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ARTICLE

Effect of Rice Bran and Wheat Fibers on Microbiological and Physicochemical Properties of Fermented Sausages during Ripening and Storage

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Abstract This study investigated the effect of rice bran fiber (RBF) and wheat fibers (WF) on microbiological and physicochemical properties of fermented sausages during ripening and storage. The experimental design included three treatments: Control, no addition; RBF, 1.5%; and WF, 1.5%. During the ripening periods, the addition of dietary fibers rapidly decreased pH and maintained high water activity values of fermented sausages ($p<0.05$). Lactic acid bacteria were more prevalent in fermented sausages with rice bran fiber than control and sausages with added wheat fiber. During cold storage, lower pH was observed in sausages with dietary fibers ($p<0.05$), and the water activity and color values were reduced as the storage period lengthened. Fermented sausages containing dietary fibers were higher in lactic acid bacteria counts, volatile basic nitrogen and 2-thiobarbituric acid reactive substance values compared to the control ($p<0.05$). The results indicate that, the addition of dietary fibers in the fermented sausages promotes the growth of lactic bacteria and fermentation, and suggests that development of functional fermented sausages is possible.

Keywords fermented sausages, dietary fibers, quality characteristics, rice bran, wheat

Introduction

Fermented sausages originated from Mediterranean countries that feature an arid climate. They are one of the most popular meat products globally. (Nassu et al., 2003; Kurćubić et al., 2014). Generally, fermented sausages are manufactured by chopping and blending raw meat, fat, salt, spices, certain additives, and starter cultures, followed by stuffing into natural or synthetic casing and ripening and drying. Fermented sausages have high biological value and are a good source of energy, vitamins, mineral, and proteins (Seong et al., 2016). Fermented sausages contain a variety of animal fats that are added during sausage manufacture to a level of 10-25%. Following ripening and drying, the content of animal fat can reach 50% (Bloukas et al., 1997; Ordóñez et al., 1999; Wirth, 1998). Fat is one of the important factors contributing to enhanced flavor, texture, juiciness, and mouthfeel of meat products (Olivares et al., 2010). However, consuming high animal fat in meat products is associated with increases risks of cardiovascular disease, obesity, hyperpiesia, and other adult diseases. Thus, the World Health Organization (WHO) recommends that consumers lower their intake of animal fat (WHO, 2003; Ham et al., 2016).

As consumers continue to focus on good health, the demand for functional foods is increasing (Choi and Chin, 2003). Dietary fiber is a functional material. Recently, dietary fiber has gained attention in the meat product industry (Choi et al., 2016). Typically, dietary fiber is defined as non-digestible oligosaccharides and fructooligosaccharides (Gibson and Roberfroid, 1995). Owing to differences in solubility, dietary fiber can be classified as water-insoluble or water-soluble (Huang et al., 2011). Water-insoluble fiber is frequently utilized in preventing and treating constipation and adult disease, as well as adjusting body weight (Nguyen et al., 2004). Water-soluble dietary fiber is beneficial in reducing serum cholesterol and blood fat, and in lessening the risk of cardiovascular diseases (Pereira et al., 2004; Schulze et al., 2004). Dietary fiber helps improve human health and wellbeing, and is an essential vital food ingredients for human health. In meat products, dietary fiber can improve the water-binding or holding capacity, viscosity, oil-binding capacity, mineral and organic molecule binding capacity, and textural properties (Tungland and Meyer, 2002; Choi et al., 2015).

Rice bran is a by-product obtained from outer rice layers during the rice milling process (Qi et al., 2016). The main ingredients in rice bran are dietary fiber, proteins (essential amino acids), oils (fatty acids), minerals, vitamins (B and E), antioxidants (tocopherols, tocotrienols, oryzanol), and phenolic compounds (Friedman, 2013; Sharif et al., 2014). In addition, rice bran is used to enrich some foods, because of its high dietary fiber content (Hu et al., 2009).

Wheat (*Triticum aestivum* L.), a member of the Poaceae (*Gramineae*) family, is a cultivated crop throughout the world and the most consumed grain globally (An, 2015). Wheat contains significant amounts of proteins, fiber, lipids, vitamins, minerals, and phytochemicals, which may contribute to a healthy diet (Shewry and Hey, 2015). Wheat also contains flavonol glycoside compounds that can be used to prevent edema and hemorrhagic disease, and relieve high blood pressure (Chung and An, 2015). The various ingredients in wheat may it useful medicinally as well as in foods.

