Enhancement of Pork Jerky Using Co-cultures of *Lactobacillus bulgaricus* and Angel Yeast

Changqing Zhao¹ · Ziyang Lu¹ · Jing Huang¹ · Sha He¹ · Hui Tan¹ · Gang Wang¹ · Dayu Liu² · Yubin Li¹

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Abstract Strains of *Lactobacillus bulgaricus* and Angel Yeast were combined to ferment raw pork and make pork jerky. After fermentation, the jerky was dried and then tested for sensory evaluation, pH and free amino acid content. The results showed that the optimal conditions for fermentation using *L. bulgaricus* and Angel Yeast were: a pH of 6.5, a 1:1 (v/v) ratio of *L. bulgaricus* to Angel Yeast, a fermentation time of 42 h and temperature of 25 °C. The results showed that the pork jerky fermented with the combined strains was not very sour which was close to the pH of 7.0 and had a higher free amino acid content which was more than 68.3 mg/100 g compared with the pork jerky fermented by either *L. bulgaricus* or Angel Yeast alone. Overall, the results demonstrate that fermentation of raw pork with combined strains of *L. bulgaricus* and Angel Yeast improves the quality and flavor of pork jerky.

Keywords Pork jerky · Fermentation · *Lactobacillus bulgaricus* · Angel Yeast

Introduction

Traditional pork jerky is usually hard and has an unappealing color due to dehydration of the meat during the cooking and baking process [1]. Moreover, nitrite, aginomoto and preservatives, which are harmful to human health, are often added to industry-produced pork jerky to improve its color and lengthen its shelf life [2]. Not only are the texture, color and flavor of pork jerky improved via fermentation, but also the stability and the safety are enhanced by comprehensive effects of microbial metabolites in the fermentation process without the need for harmful additives.

In recent years, there have been some reports on using single strains, mainly *Lactobacillus*, *Micrococcus* and *Staphylococcus*, as well as yeasts and moulds, to ferment meat [3–7]. In comparison to pure cultures, co-cultures could be more suitable for synergistic effects on product yields. More research is required for complementing the abilities of different strains to develop robust co-cultures and achieve the fermentation goal [8]. In our previous experiments, *Lactobacillus bulgaricus* or Angel Yeast, a kind of yeast widely used for industrial production for fermentation of wine, steamed bread and other fermented food, was used to ferment pork meat. Both of them were found to improve the texture, color and flavor of the pork. However, the meat products fermented with only a single strain have some flaws. The pork jerky fermented by only *L. bulgaricus* was too sour (the pH was 3.92–4.83) and the pork jerky fermented by Angel Yeast had a low free amino acid content (68.3 mg/100 g). To overcome these flaws, combined *L. bulgaricus* and Angel Yeast were selected for meat fermentation to improve the quality and the flavor of fermented pork jerky.

✉ Changqing Zhao
zhaocq2010@163.com

¹ College of Bioengineering, Sichuan University of Science and Engineering, Zigong 643000, China

² College of Bioengineering, Chengdu University, Chengdu 610106, China

The pork in the present study was fermented with *L. bulgaricus* and Angel Yeast, and then tested for sensory evaluation, pH and free amino acid content.

Materials and Methods

Materials

Biochemical-grade yeast extract and peptone were purchased from Beijing Aoboxing Biotech. Co. Ltd. (China) and Chengdu Changshou Biotech. Co. Ltd. (China), respectively. All other reagents used in this study were research grade.

Strains

The *L. bulgaricus* and Angel Yeast used in the experiments were conserved in our laboratory. Prior to use, the organisms were cultured in the liquid medium of lactic acid bacteria (MRS medium) and the liquid medium of Potato Dextrose Agar (PDA medium) at 30 °C for 12 h, respectively.

Preparation of the Pork Jerky: Pretreatment of the Pork

Fresh pork tenderloin was purchased from a supermarket and immersed in cold water several times. After 1 h, the pork was precooked on both sides for a total of 10 min to ensure that it was heated evenly. After being cooled in a sieve, the pork was cut into slices measuring 3 cm × 4 cm × 0.5 cm.

Factors Influencing the Fermentation of the Pork

Initial pH

Twenty-five milliliters of cultured *L. bulgaricus* and Angel Yeast, with initial concentrations of 10^7 and 10^5 CFU/mL, respectively, were both added to a sterilized beaker containing 25 g of pretreated pork slices. The pH (5.5–7.0) of the fermentation broth containing the *L. bulgaricus* and Angel Yeast was adjusted using 0.1 M NaOH and 0.1 M HCl solutions. The beakers were then sealed with plastic wrap and cultured statically at 30 °C for 42 h. Uninoculated pork placed in 50 mL sterilized water and cultured under the same conditions was used as a control.

