Cholesterol-lowering and lipid oxidation reduction potentials of traditional seasonings in Salchichon dry-fermented sausages

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Revised: 3 August 2016 / Accepted: 9 August 2016 / Published online: 16 August 2016
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Abstract Five different natural/traditional seasonings including *doenjang* (fermented soybean paste), *gochu-jang* (red pepper paste), fresh medium-hot and hot peppers, and garlic were used, and 1 % (w/w) each was incorporated into formulations of Salchichon fermented sausage type. After ripening for 51 days, the products were assessed for quality parameters, lipid oxidation, cholesterol content and sensory characteristics. In general, incorporation of the seasonings did not cause color or texture defects whereas it had beneficial effects on improvement of product's quality; however the effects differed depending on each type of seasonings added. Noticeably, most treatments with the seasonings significantly reduced the lipid oxidation. Additionally, incorporating *doenjang*, *gochu-jang*, medium-hot peppers, hot peppers and garlic resulted in reduction of 32.03, 28.96, 36.30, 19.53 and 33.03 mg cholesterol/100 g sample, corresponding to 26.78, 24.21, 30.35, 16.33 and 27.61 %, respectively. Higher scores for the sensory traits such as aroma, taste, color and acceptability were also observed for the samples with seasonings. The current work demonstrated that the tested seasonings represent potentially natural ingredients for producing healthier Salchichon fermented sausages.

Keywords Salchichon fermented sausage · Traditional seasonings · Lipid oxidation · Cholesterol · Sensory quality

Introduction

Dry fermented sausages are well known internationally due to their typical flavor that is the result of a subtle balance between volatile and non-volatile compounds coming from raw materials and additives/or biochemical reactions during fermentation and ripening (Stahnke 2002). The fermented meat products are generally made from lean meat (e.g., pork and beef) with or without pork back fat and starter culture, and they also have different commercial names (e.g., sausages, salami, Salchichon and Chorizo etc.) depending on the geographic locations (Rubio et al. 2008; Utrilla et al. 2014; Gonzalez-Fernandez et al. 2006). Although, the products generally have high nutritional values (Beriaín et al. 2000), however, they also possess some negative aspects such as unfavorable polyunsaturated/saturated fatty acid ratio and high cholesterol content (Muguerza et al. 2001). Researchers have reported that high blood cholesterol level is linked with coronary heart disease and mortality resulting from consumption of cholesterol-rich foods (Tanasescu et al. 2004). Besides, the products are highly susceptible to lipid oxidation which generates toxic molecules with possible hazard for human health as well as reduces shelf-life stability (Zanardi et al. 2004). So far, though the lipid oxidation in meat products can be controlled by the addition of synthetic antioxidants, however, these available synthetic antioxidants (e.g., butylated hydroxyanisole and butylated hydroxytoluene) have been suspected to cause toxicity problems that negatively affect consumer's health, therefore, these compounds have been restricted use in foods (Botterweck et al.

Electronic supplementary material The online version of this article (doi:10.1007/s13197-016-2315-8) contains supplementary material, which is available to authorized users.

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2000). Nowadays, consumers are increasingly aware of the importance of their food choices in health maintenance and disease prevention, therefore, a new trend to substitute these synthetic additives with natural functional ingredients have received the most attention by consumers and meat processors (Nassua et al. 2003; Kurćubić et al. 2014). On the other hand, the incorporation of natural additives into the conventional processing formulations makes the meat products not only healthier but also improved sensory quality (Seong et al. 2015).

Doenjang, a soybean fermented product, has long been used as a healthy food and seasoning in Korea, and this kind of product is also widely used in Asian countries such as China and Japan etc. The *doenjang* is characterized by high levels of important nutrients and bioactive compounds which exert potent biological activities such as anticancer, anti-diabetes and anti-obesity effects as proven in clinical studies (Shukla et al. 2014; Kim et al. 2005). Therefore, the product was internationally accepted food from 2009 and is now sold in markets worldwide. Like the *doenjang*, *gochu-jang*, a kind of red pepper paste made from dried pepper powder, water, salt, fermented soybean and sweet rice powder etc. is also commonly used as a seasoning in many dishes in Korea (Kwon et al. 2015; Shin and Jeong 2015). Besides, the other plant-sourced seasonings such as fresh chili peppers and garlic are also widely used due to their ability to enhance typical flavor of dishes or food products. Additionally, these fresh seasonings have also been proven to exert the beneficial effects on health such as reducing cholesterol level in blood and cardiovascular risk (Hermansen et al. 2003), antioxidant, antiatherosclerotic, hypoglycaemic, antimicrobial and hepatoprotective effects (Chung 2006; Amagase 2006; Queiroz et al. 2009).

