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Pork Jerky Fermented with *Lactobacillus bulgaricus* and Angel Yeast

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ABSTRACT

Lactobacillus bulgaricus and Angel Yeast was used to produce fermented pork jerky, and a series of indexes were measured. The results demonstrated that the pH of the fermented jerky was lower than the non-fermented jerky; the true protein content of the fermented jerky was lower than that of the non-fermented jerky; the free amino acids content of the fermented jerky was higher than the non-fermented jerky; the acid protease activity was slightly higher than the neutral protease activity in the fermented jerky; four and fifteen volatile compounds were detected for the non-fermented and fermented jerky, respectively. The important compounds affecting the flavor of fermented jerky included DL-Glutamic Acid, Phenylethyl Alcohol, 1-Octen-3-ol, Nonanal, Pentanal, 3-Methyl-Butanal, and 2-Pentyl-Furan. The study results demonstrated that the strains combined with *Lactobacillus bulgaricus* and Angel Yeast reduced the meat pH, degraded the protein in the meat by the proteases (including acid and neutral proteases) produced during fermentation, and increased the amount of free amino acids and flavored compounds. In these ways, the meat texture and taste were improved, and digestion and absorption were enhanced.

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Authors' Contribution
CZ designed the project and wrote the article. ZC performed the experimental work. YL helped to analyze the data.

Key words
Pork jerky, Fermentation, *Lactobacillus bulgaricus*, Angel Yeast.

INTRODUCTION

Pork jerky is a popular Chinese food. However, in the traditional production process, the meat jerky is dehydrated, which results in hard texture and poor color (Konieczny *et al*., 2007). However, after the fermentation process, not only is the meat tenderized with improved texture, but the acids, alcohols, and non-protein nitrogen compounds produced during fermentation give the meat a unique flavor and enhance the nutritional value. At the same time, the fermentation causes the specific bacteria, yeasts, or molds to reduce the meat pH, and the low temperature dehydration can reduce the water activity (A_w) as well, inhibiting food pathogens (such as Listeria and *Staphylococcus aureus* (Aqib *et al*., 2017)) and increasing food safety (Li and Lv, 2005). Thus, fermented pork jerky is beneficial in a variety of ways.

In recent years, there have been some reports about using single strains to ferment meat which mainly include *Lactobacillus* (Lee *et al*., 2006), *Micrococci* (Drosinos, 2006), *Staphylococcus* (Visessanguan *et al*., 2006), yeasts (Hammes and Knauf, 2001) and moulds (Li *et al*., 2004; Wang, 2013). However, no other published reports were found on pork jerky produced with fermentation. In our previous experiments, single *Lactobacillus bulgaricus* and single Angel Yeast were used to ferment pork meat and it was found that both of them had ability to improve the texture, the color and the flavor of pork. However, there are some flaws.

Lactobacillus bulgaricus alone results in an unpleasant, sour flavor, while pork jerky made through fermentation with Angel yeast alone contains too low free amino acids. In order to improve the flaws, the compound strains of the *Lactobacillus bulgaricus* and the Angel Yeast were selected for meat fermentation.

In this study, the pork was fermented with the *Lactobacillus bulgaricus* and the Angel Yeast, and some indexes were detected including pH value, free amino acids contents, protease activities of the fermented pork jerky, the non-fermented jerky, the remaining fermented medium, and the medium before fermentation.

MATERIALS AND METHODS

Materials

Biochemical-grade yeast extract, peptone and bovine serum albumin were purchased from Beijing AoBoXing Leiverseen Biotech Co. Ltd. (Beijing, China). All other reagents used were of research-grade quality.

Media

MRS solid medium

Peptone 10 g, beef extract 10 g, yeast extract 5 g, diammonium citrate 2 g, sodium acetate 5 g, K_2HPO_4 2g,

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$MnSO_4 \cdot 4H_2O$ 0.25 g, $MgSO_4 \cdot 7H_2O$ 0.58 g, glucose 20 g, tween 80 mL, agar 25 g, water 1,000 mL. The pH was adjusted to 6.2-6.4 and the medium was autoclaved at 121°C for 15 min.

Lactobacillus *medium*

Glucose 10 g, lactose 5 g, NaCl 5 g, water 1,000mL. The pH was adjusted to 6.5-6.8, and the medium was aliquoted and autoclaved at 121 °C for 20 min.

