grain, is high (5). In addition, kañiwa is rich in dietary fiber, minerals like calcium, phosphorus, and iron, and natural phenolic compounds (7,8).

Kañiwa, as a gluten-free grain, is a potential food alternative for celiac disease patients. The growing interest in gluten-free products in developed countries increased the need for substitutes with added nutritional value, and grains such as kañiwa and quinoa provide high-quality protein, fat, fiber, and physiological active compounds to the consumers. In this sense, this crop has contributed to the nutrition and a stable life for centuries and is now being studied as a suitable source for many nutrients and functional components (5,6,7,9). Additionally, previous kañiwa studies reported the manufacturing properties with respect to roasting, boiling, and extrusion (10,11). In terms of its utilization in food, the quality and functional changes as a function of kañiwa content in wheat flour have hardly been reported, and such studies are limited to bread (8,12) and pasta (13). In addition, research data that provide comparisons with respect to various aspects of existing products made with wheat flour are also rare.

In this study kañiwa flour was used to manufacture pound cakes, a common bakery product. Its applicability as a wheat substitute to improve the quality of gluten-free products was further investigated. To this end, the physical, antioxidant, rheological, and microstructural characteristics of pound cakes were measured to provide fundamental data for developing recipes for gluten-free products and to enhance the utilization of kañiwa as functional material.

Materials and Methods

Materials

The kañiwa used in the study originated from Peru, South America, and was supplied by Roland Foods (New York, US). It was washed with tap water and then freeze-dried for 30 h (FD8508, Ilshin Biobase Co., Gyeonggi, Korea) after drying in the shade for one day. The fine flour of kañiwa was obtained using a high-speed crushing machine (RT-04, Wonkangbio Co., Taipei, Taiwan) and passing it through a 40 mesh sieve. For the preparation of pound cakes, wheat flour (Beksul, Yang-san, Korea), butter (Anchor, Fonterra, New Zealand), sugar (Beksul, Incheon, Korea), eggs (Eggholic, Gyeonggi, Korea), baking powder (Sungjin, Gyeonggi, Korea), and salt (Chungjungwaon, Shinan, Korea) were purchased at a local market. All chemical reagents were of analytical grade.

Preparation of pound cake

The pound cake was prepared following the recipe reported by Joung et al. (14). The pound cake formulation consisted of 200 g wheat flour, 150 g butter, 150 g sugar, 150 g whole egg, 4 g baking powder, and 1 g salt. For samples other than the control group, the wheat flour replaced by various proportions of kañiwa flour (25, 50, 75, and 100%). The butter was creamed beforehand for 1 min at a speed setting of “4” using a KMM020 electric mixer (Kenwood, Havant, UK). Then, sugar and salt mixed with the creamed butter at a speed of “5” for 3 min. To prevent the separation of the batter, the eggs divided into eight portions and added gently to the mixture at a speed of “5” in 30 s intervals. The batter completed by mixing the sieved flour and baking powder at a speed of “2” for 30 s. Subsequently, 150 g of batter weighed into a loaf pan (120 × 40 × 40 mm) and baked for 25 min in an oven preheated to 180°C. The baked pound cakes used for further analysis after cooling for 2 h at room temperature (25°C).

Characterization of the pound cake

Batter properties. The specific gravity according to the AACC approved method 10-91 (15) was measured by dividing the density of the batter (g/ml) by the density of water (g/ml). The baking loss was calculated using the following formula:

Baking loss (%) = {(weight of batter − weight of cake)/weight of batter} × 100

Physical properties. The weight of the pound cake was measured on a digital scale (SPX2202KR, OHAUS, New Jersey, US). The height was calculated by measuring the vertical distance from the top to the bottom of the cake center using a vernier caliper. The cake volume was established using the seed displacement method described in the Approved Method 10-05.01 (15). The specific volume was determined as the volume/weight (ml/g) ratio. The moisture content of crumb parts was measured at 105°C with a moisture analyzer (MB45, OHAUS, New Jersey, US) using 5 g of sample. The water-holding capacity (WHC) of flour was analyzed using a modified method from Bchir et al. (16). An aliquot of 1 g sample was stirred with 20 ml of distilled water in a 50 ml test tube and agitated for 1 h at room temperature (25°C). The mixture was then centrifuged at 3000 rpm for 20 min (Universal 32R, Hettich, Tuttlingen, Germany). After discarding the supernatant, each tube was weighed. WHC was expressed as grams of water per gram of dry sample.