The present study investigated the effect of rice bran and wheat fibers on microbiological and physicochemical properties of fermented sausages during ripening and storage.

Materials and Methods

Formulation and processing procedure

Three different fermented sausage formulations were examined. Fermented sausages formulations and experimental design are shown in the Table 1. Hanwoo beef (top round), pork (ham), and pork back-fat were purchased in vacuum packaged containers from a butcher's shop (Chungju, Korea). The beef and pork were trimmed of visible fat and fascia. The beef, pork, and pork back-fat were cut into 3.5 cm cubes and kept frozen at -24℃ for 1 d prior to mincing using silent cutter (C50, FATOSA Co., Spain) at low speed (1,500 rpm). After wheat fiber (Vitacel WF200, J. Rettenmaier & Sohne, Germany), rice bran fiber (Organic Ineer Ricebran, Cheorwon Ecofriendly Agricultural Association Corporation, Korea), starter culture (Lyocarni VBL-73, Italy), ascorbic acid, mixed spices, and nitrite pickling salt containing nitrite 0.6% were added and mixed

Table 1. **Formulations of fermented sausages**

Ingredients (%)		Dietary fiber type		
		Control	RBF	WF
Major	Beef	33.33	33.33	33.33
	Pork	33.33	33.33	33.33
	Pork back fat	23.33	23.33	23.33
	Chicory fiber	10	10	10
Sum		100	100	100
Ascorbic acid		0.02	0.02	0.02
NPS		3.00	3.00	3.00
Starter culture		0.02	0.02	0.02
Mixed spice		6.25	6.25	6.25
Rice bran fiber		-	1.5	-
Wheat fiber		-	-	1.5

*Ascorbic acid, NPS, starter culture, mixed spice, rice bran fiber and wheat fiber were added as percentage unit for sum of major ingredients.
RBF, rice bran 1.5%; WF, wheat 1.5%; NPS, NaCl:$NaNO_2$=99:1.

at high speed (2,700 rpm). The mixture was stuffed into 28-30 mm diameter hog casings (Farmyu Co., Korea) using a model H20PA vacuum stuffer (Talsa Co., Northampton, EU). The filled sausages were transferred to a chamber capable of controlled temperature and humidity (TH-ME-100, Jeio Tech Co., Korea) for fermenting and drying. The chamber was maintained at 20℃ for 7 d were maintained. The initial humidity of 85% was reduced by 3% every day. During the ripening period, the sausages were smoked at 20±2°C for 30 min once a day. After ripening, the sausages packed in a polyethylene bag by applying vacuum and placed at 4°C for 28 d. We measured pH, water activity (a_w), color, total microbial count (TMC), and lactic acid bacteria (LAB), volatile basic nitrogen (VBN), 2-thiobarbituric acid reactive substance (TBARS) throughout the ripening period and storage periods. The analyses of each sample was repeated three times.

Analysis items

pH and water activity

pH was determined as described previously (Khalil, 2000). Ten grams of fermented sausages were homogenized in 100 mL of distilled water for 30 s using a model 400 blender (Seward, London, UK), and pH was measured using a pH meter (WTW, Germany).

Water activity (a_w) of the fermented sausages were measured in triplicate using an AquaLab series 4TE device (Decagon Devices Co, USA) at 25°C. Calibration and measurements were conducted in accordance with the instruction manual of manufacturer.

Color

The internal color of fermented sausages after removing hog casing and cutting sausages was measured using a model JX-777 spectrocolorimeter (Color Techno System Co., Japan) that was standardized to the white plate (lightness (L*), 94.04; redness (a*), 0.13; and yelllowness (b*), -0.51). At this time, a white fluorescent lamp (D65) was used as the light source, and the meat color was indicated with L*, a*, and b* value represented lightness of the Hunter lab color coordinates, redness, and yellowness, respectively.

