

# **Contribution of Some Agro-Food Processing By-products to Chicken Sausages**

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### **Introduction**

The amount of food prepared for human consumption that is wasted annually is about 1.3 billion tons (one-third of the total). By 2050, there will likely be a rise in the human population, which will raise the need for food and the amount of food waste exposed (Eliopoulos et al., 2022; Hadj Saadoun et al., 2021). Plant-derived waste is generally rich in lignocellulose, protein, fat, sugar, phytochemicals, and other valuable compounds and they could be retained/re-used as valuable products that provide economic benefit for the food, cosmetic, and pharmaceutical industries (Elleuch et al., 2011; Jin et al., 2018). Food waste in the food industry is used for advantages such as the re-evaluation of waste, recovery of the bioactive components they contain, reduction of formulation costs, and improvement of the product structure (Akşit & Gençcelep, 2021; Scarano et al., 2022). Most meat and meat products are rich in fat and salt but insufficient in complex carbohydrates such as dietary fiber, and their regular consumption is associated with various health issues such as colon cancer, obesity, and cardiovascular diseases (Mehta et al., 2015; Verma et al., 2010). Dietary fiber promotes beneficial physiological

effects, including taxation and/or lowering of blood cholesterol and/or lowering blood sugar (AACC, 2001), and combining food waste with meat products increases the fiber content of meat products. Insofar as they contain substances regarded as functional, such as dietary fiber, meat, and meat products can be categorized as functional foods (Siro et al., 2008). The effective development and marketing of such functional meat products present some challenges. A recent breakthrough is the production of fiber-enriched meat products, and more studies are needed to put forth the potential of these ingredients (Baker et al., 2022; Betoret et al., 2022).

In this study, we aimed to use some agro-food waste in sausage production as natural ingredients and produced sausages with food source additives that have high dietary fiber. We have found in our previous study that the quince, grapefruit, and tomato wastes contributed to the chickentype meat emulsion, improved the textural and rheological properties, and increased the emulsion stability (Akşit & Gençcelep, 2022, 2023) and we investigated the contributions of these food wastes to chicken type sausages in this study. Sausage samples were made with 3% quince

waste (QS3), 3% grapefruit waste (GS3), 2% tomato waste (TS2), 3% tomato waste (TS3), and no waste as control (CS). Chemical, physical, and microbiological analyses were performed on food wastes and sausage dough. TBARS, water activity, pH, purge accumulation, and microbiological analyses were carried out on sausage samples during 60 days of storage.

### **Materials and Methods**

# *Material*

Grapefruit and quince waste were obtained from the food laboratory by fruit juice production; tomato waste (the by-product of tomato paste production) was purchased from Limkon Gıda Company (Adana/Turkey). Skinless chicken breast and beef tallow were purchased from a local market in Samsun, Turkey. The spice mix (Wiss, wiscombi) used in sausage production was purchased from Özselamoğlu Gıda/Turkey. All chemicals and mediums utilized in chemical and microbiological activity were procured from Sigma Aldrich (USA), and Merck (Germany).

# *Methods*

Food waste samples were analyzed for pH, TBARS value, total dietary fiber (TDF) and microbiological analyses (TMAB, TPB, molds and yeasts). Batter analyses were done as pH value and gel and fat separation. Sausage samples analyzed for chemical composition, and sensory analyses. Samples were vacuum packaged and stored at 4°C for 60 days and pH, TBARS, water activity, purge accumulation, TMAB, TPB, and total molds-yeasts analyses were performed every 15 days (0., 15., 30., 45. and 60. days).

# *Sausage Manufacturing*

The chicken breast meat and beef tallow were cut into  $1 \text{cm}^3$  cubes and stored at  $+4$ °C the night before the production. In sausage production; breast meat with a temperature of  $+4^{\circ}$ C is placed in the cutter (MTK 661, Mado Grant, Germany) and minced; ice, beef tallow, salt and nitrite were added respectively (one after another) while processing. Process was continued additional 30 seconds for the control sample second without adding of agro-food waste; for QS3, GS3, TS2, and TS3 samples certain proportion agro-food waste was added, and the process continued for an additional 30 seconds. The whole process took places in 3 minutes at the highest cutting rate. After the batter was formed, it was taken to the filling machine (MWF 591 stuffer, Mado Patron, Germany) and filled in artificial casing (30 mm diameter, Kalle Nalo. Wursthüllen, Wiesbaden Germany), clipped (SCH 7210 poly-clip system, Gmbh & Co. KG, Germany) and heat treated in a water bath at 90±2°C for 30 minutes. Then the sausages were cooled to  $5\pm1$ °C with cold water and they were packaged with a vacuum packaging system (KOMET NG 03024, Germany) and stored at 4°C. Each sausage weights were 50±3 grams and each bulk were 1500 grams. Sausage formulations are given in Table 1. Quince (QS3), grapefruit (GS3), and tomato (TS3) samples produced with a 3% food waste level, and tomato waste used in a 2% concentration of sausages (TS2) were produced since it had the optimum values close to TS3 sample. Formulation with

no food waste was the control group (CS). Totally five different products were produced.

# *pH Analysis*

This analysis was applied to the food waste samples, batter samples, and sausage samples. 90 mL distiller water was added to a 10 gr sample and pH was measured after homogenization by immersing the pH meter (Hanna Instruments HI 2211, Romania) probe into the sample (Gökalp et al., 1993).

# *Color Analysis*

Color analysis of the samples was done by measuring the color  $(L^*, a^*, b^*)$  values in the Hunter color measurement system with a Minolta color measuring device (CR-300, Minolta Osaka, Japan). Samples were cut in 1 cm thickness and the device's probe was brought into contact with the section surface; color values were read from a digital monitor. According to this, The *L\** value is the brightness in daylight (0: black, 100: white), the *a\** value is the green-red color (-80 to 0; green, 0 to +100; red), the  $b^*$  value is the blue-yellow color (between -50 and 0; blue, between 0 and +50; yellow).

*Total Dietary Fiber (TDF) Analysis*

TDF contents of samples were determined by using a Megazyme assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland) by following AOAC-991.43 standard methods (AOAC, 2000).

TBARS Analysis

TBARS analysis in samples was performed with minor changes in the method of Zorba, Gokalp (Zorba et al., 1993). 2 g of sample was weighed in a falcon tube and 5 mL of 20% trichloro acetic acid (TCA) solution and 20 ml of distilled water were added, then the lid was closed and thoroughly shaken. The resulting mixture was filtered, and 3 mL of filtrate was mixed with 3 mL of 0.02 M 2 thiobarbituric acid (TBA) in a screw cap tube. For the blank, 3 mL of distilled water and 3 mL of TBA solution were added to another tube and mixed. Then, the tubes were kept in a water bath at 93°C for 30 minutes, then cooled in tap water and centrifuged for 5 minutes at 2000 rpm. The absorbance of the centrifuged samples at a wavelength of 532 nm was determined by a spectrophotometer (Shimadzu UV-1800, Japan). The absorbance values read were multiplied by a coefficient of 7.8 and the TBARS value was expressed as mg malonaldehyde (MA) in kg sample (Tarladgis et al., 1960).