Color. The color of crust and crumb was determined according to Hunter's color system using a colorimeter (CR-400, Konica Minolta, Tokyo, Japan). In this system, the *L* (lightness) values range from black (0) to white (+100), the *a* (redness) values range from greenness (-) to redness (+), and the *b* (yellowness) values range from blueness (-) to yellowness (+). The total color difference (*ΔE*) was calculated by the following equation:

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

Texture analysis

Texture analysis was carried out using a texture analyzer (Compac-100II, Sun Scientific CO., Tokyo, Japan). The crumb area of the pound cakes was cut to a uniform size (20 × 20 × 20 mm) and the texture profile was determined with the two-bite compression test at 25°C using a 20 mm (diameter) cylinder probe. The operating conditions of the instrument were as follows: compression level of 50%, table spe2ed of 120 mm/min, load cell (max) of 10 kg, and distance of 5 mm. The texture parameters determined were hardness, springiness, cohesiveness, fracturability, and chewiness. On an average, eight replicates were determined for each sample.

Antioxidant activity

Extraction of antioxidant compound. The pound cakes were freeze-dried at −80°C for 48 h and then ground for 1 min. The pound cakes were defatted with hexane (1:4 w/v) and agitated for 1 h with a magnetic stirrer. Thereafter the defatted samples were dried overnight at room temperature (25°C). Each defatted sample was extracted with 70% methanol (1:10 w/v) in a water bath at 40°C (BS-20, Jeio Tech, Seoul, Korea) and 185 rpm for 1 h. The sample extracts were filtered through a Whatman filter (No. 1) and used for subsequent experiments.

Total polyphenols content

The total polyphenol content was measured using the Folin-Ciocalteu's method. To 20 μl of extracted samples, 780 μl of distilled water, 50 μl of 0.9 N Folin-Ciocalteu's reagent (Junsei Chemistry, Tokyo, Japan), and 150 μl of 20% sodium carbonate solution (Merck KGaA, Darmstadt, Germany) were added sequentially. After reacting for 2 h in a darkroom (25°C), the absorbance of each sample was measured on an ELISA microplate reader (Apollo11 LB913, Berthold, Bad Wildbad, Germany) at 750 nm. A calibration curve (R^2 = 0.9998) was prepared in the range of 200–1000 μg/ml using gallic acid (Merck KGaA) as a reference material. The total polyphenol content of each sample was converted into gallic acid equivalents (mg GAE/g of sample).

Total flavonoids content

The total flavonoid content was measured according to the method of Lee et al. (17). A total of 1 ml of sample was mixed with 150 μl of 5% sodium nitrite (Junsei Chemistry) and incubated in a darkroom (25°C) for 6 min, followed by the addition of 300 μl of 10% $AlCl_3$ and an additional reaction in the darkroom (25°C) for 5 min. Finally, 1 ml of 1 N NaOH solution (Daejung Chemicals & Metals, Gyunggi, Korea) was added and the absorbance of the sample was read at 520 nm. A calibration curve (R^2 = 0.9973) was prepared in the range of 20–100 μg/ml using quercetin (Sigma-Aldrich Co., St. Louis, MO, US) as a reference. The total flavonoid content of each sample was converted into quercetin equivalents (mg QE/g of sample).

Reducing power

The reducing power was determined following the methods of Benzie and Strain (18). A volume of 250 μl of 0.2 M phosphate buffer (pH 6.6), the mixture of sodium phosphate monobasic solution and sodium phosphate dibasic solution (1:2), and 250 μl of a 1% potassium ferricyanide solution were mixed with 250 μl of each sample and reacted for 30 min at 50°C. Then, 250 μl of 10% trichloroacetic acid solution was added. Finally, 500 μl of distilled water and 100 μl of 0.1% $FeCl_3$ were mixed with 500 μl of the sample supernatant, and absorbance of the sample solution was measured at 700 nm.

Microstructure of pound cakes

To evaluate the microstructure, scanning electron microscopy (SEM) using a JSM-670F (JEOL Ltd., Tokyo, Japan) was carried out. The crumb area of a pound cakes (2 × 4 × 2 mm) was fixed separately on an aluminum mount using Nem tape and conductive graphite (Ted Pella Inc., California, US), and then freeze-dried (FD8508, Ilshin Biobase Co., Gyeonggi, Korea). The mounted samples were coated with Au using the JSM 670-1F (JEOL Ltd., Tokyo, Japan) at 10 mA for 2 min. The morphology of the samples was observed at an accelerating voltage of 10 kV with ×100 and ×250 magnification.

Sensory evaluation

The sensory evaluation was completed by 30 untrained panelists (age range 20–60 years). They were acquainted with the contents of this research through the research participation instructions. The informed consent and the collection of personal information utilization agreement were obtained for experimentation with human subjects. This study was conducted with the approval of the Korea University Institutional Review Board (Approval Number: KUIRB-2020-0205-01). The samples (20 × 20 × 20 mm) marked with a random 3-digit number were supplied to each panelist at once on a white plastic plate with a cup of water. The panelists rated the pound cakes based on their appearance, flavor, texture, sweetness, bitterness, and overall acceptability using a 9-point hedonic scale (a score of 9 indicates "extremely good" and a score of 1 indicates "extremely bad").

