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EFFECTS OF POMEGRANATE PEEL AND PROPOLIS POWDERS AND THEIR COMBINATIONS ON PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF TURKISH DRY-FERMENTED SAUSAGE (SUCUK) WITH VARIOUS NITRITE LEVELS

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Abstract

The study aimed to evaluate the effects of pomegranate peel (PP) and propolis (PR) powders (2%) and their combinations (1%PP and 1%PR) on physico-chemical and microbiological properties of sucuks produced with various nitrite (N) doses (0, 50, 100 and 150 ppm) during fermentation (10 d) and storage periods (4°C; 30 d). The results showed that the pH values of all sucuks decreased during fermentation whereas the pH increased during storage (P<0.05). Water activity (aw) decreased during both the fermentation and storage periods (P<0.05). The lowest aw was obtained in samples with PR (P<0.05), whereas nitrite doses did not have a significant effect on aw. Oxidation-Reduction Potential (ORP) increased during fermentation and storage (P<0.05). PP and PR powders and different nitrite concentrations had no effect on pH and ORP. The results indicated that L values increased with PP, and decreased with PR during fermentation and storage (P<0.05). Furthermore, a increased and b* decreased during fermentation, whereas a* and b* values decreased during storage in all sucuk groups (P<0.05). The lowest a* was determined in samples with PR (P<0.05). There was a gradual increase in TBARS in all sucuks during fermentation and storage (P<0.05). The results showed that PP and PR powders were effective in reducing the TBARS levels (P<0.05). PP inhibited TBARS formation more effectively than PR in nitrite-free samples, whereas this difference between PP and PR was not determined in samples containing different nitrite levels (P<0.05). Total mesophilic aerobic bacteria counts increased during the fermentation and storage period whereas the yeast-mould counts generally decreased at the end of the fermentation (P<0.05). Furthermore, coliform bacteria counts did not change during fermentation and storage.*

***Key words**: pomegranate, propolis, powder, nitrite, sucuk.*

INTRODUCTION

Chemical and microbiological changes are major cause of meat quality deterioration. For this purpose, food additives are natural or synthetics substances used to extend the shelf life by preserving the quality of meat products (Bobko et al., 2015). However, there are concerns and limitations about the use of synthetic additives because recent scientific studies have shown potential toxic effects and high costs, and consumer concerns about food additives are increasing. For these reasons, consumer demand for natural products has shifted the food industry to the use of natural additives in meat products (Şimşek and Kılıç, 2012).

Sucuk is one of the most popular and widely consumed dry-fermented meat products in Turkey (Bozkurt and Erkmen, 2007; Kilic, 2009). Lamb and/or beef, water buffalo meat, beef fat or tail fat, salt, sugar, nitrite and/or nitrate, garlic, and various spices such as black pepper, red pepper, and cumin are used in the sucuk production (Kilic, 2009). Sucuk is a meat product resistant to spoilage because of salt, nitrite, low pH and water activity. Nitrite is a synthetic food additive and is concerned about the consumption of nitrite-containing products due to its health effects. For this reason, some researchers are working on natural food additives that can be an alternative to nitrite (Li et al., 2013; Kurcubic et al., 2014).

The pomegranate (*Punica granatum* L.) is an edible fruit with composed of many pieces that is slightly sweet and sour and is widely grown in many tropical and subtropical countries (Yasoubi et al., 2007). Pomegranate peel and seeds are by products obtained during processing of pomegranate juice (Devatkal et al., 2011). Pomegranate peel are reported to possess a significant level of antioxidant and antimicrobial activity due to polyphenolic substances such as ellagic tannins, ellagic acid and gallic acid (Yasoubi et al., 2007; Qin et al., 2013). There are a number of studies investigating the antioxidant and antimicrobial effects of pomegranate peel or seeds in various meat products (Devatkal et al., 2010; Malviya et al., 2014; El-Nashi et al., 2015).

Propolis is a resinous, rubbery and balsamic substance collected from buds and exudates of flowers and trees by honey bees (Ali et al., 2010). Propolis has functional properties such as antioxidant, antibacterial, antifungal, local anesthetic and anti-inflammatory activities. These functional properties are due to components such as resins, aromatic and ethereal oils, flavonoid pigment, vanillin, isovanilin, caffeic, benzoic and ascorbic acids as well as benzyl alcohol and cinnamic acid (Ali et al., 2010; Temiz et al., 2011).There are numerous studies on the antioxidant and especially antimicrobial effects of propolis in meat products (Lu et al., 2005; Ali et al., 2010; Vargas-Sanchez et al., 2015). The purpose of this work is to evaluate the effects of PP and PR powders (2%) and their combinations (1% PP and 1% PR) on physico-chemical and microbiological properties of sucuks produced with various nitrite doses (0, 50, 100 and 150 ppm) during fermentation (10 d) and storage periods (4^{o}C; 30 d).