Microbiological analysis

A 10 g sample was aseptically retrieved from fermented sausages, transferred into a sterile plastic bag, and homogenized with 0.1% peptone (90 mL) solution in a 400 Lab Blender (Seward) for 90 s. Serial 10-fold dilutions were prepared from each sample using 1 mL in fluid agar, inoculated on TMC medium, and incubated at 37℃ for 48 h (Vanderzant, 1992). Lactic acid bacteria (LAB) was inoculated on De Man, Rogosa and Sharpe agar and incubated at 37℃ for 48 h. The results were expressed as Log CFU/g.

2-Thiobarbituric acid reactive substance (TBARS) value

The 2-thiobarbituric acid reactive substance (TBARS) value of fermented sausages was measured by the modified extraction method of Witte et al. (1970). Ten grams of each sample received cold 10% perchloric acid (15 mL) and triple distilled water (25 mL). After the sample was homogenized at 10,000 rpm for 10-15 s in a homogenizer (AM-Series), the homogenate was filtered using qualitative filter paper No. 2 (Advantec, Toyo Roshi Kaisha, Ltd., Japan). The 5 mL of filtrate solution was mixed with 5 mL of TBA solution (0.02 M), and its absorbance was measured at 529 nm using a spectrophotometer (DU-650, Beckman, US). Triple distilled water was used in the blank sample. TBARS values were explained as mg of malonaldehyde per 1 kg sample (mg malonaldehyde/kg). The TBA level was calculated using a standard curve, where $y=0.1975x–0.0011$ ($r=0.999$), and expressed by calculating y = absorbance and x = the TBARS value.

Volatile basic nitrogen (VBN) value

VBN was measured by microdiffusion analysis (Short, 1954) using a Conway unit. Ten grams of each sample were added to 90 mL of distilled water and homogenized at 10,000 rpm for 30 sec in a homogenizer (AM-Series). The homogenate was filtered using qualitative filter paper No. 2. One milliliter of the slurry was put in the outer place of the Conway unit, and 1 mL of 0.01 N boric acid and three drops of indicator (0.066% methyl red+0.066% bromocresol green) were put in the inner chamber. Glycerine was then applied to glue the parts to the lid and, the lid was closed. One milliliter of 50% K_2CO_3 was injected into the outer chamber and the Conway unit was closed immediately. After horizontally stirring the vessel, after incubating at 37℃ for 120 min, the boric acid in the inner chamber was titrated with 0.02 N H_2SO_4. The VBN values were expressed by converting it to mg% per 100 g sample.

$$\text{VBN} = \{(a–b) \times F \times 28.014 \times 100\}/\text{amount of sample}$$

a: Amount of injected sulfuric acid (mL)
b: Amount of sulfuric acid injected in blank (mL)
F: Standardized index of 0.02 N H_2SO_4
28.014: Amount of required to consume 0.02 N H_2SO_4 of 1 mL

Statistical analysis

The entire experiment data was analyzed using analysis of variance in the SAS program 9.4 (2012) and statistical significance was verified among the means at a 95% level using Duncan's multiple range test.

Results and Discussion

pH, a_w, and meat color values of fermented sausages during ripening period

Table 2 shows the changes in pH, water activity (a_w), and color of fermented sausages with dietary fibers during ripening period at 20℃. The pH of fermented sausages were higher than 5.70 until 3 days of ripening. After 4 day of ripening, pH of fermented sausages with dietary fibers began to decrease rapidly. In particular, the pH of RBF treatment was significantly lower than C and WF treatments until 7 days of ripening. The initial a_w of fermented sausages ranged from 0.915 to 0.926. Because of the water evaporation during ripening procedure, a_w of all fermented sausages started to gradually decrease. The a_w of RBF and WF treatments, except for day 0 and 1, were significantly higher than the control throughout the ripening period, and the final a_w of fermented sausages ranged from 0.851 to 0.857. As the ripening period increased, the Hunter L*, a* and b* values of fermented sausages significantly decreased. The initial L* values of fermented sausages were between 50.66 and 56.29. After ripening, the L* values of RBF and WF treatments significantly were higher than those of control from day 6 to 7. The initial a* values of fermented sausages were ranged from 17.08 and 20.14. The a* values of control and RBF treatment showed significantly lower values after day 1. However, a* values of WF treatment did not fluctuate significantly until day 5, and was decreased during ripening at day 6 and 7 ($p<0.05$). The initial b* values of fermented sausages ranged from 17.37 to 19.43. The b* value of RBF treatment showed significantly higher values than those of control and WF treatment during entire ripening periods (all $p<0.05$). In the b* values of WF treatment, after the 3 day, they were decreased significantly as the ripening period increased.