PDA solid medium

200 g of peeled potatoes were diced and boiled in water for 30 min. Then, they were filtered through four layers of gauze. The filtrate was mixed with 20 g of glucose, and then 20 g of agar. It was then supplemented with up to 1000 mL of water. After being heated, the filtrate was placed into a test tube and sterilized for 20 min at 121°C.

PDA liquid medium

200 g of peeled potatoes were diced and boiled in water for 30 min. Then, they were filtered through four layers of gauze. The filtrate was mixed with 20 g of glucose. It was then supplemented with up to 1,000 mL of water, placed into a triangular flask, and sterilized for 20 min at 121°C.

Bacteria strains

The *Lactobacillus bulgaricus* and Angel Yeast used in the experiment were preserved in our laboratory. Before use, the *Lactobacillus bulgaricus* was inoculated onto the MRS solid medium and cultured for 48 h. The Angel yeast was inoculated onto the PDA solid medium and cultured for 48 h.

Preparation of fermented pork jerky

1. The pre-treatment of raw pork: The inspected fine commercial pork was cut into 500 g pieces and soaked in cold water for about 1 h to remove residual blood. The pork pieces were then dried and weighted.

2. Precooking: The pork pieces were precooked with sodium chloride in water for 10 min. They were frequently flipped to ensure uniform cooking.

3. Cutting: After precooking, the pork pieces were kept in a perforated plastic container. After cooling, the meat was cut into slices measuring 2.5 cm × 1.5 cm × 0.5 cm with neat sheet-shaped and uniform thickness.

4. Fermentation: The *Lactobacillus bulgaricus* was inoculated onto the MRS solid medium, and the Angel yeast was inoculated onto the potato fluid medium. They were cultured continuously at 100RPM for 24 h at 30 °C of constant temperature. Then, 25 mL of the *Lactobacillus bulgaricus* culture solution and Angel yeast culture solution were placed into a 100 mL flask that had been dried for two hours at 160 °C in a blast drying oven. This mixture was to be used as the fermentation broth (the initial concentrations of the Lactobacillus bulgaricus and the Angel yeast were 3.8×10^7 CFU/mL and 1.5×10^5 CFU/mL, respectively). The broth's pH value was adjusted to 6.5. Then, 25 g of pretreated sliced meat was placed into the flask, which was then sealed with plastic film, labeled, and put into a constant temperature incubator at 25° C for 42 h. The fermentation medium (without meat) was cultured under the same condition as the control groups.

5. Cooking and baking: The fermented meat was transferred to spice-free boiling water to re-cook for 10 min, with frequent gentle stirring for uniform cooking. Then it was transferred to an oven and heated at 100 °C for 10 min, 80 °C for 30 min, and then 60 °C for 90 min. After cooling, the fermented pork jerky was ready. The non-fermented pork jerky was made through the same process excluding the fermentation step.

Product indices determination

The determination of pH

A solid sample of 1 g was ground finely in a mortar and then soaked in 10 mL ddH_2O for 30 min. The filtered solution was used for measurement with a pH meter. The liquid sample was directly used for pH measurement. The solid samples included non-fermented and fermented pork jerky; and the liquid samples included the remaining fermented medium, and the medium before fermentation.

Determination of true protein content

Non-fermented and fermented pork jerky samples of 10 g were weighted respectively. Then 5 times of trichloroacetic acid (TCA) solution with 10% (w/w) concentration were used to extract the true protein from the jerky (Zhou, 1996). After stirring and standing for 10 min, the samples were filtered. The residues were rinsed with 5 times of TCA solution twice and then dried in an oven at 105 °C. Finally, a 0.2 g solid sample was digested in a digestion tube and the nitrogen content was measured with an automatic Kjeldahl analyzer (HR-500, Shanghai Hua Rui Instrument Co., Ltd., China). The formula for true protein content was as follows:

$$X = W \times F$$

Where, X is the protein content of the samples (%), W is the nitrogen content measured by automatic Kjeldahl analyzer (%) and F is the nitrogen-to-protein conversion factor (6.25 was used here). This led to the calculation of W ×to obtain the protein content.

Determination of total free amino acids

The free amino acid contents of the samples, including non-fermented pork jerky, fermented pork jerky, remaining fermented medium, and the medium before fermentation, were determined using the colorimetric method of ninhydrin (Zhang, 2005). Accordingly, ninhydrin was added to the samples under acidic conditions and the absorbance of the solution was ascertained at 570 nm.