MATERIALS AND METHODS

Beef meat (*Longissimus thoracis et lumborum, LL*) and fat were purchased from a local slaughterhouse for each of two replications on separate production days. Spices, propolis powder, sodium nitrite and starter culture mix (Bitec Starter LS 25) were supplied by Arifoglu Spices and Food Industry (Istanbul, Turkey), Marmaris Balcısı (Muğla, Turkey), Merck (Germany) and Etol Aroma ve Baharat Gıda Ürünleri San ve Tic. A.Ş (Kocaeli, Turkey), respectively. Pomegranate peel were dried in an air circulatory drier (FN 500, Nüve, Turkey) at 40°C for 48 h, and ground in an analytical mill to a grain diameter of less than 0.5 mm.

Turkish dry-fermented sausage (Sucuk) production. Sucuk (approximately 1000 g each) was manufactured with respect to the traditional sucuk production method (Bozkurt and Erkmen, 2007). All sucuk samples contained beef and fat (5:1), salt (2%), garlic (1%), saccharose (0.4%), red pepper (0.7%), black pepper (0.5%), 9 g cumin (0.9%), allspice (0.25%) and starter culture mix (0.025%). Sucuk batter was formulated with different level of PP, PR and sodium nitrite (Table 1).

Table 1. Coding for pomegranate peel (PP), propolis (PR) powder and sodium nitrite (N) treatments evaluated

Groups	PP, PR and sodium nitrite treatments
Control	Without any powders and sodium nitrite
PPN0	2% Pomegranate peel powder
PRN0	2% Propolis powder
PP/PR N0	1% Pomegranate peel powder and 1% propolis powder
PPN50	2% Pomegranate peel powder and 50 ppm sodium nitrite
PRN50	2% Propolis powder and 50 ppm sodium nitrite
PP/PR N50	1% Pomegranate peel powder, 1% propolis powder, 50 ppm sodium nitrite
PPN100	2% Pomegranate peel powder and 100 ppm sodium nitrite
PRN100	2% Propolis powder and 100 ppm sodium nitrite
PP/PR N100	1% Pomegranate peel powder, 1% propolis powder, 100 ppm sodium nitrite
PPN150	2% Pomegranate peel powde rand 150 ppm sodium nitrite
PRN150	2% Propolis powder and 150 ppm sodium nitrite
PP/PR N150	1% Pomegranate peel powder, 1% propolis powder, 150 ppm sodium nitrite

The fermentation process was carried out under the following conditions: 24±1°C and relative humidity (RH) 95±1% for the first day, 22±1°C and RH 90±1% for the second day, 20±1°C and RH 85±1% for the half of the third day, 20±1°C and RH 80±1% for the other half of the third day, and 18±1°C and RH 70±1% for the last 7 days. Samples for physico-chemical and microbiological analyses were taken from sucuks immediately after stuffing (0 day), and after 5 and 10 days of ripening. Sucuks were kept at 4°C for 30 days, and samples analyzed at 0 (at the end of fermentation), 15 and 30 days of storage.

Physico-chemical analyses. The pH was measured using spear electrode (FC 200, Hanna Instruments, Germany) attached to a portable pH meter (HI 9024, Hanna Instruments, Germany). Color values of sucuk samples were measured according to CIE Lab Color System using a Minolta Colorimeter (Model CR-200, Minolta corp., Ramsey, Nj, USA). The water activity (a_w) values of the sucuks were determined at 20°C by using a Novasina LabTouch-a_w. Oxidation-reduction potential (ORP) was measured in sucuks using pH meter (WTW pH 3110, Germany) set to the millivolt scale and equipped with redox electrode. Thiobarbituric acid reactive substances (TBARS) were determined according to the extraction method of Lemon (1975) as described by Kilic and Richards (2003). The TBARS values were stated as µmol TBARS per kg of sucuk samples.

Microbiological analyses. A 10 g sample was aseptically taken from sucuks and transferred in a sterile Stomacher bag, and homogenized in 90 mL sterile 0.1% peptone water. Serial decimal dilutions were prepared using with 0.1% peptone water. Total mesophilic aerobic bacteria (TMAB), yeast and mould, and total coliform bacteria counts were determined according to the spread plate techniques on plate count agar at 30°C for 2 days, potato dextrose agar at 25°C for 2-5 days, and eosin methylene blue agar at 37°C for 2 days, respectively (Maturin and Peeler, 2001).