Blackburn et al. (1984) reported that dietary fiber was used as a substrate for lactic acid bacteria and promoted LAB growth. Therefore, it is presumed that the pH values of the fermented sausages containing dietary fiber formed lower pH values due to the production of organic acid by LAB. On the other hand, studies on meat products with added rice bran did not report significantly affected pH (Choi et al., 2008a), indicating this it was not a fermented product. In this study, the lowest pH of sausages formulated with rice bran was probably due to the excellent use of rice bran as a substrate. Cofrades et al. (2000) reported the dietary fiber addition to meat products increases the binding force between water and fat, and also absorbed minerals and other ingredients. So, we speculate that the water activity of the RBF and WF treatments is higher than the control after ripening. Choi et al. (2008a) reported that when rice bran fiber was added to the Tteokgalbi product, the water holding capacity was higher than that of the control. Huang et al. (2005) observed the same behavior. Addition of rice bran fiber reportedly decreases the L* and a* values of meat products, but increases the b* values due to the dark yellow of the rice bran itself (Choi et al., 2007; 2008b). The wheat fiber used in this study was a brilliant white powder, so that the addition of wheat fiber significantly affected the L* and a* values of fermented sausages. Fernández-Ginés et al. (2003) reported that the kind and amount of dietary fiber additives influence the color of meat products. Similar results have been reported by others (Kim et al., 2009; Pathera et al., 2017). On the other hand, the lightness, redness and yellowness in Turkish fermented sausage formulated with grape seed powder decreased significantly as the ripening and storage periods increased (Kurt, 2016). These results were similar to the present study.

Total microbial count (TMC) and lactic acid bacteria (LAB) of fermented sausages during ripening period

The changes in total microbial count (TMC) and lactic acid bacteria (LAB) of fermented sausages with dietary fibers

Table 2. Changes in pH, water activity (a_w), and color of fermented sausages with dietary fibers during ripening period at 20℃

Items		Days of ripening								
		0 day	1 day	2 day	3 day	4 day	5 day	6 day	7 day	SEM
pH	C[1)]	5.79^{Cb}	5.92^{Ab}	5.80^{Cc}	5.88^{Ba}	5.73^{Da}	5.79^{Ca}	5.78^{Ca}	5.69^{Ea}	0.01
	RBF	5.94^{Ca}	6.07^{Aa}	5.99^{Ba}	5.74^{Db}	5.02^{Hc}	5.19^{Fc}	5.16^{Gc}	5.38^{Ec}	0.01
	WF	5.82^{Bb}	5.86^{ABc}	5.90^{Ab}	5.75^{Bb}	5.11^{Fb}	5.46^{Db}	5.34^{Eb}	5.44^{Db}	0.01
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
a_w	C	0.926^{Aa}	0.908^{Bb}	0.909^{Bc}	0.895^{Cc}	0.881^{Db}	0.872^{Ec}	0.864^{Fc}	0.851^{Gb}	0.001
	RBF	0.915^{Cb}	0.925^{Aa}	0.918^{Ba}	0.901^{Da}	0.901^{Da}	0.884^{Ea}	0.867^{Fb}	0.854^{Ga}	0.001
	WF	0.918^{Ab}	0.908^{Bb}	0.916^{Ab}	0.898^{Cb}	0.897^{Ca}	0.879^{Db}	0.870^{Ea}	0.857^{Fa}	0.001
	SEM	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	
[2)]L*	C	54.19^{Ab}	44.63^{BCb}	46.56^{BCb}	45.50^{B}	44.25^{Cb}	42.14^{Db}	40.29^{Ea}	37.91^{Fa}	0.38
	RBF	50.66^{Ac}	48.89^{Ba}	46.78^{Cb}	44.43^{D}	47.24^{Ca}	42.69^{Eb}	37.20^{Fb}	36.92^{Fab}	0.43
	WF	56.29^{Aa}	47.36^{Ba}	48.22^{Ba}	44.57^{C}	43.87^{Cb}	43.52^{Ca}	36.96^{Db}	35.73^{Eb}	0.41
	SEM	0.50	0.53	0.45	0.47	0.26	0.22	0.20	0.43	
a*	C	20.14^{Aa}	17.33^{CD}	17.06^{Db}	17.24^{D}	17.89^{BCb}	15.99^{CDb}	18.14^{Ba}	16.83^{Da}	0.20
	RBF	19.04^{Aa}	17.13^{CD}	17.80^{Ba}	17.63^{BC}	19.04^{Aa}	17.02^{CDa}	16.87^{Db}	16.84^{Da}	0.21
	WF	17.08^{Ab}	16.93^{A}	16.95^{Ab}	16.83^{A}	16.77^{Ac}	16.78^{Aa}	15.78^{Bc}	15.24^{Bb}	0.20
	SEM	0.36	0.19	0.13	0.29	0.15	0.11	0.10	0.13	
b*	C	18.09^{Ab}	17.17^{Bb}	16.34^{Cb}	15.94^{Cb}	15.32^{Db}	13.76^{Eb}	14.17^{Ea}	12.01^{Fb}	0.15
	RBF	19.43^{Aa}	18.10^{Ba}	17.20^{Ca}	16.83^{CDa}	16.46^{Da}	14.71^{Ea}	13.97^{Fa}	12.30^{Ga}	0.22
	WF	17.37^{Ab}	17.08^{Ab}	17.08^{Aa}	15.59^{Bb}	14.54^{Cc}	14.55^{Ca}	12.34^{Db}	11.17^{Ec}	0.11
	SEM	0.33	0.18	0.12	0.13	0.09	0.09	0.14	0.16	