Statistical analysis

Data analysis conducted in triplicate, except for the texture analysis, and the results were expressed as the mean and standard deviation. Statistical analysis was performed by one-way analysis of variance (ANOVA) using the SPSS program (IBM SPSS Statistics 24, IBM, Armonk, NY, US). Significant differences were checked by Duncan's multiple range test at a significance level of $p < 0.05$.

Results and discussion

Quality characteristics of pound cake

The quality characteristics of pound cake were presented in Table 1. Pound cakes showed strong differences in batter properties, physical properties, and color according to the addition of the differently proportioned kañiwa flour. The specific gravity of the batter affected the quality of the cakes, as higher specific gravity resulted in heavier and smaller cakes because of the dense pores and tissue, whereas a lower gravity opened the pores forming a rough tissue, resulting in a larger volume. Substitution with the kañiwa flour reduced the gluten content, increased the water absorption, and decreased the air retention, thereby increasing the specific gravity of the batter more than that of the cake with wheat flour. The addition of the kañiwa flour made the texture of the final product rougher. The baking loss significantly decreased with the addition of kañiwa flour ($p < 0.05$). It was the highest in CON (9.38%) and declined as the kañiwa flour content increased, ranging from 6.71% to 9.36%. The baking loss is the loss caused by the expansion of water and liquids with low boiling point induced by heat penetration during baking.

Table 1. Batter and physicochemical properties of pound cakes containing various levels of kañiwa flour.

Properties	Specific gravity	Baking loss (%)	Weight (g)	Height (mm)	Volume (mL)	Specific volume (mL/g)	Moisture (%)	WHC of flour (%)
CON	0.54±0.00$^{c1)}$	9.38±0.59^{a}	127.32±0.58^{b}	63.38±0.42^{a}	137.84±4.06^{a}	1.08±0.03^{a}	21.49±0.46^{a}	59.94±1.85^{e}
KF25	0.55±0.00^{ab}	9.36±0.47^{a}	127.22±0.24^{b}	59.71±0.27^{b}	133.51±1.27^{b}	1.05±0.01^{b}	20.88±0.43^{a}	73.09±0.15^{d}
KF50	0.56±0.01^{a}	8.44±0.57^{ab}	128.46±1.12^{ab}	50.81±0.66^{c}	129.38±0.86^{c}	1.01±0.02^{c}	20.78±0.56^{a}	84.82±4.46^{c}
KF75	0.54±0.01^{bc}	8.02±0.72^{b}	129.09±1.68^{ab}	48.09±1.93^{d}	128.52±1.11^{c}	1.00±0.02^{c}	20.67±0.71^{a}	104.00±3.61^{b}
KF100	0.54±0.01^{bc}	6.71±0.70^{c}	130.79±1.82^{a}	47.08±0.59^{d}	127.72±0.22^{c}	0.98±0.01^{c}	19.23±0.87^{b}	127.00±4.58^{a}
F-value	5.767*	9.553**	4.153*	167.625***	13.431***	17.681***	5.282*	181.649***

$^{1)}$ Values are the means of triplicate determinations ± S.D.
$^{a\text{–}e}$ Different superscripts indicate there are significant differences between values in the same row according to Duncan's multiple range test (p<0.05).
* p<0.05, ** p<0.01, *** p<0.001, ^{NS} not significant.
CON, cake containing 100% wheat flour; KF25, cake containing 25% kañiwa flour; KF50, cake containing 50% kañiwa flour; KF75, cake containing 75% kañiwa flour; KF100, cake containing 100% kañiwa flour.

It can also cause structural deformation of the cake as the gas escapes. When the gluten-free kañiwa flour was added, cake expansion was suppressed and water evaporation was decreased, which reduced the baking loss. This finding agrees with the results obtained by Joung et al. (14) and Lee et al. (17). The pound cake weight significantly increased as the kañiwa flour content increased ($p < 0.05$), in contrast, the height and volume of the pound cake gradually decreased as the kañiwa flour substitution ratio increased. Further, the specific volume, which is affected by the chemical expansion ability or the degree of CO_2 generation, significantly differed according to the various levels of kañiwa flour content. The CON had the highest specific volume (1.08 ml/g), but it decreased as the kañiwa flour content increased, ranging from 0.98 ml/g to 1.05 ml/g. This agrees with results presented by Lee et al. (17), who reported the quality of muffins containing the Kamut flour. Švec et al. (12) observed that when 10% kañiwa flour was added, the specific bread volume increased (a 32% increase compared to the wheat flour bread), but in the sample prepared with 20% kañiwa flour, the specific volume decreased by about 26% compared to that in the pure wheat flour bread. According to the results of another study (8), the addition of 12.5% kañiwa flour increased the specific volume. However, at a 25% substitution level, the specific bread volume changed back to that of the original wheat level and decreased significantly as the kañiwa flour content increased again. Rosell et al. (8) stated that addition of up to 12.5% kañiwa flour increases the fermentable sugars and favors yeast fermentation. Consequently, the gas production increased, but after that, the specific volume was more affected by the reduction of the gluten content in the dough. Moisture content is one of the important factors to improve food quality, especially softness, which is critical for cake products. The crumb moisture in KF100 was the lowest among all samples and showed no statistically significant difference from CON to KF75%. Comparing the moisture content of wheat and kañiwa flour, the moisture content of kañiwa flour (4.66%) was markedly lower than that of wheat flour (12.64%) (data not shown). The moisture content of the flour itself might affect the crumb moisture, although the water holding capacity of kañiwa flour (127.00%) was higher than that of wheat flour (59.94%). According to a study by Rosell et al. (8), the moisture content of breads prepared with kañiwa flour increased with increasing wheat flour substitution, but the moisture content of pure breads with kañiwa flour only decreased to a level similar to that of pure wheat breads. The water-holding capacity (WHC) is a parameter determining the capacity for water absorption, which is measured with an external force. There was a significant difference in the WHC of the flour ($p < 0.05$), which increased in relation to the content of kañiwa flour. Dietary fiber retains water in the food matrix through chemical interactions with moisture.