RESULTS AND DISCUSSIONS

The changes in pH levels of sucuk samples are shown in table 2. The results showed that the pH values of all sucuk samples decreased during first 5 days of fermentation whereas the pH gradually increased during last 5 days of fermentation and during storage period ($P<0.05$). Similar results were reported by Kunrath et al. (2017) for the Italian-type salami samples. Researchers noted that the pH dropped rapidly during the first 6 days of maturation, and gradually increased after the 6th day. Researchers pointed out that the decline in pH over the first 6 days of maturation was probably due to the presence of lactic acid bacteria in the starter culture added to the formulation (Kunrath et al., 2017). In our study, the highest pH values were detected in groups of control and PRN150, whereas the lowest pH was detected in group of PPN0 at the beginning of fermentation ($P<0.05$). Furthermore, the pH values of the samples with PP were also found to be lower than the other treatment groups at the beginning of fermentation ($P<0.05$). Similar findings were observed by Chandralekha et al. (2012) in chicken meat balls. They reported that pomegranate rind powder extracts caused lower pH values than the other formulation groups. On the other hand, El-Nashi et al. (2015) reported that the addition of PP powder in beef sausages did not cause a significant differences in pH values. At the end of fermentation, there was not found any significant difference between all sucuk groups, whereas the lowest pH value and the highest pH value were detected in the group of PP/PRN150 and PRN150 at the end of storage, respectively ($P<0.05$). Bernardi et al. (2013) reported that propolis containing products showed similar results when compared with control in terms of pH values. In the study conducted by us, there was not detected a significant difference between the groups containing PR and the control, however, the higher pH values were determined in PR containing groups compared with control at the end of storage ($P<0.05$). Additionally, the use of various nitrite doses did not have a significant effect on pH.

Table 2. The results of pH changes of sucuk samples

	Fermentationtime (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	5.61^{aA}±0.00	4.5^{cdE}±0.01	4.72^{abD}±0.01	5.12^{aB}±0.01	5.03^{efC}±0.01
PPN0	5.31^{hA}±0.01	4.5^{cD}±0.00	4.70^{abcC}±0.01	5.06^{bB}±0.02	5.06^{bcB}±0.00
PRN0	5.56^{cA}±0.00	4.54^{bE}±0.01	4.76^{abD}±0.01	5.12^{aB}±0.01	5.06^{bcC}±0.00
PP/PR N0	5.42^{fA}±0.00	4.54^{bE}±0.01	4.69^{abcD}±0.01	4.98^{deC}±0.01	5.04^{deB}±0.01
PPN50	5.33^{gA}±0.00	4.54^{bE}±0.01	4.65^{bcD}±0.01	4.86^{hC}±0.00	5.02^{fB}±0.01
PRN50	5.59^{bA}±0.01	4.53^{bE}±0.01	4.68^{abcD}±0.01	4.99^{dC}±0.00	5.06^{cB}±0.01
PP/PR N50	5.45^{eA}±0.01	4.49^{dE}±0.01	4.78^{abD}±0.01	4.95^{fC}±0.00	5.08^{bB}±0.01
PPN100	5.33^{gA}±0.00	4.53^{bE}±0.00	4.73^{abD}±0.00	5.05^{bC}±0.01	5.06^{bcB}±0.00
PRN100	5.59^{bA}±0.01	4.58^{aE}±0.00	4.77^{abD}±0.00	5.02^{cC}±0.00	5.18^{aB}±0.00
PP/PR N100	5.46^{dA}±0.00	4.51^{cC}±0.01	4.56^{cC}±0.21	4.93^{gB}±0.01	5.05^{cdB}±0.01
PPN150	5.33^{gA}±0.01	4.40^{eE}±0.01	4.71^{abD}±0.01	4.86^{hC}±0.00	5.00^{gB}±0.01
PRN150	5.61^{aA}±0.01	4.53^{bE}±0.01	4.8^{aD}±0.00	4.96^{efC}±0.00	5.08^{bB}±0.01
PP/PR N150	5.47^{dA}±0.01	4.5^{cE}±0.00	4.79^{abD}±0.00	5.03^{cB}±0.01	4.96^{hC}±0.01

Means±standart deviation (SD). $^{a-h}$Within the column, values superscripted with different letters are significantly different (*P*<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (*P*<0.05).

The a_w values are given in Table 3. The a_w values decreased during both the fermentation and storage periods (*P*<0.05).Similarly, Kunrath et al. (2017) reported that water activity values decreased during maturation in Italian-type salami. The water activity values of the samples with PP were obtained to be lower than the control at the end of fermentation and storage (*P*<0.05). In general, the lower aw values were obtained in samples with PR compared to the other sucuk groups (*P*<0.05), whereas nitrite doses difference did not have a significant effect on aw. Kunrath et al. (2017) and Bernardi et al. (2013) indicated that the use of PR extract in Italian-type salami production did not have a significant effect on a_w values.

