Enhancing quality characteristics of salami sausages formulated with whole buckwheat flour during storage

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Abstract This study was performed to investigate the quality characteristics of salami sausages added with different levels of whole buckwheat flour (BWF) during storage. Samples included the control (Con), addition of 1% BWF (T1), 3% BWF (T2), and 5% BWF (T3). Water activity (a_w) and pH decreased with increased level of BWF. Salami sausage samples containing 5% BWF demonstrated significantly lower 2-thiobarbituric acid (TBA) values than the control. Changes in TBA values between day 0 and 21 for T2 and T3 were less than that for control. Total plate count (TPC) of all groups significantly decreased, whereas lactic acid bacteria significantly increased after 21 days. TPC of samples added with BWF was significantly lower during storage. Inclusion of BWF seemed to be an effective means of retarding lipid oxidation and enhancing storability of salami sausages.

Keywords Whole buckwheat flour · Salami sausage · Quality characteristics · Storability

Introduction

Functional foods generally provide a health benefit beyond basic nutrition in the human diet. Addition of functional ingredients might also increase the shelf life of their products by preventing rancidity as well as regulate various

body functions (Roberfroid 2000). Buckwheat (*Fagopyrum esculentum* Moench) has been used as a basic functional ingredient in food industry, such as noodles, pasta, and bread products (Bejosano and Corke 1998; Handoyo et al. 2006). Buckwheat contains a large amount of dietary fiber and essential minerals and also includes a relatively high amount of lysine and some vitamins such as B1, C, and E (Wijngaard and Arendt 2006). Furthermore, buckwheat reveals significant effects on reducing serum glucose concentrations in diabetic rats and inhibiting lipid oxidation in mouse brain during digestion (Hur et al. 2011; Kawa et al. 2003).

Buckwheat has flavonoids including rutin and quercetin, which demonstrate a higher level of antioxidant activity than ascorbic acid, α -tocopherol and β -carotene (Rice-Evans et al. 1997). The aglycones typically have much higher antioxidant activity than their corresponding glycosides (Yin et al. 2008). Quercetin (aglycone) had the higher antioxidant activity compared to rutin (glycoside). Some plant species including tomato and wheat sprout powder had powerful antioxidants such as phenols, flavonoids, and reducing glycosides, which revealed efficiently for inhibiting lipid oxidation in meat products (Georgea et al. 2004; Kim et al. 2013; Ozturk et al. 2014).

Lipid oxidation should be carefully considered as a critical quality attribute of meat products during storage because of its relatively high fat content (Jiménez-Colmenero 2000; Woo et al. 1995). The problem of the poor storage stability of meat products caused by lipid oxidation may exert a negative influence on consumers' preference and quality characteristics. Several attempts have been made to retard lipid oxidation by using some functional ingredients or artificial antioxidants (Ansorena and Astiasarán 2004; Del Nobile et al. 2009; Eburne and

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Prentice 1996; Gray et al. 1996; Mora-Gallego et al. 2013; Park et al. 2016). Although buckwheat is a good source of nutrients to provide health and functional benefits, there is limited information on the effect of whole buckwheat on the quality attributes of meat products. Thus, the purpose of this study was to evaluate the effects of whole buckwheat flour on the microbiological and functional properties, and lipid oxidation stability of the salami sausages during 4 °C storage for 21 days.

Materials and methods

Materials

The commercial whole buckwheat flour (BWF) sample was obtained from Bongpyung Memil Co., Pyengchang, Korea. The proximate chemical compositions of crude protein, ash, and crude fat for the BWF sample were 21.6, 4.08, and 7.20%, respectively. Fresh pork (*longissimus dorsi*) and pork back fat were purchased from a local market at 24 h postmortem. Samples of BWF, fresh pork, and pig back fat were stored separately at refrigerated temperature before used in experiments.