*Microbiological analyses*

To determine the microbial load of the samples, total mesophilic aerobic bacteria (TMAB), total psychrophilic aerobic bacteria (TPB), and yeast-mold analyses were performed according to ISO 7218 (ISO, 2007). For microbiological analysis, 90 mL of sterile physiological water was added to 10 g of the sample (0.85% NaCl). Appropriate dilutions were prepared from this homogenate. In the study, all analyses were done in triplicate and the results were expressed as log cfu/g.

*Determining Total Mesophilic Aerobic Bacteria (TMAB)* 

Analysis was done by spreading the method from appropriate dilutions to sterile petri dishes. Plate Count Agar (PCA, Merck) was used as the medium, and petri plates were incubated at 30°C for 72 hours. After incubation, all colonies grown in the medium were counted.





CS: Control group, QS3: Sausage with 3% quince waste addition, GS3: Sausage with 3% grapefruit waste addition, TS2: Sausage with 2% tomato waste addition, TS3: Sausage with 3% tomato waste addition; Spice mix (ingredients): White pepper, allspice, coriander, cloves, cinnamon, garlic powder, onion powder, mustard powder, hot phosphate (E450), ascorbic acid (E300), sodium ascorbate (E301), spice extracts.





MA/kg); TDF; Total Dietary Fiber

*Determining Total Psychrophilic Aerobic Bacteria (TPB)*

Analysis was done by spreading the method from appropriate dilutions to sterile petri dishes. Plate Count Agar (PCA, Merck) was used as the medium, and petri plates were incubated at 4-7°C for 10 days. After incubation, all colonies grown in the medium were counted.

### *Determining Molds and Yeasts*

Potato Dextrose Agar (PDA, Merck) medium whose pH was adjusted to 3.5 with 10% tartaric acid after sterilization was used for yeast-mold enumeration. Petri dishes were incubated for 5 days at 25°C. The number of yeast and molds were determined by counting yeast and mold colonies developed at the end of the incubation.

### *Amount of gel and fat separation from emulsion*

200 g of batter (oil in water type emulsion) was filled into glass jars with dimensions of 73×58 mm. The jars were closed and kept in a boiling water bath until the internal temperature was 90ºC (about 35 mins), and after cooling, they were stored at 4ºC for 24 hours. At the end of this period, the jars were kept at 45ºC for 1 hour and opened, and the gel and oil separated from the dough were collected in a volumetric cylinder and measured in ml (Bloukas & Honikel, 1992). The separated gel and fat amounts were calculated by the given formula.

$$
AL = (V/m) \times 100 \tag{1}
$$

AL: Amount of liquid separated from batter V: volume of separated liquid m: weighed amount of batter

# *Chemical composition of sausages*

Moisture content%, crude protein%, crude oil% and ash% were measured according to AOAC standard analyses (AOAC, 2000).

### *Sensory Analysis*

Sausage samples were evaluated sensually by 10 panelists. The panelists were informed by giving brief information for each parameter and writing the necessary

instructions on the analysis form. A random three-digit codes were given to samples. A 9-point hedonic scale was used in the evaluation, and by applying a 1-9 scoring system; 1 was evaluated as "cannot be consumed", 2 as "too bad", 3 as "bad", 4 as "slightly weak", 5 as "medium", 6 as "pretty good", 7 as "good", 8 as "very good", and 9 as "perfect". Sausage samples sliced in approximately 2 cm thickness were presented to the panelists immediately after heating the samples to 40°C. The panelists evaluated the samples sensually using water and bread between tastings. Panelists were asked to evaluate the sausage samples in terms of external appearance, cross-sectional appearance, external color, cross-section color, juiciness-firmness, flavor, foreign flavor, and general acceptance characteristics.

*Water Activity*

The analysis was carried out at room temperature with a water activity measuring device (Novasina LabStart-aw, Switzerland). When the device was ready for measurement, the appropriate amount of sample was placed in the sample cup and the result was read from the device screen.

#### *Purge accumulation*

The purge accumulation of the vacuum-packaged sausage samples was determined by the method used by Bloukas, Paneras (Bloukas & Honikel, 1992) with minor modifications. The sausages were dried with paper towels, weighed, packaged under a vacuum, and stored at 4 ºC for 60 days. At the end of each 15-day, samples removed from the packaging were dried and weighed. The purge value was calculated as a percentage of the difference between the two measurements relative to the initial weight. Three new vacuum-packaged samples were opened for each sample and each period.

 $PA = (Wm-Dm)/Wm \times 100$  (2)

### PA: Purge Accumulation

Wm: Wet mass; Weight of sausage sample before drying. Dm: Dry mass; Weight of sausage sample after drying.

#### *Statistical Analysis*

SPSS package program was used for statistical analysis (SPSS V.22). Analysis of variance (one-way ANOVA) was applied to the general analyses, and the Duncan Multiple Comparison Test was applied to the important variation sources for the storage analyses (Statistics, 2013).

#### **Results and Discussion**

# *Microbial load, TBARS, pH, TDF, and colour values of food waste samples*

Table 2 depicts the results of the TMAB, TPB, moldsyeast, TBARS, pH and TDF, *L\*, a\*, b\** colour analyses of food waste samples. According to TMAB analysis, the highest number of bacteria was found in the tomato waste at 8.96 log cfu/g, and the minimum value was found in the quince waste as 4.00 log cfu/g. TPB could not be detected in quince and grapefruit waste, while it was determined as 3.59 log cfu/g in tomato waste. According to the yeastmold analysis, it was determined that there were 3.46 log cfu/g yeast-mold in the quince waste, 5.77 log in the grapefruit waste, and 5.95 log cfu/g in the tomato waste. The highest microbial load was determined in tomato waste with all TMAB, TPB and yeast-mold, while the least microbial load was determined in quince waste. The grapefruit and quince wastes were obtained as a result of pressing the fruits in the laboratory, washing the waste part, and drying it in an oven at 40°C. Unlike quince waste, it was kept at 85°C for 5 minutes before washing to prevent browning and it is thought that fewer microorganisms develop because of heat treatment of quince waste. The low pH (4.15) of grapefruit waste also limited microbial growth in this sample. Tomato waste, which is the waste of tomato paste production, was dried under the sun for 24 hours at 39°C. The reasons why the tomato waste has a higher microbial load could be that it is exposed to high contamination because of the processes it undergoes in the factory, as well as the high-water activity of the processed tomato fruit, and its exposure to microbial growth before and during drying. Except for legumes, the interior of plants is considered sterile. However, microorganism contamination is possible as a result of hand contact, and contact with equipment and surfaces during processing, some microorganisms can potentially develop according to their susceptibly fruits or vegetables (Acar et al., 2006). In some studies, it has been reported that various pretreatments are applied to food wastes to reduce the microbial load. (de Moraes Crizel et al., 2013) used the orange juice extraction waste washed, kept in 150 ppm (mg/L) sodium hypochlorite solution for 10 minutes and sanitized. (Savadkoohi et al., 2014) subjected the tomato pulp they purchased to use in meat products to a severaltreatments such as drying, grinding, acid-base treatment, and boiling to reduce their microbial load. According to the results of TBARS analysis, which is an indicator of lipid peroxidation; it was determined that the TBARS value of quince waste was 3.16 mg MA/kg, the TBARS value of grapefruit waste was 4.25 mg MA/kg and the TBARS value of tomato waste was 16.11 mg MA/kg. The rate and extent of lipid oxidation are affected by many factors such as iron content, distribution of unsaturated fatty acids, pH, and antioxidant level (Falowo et al., 2014). Lipid oxidation can occur either autocatalytically or due to the presence of lipases (Acar et al., 2006). For these reasons, it is thought that tomato waste with a higher microbial load is exposed to more oxidation. In addition, it is estimated that the processes applied to these foods during the formation of food waste; contributed to the progression of lipid oxidation and a higher TBARS value since there is a relationship between lipid oxidation and cell membrane damage (Beltran et al., 2003). Shredding and heating processes can catalyse lipid oxidation as it disrupts cellular protective compounds found in cell membranes such as vitamin E, electron, and hydrogen donors (Keokamnerd et al., 2008).