The WHC of the product increases when ingredients with high dietary fiber content are added because of the water absorption capacity of the fiber. Kañiwa contains a higher dietary fiber content (12.56%) than other Andean crops such as quinoa (8.87%) and kiwicha (5.80%) (11). In addition, Villa et al. (9) reported whole fiber content of 6.3%, 2.1%, and 2.2% for kañiwa, wheat, and quinoa, respectively. Therefore, the high dietary fiber content of the kañiwa flour is the reason why the WHC considerably increased with respect to kañiwa addition to the flour samples.

Color

The results of crust and crumb color of pound cakes were summarized in Table 2. With respect to the color of raw materials, there was a notable darkening of both the crust and crumb. The crust color is an important parameter to determine its acceptability (19). The total color difference (ΔE) indicated the influence of the additions of kañiwa flour on the color of pound cakes. The statistical analysis revealed a significant difference between the crust of the CON and kañiwa containing cakes ($p < 0.05$), probably affected by a high quantity of added kañiwa flour (range from 25% to 100%). The crust of the cakes, rich in kañiwa flour, had a darker color characterized by low lightness (L), and as the amount of ingredients added increased, the values dropped even lower. The redness (a) and yellowness (b) significantly decreased as the amount of kañiwa flour increased, especially after the kañiwa flour substitution exceeded 75%. These results are influenced by the color of the original kañiwa ($L = 64.35$, $a = 2.45$, $b = 10.92$), and Maillard and caramelization reactions are mainly associated with these results. In terms of the crumb color, the total color variation (ΔE) differed slightly more for the CON than the crust values, which is related to the high CON lightness, compared to the crust lightness. Adding kañiwa flour reduced the lightness (L) and yellowness (b), whereas it improved the redness (a). Gómez et al. (19) stated that the crumb color is usually affected by the color of the raw ingredients because temperature of the crumb part does not reach as high as that of the crust part. This finding is consistent with the results of Rosell et al. (8) who made bread with blends of wheat-kañiwa flour in various proportions (ranging from 12.5% to 100%). As the proportion of the intense brown kañiwa flour increased, the L, a, and b values for the crust decreased. With respect to the crumb, the L and b values decreased, but the a value increased. This indicates that kañiwa-wheat flour blends can produce a range of colored bakery products.

Texture analysis

The texture profile analysis (TPA) measures the physical property of food, mimicking the mastication process of putting food in the mouth and chewing. TPA is closely correlated to the actual sensory evaluation and widely used in the food industry. The effects of the supplementation of the kañiwa flour on texture

Table 2. Color values of pound cakes containing various levels of kañiwa flour.

Properties	Crust				Crumb			
	L	a	b	ΔE	L	a	b	ΔE
CON	55.66 ± 2.95^{a}	8.79 ± 0.84^{a}	20.12 ± 1.22^{a}	45.48 ± 2.41^{c}	72.67 ± 0.85^{a}	-5.21 ± 0.21^{d}	22.15 ± 0.92^{a}	29.22 ± 1.41^{d}
KF25	41.77 ± 1.08^{b}	6.96 ± 0.18^{b}	10.87 ± 1.32^{b}	55.81 ± 0.88^{b}	40.00 ± 1.98^{b}	0.69 ± 0.06^{c}	15.75 ± 0.12^{b}	50.55 ± 0.43^{c}
KF50	40.59 ± 0.74^{b}	6.25 ± 0.14^{b}	8.28 ± 0.94^{c}	56.54 ± 0.65^{b}	34.21 ± 0.85^{c}	2.37 ± 0.05^{b}	11.59 ± 0.46^{c}	56.64 ± 0.24^{b}
KF75	36.19 ± 2.23^{c}	5.22 ± 0.10^{c}	6.08 ± 0.57^{d}	60.61 ± 2.17^{a}	28.73 ± 1.39^{d}	3.52 ± 0.09^{a}	9.26 ± 0.06^{d}	60.35 ± 0.30^{a}
KF100	34.97 ± 2.33^{c}	4.38 ± 0.58^{c}	4.26 ± 1.08^{d}	61.68 ± 2.40^{a}	27.49 ± 0.68^{d}	3.71 ± 0.27^{a}	7.30 ± 0.70^{e}	60.80 ± 0.59^{a}
F-value	48.973^{***}	38.661^{***}	103.554^{***}	35.175^{***}	1566.692^{***}	1567.688^{***}	335.081^{***}	971.307^{***}

[1)] Values are the means of triplicate determinations ± S.D.