[1)] C, Control (No addition); RBF, rice bran 1.5%; WF, wheat 1.5%.
[2)] L*, lightness; a*, redness; b*, yellowness.
Means in the same row with different letters (A-H) are significantly different (p<0.05).
Means in the same column with different letters (a-c) are significantly different (p<0.05).
SEM, standard error of the means of three replicate experiments with three samples analyzed per replicate.

during ripening period at 20°C were presented in Table 3. During 7 days of ripening, the total microbial counts of control and two treatments did not show a consistent tendency to increase or decrease with increasing ripening period, and remained at the level of 5.15 to 6.20 Log CFU/g. The initial LAB of fermented sausages on day 0 was between 6.04 and 6.07. Fermented sausages from the control and the two treatments rapidly increased lactic acid bacteria from the day 2 to 7 of ripening, and the number of LAB was significantly higher in the treatments than the control. Also, the count of LAB in fermented sausages formulated with rice bran fiber was significantly higher than that of fermented sausages containing wheat fiber during the ripening period from day 2 to 7. Presently the addition of dietary fiber had no significant effect on total microbial count. This may be due to the shorter ripening period of this study compared to the previous study. The reason for the presence of numerous LAB in the RBF and WF treatments is that dietary fibers like rice bran and wheat stimulate the growth of LAB (Blaut, 2002). Especially, the LAB of RBF treatment from day 3 to 7 day was significantly higher than the WF treatment. So, we can speculate that during ripening, rice bran promotes the growth of LAB more than does wheat fiber. Geisen et al. (1992)

Table 3. Changes in total microbial count (TMC) and lactic acid bacteria (LAB) of fermented sausages with dietary fibers during ripening period at 20℃