#### *Batter pH and Separated Gel & Fat Amount*

The pH values of the emulsion and gel and fat amount separated from the batter were given in Table 3. The pH of the batter samples varied between 5.41-6.04 and it can be said that the pH of the batter is directly affected by the pH of the waste samples. The highest pH was measured in the TS3 sample, and the lowest pH was measured in the GS3 sample in accordance with tomato waste's and grapefruit waste's pH values. Gel and oil separation refers to the total amount of liquid released from emulsions at a given temperature and is an important indicator of emulsion stability (Serdaroğlu et al., 2016). The amount of gel and fat separated from 100 g sample was measured as 1.74, 2.46, 1.73 and 1.90 in CS, GS3, TS2, and TS3 samples, respectively. There is no significant difference between these results, which differ in quantity. The sample with the least amount of water separated from the batter was measured in the QS3 sample (0.488). It has been reported in many studies that quince and especially quince seeds show high hydrocolloid properties (Kırtıl & Oztop, 2016; Ritzoulis et al., 2014) preventing liquid separation from the sample.

#### *Chemical Compositions of the Sausages*

The results of the moisture, protein, fat, and ash analysis performed after the production of the sausages are given in Table 4. The control sample has the highest moisture value (64.67%).

Table 3. Emulsion pH and separated gel  $&$  fat amount

		DS3	GS3	TS2	TS3
Batter pH	5.93 $\pm 0.02^{\circ}$	$5.81 \pm 0.01$ <sup>d</sup>	$5.41 \pm 0.01$ <sup>e</sup>	$6.02 \pm 0.02^b$	$6.04 \pm 0.01$ <sup>a</sup>
Separated gel&fat $(g/100g)$	$74 \pm 0.12^{\rm a}$	$0.49 \pm 0.41$ <sup>b</sup>	$2.46 \pm 0.52$ <sup>a</sup>	.73 $\pm$ 0.09 <sup>a</sup>	.90 $\pm$ 0.02 $^{\rm a}$

\*CS: Control group, QS3: Emulsion with 3% quince waste addition, GS3: Emulsion with 3% grapefruit waste addition, TS2: Emulsion with 2% tomato waste addition, TS3: Emulsion with 3% tomato waste addition.

Table 4. Chemical compositions of the sausages									
	O3		G3	T2	T <sup>2</sup>				
%Moisture	$64.67 \pm 0.25^{\circ}$	$63.73\pm0.31^{\circ}$	$63.75 \pm 0.07$ b	$63.50\pm0.01^{b}$	$63.46 \pm 0.24^b$				
%Protein	$11.31 \pm 0.26^a$	$11.59 \pm 0.23$ <sup>a</sup>	$11.23 \pm 0.61$ <sup>a</sup>	$11.70 \pm 00.01$ <sup>a</sup>	$12.25 \pm 0.12^a$				
$%$ Fat	$21.02 \pm 0.61$ <sup>a</sup>	$21.41 \pm 1.46^a$	$21.94 \pm 0.11$ <sup>a</sup>	$21.16\pm0.21^{\circ}$	$21.49 \pm 0.67$ <sup>a</sup>				
%Ash	$2.67 \pm 0.05$ <sup>c</sup>	$2.24\pm0.03b$	$2.12 \pm 0.05$ <sup>c</sup>	$2.69 \pm 0.05^{\text{a}}$	$2.78 \pm 0.05^{\text{a}}$				

Table 4. Chemical compositions of the sausages

CS: Control group, QS3: Sausage with 3% quince waste addition, GS3: Sausage with 3% grapefruit waste addition, TS2: Sausage with 2% tomato waste addition, TS3: Sausage with 3% tomato waste addition. \*Mean  $\pm$  standard deviation; a-c: there is a statistically significant difference in the same line ( $p < 0.05$ ).



Chart 1. Sensory analysis of the sausage samples. CS: Control group, QS3: Sausage with 3% quince waste addition, GS3: Sausage with 3% grapefruit waste addition, TS2: Sausage with 2% tomato waste addition, TS3: Sausage with 3% tomato waste addition.

The moisture contents of the food waste-added sausages are between 63.46-63.75 and there is no statistically significant difference between them. Food wastes are rich in dietary fiber and the water is held through the fiber matrix. The protein content of the sausages varies between 11.23-12.25%, but there is no statistical difference between them. Similarly, the crude fat content of the sausages is between 21.02-21.94% and there is no statistically significant difference between them. The ash contents of the TS2 and TS3 samples are higher than other samples  $(p<0.05)$ . According to the meat and meat products communiqué published by the Turkish Ministry of Agriculture and Livestock; the total meat protein of emulsified meat products is at least 10% by mass, the ratio of moisture content to total meat protein amount is below 6.5 by mass, the ratio of fat content to total meat protein amount is below 3.2, and the sum of the amount of protein and the amount of starch should be at most 5% by mass (Anonim, 2012). The contents of the sausage samples we obtained are in accordance with these values. Fernandez-Gines et al. (2003) found the moisture content of the sausages produced with 0.5%, 1%, 1.5% and 2% citrus fiber between 70.02-71.30% and they determined that the sausages with added fiber have lower moisture content shows similarity to our conclusion.