Determination of protease activity

Folin-phenol reagent (Wu *et al*., 2006) was used to measure the neutral and acid protease activities in the samples including the fermented jerky, the remaining fermented medium with meat, the fermented medium without meat, and the medium before fermentation.

Determination of flavor compounds

Extraction of volatile components by headspace solid phase microextraction (SPME)

A headspace solid-phase microextraction (HS-SPME) holder (Supelco Inc., Shanghai, China) for manual sampling, combined with gas chromatography–mass spectrometry (GC–MS) (Agilent Technologies, USA), was used to perform the experiments. Teflon covers and a 75-μm carboxen/polydimethylsiloxane fiber were purchased from Supelco Inc. Before initial use, the fiber was preconditioned for 2 h on an Agilent 6890-5975 gas chromatograph at an injector temperature of 230°C. Then, the minced samples of non-fermented pork jerky and fermented pork jerky (3 g) were respectively placed in a 15-mL vial at room temperature, and the vial was sealed with a Teflon cover, heated at 60°C in a water bath for 30 min, and mixed at intervals. The HS-SPME fiber was inserted for sampling for 40 min, which was adequate to extract the volatile compounds from the samples and introduce them into the GC–MS injector for desorption for 5 min.

GC–MS analysis

The GC–MS analysis was performed on an Agilent 6890 gas chromatograph coupled with a 5975 mass spectrometer (Agilent Technologies, USA). The carrier gas was helium with a flow rate of 1.1 mL/min. The separation was performed on a DB-WAX 30 m × 0.25 mm × 0.25 μm capillary column (Agilent Technologies, USA). The initial oven temperature was 35 °C for 1 min, which was ramped to 100 °C at the rate of 8 °C/min and held for 3 min; subsequently, the temperature was ramped to 120 °C at the rate of 3 °C/min and held for 2 min; finally, the temperature was ramped to 230 °C at the rate of 5°C/min and held for 5 min. The mass detector was operated at 230 °C in an electron impact mode at 70 eV. The ion-source temperature was 230 °C, while the transfer line temperature was 150 °C. The chromatograms were recorded by monitoring the total ion currents in the 20–350 mass range.

Data analysis

The data obtained were analyzed using MSD Productivity ChemStation Data Analysis Software (version G1701DA). Identification of volatile compounds was confirmed by comparing their mass spectra with those in the National Institute for Standards and Technology (NIST, Search Version 2.0) and Pesticides Retention Time Lock (RTLPEST, Parts number G1672AA, version A.03.00) mass spectral library. Determination of the percentage composition was based on peak area normalization (expressing the area of a given peak as a percentage of the sum of the areas of all the peaks) without the use of correction factors.

RESULTS AND DISCUSSION

pH change before and after fermentation

As shown in Figure 1A, the pH of the fermented pork jerky (4.75) was significantly lower than that of the non-fermented jerky (6.42), and the pH of the medium after fermentation (4.71) was lower than before fermentation (6.50). This indicated that during fermentation, the *Lactobacillus* produced lactic acid and acidic acid, and reduced the pH of the fermented jerky and medium. The reduced pH inhibited the growth and reproduction of adverse microorganisms, especially spoilage bacteria, thus potentially extending the shelf life of jerky and improving preservation. At the same time, the lower pH promoted the reduction of nitrite and decreased the amount of residual nitrite, thereby reducing the carcinogens – nitrosamines produced by nitrite and secondary amines.

In addition, due to the participation of the yeast, the fermented pork jerky smelled of fermented glutinous rice, which improved flavor and taste as the yeast produced significant amounts of alcohols. Compared with our previous experiment that the pH value of the pork jerky fermented at the same condition through the single *Lactobacillus bulgaricus* was 4.13 (not shown in tables or figures), the pH value of the pork jerky fermented by the *Lactobacillus bulgaricus* and the Angel Yeast was higher (pH of 4.75) which were more acceptable for people. This was because that the *Lactobacillus bulgaricus* had strong ability to produce acid but the Angel Yeast had faintish ability to produce acid and thus the compound strains not only improved the flavor of the pork but also inhibit the ability of the *Lactobacillus bulgaricus* to produce acid.