The color results (data is not presented) showed that L* values increased with PP addition to sucuks, and decreased with PR addition during fermentation and storage (*P*<0.05). Naveena et al. (2008a) and Devatkal and Naveena (2010) reported that the PP powder addition had caused lower L* values as compared to control in raw ground goat meat and cooked chicken patties, respectively. At the end of fermentation, the lowest L* values were detected in all PR containing groups (*P*<0.05). Additionally, L* values increased in PR containing groups with increasing nitrite level at the end of storage (*P*<0.05). The lowest a* values were determined in samples with PR, whereas the highest a* values were also determined in samples with PP (*P*<0.05). Naveena et al. (2008b) indicated an increase in a* values as a result of PP powder extract addition in cooked chicken patties. In general, a* values increased in PR containing groups with increasing nitrite level (*P*<0.05), whereas a similar effect did not have on PP containing groups. Furthermore, a* values increased and b* values decreased during fermentation, whereas a* and b* values decreased during storage in all sucuk groups (*P*<0.05).

The results of ORP are presented in table 4. The ORP values of sucuk samples were varied between -104.05 and -49.7 at the beginning of fermentation. The lowest ORP were detected in the group of PPN0 and PRN0, whereas the highest ORP value was determined in the group of PP/PR N50 at the beginning of the fermentation period (*P*<0.05). Results showed that the ORP values were increased during fermentation and storage period (*P*<0.05). The highest ORP values were determined in the group of PPN50 at the end of the both fermentation and storage period (*P*<0.05). The lowest ORP value was determined in the group of PPN100 at the end of fermentation, where as the lowest ORP values were determined in the groups of PPN0 and PP/PR N50 at the end of storage (*P*<0.05).

TBARS changes of sucuks are given in table 5. The TBARS values of sucuks were changed between 2.13-3.87 μmol/kg at the beginning of fermentation period. There was a gradual increase in TBARS levels in all sucuks during fermentation and storage period (*P*<0.05).

Table 3. The results of a_w values of sucuk samples

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	0.93^{aA}±0.00	0.90^{aB}±0.00	0.86^{aC}±0.00	0.71^{fgD}±0.01	0.70^{aD}±0.00
PPN0	0.93^{aA}±0.00	0.87^{efB}±0.00	0.84^{bcC}±0.00	0.69^{hD}±0.01	0.63^{bE}±0.00
PRN0	0.91^{fA}±0.00	0.87^{efB}±0.00	0.76^{eC}±0.00	0.69^{hD}±0.01	0.53^{eE}±0.00
PP/PR N0	0.92^{b-eA}±0.00	0.87^{fB}±0.00	0.82^{dC}±0.00	0.73^{cdD}±0.00	0.60^{cdE}±0.01
PPN50	0.92^{b-eA}±0.00	0.89^{dB}±0.00	0.83^{cC}±0.00	0.74^{cD}±0.00	0.59^{cdE}±0.00
PRN50	0.92^{fA}±0.01	0.88^{eB}±0.01	0.82^{dC}±0.00	0.73^{deD}±0.01	0.61^{bcE}±0.01
PP/PR N50	0.92^{efA}±0.00	0.89^{bcdB}±0.00	0.84^{bC}±0.01	0.74^{cD}±0.00	0.63^{bE}±0.01
PPN100	0.92^{b-eA}±0.00	0.89^{cdB}±0.00	0.85^{aC}±0.00	0.70^{gD}±0.00	0.60^{cdE}±0.00
PRN100	0.92^{efA}±0.00	0.89^{abB}±0.00	0.83^{cC}±0.01	0.69^{hD}±0.01	0.59^{cdE}±0.03
PP/PR N100	0.92^{defA}±0.00	0.89^{dB}±0.00	0.86^{aC}±0.00	0.77^{bD}±0.00	0.60^{cdE}±0.01
PPN150	0.92^{cdeA}±0.00	0.89^{bcdB}±0.00	0.84^{bC}±0.00	0.84^{aC}±0.00	0.59^{dE}±0.01
PRN150	0.92^{efA}±0.00	0.90^{aB}±0.00	0.86^{aC}±0.00	0.74^{cdD}±0.01	0.54^{eE}±0.00
PP/PR N150	0.92^{bcA}±0.00	0.89^{abcB}±0.00	0.86^{aC}±0.00	0.72^{efD}±0.01	0.58^{dE}±0.00

Means±standart deviation (SD). $^{a-h}$Within the column, values superscripted with different letters are significantly different (*P*<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (*P*<0.05).