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

[***] $p<0.001$.

Table 3. Texture properties of pound cake crumbs containing various levels of kañiwa flour.

Properties	Hardness (N)	Springiness (%)	Cohesiveness (%)	Fracturability (g)	Chewiness (N)
CON	2.61±0.38^{c}	81.10±3.89^{NS}	63.64±3.43^{a}	138.10±27.37^{c}	1.35±0.27^{c}
KF25	3.94±1.02^{b}	81.99±1.65	57.37±4.96^{b}	191.61±60.11^{b}	1.88±0.59^{b}
KF50	4.39±0.45^{b}	81.28±2.42	52.80±6.14^{bc}	192.16±30.50^{b}	1.89±0.30^{b}
KF75	4.92±0.96^{ab}	84.39±2.40	49.45±7.09^{c}	208.04±43.33^{ab}	2.04±0.43^{ab}
KF100	5.81±1.38^{a}	80.89±3.20	51.39±4.30^{c}	245.40±59.22^{a}	2.41±0.58^{a}
F-value	13.360***	0.739	9.021***	5.589**	5.580**

[1)] Values are the means of triplicate determinations ± S.D.

$^{a-c}$ Different superscripts indicate that there are significant differences between values in the same row according to Duncan's multiple range test (p<0.05).

** p<0.01, *** p<0.001, ^{NS} not significant.

of pound cake were presented in Table 3. Hardness, also expressed as firmness, refers to the peak force at the first compression. The crumb hardness progressively increased from 2.61 N to 5.81 N as the substitution level of kañiwa increased. Similar findings were reported in a study on breads where kañiwa was added (8). The authors noted that no significant hardening occurred until a 25% replacement, but it was observed that the hardness increased when kañiwa reached 50% in the composite. This phenomenon is attributed to the kañiwa property to provide a hard dough that is unable to retain the gas released, leading to very compact and hard bread structures. Lee et al. (17) also mentioned that this increment in hardness is related to fewer and smaller air cells inside the crumb part. Springiness is defined as the elasticity for returning to the undeformed state after removing the force applied to the sample, expressed as the ratio of the distance between the first and the second compression. The springiness did not show statistically significant differences. it seemed that the substitution with kañiwa flour induced only minor effects in terms of the springiness. Similar results were reported for sponge cakes containing black carrot flour (20) and for breads using kañiwa flour (8). Cohesiveness is the strength of internal bonds, which is an important characteristic of foods that require resistible structures under mechanical stress. Regarding the cohesiveness, this parameter decreased with addition of kañiwa flour. Similar findings were reported by Lee et al. (17), Rosell et al. (8), and Bustos et al. (13), where cohesiveness decreased increasing the proportion of the raw material. The results of this study were contrary to those obtained by Joung et al. (14) and Song et al. (20), where no significant difference in cohesiveness was observed. A too high cohesiveness requires more energy to bite, while a too low one can cause external damage during packaging or transportation. The fracturability is the force of the first significant breakage at the first bite. In case of samples with relatively rigid surfaces, this occurs because parts of the surface break first as the samples are compressed. The increase in kañiwa flour significantly increased the fracturability of the cakes from 138.10 g to 245.40 g. In general, more brittle foods tend to show low cohesiveness, and this is consistent with our result as the cohesiveness of the pound cake decreased with the increasing level of kañiwa flour. Chewiness is a secondary parameter derived from primary variables such as hardness, cohesiveness, and springiness, which means the energy needed to masticate a solid food until swallowed. The chewiness was 1.35 N in CON and as the kañiwa flour ratio increased, it significantly increased ($p < 0.05$) and reached 2.41 N in KF100, following a similar trend to hardness and fracturability. These chewiness results are similar to those where kañiwa flour was added to breads (8,12), but opposite to those adding kañiwa flour to pasta (13), as the chewiness of wheat-kañiwa pasta decreased with the increase in concentration of kañiwa flour. The results of texture analyses of this study were similar to those of other bakery products with added kañiwa flour.

Antioxidant activities

The antioxidant activities of pound cake crumbs were presented in Table 4. Polyphenols, polymers with

Table 4. Antioxidant activities of pound cakes containing various levels of kañiwa flour.