Preparation of salami sausage

The raw pork was trimmed for removing visible connective tissue and fats prior to the manufacture of the salami sausages. The salami sausage samples were processed according to the formulation, as described by Sanchez-Zapata et al. (2013). Pork sample was ground through a 5 mm plate and then the back fat was weighted before blending. The basic ingredients (10% water, 2% salt, 0.05% white pepper, 0.05% sodium ascorbate, 0.015% sodium nitrite, 0.03% potassium nitrite, and 1% lactose) and different levels of BWF (1, 3, and 5%) were added into the ground mixtures. In addition, a commercial starter culture C-P-77 (CHR Hansen, Germany) and a laboratory prepared *Lactobacillus plantarum* were added. The freeze-dried starter was a mixture of *Staphylococcus* sp. and *Lactobacillus* sp. The dose of C-P-77 was 25 g/100 kg and the cultures were mixed up with 50/50 weight ratio of *Staphylococcus* sp. and *Lactobacillus* sp. before adding the culture. The mixed samples were completely blended by hands for 5 min, and were stuffed into 16–28 mm diameter collagen casing (Shangdong Crown Collagen Casing Co., Ltd., Shandong, China). All samples were stored for 21 days in a chamber. The storage conditions for the temperature and relative humidity (RH) were 20 ± 2 °C and $90 \pm 5\%$ on the first day and 12 ± 2 °C and $75 \pm 5\%$ in the following days, respectively.

Proximate composition analysis

The proximate compositions of moisture, crude protein, crude fat, and ash were analyzed using the Association of Official Analytical Chemists (AOAC, 2002) methods.

pH and water activity

The pH values of the salami sausage samples were measured in triplicate with a pH meter (pH 900, Precisa Co, Deitikon, Switzerland). The pH values were determined in a slurry prepared by homogenizing 2 g of sample with 18 mL of distilled water for 90 s using a Bag mixer 400 (Interscience Co, St Nom la Bretèche, France). Water activity (a_w) of the samples was determined using a water activity measuring device (Aquaspector, AQS-31, NAGY, Gaeurfelden, Germany).

Color measurement

The color of salami sausage samples was measured by using handy colorimeter (NR-300, Nippon Denshoku, Tokyo, Japan). The colorimeter was calibrated with a white plate ($CIE\ L^* = +94.48$, $a^* = -0.67$, $b^* = +3.31$). Values of $CIE\ L^*$ (lightness), $CIE\ a^*$ (redness), and $CIE\ b^*$ (yellowness) were measured for the salami sausages. The salami sausage samples were stored at room temperature for 20 min prior to the color measurement.

TBARS determination

Thiobarbituric acid reactive substances (TBARS) of salami samples added with BWF were measured during storage (0, 3, 7, 14, and 21 days) by using the method of Witte et al. (1970). Two grams of sample was homogenized with 10 mL of 10% (w/v) trichloroacetic acid for 60 s. The homogenized sample was mixed with 10 mL distilled water. The mixture was filtrated through Whatman No. 1 filter paper and then the supernatant (5 mL) was added in 5 mL of 2-thiobarbituric acid (2.88 g/L H₂O). After a heat treatment process for 10 min, the absorbance was measured at 532 nm. TEP (1,1,3,3-Tetraethoxypropane) was used as a positive control for standard curve. The TBARS value was calculated as malondialdehyde (MDA) mg/kg.

Microbiological analysis

Sampling was performed during storage (0, 3, 7, 14, and 21 days). Two grams of sample and 18 mL of sterile deionized water (0.85% NaCl) were transferred to a sterile stomacher bag and homogenized for 90 s with a bag mixer (Interscience Co., France). A 10^{-1} dilution was then used for subsequent serial dilutions. An aliquot (0.1 mL) of the

appropriate dilution of sample was spread in triplicate onto agar plates. Total plate counts were enumerated on Petrifirm Aerobic Count Plates (3 M, Korea) after 48 h at 35 °C and the lactic acid bacteria (LAB) were counted on MRS agar (Oxoid, Cambridge, UK) after 24 h at 35 °C. The colony numbers were converted into a log value.

Texture analysis

Texture profile analysis (TPA) was conducted on each sample at room temperature with a TA-XT2i texture analyzer (Stable Micro Systems, Godalming, U.K.), using a TA11/1000 25.4 mm weight by 3.5 mm length cylinder probe. Samples were cut from the central portion of each sausage sample. Texture analysis was conditioned as follows: pre-test speed 2.0 mm/s, post-test speed 5.0 mm/s, maximum load 2 kg, head speed 2.0 mm/s, distance 8.0 mm, force 5 g. TPA values were calculated by graphing a curve using force and time plots. Values for hardness, springiness, cohesiveness, gumminess, and chewiness of the samples were determined as described by Bourne (1978). All measurements were performed in triplicate for the texture analysis.