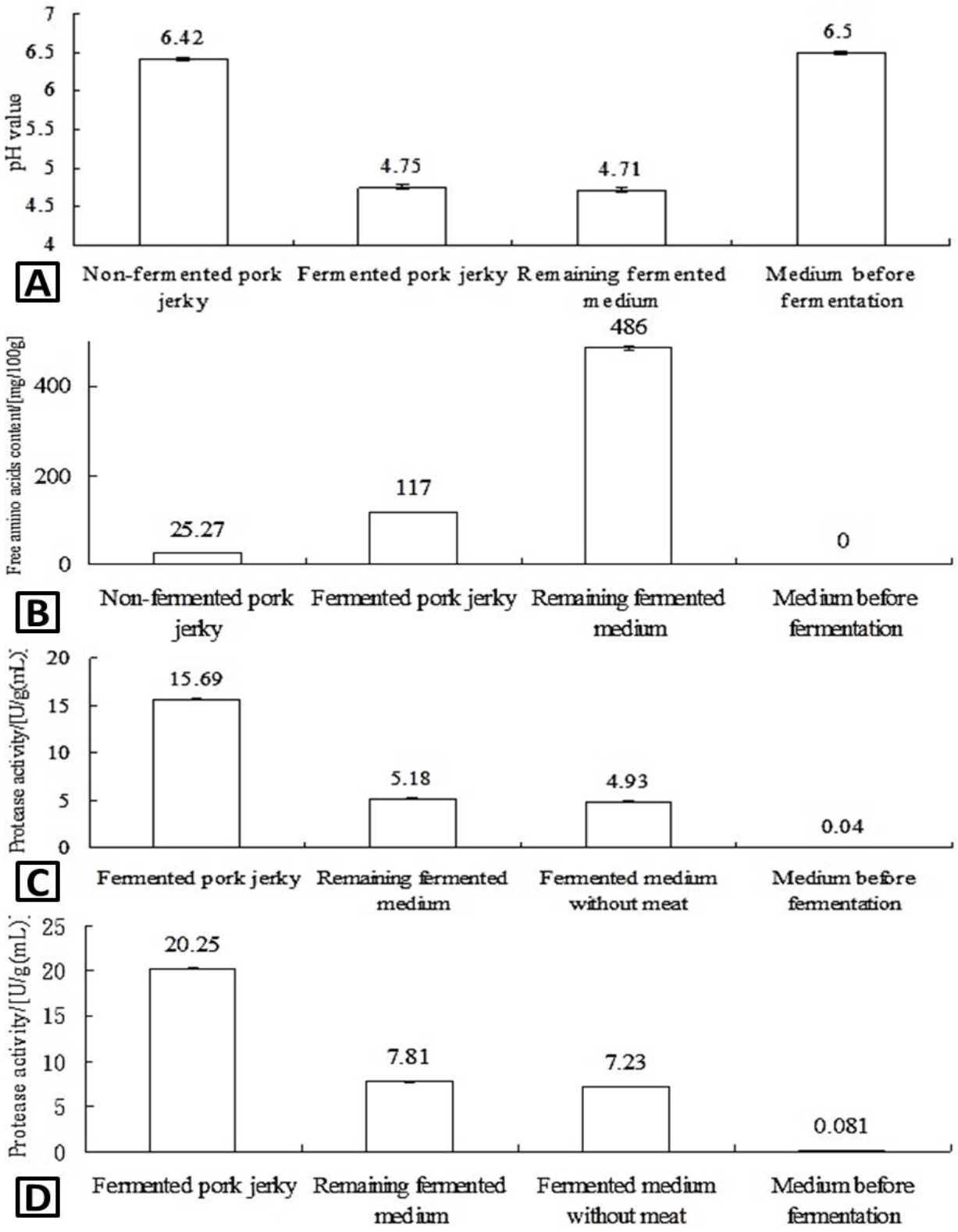

Fig. 1. pH change (A), free amino acids content (B), neutral protease activity (C) and acid protease activity (D) before and after fermentation.

True protein content change before and after fermentation

The content of protein in fresh meat is about 20g/100g (Gu *et al*., 2009). In this experiment, as the non-fermented and the fermented pork jerky were baked samples which lost water in the baking process, the higher true protein contents were obtained for the non-fermented and fermented pork jerky samples of 81.09 g/100 g and 68.50 g/100 g, respectively (Table I). From comparison, it could be seen that the fermented jerky has significantly less protein than the non-fermented jerky. This indicated that during the fermentation process, the *Lactobacillus* hydrolyzed a portion of the pork protein and produced higher proportions of polypeptides and abundant amino acids. Consequently, the fermented products became easier to digest and absorb, and offered better biochemical availability.