Properties	Total polyphenol (mg GAE/g)	Flavonoids (mg QE/g)	Reducing power
CON[1)]	0.84±0.03^{d}	7.65±0.24^{c}	3.14±0.05^{e}
KF25	0.99±0.05^{c}	9.73±0.47^{b}	3.50±0.07^{d}
KF50	1.24±0.10^{b}	10.03±0.17^{b}	4.46±0.07^{c}
KF75	1.17±0.10^{b}	10.16±0.38^{b}	5.80±0.04^{b}
KF100	1.39±0.07^{a}	11.42±0.34^{a}	6.53±0.14^{a}
F-value	25.631[***]	49.528[***]	987.545[***]

[1)] Values are the means of triplicate determinations ± S.D.

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

[***] $p<0.001$.

numerous phenolic structures, are pigment components produced by photosynthesis in plants. A phenolic compound is an electron donor that can donate electrons from the hydroxyl group of the benzene rings, and it indicates antioxidant activity as a reducing substance because the structural stabilization is achieved by the resonance of the benzene ring structure. Polyphenols are classified into several subgroups of flavonoids, phenolic acids, tannins and so on, and phenolic compounds such as flavonol, phenolic acids, and catechin are abundant in kañiwa (7). The total polyphenol contents significantly increased with increasing kañiwa flour level, ranging between 0.84–1.39 mg garlic acid equivalent (GAE)/g (p < 0.05). Repo-Carrasco et al. (11) reported that the total phenolic compounds in kañiwa grains were 29.52 mg GAE/100 g. Also, Peñarrieta et al. (7) determined that kañiwa from different ecotypes in Bolivia contains 12.4–47.0 μmol GAE/g, and these values are higher than those obtained for sweet corn (3.0 μmol GAE/g) (21), oats (1.2 μmol GAE/g), and amaranth (0.9 μmol GAE/g) (22). Flavonoids, one of the subgroups of polyphenols, are yellow-coloured pigments of food and there are more than 5,000 types worldwide. The kañiwa contains various flavonoid-based components such as quercetin and kaempferol (7). The flavonoid contents gradually increased with increasing substitution of the kañiwa flour, showing the highest value in KF100 (11.42 mg QE/g) in comparison to CON (7.65 mg QE/g). In a previous study by Peñarrieta et al. (7), it was reported that total flavonoids in kañiwa (2.2–11.0 μmol CE/g) are higher than those in sweet corn (0.2 μmol CE/g) (21), oats (0.6 μmol CE/g), and amaranth (0.5 μmol CE/g) (22). In this regard, the increasing contents of polyphenol and flavonoid in the pound cake as kañiwa flour is added can be attributed to the higher contents of those compounds in kañiwa itself. FRAP is a method of measuring the antioxidant activity of a sample using absorbance by analyzing the degree to which ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) (18). The absorbance value measured corresponds to the reducing power of the sample, and the higher the value, the higher is the antioxidant activity. Similar to the results on contents of total polyphenol and flavonoid, a significant increment in reducing power was observed as the kañiwa content in the flour increased (p < 0.05). KF100 (6.53) exhibited the highest value, which was about 2.08 times higher than that of CON. Peñarrieta et al. (7) observed that kañiwa from different ecotypes shows a 2.7–18.1 μmol trolox equivalent (TE)/g compared to other cereals such as barley (5.4 μmol TE/g), rye (2.4 μmol TE/g), buckwheat (10 μmol TE/g), and millet (1.2 μmol TE/g) (23). The antioxidant activity of kañiwa is strongly related to the amounts of phenolic components, and this activity was validated by the reducing power, which indicated that the addition of kañiwa enhanced the antioxidant activity of the products.

Microstructure of pound cakes

Micrographs of the crumb area of pound cakes made with wheat and kañiwa flours and wheat blends were shown in Fig. 1. In Figure 1 A-1 (CON), B-1 (KF25), C-1 (KF50), D-1 (KF75), and E-1 (KF100), the cake crumbs magnified at 100 times, and a variation in pore size, distribution of the air cells, and surface of the matrix structure can be seen. Figure A-1 showed that gelatinized starch buried under the gluten backbone structure closely interacted with the protein matrix, indicating that the solid phase was smoothly connected. As kañiwa flour was incorporated, a smaller pore size and a more irregular shape of starch granules