Items		Days of ripening								
		0 day	1 day	2 day	3 day	4 day	5 day	6 day	7 day	SEM
TMC (Log CFU/g)	C$^{1)}$	5.80^{Bb}	5.90^{Bab}	5.82^{B}	5.15^{Cb}	5.81^{Ba}	5.69^{B}	5.15^{C}	6.20^{Aa}	0.09
	RBF	5.76^{Ab}	5.81^{Ab}	5.77^{A}	5.81^{Aa}	5.69^{ABab}	5.88^{A}	5.45^{AB}	5.23^{Bb}	0.14
	WF	5.98^{Aa}	5.95^{ABa}	5.89^{AB}	5.66^{BCab}	5.47^{Cb}	5.92^{AB}	5.65^{BC}	5.95^{ABa}	0.08
	SEM	0.02	0.02	0.12	0.13	0.05	0.13	0.12	0.14	
LAB (Log CFU/g)	C	6.04^{D}	5.86^{Dab}	6.13^{CDb}	6.39^{Cc}	7.40^{A}	6.90^{Bc}	6.00^{Dc}	6.73^{Ac}	0.10
	RBF	6.07^{G}	5.78^{Hb}	6.60^{Fa}	7.12^{Ea}	7.89^{C}	8.16^{Aa}	8.02^{Ba}	7.65^{Da}	0.02
	WF	6.07^{F}	5.95^{Fa}	6.46^{Eab}	6.71^{Db}	7.84^{A}	7.63^{Bb}	7.73^{ABb}	7.41^{Cb}	0.05
	SEM	0.03	0.02	0.09	0.01	0.12	0.09	0.02	0.03	

$^{1)}$ C, Control (No addition); RBF, rice bran 1.5%; WF, wheat 1.5%.
Means in the same row with different letters (A-H) are significantly different ($p<0.05$).
Means in the same column with different letters (a-c) are significantly different ($p<0.05$).
SEM, standard error of the means of three replicate experiments with three samples analyzed per replicate.

reported that a high LAB viable count of fermented sausages inhibits the growth of spoilage and pathogenic bacteria, especially *Staphylococcus aureus*.

pH, a_w, and meat color values of fermented sausages during storage period

Table 4 shows the changes in pH, water activity (a_w), and color of fermented sausages with dietary fibers during storage period at 4°C. The initial pH values of fermented sausages were between 5.05 and 5.63, and the RBF and WF treatments were significantly lower than the control until day 14. However, the pH values of RBF and WF treatments from day 21 to 28 were significantly higher than the control. The a_w of fermented sausages decreased with the length of cold storage; a_w of all fermented sausages at day 7 to 28 day were decreased compared to day 0. The initial a_w of fermented sausages ranged from 0.840 to 0.853, and a_w of fermented sausages on day 28 were between 0.821 and 0.828. The initial L* values of fermented sausages during cold storage ranged from 40.49 to 41.12. The L* values of all fermented sausages during storage from day 7 to 28 were significantly lower compared to day 0, and there was no significant difference between treatments during the cold storage period. The initial a* values of fermented sausages ranged from 16.17 to 16.65. As the storage period increased, the redness values of control and treatments with dietary fibers were reduced significantly. The a* values of the RBF treatment were significantly higher than those of control and WF treatment during cold storage period, except for day 0. As the storage period increased, the b* values of fermented sausages during storage for 28 d at 4°C showed a statistically significant change, but there was no consistent tendency and the impact on yellowness of fermented sausages by storage period and dietary fiber addition.

Sancho et al. (1994) reported that the increased pH values for fermented sausages at the end of storage due to the accumulation of peptides, amino acids, and ammonia as a result of proteolytic reactions. Similar results were observed for pH value of short-ripened salami (Lee et al., 2009). Kargozari et al. (2014) reported that lower a_w helps control microbial growth and over-fermentation in fermented products. Lee et al. (2009) reported that a_w of short-term fermented salami during cold storage for 120 days decreased with increasing storage period. These results were similar to the present study. Papastamatiou

Table 4. **Changes in pH, water activity (a_w), and color of fermented sausages with dietary fibers during storage period at 4℃**