Table I.- True protein content of pork jerky before and after fermentation.

Sample	Protein content (g/100 g)
Non-fermented pork jerky	81.09
Fermented pork jerky	68.50

Total free amino acids before and after fermentation

The content of free amino acids (which can be easily assimilated by humans) is an important parameter for the evaluation of the nutritional value of pork (Chen and Liu, 2004). The levels of free amino acids in the jerky and media before and after fermentation were shown in Figure 1B. As could be seen, there was a significant difference in the free amino acids content between the fermented and non-fermented jerkies. The amino acid content in the fermented jerky (117.0 mg/100 g) was 3.5 times that of the non-fermented jerky (25.27 mg/100 g). It was also discovered that the free amino acids content was higher in the medium after fermentation than before fermentation. Combined with the protein content change of the jerkies before and after fermentation, the results indicated that as the protein in the fermented jerky decreased, the free amino acids increased. This was because the protein was degraded and produced free amino acids, part of which remained in the jerky to improve the flavor, and part of which was left in the fermentation medium. Thus, the medium after fermentation had much a higher free amino acids content than before fermentation (0 mg/100 g). These free amino acids by themselves can enhance fermented meat flavor, and they can also serve as substrates for further production of flavor compounds, improving a product's flavor and taste.

In addition, the content of free amino acids in the pork jerky fermented with the compound strains (117.0 mg/100g) was all more than the content of the raw pork (64.0 mg/100g) and the content of the pork jerky fermented by the single Angel Yeast (68.3 mg/100g) or the single *Lactobacillus bulgaricus* (62.0 mg/100g) (results of previous experiments). It showed that the compound strains were with strong ability to produce free amino acids in the fermentation when fermentation with the *Lactobacillus bulgaricus* and the Angel Yeast.

Protease activity changes before and after fermentation

As shown in Figure 1C and D, the neutral and acid protease activities in the fermented jerky were much higher than in the medium both before and after fermentation. The protease activities were in the following order: fermented jerky > remaining fermented medium > fermented medium without meat > medium before fermentation (inoculation medium). This was because most of the *Lactobacillus* were immobile. During fermentation, the media were kept still in the incubator without shaking. After mass consumption of the surrounding nutrition in the medium, the immobile *Lactobacillus* used substances inside and close to the meat, changed the nutrients and texture of the meat, and tenderized the meat. Meanwhile, the remote *Lactobacillus* would autolyse due to nutritional deficiencies and aging. Therefore, the fermented jerky had higher protease activities than the remaining fermented medium.

Furthermore, acidic protease activities in the fermented jerky (20.25 U/g), and the remaining fermented medium (7.81 U/mL) were both a little higher than the neutral protease activities (15.69 U/g and 5.18 U/mL, respectively). This was because the optimal fermentation condition for *Lactobacillus bulgaricus* and Angel Yeast was both acidic (pH=6.5 and 4.5, respectively), which also meant that it was appropriate for *Lactobacillus bulgaricus* to produce fermented jerky under acidic conditions.

Changes in flavor substances before and after fermentation

Meat fermentation is accompanied by complex chemical changes, primarily including carbohydrate, protein, and fat degradation. Precursors in meat such as sugars, amino acids, sulfur-containing amino acids, lipids, thiamine, nucleotides, and peptides undergo a series of changes to generate volatile and nonvolatile components, which then interact to form final flavor substances (Zhang *et al*., 2013). Meat flavors refer to the fresh meat smell, and the aroma and taste of heated meat and meat products. They result from the various organic compounds generated from complex physiological and biochemical changes in components inherent in meat; meat aroma is primarily reflected by sulfur-containing compounds (Xia, 2008). Whether a compound can generate smell and taste depends on its odor threshold; the contribution of a volatile substance to the aroma and total meat flavor depends on

the ratio of the substance's concentration to odor threshold (OVA). A substance can be detected and called a flavor substance only when its OVA is greater than 1 (Fan and Xu, 2014).

As shown in Table II, gas chromatography and mass spectrometry revealed that the non-fermented pork jerky contained four volatile flavor substances, while the fermented pork jerky contained fifteen volatile flavor substances, including two acids (0.67% total content), six alcohols (12.16% total content), five aldehydes (71.94% total content), two alkanes (2.01% total content), and two other compounds (1.54% total content).