Ratio of Strains

A total of 50 mL of the cultured *L. bulgaricus* and Angel Yeast at different ratios [1.5:1, 1:1, 1:1.5 and 1:2 (v/v)] was added to a sterilized beaker containing 25 g of pretreated

pork slices. After the fermentation, broth was adjusted to pH 6.5, the beakers were cultured statically at 30 °C for 42 h.

Time

Twenty-five milliliters of both the cultured *L. bulgaricus* and Angel Yeast were added to a sterilized beaker containing 25 g of pretreated pork slices. The fermentation broth containing the *L. bulgaricus* and Angel Yeast was subsequently adjusted to pH 6.5. The beakers were then sealed with plastic wrap and cultured at 30 °C for 24, 30, 36, 42, 48, 54, 60 and 72 h.

Temperature

Twenty-five milliliters of both the cultured *L. bulgaricus* and Angel Yeast were added to a sterilized beaker containing 25 g of pretreated pork slices. The fermentation broth was adjusted to pH 6.5. The beakers were then sealed with plastic wrap and cultured at 20, 25, 30, 35 and 40 °C for 42 h.

Recoction and Baking of the Pork

After removal from the fermentation broth, the fermented meat was boiled for 10 min and agitated gently during the recoction to ripen the pork evenly. The samples were then baked at 50 °C for 120 min. The pork jerky was prepared after the samples had cooled. The fresh pork sample was nonfermented pork that had been recocted and baked.

Determination of Indexes for the Pork Jerky: Sensory Evaluation

The sensory indexes of the fermented pork jerky and the fresh pork, including flavor, mouthfeel, structure and color, were evaluated by eight professionals. This group scored samples according to an evaluation standard [2].

Determination of pH

The pH of the samples, including the fresh pork, pork jerky, and the inoculated medium before and after fermentation, was determined. One gram of the solid samples was ground in a mortar, added to 10 mL of distilled water and then soaked for 30 min. The samples were subsequently filtered, and the pH of the filtrate and liquid samples was measured using a pH meter (pHS-3C, Chengdu Fangzhou Technology Experiment Equipment Co., China).

Determination of the Free Amino Acid Content

The free amino acid content of samples of the unfermented raw pork (control) and the pork jerky was detected using

the ninhydrin colorimetric method [9]. Accordingly, ninhydrin was added to the samples under acidic conditions, and the absorbance of the solution was ascertained at 570 nm using a UV/VIS spectrophotometer (Hong Qiao High-tech Instrument Co., Shanghai, P. R. China). All experiments were repeated three times.

Results and Discussion

Sensory Evaluation of the Pork Jerky with Different Initial pH

As shown in Table 1, when the initial pH of the fermented pork jerky was 6.5 and 6.0, the total scores for the sensory evaluation were 93.1 and 92.3, respectively. These values were higher than those at pH 5.5 and 7.0. When the initial pH was 6.5, the pork jerky had a strong meat flavor, was chewable, parts of muscle fibers appeared fractured, and the jerky was loose but formed. When the initial pH was 6.0, the sensory evaluation indexes were a little higher than those at pH 6.5, except for flavor. As the flavor was the main factor to be considered for the sensory evaluation, and the flavor at pH 6.5 was rated better than that at pH 6.0, the sensory evaluation for the initial pH of 6.5 was the best.

pH of the Pork Jerky with Different Initial pH

As the initial pH increased, the pH of the pork jerky gradually increased to between 6.37–6.80 (Fig. 1). Compared with our previous experiment showing that the pH of pork jerky fermented by only *L. bulgaricus* was 3.92–4.83, the pH of the pork jerky fermented by the combined *L. bulgaricus* and Angel Yeast was close to the pH of the unfermented raw pork (pH 6.42) (data not shown) and more palatable for people. This was because the *L. bulgaricus* had a stronger ability to produce acid compared to the Angel Yeast. As the optimal pH of the Angel Yeast for meat fermentation in our previous experiments was 6.5, the pH of pork jerky fermented with the co-cultures of Angel

Yeast and *L. bulgaricus* was close to 6.5, which may stem Angel Yeast's important role in the fermentation process. Thus, the combined strains not only improved the flavor of the pork but also inhibited the ability of the *L. bulgaricus* to produce acid.