Items		Days of storage					
		0 day	7 day	14 day	21 day	28 day	SEM
pH	C[1)]	5.63^{Ba}	5.74^{Aa}	5.63^{Ba}	5.28^{Db}	5.47^{Cb}	0.01
	RBF	5.11^{Db}	5.16^{Cc}	5.09^{Dc}	5.30^{Ba}	5.60^{Aa}	0.01
	WF	5.05^{Dc}	5.44^{Bb}	5.41^{Bb}	5.31^{Ca}	5.61^{Aa}	0.02
	SEM	0.01	0.02	0.01	0.01	0.01	
a_w	C	0.840^{Ac}	0.818^{Ec}	0.823^{Db}	0.833^{Ba}	0.828^{Ca}	0.001
	RBF	0.844^{Ab}	0.824^{Db}	0.832^{Ca}	0.834^{Ba}	0.821^{Ec}	0.001
	WF	0.853^{Aa}	0.834^{Ba}	0.822^{Db}	0.831^{Cb}	0.824^{Db}	0.001
	SEM	0.001	0.001	0.001	0.001	0.001	
L*[2)]	C	41.12^{Aa}	32.22^{B}	31.82^{BCb}	31.31^{BCb}	31.13^{C}	0.33
	RBF	40.51^{Ab}	31.58^{B}	32.00^{Bab}	31.79^{Bb}	31.52^{B}	0.49
	WF	40.49^{Ab}	32.54^{BC}	33.35^{Ba}	33.29^{Ba}	31.93^{C}	0.27
	SEM	0.12	0.39	0.45	0.47	0.34	
a*	C	16.17^{A}	15.25^{Bb}	14.57^{Cb}	14.10^{Cb}	12.97^{Db}	0.17
	RBF	16.65^{A}	16.28^{Aa}	15.95^{ABa}	15.84^{ABa}	14.86^{Ba}	0.37
	WF	16.37^{A}	15.25^{Bb}	14.93^{Bb}	13.69^{Cb}	12.70^{Db}	0.14
	SEM	0.15	0.20	0.27	0.25	0.33	
b*	C	11.77^{ABc}	11.28^{Cb}	12.23^{Aab}	12.06^{Ab}	11.11^{C}	0.16
	RBF	12.95^{a}	12.45^{a}	12.92^{a}	12.84^{a}	12.11	0.39
	WF	12.40^{Ab}	12.19^{Aa}	11.61^{Bb}	11.61^{Bb}	10.98^{C}	0.15
	SEM	0.08	0.21	0.22	0.22	0.44	

[1)] C, Control (No addition); RBF, rice bran 1.5%; WF, wheat 1.5%.
[2)] L*, lightness; a*, redness; b*, yellowness.
Means in the same row with different letters (A-E) are significantly different ($p<0.05$).
Means in the same column with different letters (a-c) are significantly different ($p<0.05$).
SEM, standard error of the means of three replicate experiments with three samples analyzed per replicate.

et al. (2007) reported that the decrease of L* values in sausages during storage is due to the browning reaction and concentration of color by the drying of sausages in the air flow. Also, Kurt (2016) reported that the lightness of Turkish fermented sausage decreased as the storage period increased. In the previous studies, addition of dietary fibers decreased the redness of meat products during cold storage (Choi et al., 2007; 2008b). For this reason, Shon and Chin (2008) reported that lipid oxidation may initiate the oxidation of myoglobin to metmyoglobin, and changed the* meat color from red to an unattractive brown. Although this can increase yellowness, presently the yellowness did not show any significant change. Harms et al. (2003) reported that the changes of b* values in meat products during storage period is associated with fat oxidation.

Total microbial count (TMC), lactic acid bacteria (LAB), volatile basic nitrogen (VBN), and 2-thiobarbituric acid reactive substance (TBARS) of fermented sausages during storage period

The changes in total microbial count (TMC), lactic acid bacteria (LAB), 2-thiobarbituric acid reactive substance (TBARS) and volatile basic nitrogen (VBN) of fermented sausages with dietary fibers during storage period at 4℃ were presented in Table 5. The initial TMC in the fermented sausages on day 0 was about 5 Log CFU/g, which was maintained until day 28. There were no significant differences in TMC during all storage periods and treatments in the fermented sausages. The initial LAB of fermented sausages on the 0 day was between 6.99 and 8.00 Log CFU/g, and the RBF and WF treatments on the 0 day were significantly higher than control. Also, the LAB of RBF treatments on day 14 was significantly higher than the control. The LAB count of fermented sausages was about 7 Log CFU/g during cold storage, with no significant differences during all storage periods. The VBN values of the RBF and WF treatments were significantly higher than the control during all storage periods. The VBN values of treatments were significantly higher than the control during all storage period. The TBARS values of treatments with dietary fibers also were higher than the control during cold storage period, and the RBF treatment on day 7 and 28 had significantly higher TBARS values than those of WF treatment.