Table 4. The results of ORP values of sucuk samples

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	-92.8^{jE}±0.14	-52.85^{kD}±0.07	48.30^{dC}±0.00	64.70^{dB}±0.14	92.65^{bA}±0.07
PPN0	-103.75^{kE}±2.05	-77.35^{lD}±0.21	15.85^{lC}±0.07	39.25^{kB}±0.91	56.35^{kA}±0.78
PRN0	-104.05^{kE}±0.07	-42.60^{jD}±0.14	25.15^{iC}±0.21	82.85^{aB}±0.21	73.95^{dA}±0.07
PP/PR N0	-73.65^{fE}±0.07	-20.85^{fD}±0.07	48.65^{cB}±0.21	37.70^{lC}±0.14	57.95^{jA}±0.07
PPN50	-87.55^{hE}±0.07	-33.65^{gD}±0.07	76.55^{aB}±0.07	70.45^{cC}±0.07	95.75^{aA}±0.07
PRN50	-90.9^{iE}±0.14	-11.55^{dD}±0.07	39.05^{fC}±0.07	79.75^{bA}±0.07	64.25^{gB}±0.07
PP/PR N50	-49.7^{aE}±0.14	-7.85^{bD}±0.07	50.00^{bC}±0.00	61.95^{eA}±0.07	56.55^{kB}±0.07
PPN100	-60.95^{dE}±0.07	-35.95^{hD}±0.07	4.65^{mC}±0.07	45.85^{jB}±0.21	60.95^{iA}±0.07
PRN100	-73.6^{fE}±0.14	-10.35^{cD}±0.07	33.85^{gC}±0.07	60.45^{fB}±0.07	63.20^{hA}±0.14
PP/PR N100	-54.3^{cE}±0.14	-7.75^{bD}±0.07	22.45^{jC}±0.07	56.40^{hB}±0.00	68.10^{fA}±0.14
PPN150	-79.45^{gE}±0.07	-39.55^{iD}±0.07	18.10^{kC}±0.14	48.50^{iB}±0.14	70.60^{eA}±0.14
PRN150	-68.5^{eE}±0.14	-5.25^{aD}±0.07	32.45^{hC}±0.07	59.45^{gB}±0.07	62.75^{hA}±0.07
PP/PR N150	-51.65^{bE}±0.07	-14.90^{eD}±0.14	45.75^{eC}±0.07	60.75^{fB}±0.07	89.95^{cA}±0.07

Means±standart deviation (SD). $^{a-m}$Within the column, values superscripted with different letters are significantly different (*P*<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (*P*<0.05).

At the end of fermentation and storage period, the lower (*P*<0.05) TBARS levels were detected in PP or PR containing groups compared to control group. Similarly, Han and Park (2002) indicated that the addition of PR extract to cured pork sausages resulted in lower TBARS levels than the control groups. Additionally, Ali et al. (2010) stated that the addition of PR lowered the TBA levels in fresh oriental sausages. El-Nashi et al. (2015) pointed out that the addition of PP powder reduced values of TBA in beef sausage samples as compared to control during refrigerated storage. Similarly, Borah et al. (2014) stated that the lower TBARS values as compared to control was obtained in chicken meatball with PP powder extracts during refrigerated storage. There are some other studies showing the effect of PP addition on reducing oxidation levels of meat products (Naveena et al., 2008a; Devatkal et al., 2010; El-Gharably and Ashoush, 2011). Our study results showed that PP and PR powders were effective in reducing the TBARS levels (*P*<0.05). PP inhibited TBARS formation more effectively than PR in nitrite-free samples, whereas this difference between PP and PR was not determined in samples containing different nitrite levels (*P*<0.05).

Total mesophilic aerobic bacteria (TMAB) counts were varied between 6.01 and 6.73 Log_{10} CFU/g at the beginning of fermentation (Table 6). The highest TMAB counts were detected in groups of PPN50 and PPN100 at the end of fermentation, whereas the highest TMAB counts was found in control group at the end of storage (*P*<0.05).