Free Amino Acid Content of the Pork Jerky with Different Initial pH

The content of free amino acids that are easily absorbed by people is an important index for evaluating the nutritional value of pork [2]. The free amino acid content of the unfermented raw pork was 64.0 mg/100 g. As can be seen from Fig. 1, the free amino acid content of the pork jerky fermented with the combined strains was greater than that of the raw pork (64.0 mg/100 g) and that of the pork jerky fermented by only Angel Yeast (68.3 mg/100 g) (result of previous experiments). These results indicated that many proteins in the pork were degraded to free amino acids by the protease produced from *L. bulgaricus* and Angel Yeast through fermentation. Moreover, the free amino acid content was the highest (117.0 mg/100 g) in the pork jerky when the initial pH was 6.5, which showed that the combined strains had a strong ability to produce free amino acids during fermentation at an initial pH of 6.5.

Examining the aforementioned indexes for pork jerky fermented at different initial pH, the sensory evaluation was the best and the free amino acid content was the highest at pH 6.5. Therefore, pH 6.5 was selected as the optimal initial pH to ferment raw pork in the subsequent experiments.

Sensory Evaluation of the Pork Jerky with Different Ratio of Strains

As shown in Table 2, the total score for the sensory evaluation of the fermented pork jerky was the highest (93.1) when the ratio of the *L. bulgaricus* and Angel Yeast was 1:1 (v/v). At a ratio of 1.5:1, the pork jerky was difficult to chew and stuck to the testers' teeth. At a ratio of 1:1.5, the pork jerky exhibited a pale, sauce-like colour. At a ratio of 1:2, the pork jerky had a strong odor of alcohol. Thus, the sensory evaluation of the pork jerky was the best when the ratio of the *L. bulgaricus* and Angel Yeast was 1:1 (v/v).

pH of the Pork Jerky with Different Ratio of Strains

At different ratios of *L. bulgaricus* and Angel Yeast, the pH of the pork jerky ranged from 6.15 to 6.62 (Table 2), which was close to a neutral pH of 7.0. There were no obvious differences in the jerky, and testers found them palatable.

Table 1 Sensory evaluation of the pork jerky at different initial pH

Evaluation	pH 5.5	pH 6.0	pH 6.5	pH 7.0
Flavor (total score 60)	52.6	54.5	58.3	51.2
Mouthfeel (total score 20)	15.2	18.2	17.5	14.6
Structure (total score 10)	6.3	9.7	8.4	7.5
Colour (total score 10)	4.1	9.9	8.9	8.1
Total score	78.2	92.3	93.1	81.4

According to the evaluation standard [9], the trained panel of eight people scored the flavor (total score of 60), mouthfeel (total score of 20), structure (total score of 10), and colour (total score of 10) of the samples

Fig. 1 pH and free amino acid content of pork jerky at different initial pH

Table 2 Sensory evaluation, pH and free amino acid content of the pork jerky at different strain ratios (v/v)

Evaluation	1.5:1	1:1	1:1.5	1:2
Flavor (total score 60)	54.2	58.3	56.9	49.8
Mouthfeel (total score 20)	15.4	17.5	12.5	12.0
Structure (total score 10)	9.6	8.4	8.4	5.7
Colour (total score 10)	9.1	8.9	5.6	5.1
Total score	88.3	93.1	83.4	72.6
pH	6.28	6.62	6.16	6.15
Free amino acid content (mg/100 g)	72.2	117.0	86.5	81.7

Free Amino Acid Content of the Pork Jerky with Different Ratio of Strains

As can be seen from Table 2, the free amino acid content of the fermented pork jerky was the highest (117.0 mg/100 g) when the ratio of *L. bulgaricus* and Angel Yeast was 1:1 (v/v), which was higher than for the other three ratios (1.5:1, 1:1.5 and 1:2). This indicates that the ability to degrade the proteins in the pork to free amino acids was the strongest when the ratio of *L. bulgaricus* and Angel Yeast was 1:1 (v/v).

Because the sensory evaluation was the best and the free amino acid content was the highest at the strain ratio of 1:1 (v/v), this ratio was selected as the optimal ratio for the two strains to ferment the raw pork in the subsequent experiments.

Sensory Evaluation of the Pork Jerky with Different Time

As shown in Table 3, the fermentation time had a larger effect on the quality of the fermented pork jerky. When

fermented for 24, 30 and 36 h with the combined strains, the pork jerky had a strong odor of alcohol, a light odor of meat, tawny color and compacted structure. This was because the Angel Yeast played an important part in the initial stage of fermentation and because the fermentation time was too short.