#### **Sensory Analysis**

Ten panelists performed sensory analysis and brief training was given to the panelists on the interpretation of sensory characteristics before the analysis. The sensory analysis results of the sausage samples are given in Chart

1. According to the results, no difference was determined between the external appearances of sausage samples statistically. For the external appearance feature, it is desired that the samples are smooth, shiny and that there is no water on the surface. Control and waste added samples were not statistically different from each other ( $p > 0.05$ ) and samples scored between 6.50-7.20 out of 9. For the section view, it is important that the oil of the samples is homogeneously dispersed, not in a perforated structure, not cracked and not scattered. For this feature, the control and application samples were not statistically different from each other (p>0.05) and the samples scored between 6.80-7.30 out of 9. The panelists could not see any difference between the sausage samples for the external color and cross-section color characteristics, which were desired to be pinkish-red. Samples scored between 6.60-7.20 for the exterior color feature, while their scores for the section color were between 6.80-7.0. Although the scores given by the panelists for the juiciness-firmness feature were between 6.20-7.30, there was no statistical difference between the samples ( $p>0.05$ ). For the taste feature, the pleasing sausage taste, smell, and aroma are sought and the GS3 sample got an average of 4.30 points for this parameter. Other samples are of between 6.90-7.50 and there is no statistical difference between them  $(p>0.05)$ . Scale 1 represents no foreign flavor is felt and scale 9 means there is an inexhaustible foreign flavor for the perceived foreign taste feature. GS3 sample which scored the lowest in taste, received the highest score (7.50) in terms of foreign flavor, in parallel. The scores of all other samples with foreign flavor characteristics are between

1.70-2.30 and are statistically indistinguishable from one another. Evaluating the samples in terms of general acceptability, the panelists gave high scores between 7.00- 7.40 to the samples other than the GS3 sample.

In summary, according to the evaluation results of the panelists, there was no difference between the CS, QS3, TS2, and TS3 samples in terms of all sensory properties. The taste value of the GS3 sample is lower than the other four types of sausage. Again, the more foreign taste was felt in the GS3 sample than in the other sausage samples, and this feature caused the GS3 sample to have a general acceptability value of 4 out of 10. QS3, TS2, and TS3 samples were evaluated by the panelists as indistinguishable from each other and having the same sensory characteristics as the control group. Dietary fibers should have a neutral taste. The amount of dietary fiber added to food is limited because it can cause undesirable changes in terms of color and texture. There are significant amounts of bitterness substances (flavanoglycosides) in the composition of citrus fruits such as naringin, limonin and neohesperidin. The substance that gives bitterness to the grapefruit fruit is naringin and is especially found in the skin of the fruit, in the slice membranes and the axis of the fruit (Drewnowski & Gomez-Carneros, 2000). The grapefruit waste has a bitter taste because it contains slice membranes, fruit axes, and fruit seeds, and caused less taste, much foreign flavor, and lower general appreciation for GS3 samples compared to the other sausage samples. Çoksever (2009) added raw and cooked citrus albedos to sausage in different ratios and reported that the overall acceptability of row albedo-added sausages decreased due to the bitterness of the sourdough albedos like our results. Fernandez-Gines et al. (2004) reported that the addition of lemon albedo did not affect the odor and residual flavor value whether it was raw or cooked. Another study made

by the same researchers reported that using citrus fiber at 0.5%, 1%, 1.5%, and 2% ratios in sausages reduces juiciness, and odor regardless of the added dose (Fernandez‐Gines et al., 2003).

### *Sausages and Storage Analyses*

Water activity, pH, TBARS, and purge accumulation of the sample values measured during the storage period are given in Table 5.

# *Water Activity (aw)*

The water activity values measured during the storage period are given in Table 5. The  $a_w$  value varied between 0.953-0.976 during storage and the increase/decrease in the values was not regular but slightly fluctuated. During storage, the highest  $a_w$  value belonged to the CS sample and the lowest aw value belonged to the QS3 sample. In addition, the amount of liquid separated from the sausage batter was lowest in the QS3 sample (Table 3). According to the variance analysis, sample type (St) and storage period (Sp) showed a significant ( $p$ <0.05) effect from the sources of variation. The effect of the interaction of St×Sp on aw was not found to be statistically significant. Water activity is affected by the dry matter amount of the food, the amount of water added to the product, the product pH, temperature, preservatives, and fillers used. It is expected that the water activity of the application samples will be lower than the control group. The high hydration properties of the food waste-added samples ensured that the  $a_w$  values of these samples were lower than the control. Microbial growth could lead to an increase in aw value, also purge accumulation was observed during storage and this leakage was thought to be effective on  $a_w$  value. Andres et al. (2006) prepared low-fat (0%, 2%, 5%) sausage with chicken breast meat.

Table 5. aw, pH, TBARS and Purge Accumulation Analyses of the sausages during storage