Statistical analysis

Analysis of variance (ANOVA) was employed to assess the contribution of BWF to data variability of quality parameters using a 4 treatment $\times$ 5 storage periods factorial design. As an ANOVA simple test was carried out only for BWF content, storage periods were regarded as repeated measurements in this study. All data were analyzed at least in triplicate and mean values were reported. Statistical analyses were conducted with the General Linear Model (GLM) procedure of the SPSS version 18.0 using one-way ANOVA. The significant difference means of all data among the groups and the storage periods were determined using Tukey test ($P < 0.05$).

Results and discussion

Proximate composition

The initial moisture content of all fermented salami sausage samples ranged from 64.45 to 68.85%, which reduced to 20.75–28.10% in the final products (Table 1). In the initial phase, the moisture content for T2 and T3 groups was significantly lower than the control ($P < 0.05$). The moisture content significantly decreased as added BWF content increased in the final phase of storage ($P < 0.05$).

Crude fat concentration among all groups was not significantly different at 0 d ($P > 0.05$), while crude fat

content of T1, T2, and T3 did show significantly lower at 21 d ($P < 0.05$) when compared to the control. Both crude protein and fat contents of all groups increased during storage. Although crude protein content among all groups was not significantly different at 0 d, crude protein content for T1 group was significantly higher than T3 in the final product ($P < 0.05$). Ash content of salami sausages did not show the significant differences among the samples in the initial storage, whereas they displayed the significant differences in the final phase of storage ($P < 0.05$). The ash concentration for the fermented salami sausage was decreased with the increased amounts of buckwheat added only at 21 days. Moreover, crude fat content of salami sausage samples treated with BWF was also significantly lower than that of the control group at 0 and 21 days ($P < 0.05$). Accordingly, ash and crude protein contents of salami sausage samples formulated with BWF might be decreased throughout the drying and storage process when compared to the control group.

Water activity (a_w) and pH value

The a_w of all four groups significantly decreased between day 0 and 21 ($P < 0.05$, Table 2). The salami sausage samples produced with BWF had significant lower a_w than the Con sample on 21 day of storage ($P < 0.05$).

The pH values of all groups significantly reduced from the initial 0–3 day of storage (Table 2). The pH values of salami sample decreased during storage when added BWF contents increased. There was a higher decrease in T3 between day 0 and 3 when comparing to the other groups. Decrease in the pH of salami sausage sample during storage could be related to the growth of LAB (Drosinos et al. 2005). This was possibly because of increased lactic acid production caused by elevated addition of BWF during storage. The pH value of 5% BWF added salami sausage was significantly lower than that of other groups on each 3, 7, 14, and 21 day of storage, respectively.

Color

Changes in the color of salami sausage during storage are summarized in Table 3. Initially, all samples had a range of 69.0–71.6 for the lightness (L^*) values and significantly decreased to 44.7–48.5 in the final phase of storage ($P < 0.05$). This reduction of the L -value could result in dark coloration because of browning reaction in all salami sausage samples. Previous study indicated that metmyoglobin generated by pigment oxidation could reduce the lightness value of meat products during storage period (Kayaardı and Gök 2003). The pigment oxidation could be promoted by the interaction of the secondary lipid oxidation products (Faustman et al.

Table 1 Proximate composition of salami sausage added with whole buckwheat flour

Proximate Composition (%)	Storage period (days)	Treatments			
		Con	T1	T2	T3
Moisture	0	68.85 ^{Aa}	69.03 ^{Aa}	64.45 ^{Ab}	66.39 ^{Ab}
	21	28.10 ^{Ba}	20.75 ^{Bb}	22.18 ^{Bb}	23.63 ^{Bb}
Crude protein	0	17.45 ^B	18.38 ^B	17.78 ^B	17.72 ^B
	21	40.15 ^{Aab}	43.22 ^{Aa}	38.47 ^{Aab}	35.65 ^{Ab}
Crude fat	0	6.31 ^B	5.46 ^B	5.74 ^B	5.29 ^B
	21	19.22 ^{Aa}	18.11 ^{Ab}	17.53 ^{Ab}	16.13 ^{Ab}
Ash	0	2.75 ^B	2.93 ^B	2.75 ^B	2.80 ^B
	21	6.57 ^{Aa}	5.84 ^{Ab}	5.68 ^{Abc}	5.30 ^{Ac}