Though some of the seasonings (e.g., garlic and chili) have been used in meat products (Deda et al. 2007; Lorenzo et al. 2013) but most of them are added into the meat products usually in the form of dry powder. These are subjected to various cooking process and partly loss their typical flavors and bioactivities. Therefore, it is still unknown whether these natural seasonings could exert their bioactivities (e.g., anti-oxidation) in uncooked meat products such as fermented sausages because application of functional ingredients from plant sources for improving sensory quality as well as health benefit of meat products requires the evaluation of efficacy within each type of meat product.

In previous studies, authors have demonstrated that the addition of natural/traditional ingredients such as mushroom or *kimchi* powder significantly improved the lipid oxidation stability and sensory quality of meat products (Seong et al. 2015; Lee et al. 2008). While the seasonings

from natural sources like *doenjang*, *gochu-jang*, fresh garlic and chili peppers are abundant, inexpensive and widely used for multi-purposes as mentioned above, however, no attention has been paid to the investigation of effects of their addition on the quality of meat products. Therefore, the objective of the present study was to assess the impacts of incorporation of some natural/traditional seasonings on the quality characteristics, cholesterol content and sensory perception of Salchichon dry-fermented sausages type.

Materials and methods

Materials

Pork hams (*semimembraneous*, *semitendinous adduct*, *biceps femoris*, *quadriceps femoris*, *middle gluteal* and *gracilis* muscles) were obtained from a local slaughterhouse (Suwon, Korea) 24 h after slaughter. Seasonings including: *Doenjang* (made with soybean, wheat flour, salts, meju power and starters), *gochu-jang* (made with hot pepper powder, sweet rice powder, salt, water, corn syrup, garlic and onion), fresh green medium-hot peppers, green hot peppers and fresh garlic cloves were purchased from a local market in Suwon, South Korea. Sodium nitrite and sodium nitrate were purchased from Sigma Chemical Co. (Louis, MO, USA). Sodium ascorbate, sodium chloride and sugar were purchased from Shinyo Pure Chemicals Co. (Osaka, Japan), Hanju Co. (Ulsan, Korea) and Beksul Food Co. (Seoul, Korea), respectively.

Salchichon dry-fermented sausage formulation and processing

The formulations of Salchichon sausages in the present study were referred to the previously developed formulations by Casquete et al. (2012) with some modifications. Fresh peppers (both medium-hot and hot types) and garlic cloves were washed to remove any impurities, left to drain the water, cut and then ground separately using a blender (HMF 3160S, Hanil electric, Co, Seoul, Korea). The initial pH values of *doenjang*, *gochu-jang*, ground medium-hot peppers (GMP), ground hot peppers (GHP) and ground garlic (GG) were 5.70, 5.45, 5.65, 5.63 and 5.80, respectively.

Six different treatments of traditional Salchichon fermented sausages (20 kg each) were prepared in the present study. All treatments (T) were formulated with only lean meat, 1.6 % sodium chloride, 1.5 % sugar, 0.2 % black pepper, 0.03 % sodium ascorbate, 0.01 % sodium nitrite

and 0.005 % sodium nitrate. Besides, 1 % (w/w) of each test seasonings such as *doanjang*, *gochu-jang*, GMP, GHP and GG was added to the T1, T2, T3, T4 and T5, respectively. The batch made without the added test seasonings was served as the control (C). The processing procedure of fermented sausages was performed as follows: The refrigerated meat was first trimmed off of all connective tissue and visible fats and then cut into small pieces (40 mm × 30 mm × 10 mm). The pieces were then mixed with all of the ingredients (except for the test seasonings) and left in a chilling room for overnight. The following day, for each treatment, the meat mixture was placed in a meat mixer (model CE93, RUHLE GMBH, Grafenhausen, Germany) and then the test seasoning was also added to the corresponding treatment and mixed for further 15 min at 4 °C. After mixing, the meat mixtures were immediately stuffed into 65-mm diameter collagen casings (Naturin Viscofan Co, Tajonar-Navarra, Spain) using a vacuum stuffer (Model VF610, Handtmann Co, Biberach, Germany), resulting in sausages of ca. 1.5 kg. The filled sausages were then hung vertically on metal rods, set on the cart and transferred to a fermenting room. Temperature and relative humidity (RH) conditions in the room during curing, fermentation, ripening and drying were set as follows: 4 °C/85 % RH for 14 days, 8 °C/75 % RH for 7 days, 12 °C/65 % RH for 30 days. At the end of the ripening/drying (30 days), the sausage samples ($n = 12$ for each treatment) were collected used for analyses of proximate composition, quality traits, cholesterol content, and sensory evaluation. The weights of samples taken after filling into casings and at the end of ripening were used for weight loss determination.