Table 5. The results of TBARS values of sucuk samples (μmol TBARS per kg sucuk)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	$3.50^{abcE}\pm0.47$	$4.94^{aD}\pm0.43$	$6.32^{aC}\pm0.39$	$9.25^{aB}\pm0.04$	$11.25^{aA}\pm0.04$
PPN0	$3.24^{a\text{-}dC}\pm0.04$	$3.83^{bcdC}\pm0.40$	$3.68^{cC}\pm0.43$	$5.57^{bcdB}\pm0.24$	$7.69^{cdA}\pm0.12$
PRN0	$3.87^{aC}\pm0.09$	$4.41^{abC}\pm0.09$	$4.82^{bC}\pm0.67$	$6.44^{bB}\pm0.36$	$9.69^{bA}\pm0.28$
PP/PR N0	$3.29^{abcC}\pm0.44$	$3.53^{c\text{-}fC}\pm0.58$	$4.84^{bB}\pm0.11$	$5.60^{bcdB}\pm0.42$	$8.30^{cA}\pm0.29$
PPN50	$3.58^{abC}\pm0.35$	$4.19^{abcBC}\pm0.29$	$4.19^{bcBC}\pm0.26$	$4.49^{eB}\pm0.46$	$6.42^{fA}\pm0.27$
PRN50	$3.17^{a\text{-}dC}\pm1.12$	$3.47^{c\text{-}fC}\pm0.07$	$4.58^{bcBC}\pm0.20$	$5.56^{bcdAB}\pm0.60$	$7.51^{cdeA}\pm0.11$
PP/PR N50	$2.64^{bcdC}\pm0.41$	$3.22^{defC}\pm0.18$	$4.37^{bcB}\pm0.10$	$5.09^{cdeB}\pm0.37$	$7.06^{defA}\pm0.44$
PPN100	$2.38^{cdC}\pm0.51$	$3.42^{c\text{-}fBC}\pm0.15$	$4.38^{bcB}\pm0.13$	$4.40^{eB}\pm0.38$	$6.90^{defA}\pm0.80$
PRN100	$2.43^{cdE}\pm0.05$	$3.73^{b\text{-}eD}\pm0.08$	$4.61^{bcC}\pm0.15$	$5.71^{bcB}\pm0.24$	$7.42^{cdeA}\pm0.04$
PP/PR N100	$3.12^{a\text{-}dB}\pm0.67$	$3.30^{defB}\pm0.53$	$4.43^{bcAB}\pm0.77$	$4.99^{cdeA}\pm0.62$	$6.94^{defA}\pm0.38$
PPN150	$2.73^{bcdC}\pm0.37$	$2.96^{efC}\pm0.32$	$3.68^{cBC}\pm0.47$	$4.71^{deAB}\pm0.41$	$6.69^{efA}\pm0.49$
PRN150	$2.13^{dD}\pm0.14$	$2.74^{fC}\pm0.08$	$3.75^{cB}\pm0.44$	$4.35^{eB}\pm0.22$	$7.27^{defA}\pm0.07$
PP/PR N150	$2.37^{cdD}\pm0.06$	$3.29^{defCD}\pm0.56$	$4.19^{bcBC}\pm0.57$	$4.60^{eB}\pm0.01$	$7.03^{defA}\pm0.62$

Means±standart deviation (SD). $^{a\text{-}f}$Within the column, values superscripted with different letters are significantly different (*P*<0.05). $^{A\text{-}E}$Within the row, values superscripted with different letters are significantly different (*P*<0.05).

Table 6. The results of total mesophilic aerobic bacteria counts of sucuks (Log_{10} CFU/g)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	$6.34^{bcD}\pm0.05$	$7.75^{eC}\pm0.21$	$8.78^{bcB}\pm0.00$	$10.11^{aA}\pm0.00$	$10.15^{aA}\pm0.05$
PPN0	$6.54^{abD}\pm0.04$	$8.04^{dC}\pm0.27$	$8.13^{dBC}\pm0.03$	$8.44^{dAB}\pm0.04$	$8.57^{bcA}\pm0.04$
PRN0	$6.47^{abE}\pm0.06$	$8.54^{aA}\pm0.09$	$8.14^{dB}\pm0.13$	$7.90^{gC}\pm0.00$	$7.30^{efD}\pm0.00$
PP/PR N0	$6.44^{bcD}\pm0.04$	$8.29^{a\text{-}dB}\pm0.13$	$8.19^{dBC}\pm0.11$	$7.91^{gC}\pm0.18$	$8.73^{bA}\pm0.00$
PPN50	$6.49^{abE}\pm0.04$	$8.47^{abD}\pm0.01$	$9.83^{aA}\pm0.00$	$9.08^{bB}\pm0.00$	$8.87^{bC}\pm0.09$
PRN50	$6.46^{abC}\pm0.16$	$8.22^{a\text{-}dA}\pm0.06$	$8.69^{bcA}\pm0.30$	$8.26^{eA}\pm0.00$	$7.15^{efB}\pm0.21$
PP/PR N50	$6.73^{aC}\pm0.04$	$8.51^{aA}\pm0.00$	$8.49^{bcdA}\pm0.01$	$8.48^{dA}\pm0.00$	$7.99^{dB}\pm0.00$
PPN100	$6.31^{bcD}\pm0.17$	$8.14^{cdC}\pm0.19$	$9.53^{aA}\pm0.06$	$8.86^{cB}\pm0.00$	$8.18^{cdC}\pm0.00$
PRN100	$6.01^{dC}\pm0.08$	$8.40^{abcA}\pm0.20$	$8.43^{cdA}\pm0.00$	$8.18^{efA}\pm0.00$	$7.00^{fB}\pm0.00$
PP/PR N100	$6.41^{bcD}\pm0.04$	$8.33^{a\text{-}dC}\pm0.04$	$8.52^{bcdB}\pm0.09$	$8.84^{cA}\pm0.09$	$8.75^{bA}\pm0.01$
PPN150	$6.36^{bcC}\pm0.25$	$8.22^{a\text{-}dB}\pm0.06$	$8.62^{bcAB}\pm0.40$	$8.88^{cA}\pm0.04$	$8.79^{bA}\pm0.06$
PRN150	$6.42^{bcD}\pm0.13$	$8.49^{abAB}\pm0.01$	$8.88^{bA}\pm0.04$	$8.12^{fBC}\pm0.05$	$7.43^{eC}\pm0.60$
PP/PR N150	$6.18^{cdB}\pm0.14$	$8.17^{bcdA}\pm0.02$	$8.48^{bcdA}\pm0.28$	$8.12^{fA}\pm0.05$	$8.10^{dA}\pm0.02$