Con Control group, T1 addition of 1% whole buckwheat flour (BWF), T2 addition of 3% BWF, T3 addition of 5% BWF

^{a–d} Means within the row with different letters are significantly different ($P < 0.05$)

^{A–B} Means within a column for each treatment with different letters are significantly different ($P < 0.05$)

Table 2 Water activity (a_w) and pH of salami sausage added with whole buckwheat flour during storage

Parameter	Storage period (days)	Treatments			
		Con	T1	T2	T3
a_w	0	1.00 ^A	1.00 ^A	1.00 ^A	1.00 ^A
	3	0.99 ^{Bab}	0.98 ^{Bb}	1.00 ^{Aa}	0.99 ^{Aab}
	7	0.91 ^{Db}	0.94 ^{Ca}	0.94 ^{Ba}	0.93 ^{Ba}
	14	0.94 ^{Cb}	0.94 ^{Cab}	0.95 ^{Ba}	0.94 ^{Bab}
	21	0.87 ^{Ea}	0.81 ^{Db}	0.83 ^{Cb}	0.82 ^{Cb}
pH	0	5.94 ^A	5.91 ^A	6.06 ^A	6.07 ^A
	3	5.01 ^{Ba}	5.04 ^{Da}	4.82 ^{Cb}	4.68 ^{Ec}
	7	5.18 ^{Ba}	5.17 ^{Ca}	5.04 ^{Bb}	4.77 ^{Cc}
	14	5.11 ^{Ba}	4.90 ^{Ec}	5.04 ^{Bb}	4.73 ^{DEd}
	21	5.14 ^{Ba}	5.23 ^{Ba}	5.09 ^{Bb}	4.97 ^{Bc}

Con Control group, T1 addition of 1% whole buckwheat flour (BWF), T2 addition of 3% BWF, T3 addition of 5% BWF

^{a–e} Means within the row with different letters are significantly different ($P < 0.05$)

^{A–D} Means within a column for each treatment with different letters are significantly different ($P < 0.05$)

2010). Accordingly, higher lightness value of the BWF added groups might be occurred by prevention of pigment and lipid oxidation during storage when compared to the control sample. The redness (a^*) values of T2 and T3 significantly increased during 21 days of storage ($P < 0.05$). The a^* values of all groups increased up to 7 day of storage, and did not consistently increase after 7 days. Kayaardi and Gök (2003) also reported that a -values of meat products increased to the certain storage period, and inconsistent a -value changes were examined during further storage. No significant differences in their respective storage periods were observed for the a -values among the four groups. This indicated that redness (a^*) of the salami sausage products on each storage day was not affected by introduction of BWF. The decrease in lightness and the increase in redness of noodle products

produced with fermented buckwheat were also reported by Handoyo et al. (2006).

The yellowness (b^*) value of the salami sausage ranged from 10.9 to 18.1 during the entire storage. The yellowness values of salami sample increased during storage as BWF concentration increased. Similar result of increased yellowness in meat product during storage was also proved by Choi and Chin (2003). Significant changes in the b^* values were observed among the groups during storage.

Thiobarbituric acid reactive substances (TBARS) of salami sausage during storage

Thiobarbituric acid (TBA) values of salami sausage produced with different levels of BWF are shown in Table 4. TBA values of all samples significantly increased until day

Table 3 Lightness (L^*), redness (a^*), and yellowness (b^*) of salami sausage added with whole buckwheat during storage