Proximate composition

Moisture, fat and protein contents were analyzed using the method of the Association of Official Analytical Chemists (Anderson 2007). Moisture and fat content were determined by using a moisture & fat analyzer (SMART Trac, CEM Corp, USA); while, the nitrogen content was determined by using a nitrogen analyzer (Rapid N cube, Elementar, Germany) and then converted into protein content ($N \times 6.25$). To determine energy, the sample was homogenized in a blender then the homogenate was used for measurement of calorie content by using a calorimeter (model 6400, Parr instrument, USA). Calories were expressed as kcal/100 g of the sample. Three repetitions were applied for each sample in each analysis.

pH and water activity (a_w)

The pH values of samples were determined in triplicates using a pH meter (Model 340, Mettler-Toledo GmbH,

Schwerzenbach, Switzerland) that was calibrated with 3 different standard pH solutions (4.0, 7.0 and 9.25). The pH was measured after homogenizing 3 g of each sample with 27 mL of distilled water for 30 s using a homogenizer. Water activity (a_w) of the fermented sausages was determined at 25 °C with a Novasina measuring instrument; model AW SPRINT-TH 300 (Pfaffikon, Switzerland). Calibration was done by using several saturated solutions of known a_w .

Instrumental color measurement

Color was determined at three defined areas on the cut surface of each sample using a Minolta Chroma Meter (model: CR-400, 8 mm measuring aperture; Illuminant condition: CIE: C, D65; CIE: 2° Standard Observer. Minolta Camera Co., Ltd., Osaka, Japan) that was standardized with a white plate ($Y = 86.3$, $X = 0.3165$ and $y = 0.3242$). Special care was taken to avoid scanning areas of fat in the sample. Color was expressed according to the Commission International de l'Eclairage (CIE) system and reported as CIE L^* (lightness), CIE a^* (redness), CIE b^* (yellowness), chroma and hue angle (h°). In which the chroma and hue angle were calculated as $(a^{*2} + b^{*2})^{0.5}$ and $\tan^{-1}(b^*/a^*)$, respectively.

Texture profile analysis (TPA)

The TPA was performed using a puncture probe (7 mm diameter) attached to a texture Analyzer (Model 4465, Instron Corp, UK). For texture analysis, the samples from each treatment were cut into 2.5-cm long pieces (3 pieces for each sausage) which were then axially compressed twice until reaching each time 80 % of its initial height. The speed of load cell was set at 120 mm/min and the following parameters were calculated: hardness (kg), cohesiveness (kg*mm), springiness (mm), gumminess (kg) and chewiness (kg*mm).

Thiobarbituric acid reactive substances (TBARS) measurement

The content of TBARS was determined to evaluate the lipid oxidation level in samples between the treatments, using the method of Pikul et al. (1989). The TBARS concentrations were calculated by multiplying the absorbance by a constant coefficient of 5.5 which obtained from standard curves and known dilutions (0.1–0.8 μ M) of 1,1,3,3 tetramethoxypropane (Aldrich, Louis, MA, USA). The TBARS value was expressed as mg malonaldehyde/kg (MA/kg) of sample. Three repetitions were applied for each sample in each treatment.

Cholesterol, monosaccharide, saturated fat and sodium compositions

The cholesterol, saturated fat, carbohydrate and sodium contents were determined in triplicates using the methods standardized by the Ministry of Food and Drug Safety of Korea (MFDS 2014). The levels of the examined contents were calculated into milligram (mg) or gram (g) in 100 g sample. For the cholesterol, the reduction capacity of this content were also calculated using the concentrations of samples in each treatment: reduction capacity (%) = $100 \times (\text{control} - \text{treatment})/\text{control}$.

Sensory evaluation

Sensory evaluation was performed using a panel consisted of 12 assessors. The panelists with average age of 35 years old were the staffs of the department of animal products processing and utilization, National Institute of Animal Science, Korea, and were chosen on the basis of previous experience in sensory evaluation of meat products. Briefly, each sausage sample was cut into 0.3 cm thick pieces, placed onto dishes and coded with random numbers. The panelists were laid to seat in private seats under fluorescent lighting and were served with the sensory samples in a random manner. Five major sensory traits including flavor, color, taste and overall acceptability were used and the samples were then evaluated for the aforementioned traits using a 7-point scale (1 point = extremely undesirable, 7 point = extremely desirable) as described by Meilgaard et al. (1991). The panelists were asked to refresh their mouth with the drinking distilled water and salt-free crackers between samples. The sensory evaluation was carried out in the sensory panel booth room equipped with white lighting at a constant temperature of about 20 °C.