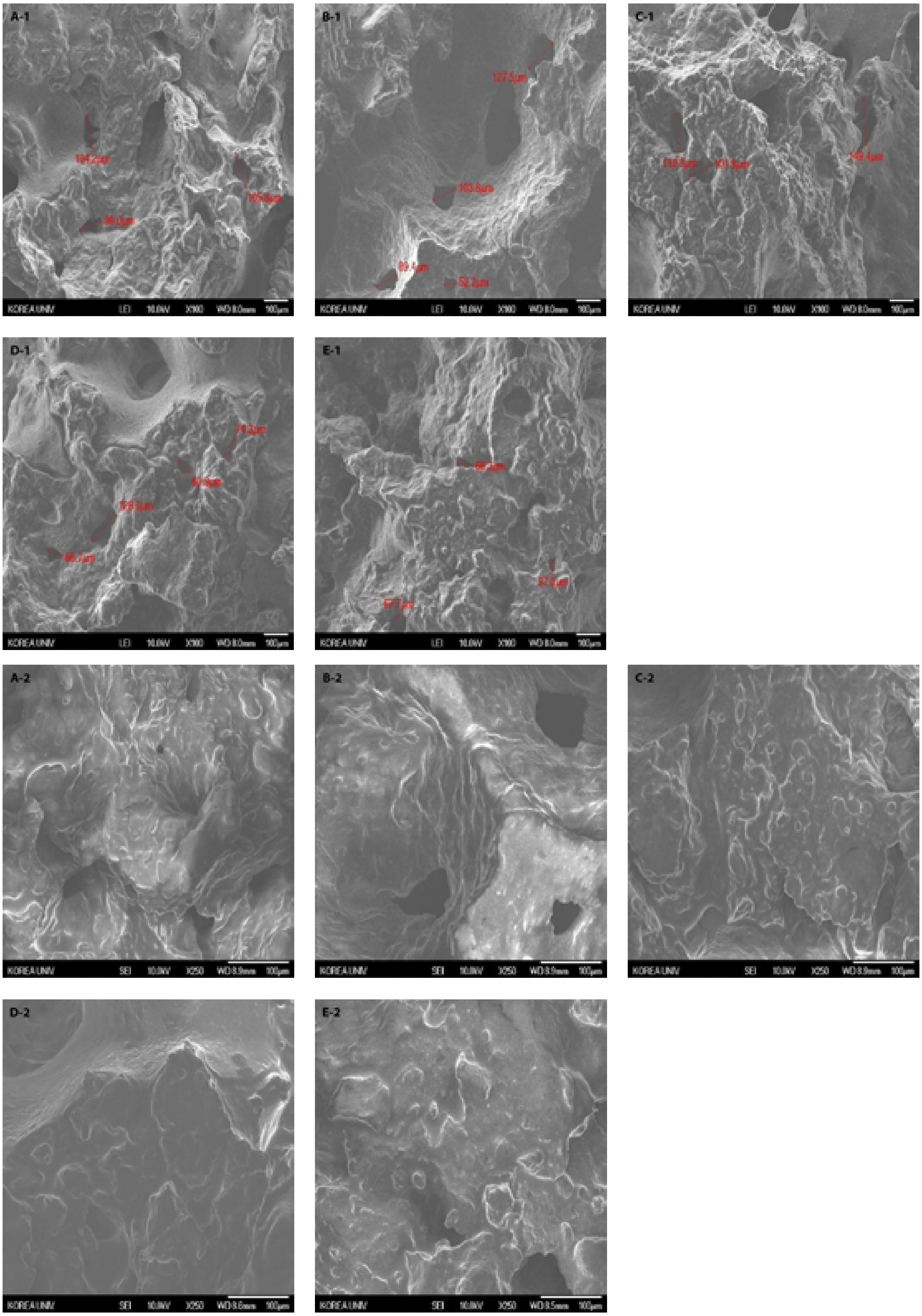

Figure 1. Scanning electron micrograph of pound cake crumbs with various levels of kañiwa flour.
Top line: SEM micrograph magnified 100×. Bottom line: SEM micrograph magnified 250×.
A(CON), cake containing 100% wheat flour; B(KF25), cake containing 25% kañiwa flour; C(KF50), cake containing 50% kañiwa flour; D(KF75), cake containing 75% kañiwa flour; E(KF100), cake containing 100% kañiwa flour.

were observed. Diaz et al. (10) reported that the pore size of extrudates progressively decreased as the content of kañiwa flours increased, resulting in much smaller pores than those of amaranth or quinoa. The main role of gluten is to form a three-dimensional network, which is created by connections to starch granules. It is known that adding non-gluten flours containing ample dietary fiber and lipids dilutes gluten protein and weakens the food structure (13). A previous study reporting pasta made with wheat-kañiwa blends revealed that kañiwa-enriched pasta consists of a disrupted protein matrix with a more irregular structure (13). In this study, an irregular pore distribution was observed, and the number of small-sized pores increased. It is suggested that the gluten dilution caused a decrease in bubble retention capacity reducing the pore expansion and increasing the number of small pores. This is consistent with the results obtained in this study, where hardness and fracturability increased and cohesiveness decreased. Panel A-2 (CON), B-2 (KF25), C-2 (KF50), D-2 (KF75), and E-2 (KF100) in Figure 1 showed micrographs of cake crumbs magnified at 250 times. Panel B-2 in Figure 1 exhibited a smooth continuous matrix surface similar to that of CON (Panel A-2 in Fig. 1), and the form of starch granules did not differ much. Panel C-2 in Figure 1 displayed the shape of a noticeably small granule, and the surface of the protein matrix was rough and uneven. This was related to the irregular, polygonal, and dented shape of kañiwa displaying a small size of starch granules below 2.0 μm (6). Panel D-2 and E-2 in Figure 1 showed that as the kañiwa flour level increased, the gelatinized starch granules increased in size, and the continuity of the protein matrix was disrupted.