The sensory evaluation of the pork jerky fermented for 42 h was the best. When the fermentation time was equal to or greater than 48 h, there was some meat powder in the fermentation broth, and the pork jerky had a strong odor of meat. At 72 h, the pork was rotten (data not shown). This pork was likely degraded by microorganisms not beneficial to fermentation of the pork.

pH of the Pork Jerky with Different Time

Different fermentation times also varied the pH of the pork jerky. The pH of the pork jerky increased as the fermentation time increased from 24 to 42 h and decreased from 42 to 60 h (Fig. 2). However, the pH was close to neutral, with no distinct differences in the jerky, and testers finding each palatable.

Free Amino Acid Content of the Pork Jerky with Different Time

As can be seen from Fig. 2, the free amino acid content of the fermented pork jerky increased with the increase in fermentation time. For fermentation times of 42–60 h, the free amino acid content was nearly the same (115.8–118.2 mg/100 g).

Considering the shortest fermentation time that gave the best sensory evaluation of the pork jerky, 42 h was the

Table 3 Sensory evaluation of the pork jerky at different fermentation times

Evaluation	24 h	30 h	36 h	42 h	48 h	54 h	60 h
Flavor (total score 60)	52.3	53.2	53.8	58.3	56.3	56.5	54.4
Mouthfeel (total score 20)	14.2	12.6	13.6	17.5	16.5	14.1	15.2
Structure (total score 10)	6.3	7.4	6.4	8.4	7.3	8.2	8.3
Colour (total score 10)	4.5	5.5	4.7	8.9	8.2	9.4	8.4
Total score	77.3	78.7	76.5	93.1	88.3	88.2	86.3

Fig. 2 pH and free amino acid content of the pork jerky at different fermentation times

selected as the optimal time for fermentation of the pork using *L. bulgaricus* and Angel Yeast.

Sensory Evaluation of the Pork Jerky with Different Temperature

As shown in Table 4, the total scores of the sensory evaluation for the fermented pork jerky were 93.4 and 93.1, respectively, when the fermentation temperatures were 25 and 30 °C, which were obviously higher than the scores at other temperatures. When the fermentation temperature was 25 °C, the total score of the sensory evaluation was 93.4, which was nearly the same as the score for 30 °C (93.1). The main difference between them was the mouthfeel; the jerky was softer after fermentation at 25 °C. Thus, 25 °C was considered the optimal fermentation

temperature based on the sensory evaluation of the pork jerky.

pH of the Pork Jerky with Different Temperature

At different fermentation temperatures, the pH of the pork jerky ranged from 6.12 to 6.62 (close to a neutral pH of 7.0) (Fig. 3), with no distinct differences in the jerky, and testers found each palatable.

Free Amino Acid Content Before and After Fermentation with Different Temperature

As can be seen from Fig. 3, the free amino acid content in the fermented pork jerky reached a maximum of 128.2 mg/100 g at a fermentation temperature of 25 °C. This is because the fermentation ability of the combined strains is the best at this temperature.

Because the sensory evaluation was the best and free amino acid content was the highest at a fermentation temperature of 25 °C, this temperature was selected as the optimal temperature for the combined strains to ferment the raw pork.

The combined strains were found to overcome the flaws of fermenting pork with only *L. bulgaricus*, which made the resultant jerky too sour (pH 3.92–4.83) and only Angel Yeast, which produced jerky with a low free amino acid content (68.3 mg/100 g).

Table 4 Sensory evaluation of the pork jerky at different fermentation temperatures

Evaluation	20 °C	25 °C	30 °C	35 °C	40 °C
Flavor (total score 60)	50.1	58.4	58.3	54.5	54.3
Mouthfeel (total score 20)	16.0	18.2	17.5	18.2	12.5
Structure (total score 10)	7.9	8.2	8.4	9.7	8.4
Colour (total score 10)	6.2	8.6	8.9	8.1	6.0
Total score	80.2	93.4	93.1	90.5	81.2

Fig. 3 pH and free amino acid content of pork jerky at different fermentation temperatures

Compared with pork jerky only fermented using *L. bulgaricus*, pork fermented with the combined strains was not sour and suited peoples' tastes. Compared with pork jerky only fermented using Angel Yeast, the increased free amino acid content of pork fermented with combined strains is more nutritious. Considering taste and nutrition, using combined strains to ferment raw pork is recommended.

Conclusion

Combined strains of *L. bulgaricus* and Angel Yeast are effective for fermenting raw pork to make pork jerky. Fermentation using the combined strains increased the free amino acid content of the resultant jerky, making it more nutritious. The higher pH also made the jerky more palatable. Thus, a fermentation method using combined strains can be used to improve the quality and flavor of pork jerky.

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Appendix

See Tables 3 and 4.

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