Statistic analysis

The data were subjected to statistical analysis using the Statistic Analysis System (SAS) package (SAS Institute, Cary, NC, USA, 2007). All data were analyzed by the General Linear Model procedure considering treatment the main effect. Means were compared using Duncan's Multiple Range Test, with a significance of $p < 0.05$. Principal component analysis (PCA) was determined to explore relationships between multiple physicochemical compositions, technological traits, sensory attributes and fermented sausages formulated with different seasonings.

Results and discussion

In the present study, a type of healthier Salchichon dry sausage with lowered cholesterol content was successfully produced with the addition of some natural/traditional

seasonings (*doen-jang*, *gochu-jang*, ground peppers and garlic). Total processing time was 58 days and some representative images showing the cross-section of the products at the end of ripening were also taken (supplementary Fig. 1).

Effect of seasonings addition on proximate composition and some technological quality trait

In general, the addition of seasonings had minor effects on the proximate compositions (Table 1). The moisture content in sausages ranged between the control and treatments from 34.01 to 38.10 %. No differences in the moisture were found between the control and GHP (T4) or GG (T5) treatment ($p > 0.05$), whereas the control samples showed a significantly ($p < 0.05$) lower moisture compared to those of T1, T2 and T3. Moisture content is one of the important water-related criteria used to indicate the shelf-life stability of the dry cured meat products. The levels of moisture in all samples were almost similar to the level (37.24 %) in industrial Salchichon ripened for 55 days (Casquete et al. 2012), but lower than the levels (41.01–49.14 %) in the same product type ripened for 28 days (Utrilla et al. 2014). The contrasting results could be due to the differences in the drying/ripening conditions among the studies. Similarly, the fat levels in samples made with *doenjang*, GHP and GG were not significantly different from the control. Regarding protein content, it's level in the control samples was similar to the levels in samples from the T1, T3 and T5, but was higher than those in T2 and T4, this could be due to the lower moisture contents of the control samples. For energy, most treatments with the seasonings had levels similar to that of the control samples.

The weight loss ranged between the treatments from 44.54 to 49.52 %, no differences occurred between the control and GG treatment ($p > 0.05$), however, the treatments with both types of peppers had significantly higher levels compared to the control. The levels of weight loss in all samples in this study were almost similar to the levels (46.31–49.60 %) reported for the Salchichon sausages by Utrilla et al. (2014). The pH values ranged between the control and treatments from 4.75 to 4.95, in which most treatments with the seasonings such as *gochu-jang* and peppers had significantly lower values in comparison with the control, this could be due to higher level of lactic acid produced in these treated samples. The pH values in all samples in present study were much lower than the value (6.70) reported for the Salchichon sausages ripened for 60 days (Jung et al. 2015), and also lower than the values (5.34–5.39) reported for the same product type ripened for 55 days in literature (Casquete et al. 2012). These contrasting results could be due to the differences in initial pH

Table 1 Proximate composition and technological quality traits of Salchichon dry-fermented sausages formulated with natural seasonings

Treatment	Proximate composition			Technological quality traits				
	Moisture (%)	Fat (g/100 g)	Protein (g/100 g)	Energy (kcal/100 g)	Weight loss (%)	pH	a_w	TBARS (mg MDA/kg sample)
Control (C)	34.01 ± 0.58c ¹	11.82 ± 0.48a	42.53 ± 1.35a	296.98 ± 5.16b	48.73 ± 0.30b	4.86 ± 0.01bc	0.822 ± 0.01b	0.50 ± 0.02b
1 % <i>doenjang</i> (T1)	36.03 ± 0.50ab	11.85 ± 0.59a	39.63 ± 1.12ab	300.54 ± 4.32b	45.55 ± 0.21c	4.95 ± 0.02a	0.81 ± 0.01b	0.40 ± 0.01c
1 % <i>gochu-jang</i> (T2)	37.50 ± 0.56ab	9.40 ± 0.37b	37.60 ± 0.78b	284.24 ± 6.31c	44.54 ± 0.13d	4.78 ± 0.01d	0.81 ± 0.04b	0.32 ± 0.01d
1 % GMP (T3)	38.10 ± 0.46a	10.33 ± 0.52b	40.53 ± 2.78ab	295.12 ± 4.35b	49.40 ± 0.22a	4.75 ± 0.01d	0.83 ± 0.01a	0.51 ± 0.04b
1 % GHP (T4)	35.79 ± 0.84bc	13.20 ± 0.53a	38.00 ± 0.66b	295.26 ± 7.29b	49.52 ± 0.21a	4.80 ± 0.02d	0.82 ± 0.03b	0.40 ± 0.01cd
1 % GG (T5)	35.64 ± 0.55bc	12.16 ± 0.77a	40.18 ± 1.56ab	309.88 ± 5.56a	48.51 ± 0.13b	4.92 ± 0.01ab	0.82 ± 0.02b	0.86 ± 0.01a