The reason for the higher number of LAB in the treatments compared to the control is the final LAB number was higher in the treatments during the ripening period. Therefore, the microbial counts seemed to be maintained during cold storage. In study of Ham et al. (2016), the total microbial bacteria counts and LAB counts of fermented sausages with dietary fiber significantly increased as cold storage period increase. These results were similar to the present study.

In addition, Fista et al. (2004) reported that high LAB in sausages inhibits the growth of pathogenic bacteria and spoilage

Table 5. Changes in total microbial count (TMC), lactic acid bacteria (LAB), 2-thiobarbituric acid reactive substance (TBARS) and volatile basic nitrogen (VBN) of fermented sausages with dietary fibers during storage period at 4℃

Items		Days of storage					
		0	7	14	21	28	SEM
TMC (Log CFU/g)	C[1)]	5.40	5.38	5.15	5.45	5.15	0.12
	RBF	5.30	5.03	5.00	5.15	5.53	0.16
	WF	5.23	5.19	5.38	5.38	5.30	0.15
	SEM	0.12	0.16	0.10	0.13	0.18	
LAB (Log CFU/g)	C	6.99^{c}	7.02	7.15^{b}	7.53	7.30	0.24
	RBF	8.00^{a}	7.65	7.81^{a}	7.45	7.38	0.18
	WF	7.63^{b}	7.40	7.45^{ab}	7.30	7.38	0.16
	SEM	0.03	0.25	0.12	0.19	0.28	
VBN (mg%)	C	21.45^{Ac}	16.70^{Cc}	18.57^{Bc}	19.08^{Bc}	16.79^{Cc}	0.53
	RBF	32.53^{Aa}	26.58^{Ba}	31.93^{Aa}	20.45^{Cb}	29.97^{Aa}	0.78
	WF	29.23^{Ab}	18.71^{Cb}	23.7^{Bb}	23.01^{Ba}	19.81^{Cb}	0.36
	SEM	0.62	0.54	0.39	0.36	0.86	
TBARS (mg MA/kg)	C	0.19^{B}	0.16^{Bc}	0.43^{A}	0.15^{B}	0.08^{Bb}	0.06
	RBF	0.39^{A}	0.45^{Aa}	0.48^{A}	0.19^{B}	0.17^{Ba}	0.04
	WF	0.44^{B}	0.32^{BCb}	0.68^{A}	0.17^{CD}	0.09^{Db}	0.05
	SEM	0.07	0.03	0.08	0.01	0.01	

[1)] C, Control (No addition); RBF, rice bran 1.5%; WF, wheat 1.5%.
Means in the same row with different letters (A-D) are significantly different (p<0.05).
Means in the same column with different letters (a-c) are significantly different (p<0.05).
SEM, standard error of the means of three replicate experiments with three samples analyzed per replicate.

bacteria as well during the storage. The increase of VBN is highly associated with microbial growth (Kruk et al., 2011). Also, increased microbial degrades nutrients, such as proteins and fat, as enzymes, resulting in reduced quality of the meat products. Jin et al. (2015) reported that protein denaturation and fat oxidation of sausages together with inhibition of microbial growth decreased. Also, Choi et al. (2008b) reported that rice bran has considerable quantities of lipid and lipase, and especially the presence of enzymes, such as lipase, causes rancidity and rapidly degrades sausage quality during storage. In this study, the fermented sausages with rice bran tended to have high VBN values and TBARS values. Lee et al. (2009) reported that the VBN and TBARS values increased with increasing storage period in short-term fermented sausages. Also, Kurt (2016) reported that the TBARS values of fermented sausage added with grape seed flour increased significantly as the ripening and storage period increased.

Conclusion

The purpose of this study was to investigate the effects of rice bran and wheat fibers on microbiological and physicochemical properties of fermented sausages during ripening and storage, and to develop functional meat products. The addition of rice bran and wheat fiber to fermented sausages during ripening decreased pH and accelerated fermentation by the rapid growth of LAB with a quality not markedly different from that of the control even though there were rise of VBN and TBARS values during cold storage. In conclusion, rice bran and wheat fibers promoted the fermentation of sausages. Thus, these fibers are excellent additives for the production of fermented sausage and are expected to be applied to the development of various functional meat products.

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