Sensory evaluation

Results of the sensory evaluation of the pound cakes were provided in Table 5. The sensory score for appearance, flavor, texture, sweetness, bitterness, and overall acceptability significantly decreased as the kañiwa flour level increased ($p < 0.05$). The appearance is one of the important parameters in sensory evaluation and is visually perceived judging the form and color of food. In this study, the score for appearance was the highest for CON and both KF25 and KF50 scored higher than other samples containing kañiwa flour. The lowest score for KF100 was the result of a too low volume and the dark color induced by the Maillard reaction and the inherent color of kañiwa. Regarding flavor and texture, except for CON, KF25 scored the highest values, and other samples did not exhibit statistically significant difference ($p < 0.05$). Sensorial properties are significantly influenced by the crumb texture, which is noticeably related to the increase in hardness or fracturability that results from the addition of kañiwa flour. With respect to sweetness and bitterness, KF25 achieved the highest scores, except for CON. Kañiwa provides a bitter taste because it contains saponins. In our study, a lower kañiwa content was preferred with respect to bitterness, however there was no statistically clear difference at kañiwa levels above 50%. In sensory profiling of extruded snacks containing kañiwa (10), there was no change in

Table 5. Sensory evaluation of pound cakes containing various levels of kañiwa flour.

Properties	Appearance	Flavor	Texture	Sweetness	Bitterness	Overall acceptability
CON	7.13 ± 1.61^{a}	7.10 ± 1.45^{a}	6.37 ± 1.63^{a}	6.90 ± 1.37^{a}	7.27 ± 1.44^{a}	7.00 ± 1.53^{a}
KF25	6.30 ± 1.47^{ab}	5.70 ± 2.00^{b}	5.83 ± 1.56^{ab}	6.03 ± 1.45^{b}	6.77 ± 1.55^{ab}	6.03 ± 1.79^{b}
KF50	6.57 ± 1.57^{ab}	5.00 ± 1.29^{b}	4.87 ± 1.89^{b}	5.43 ± 1.68^{b}	6.27 ± 1.82^{b}	5.43 ± 1.79^{bc}
KF75	5.80 ± 1.71^{b}	5.47 ± 1.87^{b}	5.07 ± 2.07^{b}	5.97 ± 1.67^{b}	5.90 ± 1.94^{b}	5.17 ± 1.82^{bc}
KF100	5.73 ± 1.82^{b}	4.97 ± 1.75^{b}	4.90 ± 2.14^{b}	5.83 ± 1.80^{b}	5.87 ± 1.91^{b}	4.90 ± 1.95^{c}
F-value	3.729^{**}	7.927^{***}	3.790^{**}	3.377^{*}	3.557^{**}	6.594^{***}

1) Values are the means of triplicate determinations ± S.D.

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

* p<0.05, ** p<0.01, *** p<0.001.

sweetness, but the bitterness and overall taste intensity were affected in samples that contained 50% kañiwa. With respect to overall acceptability, apart from CON, KF25 scored the highest among all samples containing kañiwa and acceptability decreased at levels exceeding 50%. These results are similar to those reported by Rosell et al. (8), where the overall acceptance of kañiwa bread was not significantly different than that of wheat bread with blends up to 25% kañiwa. According to Švec et al. (12), 20% kañiwa worsened the overall consumer acceptance of wheat bread, and therefore, they proposed a tolerable kañiwa dosage of between 10 and 15%. Derived from our study, up to 25% kañiwa flour in a pound cake is thought to be a suitable substitution level for practical applications.

Conclusion

The addition of gluten-free material such as kañiwa considerably affected the textural and physical properties and sensory properties of pound cakes. As the replacement level of high dietary fiber-containing kañiwa flour increased, the texture and structure of crumb were altered. Compared to wheat flour cake, the hardness, chewiness, and fracturability increased, but the cohesiveness decreased. Moreover, with the increasing content of kañiwa flour, larger starch granules and a disrupted protein matrix in the crumb surface were observed in micrographs. In addition, the substitution with kañiwa flour improved the antioxidant activities of the cakes because of the higher contents of phenolic compounds. Regarding the sensory properties, pound cake made with 25% kañiwa flour was found to be the least statistically different from that made with wheat flour. In conclusion, kañiwa substitution up to 25% proportion in pound cakes is likely to be appropriate. This study shows that kañiwa flour is an alternative for wheat flour in pound cakes, and is beneficial to health because of its physiological activity. Furthermore, it provides the potential to increase the value-added production of gluten-free bakery products.

Acknowledgements: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest: The authors declare no conflict of interest.

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