$\mathbf{A}$	<b>ST</b>		Storage Period (Sp)				Avarage (St)	Interactions		
		$0.$ day	$15.$ day	$30.$ day	45.day	$60 \text{.day}$				St Sp $St \times Sp$
WA		CS $0.970\pm0.00^{ABa}$ $0.975\pm0.00^{Aa}$ $0.973\pm0.00^{Aa}$ $0.976\pm0.01^{Aa}$ $0.965\pm0.01^{Aa}$ $0.972\pm0.01^{A}$								
		QS3 0.970±0.00 <sup>ABa</sup> 0.967±0.00 <sup>Da</sup> 0.961±0.00 <sup>Aa</sup> 0.953±0.01 <sup>Ba</sup> 0.960±0.01 <sup>Aa</sup> 0.962±0.01 <sup>C</sup>								
		GS3 $0.968\pm0.00^{Ba}$ $0.970\pm0.00^{BCa}$ $0.953\pm0.02^{Aa}$ $0.960\pm0.00^{ABa}$ $0.972\pm0.01^{Aa}$ $0.964\pm0.01^{BC}$						$\ast$	*	ns
							TS2 0.968±0.00 <sup>Bab</sup> 0.969±0.00 <sup>CDa</sup> 0.957±0.01 <sup>Abc</sup> 0.957±0.01 <sup>ABc</sup> 0.976±0.00 <sup>Aa</sup> 0.965±0.01 <sup>ABC</sup>			
		TS3 $0.972 \pm 0.00$ <sup>Aa</sup>	$0.972 \pm 0.00^{\rm Ba}$		$0.969\pm0.00^{Aa}$ $0.969\pm0.00^{ABa}$ $0.971\pm0.01^{Aa}$ $0.970\pm0.01^{AB}$					
		$CS$ 6.37 $\pm$ 0.01 <sup>Bc</sup>	$6.37 \pm 0.01$ <sup>Bc</sup>	$6.40\pm0.01^{Ab}$	$6.47 \pm 0.01$ <sup>Ba</sup>	$6.48 \pm 0.01$ <sup>Ba</sup>	$6.42 \pm 0.05^{\rm B}$			
		QS3 $6.25 \pm 0.01$ <sup>Ca</sup>	$6.23 \pm 0.00$ <sup>Cb</sup>	$6.23 \pm 0.01^{Bb}$	$6.17 \pm 0.01^{Dc}$	5.94 $\pm$ 0.01 <sup>Dd</sup>	$6.16 \pm 0.12^D$			
pH		GS3 5.92±0.01 <sup>Da</sup>	$5.92 \pm 0.01^{Da}$	$5.84 \pm 0.01$ <sup>Cb</sup>	$5.56 \pm 0.01$ <sub>Ec</sub>	$5.55 \pm 0.01$ <sup>Ec</sup>	$5.76 \pm 0.18$ <sup>E</sup>	** **		**
		TS2 $6.39 \pm 0.01$ <sup>Ab</sup>	$6.37 \pm 0.01$ <sup>ABc</sup>	$6.41 \pm 0.01$ <sup>Aa</sup>	$6.42 \pm 0.01$ <sup>Ca</sup>	$6.32 \pm 0.01$ <sup>Cd</sup>	$6.38 \pm 0.04^{\circ}$			
		TS3 $6.38 \pm 0.01$ <sup>Bc</sup>	$6.37 \pm 0.01$ <sup>Ac</sup>	$6.40\pm0.01^{Ab}$	$6.49 \pm 0.01$ <sup>Aa</sup>	$6.50 \pm 0.01$ <sup>Aa</sup>	$6.43 \pm 0.06$ <sup>A</sup>			
	CS.	$0.24 \pm 0.05^{Bb}$	$0.23 \pm 0.07$ <sup>Cb</sup>	$0.23 \pm 0.03^{Db}$	$0.31 \pm 0.05^{\text{Cb}}$	$0.53 \pm 0.05$ <sup>Ca</sup>	$0.31 \pm 0.12^D$			
		QS3 0.53±0.09 <sup>Ab</sup>	$0.55 \pm 0.02^{Ab}$	$0.32 \pm 0.09^{\rm CDc}$	$0.37 \pm 0.07$ <sup>BCcb</sup>	$1.03 \pm 0.16$ <sup>ABa</sup> $0.56 \pm 0.27$ <sup>B</sup>				
<b>TBARS</b>		GS3 $0.29 \pm 0.09^{Bb}$	$0.31 \pm 0.02$ <sup>BCb</sup>	$0.37 \pm 0.05^{\text{BCb}}$	$0.44 \pm 0.04$ <sup>Bb</sup>	$0.93 \pm 0.16^{Ba}$	$0.47 \pm 0.26$ <sup>C</sup>		** **	**
		TS2 $0.34 \pm 0.05$ <sup>Bb</sup>	$0.34 \pm 0.08$ <sup>Bb</sup>	$0.44 \pm 0.04^{ABb}$	$0.46 \pm 0.06$ <sup>ABb</sup>	$1.04 \pm 0.07$ <sup>ABa</sup> $0.52 \pm 0.28$ <sup>B</sup>				
		TS3 $0.47 \pm 0.04$ <sup>Ab</sup>	$0.51 \pm 0.00^{Ab}$	$0.53 \pm 0.06$ <sup>Ab</sup>	$0.55 \pm 0.02$ <sup>Ab</sup>	$1.17 \pm 0.08$ <sup>Aa</sup>	$0.65 \pm 0.28$ <sup>A</sup>			
	CS.	$3.41 \pm 0.70$ <sup>Aa</sup>	$3.48 \pm 0.88$ <sup>Aa</sup>	$3.79 \pm 0.96$ <sup>Aa</sup>	$3.85 \pm 0.34$ <sup>Aa</sup>	$3.95 \pm 2.66$ <sup>Aa</sup>	$3.70\pm1.04^{\rm A}$			
		QS3 $0.99 \pm 0.36$ <sup>Ba</sup>	$1.18 \pm 0.04$ <sup>Ba</sup>	$1.23 \pm 0.03^{Ba}$	$1.25 \pm 0.62$ <sup>Ba</sup>	$1.26 \pm 0.09$ <sup>Aa</sup>	$1.18 \pm 0.26$ <sup>C</sup>			
PA		GS3 $1.93 \pm 0.30$ <sup>Ba</sup>	$1.95 \pm 0.32$ <sup>Ba</sup>	$2.33 \pm 0.56$ <sup>Ba</sup>	$2.38 \pm 0.84$ <sup>Ba</sup>	$2.32 \pm 0.08$ <sup>Aa</sup>	$2.18 \pm 0.42^B$		$**$ ns	ns
		TS2 1.39±0.37 <sup>Bb</sup>	$1.72 \pm 0.09$ <sup>Bab</sup>	$2.26 \pm 0.34$ <sup>Ba</sup>	$2.33 \pm 0.09$ <sup>Ba</sup>	$2.39 \pm 0.26$ <sup>Aa</sup>	$2.02\pm0.46^{\rm B}$			
		TS3 $1.33 \pm 0.21$ <sup>Bb</sup>	$1.36 \pm 0.11^{Bb}$	$2.13 \pm 0.43$ <sup>Ba</sup>	$2.03 \pm 0.23$ <sup>Bab</sup>	$2.25 \pm 0.26$ <sup>Aa</sup>	$1.82 \pm 0.46$ <sup>B</sup>			

A: Analyses; WA: Water Activity; TBARS: TBARS (mg MA/kg); PA: Purge Accumulation (mL/kg); ST: Sample Type (St);CS: Control group, QS3: Sausage with 3% quince waste addition, GS3: Sausage with 3% grapefruit waste addition, TS2: Sausage with 2% tomato waste addition, TS3: Sausage with 3% tomato waste addition. \*Mean ± standard deviation. A-E: There is a statistically significant difference in the same column, a-d: there is a statistically significant difference in the same line (P<0.05). \*\*: P<0.01, \*: P<0.05, ns: not significant

They stated that at the end of the 50-day storage, the  $a_w$ value of the control sample decreased from 0.961 to 0.949, and the  $a_w$  value of the 5% oil application sample decreased from 0.963 to 0.943. In our study, a significant decrease in aw value was not observed for the mentioned reasons. de Avila et al. (2014) reported that the water activity of samples produced with chicken breast meat was 0.973 in their study on 14 different meat products.

# *pH Analysis*

The pH values of the samples measured during the storage period are given in Table 5. The pH value varied between 5.92-6.39 at the beginning of storage, and it was between 5.55-6.50 at the end of storage (60th day). The sausage samples made with grapefruit waste had a lower pH value during storage ( $p$ <0.05). It was determined that the pH values of the sausage samples were closely related to the pH values of the food waste. According to the variance analysis results, the St, Sp, and St×Sp interactions had a very significant ( $p<0.01$ ) effect on the pH value of the sausages. The highest average pH value was measured in the TS3 sausage sample (6.43) and the lowest average pH value in the GS3 sample (5.76) related with the pH of the waste types. When the effect of storage periods on pH value is examined; it was observed that the overall pH values decreased with the increase of storage time. As a consequence, lipid hydrolysis increased free fatty acidity and decreased the pH. According to the results of TBARS analysis in the same table, significant increases in the amount of lipid oxidation were detected with the increase in storage time. For this reason, it is thought that the increase in free acidity is effective in decreasing the pH values. It has also been reported that during the storage of meat products causes lactic acid bacteria to produce lactic acid from the product, which leads to a gradual decrease in pH (Viuda-Martos et al., 2010). There was also an increase the in the pH value of the CS and TS3 samples at the end of storage. It is possible that the cooking process breaks down the cell buffers of the meat and raises the pH due to the release of free fat (Fernandez-Gines et al., 2004). During the storage some microorganisms metabolize amino acids, resulting in amine, ammonia, mercaptan, metabisulfite, and putrefaction in meat stored in vacuum packaging and cold storage results raising the pH of the meat generally (Erkmen, 2013; Seol et al., 2013).