Butanoic acid (No.1) detected in the non-fermented pork jerky wass not found in the pork jerky. Butanoic acid has strong, unpleasant smells of cream and cheese, and it has a creamy taste. The odor threshold of butanoic acid in a 12% solution of alcohol in water is 10000 ug/L (Guth, 1997). The non-fermented pork jerky contained a small amount of butanoic acid (2.912%). After fermentation, butanoic acid disappeared, which improved the meat's flavor. DL-glutamic acid (No. 3) is sour and has an odor threshold of 50 ng/L; D-glutamic acid is tasteless and primarily used as a flavor enhancer (Fan and Xu, 2014).

1-Hexanol (No. 5), which has an odor threshold of 2,500μg/kg, can be used in the food industry in baked foods, pudding, and meat products (Fan and Xu, 2014). Phenylethyl Alcohol (No. 6), which has an odor threshold of 12-24 μg/L in the air and an odor threshold of 1,000 μg/L in water, can be used for preparation of roses, caramel, honey, and various liquor and tobacco flavors. It is also an indispensable substance in the scents of roses and other plants. Ethyl alcohol (No. 7), which has an odor threshold of 100,000μg/kg, is used to manufacture acetic acid, beverages, flavors, dyes, fuels, *etc*. (Fan and Xu, 2014). 1-Octen-3-ol (No. 8), which has a smell of unprocessed mushrooms and is also known as Mushroom Alcohol, has an odor threshold of 0.01 mg/kg; it is one of the key components of the overall flavor of Camembert cheese (Hou *et al*., 2014). 1-Pentanol (No. 9), which has an odor threshold of 4,000 μg/kg, is used for organic synthesis and has a slight smell (Zhang *et al*., 2013). Nonanal (No. 10), which has an odor threshold of 1 μg/kg, plays a significant role in pork flavor. Pentanal (No. 11), which has an odor threshold of 20 μg/kg, is primarily used as a flavor, intermediate for organic synthesis, and rubber accelerator (Mo *et al*., 2007). 3-Methyl-Butanal (No. 12), which has an odor threshold of 1 μg/kg, is used as a food material, flavor, reagent, *etc*. (Zhang *et al*., 2013) (Table II).

Table II.- Volatile compounds of fermented and non-fermented pork jerkies.

No.	Volatile compounds	Retention time	Pork jerkies	
			Non-fermented	Fermented
Acids				
1	Butanoic acid	26.944	2.912%	
2	2-(14-Carboxytetradecyl)-2-ethyl-4, 4-dimethyl-1,3-oxazolidine-N-oxyl	14.6329		0.16%
3	DL- Glutamic acid	12.8385		0.51%
Alcohols				
4	1-Nonanol	13.3762		0.21%
5	1-Hexanol	13.7025		2.53%
6	Phenylethyl Alcohol	21.267		0.15%
7	Ethyl Alcohol	6.3132		5.38%
8	1-Octen-3-ol	15.0136		1.99%
9	1-Pentanol	12.1799	1.14%	1.90%
Aldehydes				
10	Nonanal	14.349		0.88%
11	Pentanal	7.0805		7.93%
12	3-Methyl-Butanal	10.9594		2.02%
13	Benzaldehyde	16.367	0.78%	0.93%
14	Hexanal	9.1287	63.58%	60.18%
Others				
15	2-pentyl- Furan	11.5213		1.37%
16	1-methyl-4- (1-methylethyl)- Benzene	12.331		0.17%

2-Pentyl- Furan (No. 15) is a primary flavoring substance with an odor threshold of 270 ng/L in the air and an odor threshold of 6 μg/L in water (Fan and Xu, 2014). It is a primary flavoring substance in meat that smells of caramel and fruit. It is also found in many foods such as fruits, vegetables, meats, baked foods, coffee, cocoa, tea, and fish, and even spirits (Zhang *et al*., 2013) (Table II).

Analysis revealed that DL-Glutamic Acid (No.3), Phenylethyl Alcohol (No. 6), 1-Octen-3-ol (No. 8), Nonanal (No. 10), Pentanal (No. 11), 3-Methyl-Butanal (No. 12), and 2-Pentyl- Furan (No. 15) play important roles in pork jerky production (Table II).

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Statement of conflict of interest

Authors declare that there is no conflict of interests regarding the publication of the manuscript.

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