Parameter	Storage (days)	Treatments			
		Con	T1	T2	T3
L^*	0	71.6 ^{Aa}	69.2 ^{Aab}	71.1 ^{Aab}	69.0 ^{Ab}
	3	65.1 ^{Bab}	62.2 ^{Bb}	67.6 ^{Aa}	66.7 ^{Aa}
	7	48.1 ^{Cc}	51.0 ^{Cbc}	55.6 ^{BCa}	53.5 ^{Cab}
	14	48.2 ^{Cb}	51.0 ^{Cb}	57.8 ^{Ba}	58.7 ^{Ba}
	21	44.7 ^C	47.6 ^D	48.5 ^C	46.8 ^D
a^*	0	8.3	8.2	7.0 ^C	7.3 ^B
	3	9.3	9.3	8.2 ^{BC}	9.0 ^{AB}
	7	9.3	11.0	9.2 ^{BC}	11.1 ^{AB}
	14	12.0	11.0	10.2 ^{AB}	9.3 ^{AB}
	21	10.6	11.1	12.1 ^A	12.5 ^A
b^*	0	14.0 ^{AB}	13.4 ^B	14.6 ^B	14.7 ^B
	3	11.4 ^{Cb}	12.4 ^{Bab}	13.2 ^{BCa}	13.6 ^{Ba}
	7	12.3 ^{BCb}	13.0 ^{Bab}	11.9 ^{Cb}	16.0 ^{ABa}
	14	10.9 ^{Cb}	14.4 ^{ABa}	14.5 ^{BCa}	13.5 ^{Ba}
	21	14.7 ^A	16.2 ^A	18.1 ^A	18.00 ^A

^{a-c} Means within the row with different letters are significantly different ($P < 0.05$)

^{A-D} Means within a column for each treatment with different letters are significantly different ($P < 0.05$)

Con Control group, T1 addition of 1% whole buckwheat flour (BWF), T2 addition of 3% BWF, T3 addition of 5% BWF

Table 4 Changes in thiobarbituric acid value (TBARS) of salami sausage added with whole buckwheat flour during storage (malondialdehyde mg/kg)

Storage period (days)	Treatments			
	Con	T1	T2	T3
0	0.05 ^{Cb}	0.05 ^{Cb}	0.05 ^{Cb}	0.10 ^{Ca}
3	0.21 ^B	0.23 ^B	0.24 ^{BC}	0.30 ^B
7	0.47 ^{Ab}	0.48 ^{Ab}	0.59 ^{Aa}	0.55 ^{Bab}
14	0.40 ^{Aab}	0.46 ^{Aa}	0.42 ^{ABa}	0.30 ^{Bb}
21	0.44 ^{Aa}	0.37 ^{Aab}	0.31 ^{Bab}	0.22 ^{ABb}
Δ TBA (0–21 day)	0.36 ^a	0.40 ^a	0.24 ^b	0.17 ^b

Con Control group, T1 addition of 1% whole buckwheat flour (BWF), T2 addition of 3% BWF, T3 addition of 5% BWF

^{a-c} Means within the row with different letters are significantly different ($P < 0.05$)

^{A-D} Means within a column for each treatment with different letters are significantly different ($P < 0.05$)

7 of storage ($P < 0.05$), which corroborates the results of a previous study by Fanco et al. (2002). The 3 and 5% BWF added salami sausage samples showed significantly lower changes in TBA values than the Con ($P < 0.05$) during

21 days of storage. This result might be explained by the antioxidant effects of buckwheat that prevents lipid oxidation (Jiang et al. 2007). The inhibition of lipid oxidation in samples treated with BWF during storage might be associated with the increased the free radical scavenging ability by the fermentation that could result in producing more total phenolic compounds and flavonoids in the meat products. Park et al. (2016) and Kariluoto et al. (2006) indicated that fermentation enhanced the total phenolic content and antioxidant activity in cereals. Other research found that the growth of *Lactobacillus* could be promoted when some flavonoid glycosides were converted to aglycones (Pham and Shah 2008). Also, previous studies reported that a substantial amount of rutin (glycoside) and quercetin (aglycone) were included in buckwheat and quercetin had more powerful antioxidant effect than rutin (Yang et al. 2008; Quettier-Deleu et al. 2000). Maximum malondialdehyde (MDA) values were observed in all samples on day 7 and then TBA values decreased up to day 21 of storage. This could be attributed to decomposition of MDA by bacteria, which could selectively breakdown and utilize carbonyl compounds such as MDA (Smith and Alfod 1968; Moerck and Ball 1974). In addition, the decreased TBA values on day 21 might be related to some by-products of lipid oxidation such as organic acids and alcohols, which did not produce the colored chemical compounds when TBA reacted with MDA (Stapelfedt et al. 1992).