### *TBARS analysis*

Lipid oxidation is one of the most important parameters for the quality and acceptance of meat and poultry. This reaction occurs easily in poultry with unsaturated fat. Heat treatment is also known to increase lipid oxidation. TBARS analysis is a widely used technique to evaluate lipid oxidation (Beltran et al., 2003). TBARS values are given in Table 5. were between 0.24-0.53 mg MA/kg at the beginning of storage and between 0.53-1.17 mg MA/kg at the end of storage. TBARS values increased with increasing storage time in CS, GS3, TS2, and TS3 samples. In the QS3 sample, fluctuations in the form of increase and decrease were determined. According to the variance analysis results of TBARS values, the effect of St, Sp, and St×Sp interactions is very important ( $p$ <0.01). According to Duncan's multiple comparison test results, there was no statistically significant difference between the mean TBARS values of TS2 and QS3 samples (p>0.05); Mean TBARS values of CS, GS3, and TS3 samples were found to be different from these samples and each other  $(p<0.05)$ . At the end of storage, QS3, TS2 and TS3 samples exceeded the acceptable TBA<1.0 mg MA/kg limit (Oruç et al., 2005). In the GS3 sample, it is thought that the low pH (4.15) of grapefruit waste limited the oxidation. For the control sample, the absence of microbial load and lipid oxidation from a waste source allowed this sample to have a lower TBARS value. Lipid oxidation in the products increased during storage due to reasons such as the unsaturated fat content of the raw materials, the use of salt, microorganism activities, the pH of the environment, and the heat treatment applied. It has been mentioned before the used food wastes already had a definite level of TBARS value due to their processes and microbial load. It is thought that the oxidation values of the waste increased with the formation and cooking of sausage butter and with the increase in storage time. It is reported that chicken meat is more prone to oxidation compared to other types of meat (Rhee et al., 1996). TBARS values are expected to increase when sodium chloride is added due to the pro-oxidant effect of salt (Beltran et al., 2003). At the same time, cooking or grinding exposes the reactive lipid components to O2 and other oxidative catalysts in the meat (Keokamnerd et al., 2008).

Urgu (2013) stated that the TBA values of the sausages produced using hazelnut flour and hazelnut oil were between 0.04-0.18 mg MA/kg during the 60-day storage period, and the TBA values of the samples remained within the acceptable limits during the storage. (Fernandez-Lopez et al., 2004) produced sausages using different ratios of citrus fiber for 28 days and found that the TBA values of the samples containing 0.5%, 1%, 1.5% and 2% fiber were 8.10, 7.49, 7.30 and 7.32 mg MA/kg sample respectively, while the TBA value of the control sample was 7.99 mg MA/kg sample. Rhee et al. (1996) stored the meatballs made with chicken, beef, and pork meat as raw and cooked. They determined that the TBA value of chicken meatballs was much higher than the others among both raw and cooked samples, and they stated that the reason was the excess amount of polyunsaturated fatty acids (PUFA) in chicken meat.

#### *Purge accumulation*

In meat emulsions, it is a serious problem that some oil and water separate from the vacuum-packaged products and leak into the package. The amount of leakage varies according to the volume and shape of the sample, the processing applied, the storage temperature, and the storage time. Leakage during the cooking process is a significant change in meat products with high moisture content, may change the nutritional value of the product due to the release of soluble vitamins and amino acids, have negative effects on texture and juiciness, and cause economic losses (López-Vargas et al., 2014; Uysal, 2011).

Purge accumulation values measured during the storage period of the sausage samples are given in Table 5. When the data are examined, even if more purge accumulation is observed with the increase in the storage time, St, and  $St \times Sp$  interactions do not have a significant effect on the amount of leakage statistically. According to Duncan's multiple comparison tests, the highest average purge accumulation was detected in the control sample (CS). Statistically, there is no significant difference between the purge accumulation values of the GS3, TS2, and TS3

samples during storage  $(p>0.05)$ . The lowest value was detected in the QS3 sample. The dietary fiber and pectin content of food waste we used in sausage production prevent increasing purge accumulation. Pectin is present in both raw and by-products of the fruit and vegetable processing industries (Neckebroeck et al., 2021) Also quince, grapefruit and tomato contain a significant amount of pectin (Anthon et al., 2008; Thomas et al., 2003). Pectin and dietary fibers are known to reduce syneresis and increase viscosity in foods (do Nascimento et al., 2016; Elleuch et al., 2011). In addition, fibers with high water holding capacity are used to prevent water release in foods and to improve viscosity and texture. The fact that the waste types we use in the study reduced the amount of leakage means that the shelf life of the product is longer. Although the amount of water and gel separated from the sausages is considered as the loss of soluble components, it is a suitable environment for microorganism activities. In addition, purge accumulation negatively affects the microbiological, chemical, and sensory properties of the product, and causes an undesirable appearance. Compared to the control sample, samples produced with the addition of quince, grapefruit and tomato waste are free of these disadvantages.

*Color Analysis* 

The inner section and outer surface *L\**, *a\**, and *b\** values of the samples measured during the storage period are given in Table 6. The internal section *L\** values of the samples ranged between 67.98 and 74.87; the outer surface *L\** values ranged between 67.61 and 75.78 during storage. According to Duncan's multiple comparison test, the highest outer surface *L\** value was determined in the GS3 sample, and the inner section *L\** value was determined in the QS3 and GS3 samples among the application samples. The high *L\** value of grapefruit waste-added samples is due to the high gloss of grapefruit albedo. In general, the addition of food waste to sausages caused a decrease in the brightness of the products, and fluctuations in *L\** values were observed during storage. The fiber content, functional characteristics, and microbiological quality of agri-food waste can all be impacted by different technological treatments. The fiber begins to brown because of the drying process (*a\** increases and *L\** decreases). Washing before drying can prevent the fibers from browning, possibly due to the removal of sugars (Lario et al., 2004). The internal section *a\** values of the samples varied between 10.54 and 9.17; the outer surface *a\** values varied between 11.56 and 10.02 during storage. According to Duncan's multiple comparison test, the highest outer surface and inner surface *a\** value among the storage application samples was determined in the QS3 sample. Anthocyanin presence in quince lead to higher *a\** values, in addition heat treatment

applied to quince waste may not have completely prevented browning. The internal cross-section *b\** values of the samples were between 21.16 and 26.79; the outer surface *b\** values were between 23.10 and 26.56 during storage. According to Duncan's multiple comparison test, the highest internal and external *b\** values were determined in the TS3 sample. This sample was followed by the TS2. In general, the addition of food waste caused the sausages to have a higher *b\** value due to the carotenoid content of waste.

# *Microbiological analyses*

Microbial and chemical spoilage in meat causes foodborne diseases, economic losses, food insecurity, and, as a result, wastage in meat (Falowo et al., 2014). Despite the heat treatment applied to meat products such as sausage, bologna, ham, sausage, and bacon, microbiological deterioration is frequently observed. In the mincing process, all microorganisms in the microflora are dispersed into the product. Microorganisms can be transmitted from equipment, personnel, air, water, raw materials, and additives (Andres et al., 2006; Erkmen, 2013). Sausage samples were subjected to TMAB, TPAB and mold-yeast analyses on the 0th, 15th, 30th, 45th, and 60th days of storage.