GMP ground medium-hot peppers, GHP ground hot peppers, GG ground garlic

¹ Values are expressed as mean ± standard deviationMeans with different letters (a–d) in the same column differ significantly ($p < 0.05$)

values of the raw materials or fermentation conditions used among the studies. It is well known fact that the shelf-life of meat products is affected by a number of factors including pH condition; the lower the pH the higher the acidity and most bacteria that contribute to food spoilage do not prosper in acidic conditions. Practically, in order to be considered “shelf-stable” the ultimate pH of finished products must be 5.3 or lower. Thus, these pH results could consider enough to ensure the safety of meat products (Papadima and Bloukas 1999), and also suggested that the incorporation of these seasonings may partly enhance the shelf-life stability of fermented sausage products. The water activity (a_w) in most treatments were not significantly different from the control ($p > 0.05$), suggesting that the treatments with seasonings did not affect of the rate of water activity reduction. The shelf-life stability of food products usually increases with decreasing a_w , whereas high a_w (e.g., around 0.95) can form an ideal medium for bacterial growth on the surface that influences the microbiological stability (Fernandez-Salguero et al. 1993). The a_w -values in samples made with seasoning in the present were generally lower than the value (0.86) in the same product type ripened for 55 days (Casquete et al. 2012).

Regarding the lipid oxidation, it was observed that the treatments with *doenjang*, *gochu-jang* and GHP significantly ($p < 0.05$) reduced the TBARS content as compared to the control, indicating that the lipid oxidation was inhibited by the added *doenjang*, *gochu-jang* and GHP. The obtained results could be explained due to the actions of antioxidants present in these seasonings. Regarding this, earlier workers have shown that *doenjang* and *gochu-jang* possess high antioxidant activity in vitro and in vivo studies (Shukla et al. 2014; Kim et al. 2005), and the peppers contain bioactive compounds such as phenols that are powerful antioxidants (Ogiso et al. 2008). When compared to TBARS levels (0.73–1.21 mg MA/kg sample) of Salchichon sausages ripened for 28 days (Utrilla et al. 2014), all the samples in the present study had lower levels.

Effect of seasonings addition on color and texture

The color and texture traits of Salchichon sausages as affected by the additions of seasonings are presented in Table 2. Regarding L* (lightness), the treatments with peppers (T3 and T4) had the L*- values similar to that of the control, whereas the treatment with other remaining seasonings had lower values in comparison to the control samples ($p < 0.05$). Interestingly, the a*-values of samples in all treatments with seasonings (T1–T5) were significantly higher than that of the control. Furthermore, comparing the redness among the treatments shows that the sausages produced with *gochu-jang* had significantly ($p < 0.05$) higher value, probably result from the

Table 2 Color and texture traits of Salchichon dry-fermented sausages formulated with natural seasonings

Treatment	Color			Texture						
	L* (lightness)	a* (redness)	b* (yellowness)	Chroma	Hue angle	Hardness (kg)	Cohesiveness	Springiness (mm)	Gumminess (kg)	Chewiness (kg mm)
Control (C)	48.58 ± 1.10a ¹	4.48 ± 0.46c	5.48 ± 0.64c	8.62 ± 0.86b	50.69 ± 5.32	2.60 ± 0.15b	1.27 ± 0.28	18.85 ± 0.84ab	3.34 ± 1.44b	61.18 ± 5.76c
1 % <i>doenjang</i> (T1)	41.64 ± 0.68c	5.99 ± 0.36b	7.14 ± 0.53ab	8.41 ± 0.55b	41.95 ± 6.11	4.34 ± 0.47a	1.40 ± 0.32	19.32 ± 0.94ab	6.12 ± 0.94ab	117.54 ± 10.17ab
1 % <i>gochujang</i> (T2)	39.77 ± 0.80c	11.55 ± 0.19a	7.43 ± 0.52ab	13.49 ± 0.78a	45.93 ± 4.15	3.32 ± 0.22ab	1.22 ± 0.24	19.26 ± 0.65ab	4.06 ± 0.36b	77.53 ± 5.46bc
1 % GMP (T3)	46.81 ± 0.32ab	5.73 ± 0.16b	8.46 ± 0.90a	7.68 ± 0.63b	47.41 ± 3.67	4.04 ± 0.40a	1.86 ± 0.14	18.51 ± 0.44b	7.70 ± 0.78a	138.00 ± 11.32a
1 % GHP (T4)	47.06 ± 0.16ab	5.84 ± 0.51b	6.25 ± 0.45bc	7.23 ± 0.52b	40.86 ± 3.26	3.50 ± 0.32ab	1.35 ± 0.36	20.36 ± 0.38a	4.69 ± 0.45ab	95.35 ± 7.56ab
1 % GG (T5)	45.21 ± 0.36b	5.71 ± 0.33b	5.53 ± 0.23c	7.13 ± 0.70b	48.79 ± 4.12	3.80 ± 0.17a	1.63 ± 0.12	18.96 ± 0.50ab	6.07 ± 0.56ab	112.51 ± 8.06ab