Microbiological assessment

Microbiological analyses of the salami sausages during the 21-day storage period are presented in Table 5. Total plate counts increased during the 7 day of storage and then decreased until day 21. This decline tendency from 7 d to 21 d could be associated with decreased water activity during the drying and storage process. Total aerobic bacterial counts were significantly lower in salami sausage containing 5% BWF compared to the control at day 3, 7, and 14 storage period. This result might be due to the antimicrobial activity of buckwheat having some representative flavonoids with antibacterial activity (Hong et al. 2006; Narayana et al. 2001). The number of LAB increased by day 7 and then reduced in all salami sausages until day 21. Visible LAB counts in the 1, 3 and 5% BWF added samples were significantly higher than those in the control sample at day 7 and 21 storage period. The LAB of salami samples increased during storage when added BWF contents increased. Other reports indicated that buckwheat has a large amount of carbohydrate (68.6–79.5%) which can be used for producing lactic acid by lactic acid bacteria during storage (Kandler 1983; Li and Zhang 2010).

Table 5 Microbial counts in salami sausage added with whole buckwheat during storage

Composition (log CFU/g)	Storage period (days)	Treatments			
		Con	T1	T2	T3
Total aerobes	0	3.08 ^D	2.98 ^D	3.03 ^D	2.88 ^D
	3	6.24 ^{Aa}	6.20 ^{Aa}	5.50 ^{Bb}	4.94 ^{Bc}
	7	6.44 ^{Aa}	6.35 ^{Aab}	6.31 ^{Ab}	6.20 ^{Ac}
	14	5.63 ^{Ba}	4.83 ^{Bb}	5.07 ^{Bbc}	4.20 ^{Cc}
	21	4.17 ^C	3.49 ^C	3.96 ^C	3.53 ^{CD}
Lactic acid bacteria	0	3.06 ^C	3.10 ^D	3.13 ^C	3.35 ^C
	3	6.42 ^{Aab}	6.41 ^{Ba}	6.69 ^{Ab}	6.55 ^{Aab}
	7	6.53 ^{Ab}	6.69 ^{Aa}	6.76 ^{Aa}	6.67 ^{Aa}
	14	5.98 ^B	6.30 ^B	6.33 ^{AB}	6.42 ^A
	21	5.71 ^{Bb}	5.87 ^{Cab}	5.91 ^{Ba}	5.98 ^{Ba}

Con Control group, T1 addition of 1% whole buckwheat flour (BWF), T2 addition of 3% BWF, T3 addition of 5% BWF

^{a–c} Means within the row with different letters are significantly different ($P < 0.05$)

^{A–D} Means within a column for each treatment with different letters are significantly different ($P < 0.05$)

Table 6 Textural properties of salami sausage added with the different levels of whole buckwheat flour

Parameter	Treatments			
	Con	T1	T2	T3
Hardness (g)	6.67	6.55	5.86	6.93
Springiness (mm)	1.96 ^a	1.7 ^b	1.6 ^b	1.7 ^b
Cohesiveness	0.77 ^a	0.69 ^b	0.69 ^b	0.69 ^b
Gumminess (g)	4.44	4.91	4.64	5.16
Chewiness (mJ)	87.40	90.07	80.17	95.13

Con Control group, T1 addition of 1% whole buckwheat flour (BWF), T2 addition of 3% BWF, T3 addition of 5% BWF

^{a–d} Means within the row with different letters are significantly different ($P < 0.05$)

Texture profile analysis

The effects of BWF on the textural properties of salami sausages were shown in Table 6. The introduction of BWF did not influence the hardness, gumminess, and chewiness of the salami sausage samples compared to the control sample. The addition of BWF had contributed to decrease the springiness and the cohesiveness of salami sausages. In this study, the textural characteristics of the salami sausages, such as springiness and cohesiveness were reduced by the BWF addition.

Conclusion

The introduction of whole buckwheat showed the changes in functional and microbiological properties of the salami sausage during storage. The a_w , pH, and TBA values of the

salami sausages formulated with BWF were lower than the control sample. Salami sausages containing BWF had the reduced total aerobes and increased LAB during storage. In conclusion, the addition of BWF could be efficiently used as a natural ingredient for enhancing the quality attributes and storability of meat products by retardation of lipid oxidation during storage.

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