# *TMAB during storage*

Total Mesophilic Aerobic Bacteria (TMAB) analysis results are given in Table 7. According to results, it was determined that the average TMAB values in the control samples were lower than 2.00 log cfu/g while increasing in the food waste-added samples during storage. Variance analysis of TMAB values indicated that the effect of St and SP on TMAB is very important  $(p<0.01)$ . In addition, the interaction of St×Sp had a great effect on TMAB values (p<0.01). For the results of the average TMAB values during the storage period, the highest TMAB was determined in the sausages that used tomato waste, and there was no statistical difference between the TMAB values of the TS2 and TS3 samples. The lowest TMAB count was found in GS3 sausages with an average of 4.359 log cfu/g although there was no statistical difference between the TMAB values of the QS3 and GS3 samples. The number of TMAB increased during the storage period which was 3.539 log cfu/g on day 0, increased to an average of 6.111 log cfu/g on day 60 of storage.

The microbial load of the food wastes affected the microbiological quality of the final products. When the microbial load of the waste is examined (Table 2), the lowest TMAB and yeast mold counts were determined in quince waste, and the highest TMAB and yeast-mold counts were determined in tomato waste. In this direction, it is not surprising for TS2 and TS3 samples to have the highest TMAB value.

Table 6. Average *L\*, a\*, b\** values of the sausages during storage

$1.0015$ of $1.101$ and $1.001$ and $1.00$									
<b>Samples</b>		$L^*$		$a^*$	h*				
	Internal	External	Internal	External	Internal	External			
CS.	$73.84 \pm 0.7^{\rm a}$	$74.40 \pm 0.9^{\rm a}$	9.95 $\pm$ 0.3 <sup>bc</sup>	$10.99 \pm 0.6^{\text{a}}$	$21.621 \pm 0.6^e$	$23.469 \pm 0.5$ °			
OS3	$71.85 \pm 0.9^b$	$72.04 \pm 1.2$ °	$10.40 \pm 0.3^{\text{a}}$	$11.01 \pm 0.5^{\text{a}}$	$24.334\pm0.6^{\circ}$	$24.573 \pm 0.7^{\rm b}$			
GS3	$72.13 \pm 0.9^b$	$72.76 \pm 0.7^{\rm b}$	9.73 $\pm$ 0.4 <sup>cd</sup>	$10.79 \pm 0.5^{ab}$	$23.365 \pm 0.5^{\rm d}$	$24.350\pm0.9b$			
TS <sub>2</sub>	$69.15 \pm 1.0^{\circ}$	$70.73 \pm 1.6^d$	$10.22 \pm 0.5^{ab}$	$10.35 \pm 0.5$ <sup>c</sup>	$25.287\pm0.8^{b}$	$24.909\pm0.9^b$			
TS3	$68.65 \pm 0.7$ °	$68.26 \pm 1.0^e$	9.55 $\pm$ 0.4 <sup>d</sup>	$10.51 \pm 0.4$ <sup>cb</sup>	$26.334 \pm 0.7^{\circ}$	$26.410\pm0.8^{\rm a}$			

CS: Control group, QS3: Sausage with 3% quince waste addition, GS3: Sausage with 3% grapefruit waste addition, TS2: Sausage with 2% tomato waste addition, TS3: Sausage with 3% tomato waste addition. \*Mean ± standard deviation. a-d: There is a statistically significant difference in the same column ( $p \le 0.05$ ).

	<b>ST</b>		Storage Period (Sp)					Interactions		
		$0.$ day	$15.$ day	$30.$ day	$45.$ day	$60 \text{.day}$	Average	St	Sp	StxSp
	CS	$\leq$ 2	$\leq$ 2	2.00	2.00	$2.50\pm0.71$				
	QS3			2.74±0.37 <sup>Bd</sup> 3.29±0.02 <sup>Cc</sup> 4.88±0.07 <sup>Bb</sup> 5.26±0.14 <sup>Ab</sup> 6.22±0.19 <sup>Aa</sup> 4.48±1.36 <sup>B</sup>						
<b>TAMB</b>	GS3			2.65±0.49 <sup>Bc</sup> 3.06±0.08 <sup>Dbc</sup> 4.04±0.27 <sup>Cb</sup> 5.81±0.72 <sup>Aa</sup> 6.24±0.13 <sup>Aa</sup> 4.36±1.55 <sup>B</sup> **					$***$	$***$
	TS2			4.32±0.03 <sup>Ac</sup> 4.61±0.01 <sup>Bbc</sup> 5.10±0.14 <sup>Bb</sup> 6.50±0.50 <sup>Aa</sup> 6.11±0.22 <sup>Aa</sup> 5.33±0.91 <sup>A</sup>						
	TS3			4.45±0.04 <sup>Ad</sup> 5.29±0.02 <sup>Ac</sup> 5.66±0.06 <sup>Ab</sup> 5.76±0.03 <sup>Ab</sup> 5.87±0.41 <sup>Aa</sup> 5.41±0.55 <sup>A</sup>						
	CS	$<$ 2	$\langle$	$\langle$	$\mathord{<}$	</td <td><?</td><td></td><td></td><td></td></td>	</td <td></td> <td></td> <td></td>			
	QS3	$<$ 2		$3.53\pm0.03^{Cc}$ 4.11 $\pm$ 0.29 <sup>Bbc</sup> 4.76 $\pm$ 0.16 <sup>Cb</sup> 5.96 $\pm$ 0.41 <sup>Aa</sup> 4.59 $\pm$ 0.98 <sup>D</sup>						
<b>TPB</b>	GS3	$<$ 2		$5.04\pm0.05^{Ac}$ $5.30\pm0.12^{Abc}$ $5.53\pm0.13^{Bb}$ $6.32\pm0.15^{Aa}$ $5.55\pm0.52^{A}$ **					**	$***$
	TS <sub>2</sub>	$<$ 2		$4.04\pm0.19^{Bc}$ $4.38\pm0.10^{Bb}$ $5.98\pm0.03^{Aa}$ $6.11\pm0.09^{Aa}$ $5.13\pm0.99^{B}$						
	TS3	$\leq$ 2		4.26±0.06 <sup>Bd</sup> 4.53±0.08 <sup>Bc</sup> 5.00±0.06 <sup>Cb</sup> 5.93±0.04 <sup>Aa</sup> 4.93±0.68 <sup>C</sup>						
	CS	$<$ 2	$\leq$ 2	$\leq$ 2	$\leq$ 2	$\leq$ 2				
	QS3	$<$ 2	$\leq$ 2	$\leq$ 2	$\leq$ 2	$\leq$ 2				
MY	GS3	$\leq$ 2	$<$ 2	$<$ $\!\!2$	$\leq$ 2	$\leq$ 2		ns	ns	ns
	TS <sub>2</sub>	$\leq$ 2	$<$ 2	$<$ 2	$\leq$ 2	$\leq$ 2				
	TS3	$\leq$ 2	$<$ 2	$<$ 2	$<$ 2	$\leq$ 2				