GMP ground medium-hot peppers, GHP ground hot peppers, GG ground garlic

¹ Values are expressed as mean ± standard deviationMean with different letters (a–c) in the same column differ significantly ($p < 0.05$)

suppression of the product's natural color owing to the addition of *gochu-jang*. The a^* -value of the *gochu-jang* treated samples in the present study was nearly two times greater than the values (5.69–6.85) reported for same product type (Utrilla et al. 2014). For the b^* (yellowness), its values in T1 and T2 samples were significantly ($p < 0.05$) higher compared to that of the control samples. While, no differences in chroma and hue angle values occurred among the control and treatments, except the *gochu-jang* treated samples (T2) which presented higher chroma values compared to the control. Color is the single most important factor of meat and meat products, that affects the consumer purchasing decision, and nitrite has long been used to enhance the red color of these products, however, the use of nitrite in meat product is also associated with health risk (Chow and Hong 2002). Researchers therefore have been attempting to reduce the added nitrite content in meat products by partially replacing it with natural additives (Deda et al. 2007; Ruiz-Capillas et al. 2015). Thus, it could be said that the additions of the seasonings did not cause color defects in the finish products. Additionally, the incorporation of *gochu-jang* improved the red color of the products, suggesting that the addition of *gochu-jang* to fermented sausages may be an alternative way to instead of nitrate or reduce its added level.

Texture is one of the important characteristics of a food. Table 2 shows that the additions of seasonings did not affect the cohesiveness, gumminess and chewiness ($p > 0.05$). Whereas, the treatments with *doenjang* (T1) or GMP (T3) and GG (T5) showed significantly ($p < 0.05$) higher hardness and chewiness values compared to the control samples. These results suggest that the incorporation of seasonings seemed not to cause a texture defect of the finished products.

Effect of seasonings addition on cholesterol, monosaccharide and sodium contents

Saturated fat, cholesterol, Na and carbohydrate contents of Salchichon dry-fermented sausages as affected by the seasonings incorporation are presented in Table 3. No differences in the saturated fat contents occurred among the control and all treatments ($p > 0.05$), except for the treatment with GG (T5) which showed higher level.

Regarding the cholesterol content, the outcome of our analysis showed that the additions of the seasonings produced beneficial effects on reduction of cholesterol contents in fermented sausages. Particularly, incorporating *doenjang*, *gochu-jang*, GMP, GHP and GG resulted in reduction of 32.03, 28.96, 36.30, 19.53 and 33.03 mg cholesterol/100 g samples, corresponding to 26.78, 24.21, 30.35, 16.33 and 27.61 %, respectively as compared with

Table 3 Saturated fat, cholesterol, Na and carbohydrate contents of Salchichon dry-fermented sausages formulated with natural seasonings

Treatment	Saturated fat (g/100 g)	Cholesterol (mg/100 g)	Na (mg/100 g)	Carbohydrate (g/100 g)
Control (C)	4.62 ± 0.37bc ¹	115.25 ± 7.25a	1329.48 ± 157.37	1.14 ± 0.14c
1 % <i>doenjang</i> (T1)	4.76 ± 0.38abc	88.42 ± 2.78bc	1539.50 ± 132.03	1.88 ± 0.13b
1 % <i>gochu-jang</i> (T2)	4.22 ± 0.18c	92.15 ± 3.16bc	1778.28 ± 136.20	3.06 ± 0.27a
1 % GMP (T3)	4.98 ± 0.77abc	83.97 ± 1.90c	1405.48 ± 109.32	1.48 ± 0.12c
1 % GHP (T4)	5.52 ± 0.36ab	103.7 ± 4.12ab	1644.48 ± 111.13	1.12 ± 0.10c
1 % GG (T5)	6.00 ± 0.45a	89.08 ± 4.55bc	1374.92 ± 98.72	1.16 ± 0.11c