Means±standart deviation (SD). $^{a\text{-}f}$Within the column, values superscripted with different letters are significantly different (*P*<0.05). $^{A\text{-}E}$Within the row, values superscripted with different letters are significantly different (*P*<0.05).

The lowest TMAB counts were determined in groups of PRN0, PRN50, PRN100 and PRN150 at the end of storage (*P*<0.05). The increase in the nitrite doses did not have a significant effect on the TMAB counts. In general, TMAB counts increased during the fermentation and storage period (*P*<0.05). The lower (*P*<0.05) TMAB counts were detected in PP or PR containing groups compared to control group at the end of fermentation and during storage. El-Nashi et al. (2015) indicated that PP powder addition reduced TMAB counts in beef sausages as compared to control during refrigerated storage. Similar results regarding the reduction of the TMAB counts of PP powder extract additions were reported by Chandralekha et al. (2012).

The yeast-mould counts generally decreased during fermentation and storage period in all groups except for control (*P*<0.05). Whereas the yeast-mould counts did not significantly change during the fermentation in control group, its increased during storage period (*P*<0.05; Table 7). At the end of fermentation, the highest yeast-mould counts were detected in groups of PPN0 and control (*P*<0.05). On the other hand, the highest yeast-mould counts were obtained in the control group at the end of storage (*P*<0.05). The lower yeast-mould counts were obtained in PP and PR containing groups with increasing nitrite levels in the 15th and 30th days of storage (*P*<0.05). El-Nashi et al. (2015) stated that PP powder decreased yeast and mould counts in beef sausages as compared to control during refrigerated storage, and this reducing effect of PP powder addition on yeast and mould counts also increased with increasing powder levels.

Table 7. The results of yeast and mould counts of sucuk samples (Log_{10} CFU/g)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	4.64^{defC}±0.09	4.78^{fgC}±0.04	4.59^{aC}±0.09	5.00^{aB}±0.00	5.43^{aA}±0.04
PPN0	4.61^{efB}±0.02	5.31^{bcA}±0.04	4.63^{aB}±0.02	3.95^{eC}±0.07	3.47^{cdD}±0.18
PRN0	4.51^{fB}±0.23	4.99^{defA}±0.01	4.06^{bcdC}±0.03	4.60^{cB}±0.00	4.23^{bC}±0.02
PP/PR N0	5.00^{bB}±0.06	5.79^{aA}±0.04	4.39^{abC}±0.04	4.73^{bBC}±0.01	3.24^{dD}±0.34
PPN50	4.79^{cdeA}±0.05	4.30^{hA}±0.09	3.65^{eB}±0.50	3.00^{iC}±0.00	3.22^{dBC}±0.02
PRN50	4.97^{bcA}±0.09	5.06^{cdeA}±0.35	4.06^{bcdB}±0.03	3.60^{fB}±0.00	2.98^{dC}±0.18
PP/PR N50	5.27^{aA}±0.06	5.06^{cdeB}±0.01	3.00^{fD}±0.00	3.13^{hC}±0.07	3.00^{dD}±0.00
PPN100	4.55^{fB}±0.01	5.68^{aA}±0.06	3.72^{deC}±0.33	4.30^{dB}±0.00	3.14^{dD}±0.09
PRN100	4.56^{fB}±0.02	5.54^{abA}±0.09	3.00^{fE}±0.00	3.48^{gD}±0.00	3.86^{bcC}±0.11
PP/PR N100	4.54^{fA}±0.04	4.68^{gA}±0.01	3.65^{eB}±0.07	3.00^{iC}±0.00	2.30^{eD}±0.42
PPN150	4.47^{fA}±0.09	4.26^{hA}±0.21	4.13^{bcA}±0.03	3.00^{iB}±0.00	2.15^{eC}±0.21
PRN150	4.63^{defA}±0.10	4.70^{fgA}±0.10	3.88^{cdeB}±0.04	3.00^{iC}±0.00	2.39^{eD}±0.13
PP/PR N150	4.84^{bcdA}±0.05	4.87^{efgA}±0.14	3.95^{cdeB}±0.00	3.48^{gC}±0.00	2.24^{eD}±0.34