Table 7. Microbiological Analyses of Storage

TAMB: TAMB (log cfu/g); TPB: TPB (log cfu/g); MY: Molds&yeast (log cfu/g); ST: Sample Type (St); CS: Control group, QS3: Sausage with 3% quince waste addition, GS3: Sausage with 3% grapefruit waste addition, TS2: Sausage with 2% tomato waste addition, TS3: Sausage with 3% tomato waste addition. \*Mean ± standard deviation. A-D: There is a statistically significant difference in the same column, a-d: there is a statistically significant difference in the same line ( $p \le 0.05$ ). \*\*:  $p \le 0.01$ , ns: not significant

The lower TMAB value of quince waste and the lower pH value of grapefruit waste resulted in lower TMAB values of the QS3 and GS3 samples. The reasons of lowest TMAB values of the CS sample which did not contain any food waste that there was no microbial load or lipid oxidation caused by food waste. Water activity is also an important parameter for microbial growth. The fact that the average water activity of the sausage samples did not decrease below 0.962 created a suitable environment for microbial development. QS3 and GS3 samples reached the same level as TS2 and TS3 samples on the 60th day of storage. TS2 sample reached the highest number of TMAB on the 45th day, and this value decreased on the 60th day. For this sample, it is thought that after the 45th day, the microorganisms pass from the stationary phase to the death phase. During the death period, the nutrients in the environment deplete, the metabolic waste concentration increases, and the environment becomes obstructive to development. The time required for bacteria to die varies depending on the type of bacteria (Tayar & Hecer, 2010). The most important microorganisms causing spoilage in chicken meat are Pseudomonas, Acinetobacter, Alcaligenes, Aeromonas, Moraxella and Alteromonas. Most of these microorganisms are also lipolytic bacteria (Erkmen, 2013) so microbial load and TBARS values paralleled in this study. Cardoso et al. (2008) added dietary fiber to fish sausages and found that the TMAB count of the products was low during storage, the control sample was between 2.0 - 2.1 log cfu/g on days 0 and 80, and the application samples were between 1.9-2.3 log cfu/g on day 0 and 80. They stated that these results were unexpected as the applied heat treatment should prevent the survival of vegetative cells present in the raw material.

#### *Total Psychrophilic Aerobic Bacteria (TPB)*

The TPB values of the sausage samples are given in Table 7. While the TPB of all samples was determined as  $\leq$ 2.00 log cfu/g on day 0, the TPB value of all groups except the control group increased during storage. During storage, the highest TPB value was determined as 6.32 log cfu/g in the sausage sample of GS3 on the 60th day. According to the results of the variance analysis, the effects of St and Sp on TPB are very important  $(p<0.01)$ . In addition, the interaction of  $St \times Sp$  showed a significant effect on TPB ( $p<0.01$ ). The average highest and lowest TPB counts were determined in the GS3 sample with 5.55 log cfu/g and in the QS3 sample with 4.93 log cfu/g, respectively. It is seen in Table 7 that TPB values increase during the storage period. It was determined that the number of psychrophilic microorganisms in the first period of storage was below 2 log cfu/g. However, high TPB values detected on the 15th day and rapid growth was observed due to the environment being suitable for the growth of psychrophilic bacteria. In the QS3, GS3, TS2, and TS3 samples, a slight increase was observed in their growth with increasing storage time between the 15th and 45th days, while it was observed that they developed faster on the 60th day. Psychrotrophic microorganisms are the primary spoilage factor in animal products that are kept cold or frozen. Chicken meat contains several hundred types of microorganisms immediately after slaughter. Psychrophilic microorganisms predominate in cold storage and signs of deterioration are observed when they reach 6- 8 log cfu/g (Şahin & Başoğlu, 2011). Fernandez‐Gines et al. (2003) reported that no psychrophilic bacteria growth was observed in the sausage samples produced using different ratios of citrus fiber during the 28-day storage period similar with our study. They also reported that the TMAB values of the samples remained below the limit to cause deterioration.

#### *Yeast-mold count*

Yeast-mold count results of the samples determined during storage are given in Table 7. According to the table, yeast-mold counts of all groups were found to be less than 2.00 log cfu/g during storage. Yeast and molds are generally less important in spoiling poultry meat. In addition, lactic acid bacteria become the dominant flora with vacuum packaging of meats (Erkmen, 2013). Molds are generally obligatory aerobic, and yeasts are facultative microorganisms, but they cannot grow in an environment where there is no oxygen. Yeast and molds could not grow

due to vacuum packaging and bacteria population of the products. The high TMAB and yeast-mold counts of the food wastes used in this study affected the quality of the obtained product. Especially high microbial load of tomato waste caused TS2 and TS3 samples to have a high microbial load. The heat treatment applied during production was sufficient for the limiting microorganisms for control sample. It is known that bacteria become common microorganisms by suppressing yeast-mold when an environment is suitable for both bacteria and yeast-mold growth. In addition, the obligatory aerobic molds could not find the oxygen they needed in the vacuum packaging environment. In our study, it is thought that bacteria that develop under vacuum packaging and cold storage conditions suppress yeast-molds. Applying techniques to reduce the microbial load of the food waste before production is recommended in terms of reducing the microbial load of the product, limiting lipid oxidation and protein deamination, preventing the formation of bad aroma, and prolonging the shelf life.

According to (Cardoso et al., 2008), the products' microbiological quality was improved by vacuum packaging, cold storage, heat treatment (10 min at 90°C), and salt addition to dietary fibers added to fish sausages. Andres et al. (2006) prepared sausages with chicken breast meat and low fat (0%, 2%, 5%). They determined that yeast and mold were always less than 2 log cfu/g during storage which is like our study.

### **Conclusion**

In the food sector, food waste is utilized for benefits such waste re-evaluation, recovery of the bioactive components they contain, decreased formulation costs, and enhanced product structure. Agro-food by-products used in the study are exposed to some factor that increased microbial load during obtaining; especially tomato waste is remaining part of paste production, exposing contamination elements. Microbial load of the food waste affected the microbiological quality of the produced product, and TBARS values were found to be directly affected by microbiological load. For this reason, it is estimated that the application of methods to reduce the microbial load on waste will also reduce lipid oxidation by lowering TBARS values. There was no difference between the control sample and QS3, TS2 and TS3 samples in terms of the sensory analysis criteria. However, in the GS3 sample foreign flavor was perceived by the panelists and therefore received a low overall score. Removing the bitter substances such as naringin in grapefruit fruit, combining it with different additives to neutralize the foreign flavor or using in lower concentrations can be considered as a solution to this problem. Although lipid oxidation increased because of waste adding, using these by-products in sausage production reduced the purge accumulation of sausages during storage. It is predicted that sausages could have lower lipid oxidation and have higher microbial quality and longer shelf life by applying suitable pasteurization technique to these wastes. Manufacturing meat products with food waste and dietary fiber is a novel development, but there are some difficulties in processing and marketing such functional meat products. Research in

this area helps to eliminate problems, remove question marks, and take full advantage of food waste.

### **Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

#### **Author Contributions**

Zeynep Akşit: Conceptualization, Investigation, Formal analyses, Data curation, Writing&editing

Hüseyin Gençcelep: Supervision, Methodology, Writing, Resources.

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