GMP ground medium-hot peppers, GHP ground hot peppers, GG ground garlic

¹ Values are expressed as mean ± standard deviation

Means with different letters (a–c) in the same column differ significantly ($p < 0.05$)

the control ($p < 0.05$). The fermented meat products also have some negative aspects such as the high cholesterol level which was the main cause of cardiovascular disorder disease, high blood pressure and mortality in the world (Tanasescu et al. 2004). When compared to the cholesterol level (110.5 mg/100 g sample) reported for fermented sausages in literature (Muguerza et al. 2001), all of the sausage samples made with seasonings in the present study had lower levels whereas those made without the test seasonings (control) had higher level. Regarding this, studies with human subject have shown the similar results, authors have concluded that consumption of soya products or garlic, soluble fiber and plant sterol esters for several months significantly reduced serum concentrations of total cholesterol and LDL cholesterol up to 27 % (Hermansen et al. 2003; Taku et al. 2007). A recent report showed that consuming 15–25 g of soya protein or soybean processed products per day could reduce LDL cholesterol by 10 % (Baic 2015). However, the possible mechanism of cholesterol-lowering effect of plant-sourced products in these studies still remains unclear. The present work, reflected the cholesterol-lowering effect of traditional/natural seasonings in Salchichon fermented sausages. Despite we cannot yet offer a satisfactory explanation for this phenomenon, however, this is probably due to the bioactive components/or enzymes originated from microorganisms present in these seasonings which degraded the cholesterol in the products. Lee et al. (1997) demonstrated that some microorganisms were capable of degrading cholesterol or β -sitosterol. The present result suggested that addition of natural/traditional seasonings could be used to reduced cholesterol content in fermented sausages. Na content, control and treatments did not show significant differences ($p > 0.05$). Sodium is an essential nutrient found in salt and many foods, and our bodies need a small amount of Na to be healthy, but too much salt intake can lead to high blood pressure, stroke and kidney disease. When compared to the Na levels (1.1–1.8 g/100 g) reported for most types

of dry-cured meat products (Jimenez-Colmenero et al. 2010) all the samples made with or without seasonings in the present study had similar levels.

For the carbohydrate content, whose levels in samples in the treatments with peppers (T3 and T4) and garlic (T5) were similar to that of the control samples ($p > 0.05$), whereas the levels in samples treated with *doenjang*, *gochu-jang* were higher in comparison with the control. The addition of these two seasonings could be the reason responsible for the increased carbohydrate contents in the treated samples.

Effect of seasonings addition on sensory quality

The impact of the seasonings incorporation on the sensory characteristics of the products is shown in Table 4. In general, the addition of seasonings had positive effects on the sensory quality improvement of the products; however, the effects differed depending on type of seasonings added and sensory traits evaluated. Regarding flavor, higher scores were given for the samples added with seasonings compared to the control, but statistical differences ($p < 0.05$) were observed for the treatments with *doenjang*, *gochu-jang* and GMP. Similarly, the panelists gave higher taste scores for all treated samples than for the control, however, differences ($p < 0.05$) were observed for *doenjang* and *gochu-jang* treatments. The reason making the panelists gave higher flavor and taste scores for the samples made with the seasonings could be due to the contributions of the flavor enhancers and taste active compounds that exist within these seasonings. Additionally, the seasonings such as peppers and garlic have widely been used as flavor enhancers to enhance the flavor of many dishes for centuries due to their abundance and inexpensiveness. In meat industry, some of these ingredients are also used in meat products (Deda et al. 2007; Lorenzo et al. 2013), however, most of them when added to the meat products are usually in the form of dry powder which undergone the processing

Table 4 Sensory characteristics of Salchichon dry-fermented sausages formulated with natural seasonings

Treatment	Flavor	Taste	Color	Acceptability
Control (C)	4.17 ± 0.37d ¹	4.57 ± 0.28b	4.14 ± 0.35c	4.32 ± 0.24c
1 % <i>doenjang</i> (T1)	5.46 ± 0.26a	5.32 ± 0.14a	4.94 ± 0.33a	5.32 ± 0.10a
1 % <i>gochu-jang</i> (T2)	4.91 ± 0.39b	5.14 ± 0.17a	5.22 ± 0.36a	4.96 ± 0.26ab
1 % GMP (T3)	4.80 ± 0.18bc	4.86 ± 0.22ab	4.29 ± 0.21c	4.68 ± 0.16bc
1 % GHP (T4)	4.34 ± 0.36d	4.57 ± 0.21b	4.91 ± 0.17ab	4.18 ± 0.23c
1 % GG (T5)	4.51 ± 0.10 cd	4.82 ± 0.13ab	4.25 ± 0.11bc	4.61 ± 0.12bc