Means±standart deviation (SD). $^{a-i}$Within the column, values superscripted with different letters are significantly different (*P*<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (*P*<0.05).

Table 8. The results of coliform bacteria counts of sucuk samples (Log_{10} CFU/g)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	4.32^{aA}±0.16	4.30^{abA}±0.42	4.11^{eA}±0.10	4.18^{b-eA}±0.00	4.04^{bcdA}±0.00
PPN0	4.07^{aBC}±0.17	4.45^{abA}±0.21	4.30^{bcdAB}±0.00	4.50^{abA}±0.02	3.93^{b-eC}±0.04
PRN0	4.35^{aB}±0.00	4.59^{abA}±0.16	4.35^{bcB}±0.01	4.15^{b-eC}±0.05	4.12^{bcdC}±0.05
PP/PR N0	4.26^{aAB}±0.09	5.26^{aA}±0.79	4.30^{bcdAB}±0.06	4.60^{aAB}±0.57	3.87^{cdeB}±0.12
PPN50	4.15^{aA}±0.13	4.15^{bA}±0.21	4.19^{deA}±0.01	4.00^{deA}±0.00	4.19^{abA}±0.11
PRN50	3.95^{aA}±0.04	4.35^{abA}±0.50	3.95^{fA}±0.00	3.98^{eA}±0.04	4.04^{bcdA}±0.06
PP/PR N50	4.16^{aB}±0.11	4.65^{abA}±0.07	4.55^{aA}±0.03	4.15^{b-eB}±0.00	3.69^{eC}±0.13
PPN100	4.10^{aB}±0.09	4.15^{bAB}±0.21	4.45^{abA}±0.00	4.42^{abcA}±0.01	4.44^{aA}±0.11
PRN100	4.11^{aC}±0.03	4.60^{abA}±0.00	4.41^{bB}±0.04	4.28^{a-eB}±0.03	3.93^{b-eD}±0.11
PP/PR N100	4.17^{aB}±0.15	4.63^{abA}±0.21	4.31^{bcdAB}±0.01	4.00^{deBC}±0.00	3.69^{eC}±0.13
PPN150	4.44^{aB}±0.03	4.74^{abA}±0.06	4.34^{bcdB}±0.03	4.39^{a-dB}±0.09	4.14^{bcC}±0.09
PRN150	3.07^{bA}±1.09	4.00^{bA}±0.00	4.25^{cdeA}±0.19	4.09^{cdeA}±0.13	3.82^{deA}±0.31
PP/PR N150	4.01^{aA}±0.04	5.03^{abA}±1.03	4.25^{cdeA}±0.02	4.27^{a-eA}±0.01	4.11^{bcdA}±0.10

Means±standart deviation (SD). $^{a-f}$ Within the column, values superscripted with different letters are significantly different (*P*<0.05). $^{A-E}$ Within the row, values superscripted with different letters are significantly different (*P*<0.05).

At the beginning of fermentation, whereas the lowest coliform count was determined in group of PRN150 (*P*<0.05), there was not found a significant difference between the other groups (Table 8). In general, there was no significant changes in all sucuk groups during fermentation, whereas the lowest number of coliform was determined in the group of PRN50 at the end of fermentation (*P*<0.05). There was a decrease in the groups of PPN0, PRN0, PP/PRN50, PRN100, PP/PRN100 and PPN150 during storage (*P*<0.05), whereas there was also no significant changes in other groups during storage. The highest numbers of coliform were determined in PPN50 and PPN100 groups at the end of storage (*P*<0.05). The results of coliform analysis shown that the changes in nitrite doses had no significant effect on the coliform counts.

CONCLUSIONS

Oxidative stability of sucuks was enhanced with the use of PP or PR or their combination.
Additionally, oxidative stability is also further improved by the use of PP or PR powders in combination with nitrite in sucuks. However, doses of nitrite did not created any difference.
PR powder was significantly effective in inhibiting of microbial growth.
The PP or PR powders did not have a negative effect on other physicochemical properties of sucuks.
The use of these powders are recommended as a natural antimicrobial and especially antioxidant additives in sucuk production.

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