GMP ground medium-hot peppers, GHP ground hot peppers, GG ground garlic

¹ Values are expressed as mean ± standard deviation

Flavor, taste, color and acceptability, 7-point scales: 1 point = extremely undesirable; 7 point = extremely desirable

Means with different letters (a–d) in the same column differ significantly ($p < 0.05$)

treatment therefore making their typical flavors partly lost. For color, significantly ($p < 0.05$) higher scores were found in the treatments with *doenjang*, *gochu-jang* and GHP compared to that of the control. Overall acceptance is the sum of all sensory traits, our results showed that most treatments with seasonings had generally higher acceptability scores than the control, but statistically significant ($p < 0.05$) differences were observed for *doenjang* and *gochu-jang* treatments. The higher acceptability scores given by the panelists for these treated samples could be associated with the synergistic effect of their higher aroma, taste and color scores. Similarly, previous studies have also showed that the addition of natural ingredients (e.g., mushroom, tomato paste) had positive effects on improvement of sensory quality of sausages (Lorenzo et al. 2013; Seong et al. 2015). Hence our results suggest the potential utilization of these natural seasonings for improving sensory quality of meat products in general and Salchichon fermented sausages in particular.

Principal component analysis (PCA)

Principal component analysis (PCA) was conducted in order to explore relationships between multiple quality traits and fermented sausages formulated with different seasonings. The PCA showed that about 70.96 and 16.36 % of the variability was explained by the first two PCs and third PC, respectively (supplementary Fig. 2a, b). The correlation matrix for this model has a determinant close to zero indicating significant correlations among variables and suitability for PCA (Table 5). PC1 was positively related to saturated fat, cholesterol, weight loss, pH, a_w , fat, protein, energy and L^* . PC1 was inversely related to a^* , b^* , moisture, carbohydrate, Na, flavor, taste, color and acceptability. Fermented sausages from T3, T4 and T5 were on the positive PC1 axis, while the samples from T1 and T2 were on the negative PC1 axis. PC2 was inversely related to cholesterol, moisture, a_w , a^* , L^* and b^* .

Table 5 Factor loadings for parameters determined on the first three principal components

	PC1	PC2	PC3
Flavor	−0.761	0.421	−0.416
Taste	−0.842	0.431	−0.320
Color	−0.737	0.202	0.583
Acceptability	−0.770	0.477	−0.400
Saturated fat	0.613	0.229	−0.120
Cholesterol	0.374	−0.082	0.864
Na	−0.771	−0.145	0.526
Carbohydrate	−0.930	−0.156	0.171
Weight loss	0.881	−0.344	−0.148
pH	0.176	0.972	−0.038
a_w	0.362	−0.852	−0.347
Moisture	−0.658	−0.468	−0.468
Fat	0.695	0.451	0.317
Protein	0.659	0.098	−0.388
Energy	0.617	0.627	−0.353
L^*	0.933	−0.304	−0.047
a^*	−0.865	−0.246	0.273
b^*	−0.635	−0.426	−0.536

Fermented sausages from T1 and T5 were on the positive PC2 axis, while the ones from T2, T3 and T4 were on the negative PC2 axis. Finally, PC3 was positively related to fat, color, cholesterol, Na, a^* and carbohydrate, and inversely related to the remaining parameters determined. The fermented sausages from C, T2 and T3 were on the positive PC3 axis, while the ones from T1, T4 and T5 on the negative PC3 axis. PC1 shows a clear difference between control and the other treatments as it obtained higher score for cholesterol content. While, PC2 shows a clear difference between T1 (treated with *doenjang*) and the other remaining treatments as it obtained higher scores for the all sensory attributes determined such as flavor, taste and acceptability. On the other hand, PC3 allows

separation of the treatments according to a^* (redness) scores, discriminating T2 from the C and other remaining treatments. These differences were also observed in the results for physicochemical parameters (Tables 1, 2 and 3) and sensory characteristics (Table 4).

Conclusion

The present work showed the effects of addition of some natural/traditional seasonings on the quality characteristics of Salchichon fermented sausage type. The additions of seasonings did not cause quality defects whereas partly improved some quality traits of the products. Additionally, the incorporation of seasonings showed beneficial effects on lipid oxidation reduction and cholesterol lowering in the finished products. It can be suggested that the test natural/traditional seasonings could be added as the functional ingredients for reducing cholesterol content and improving lipid oxidation stability of Salchichon fermented sausage type. Insights on the cholesterol-lowering effect as well as the mechanisms underlying this phenomenon will be investigated in future study.

Acknowledgments This work was supported by “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ00984805, Development of Korean style fermented meat products)” Rural Development Administration, Republic of Korea.

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