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STUDY ON APPLICATION OF PUMPKIN SEED PROTEIN ISOLATE IN SAUSAGE PRODUCTION PROCESS

The object of the research is sausage added with pumpkin seed protein isolate. Recently, plant proteins such as soybean protein and peanut protein are widely applied in meat products. Plant proteins have a lower price and less fat than animal meat, which is benefit for human health. Pumpkin seed protein is one of the new plant proteins, which contained balanced amino acids for human beings, was attracted an increasing interest in food industry. In this study, a new type of sausage was developed by single-factor experiments and orthogonal test. According to the single factor results, the added amount of the pumpkin seed protein isolate (1.5 g/100 g, 2.25 g/100 g, 3.0 g/100 g), lean meat (60 g/100 g, 70 g/100 g, 80 g/100 g), cooking time (35 min, 40 min, 45 min), and baking time (2.5 h) were determined to do the orthogonal test. The orthogonal test showed that the addition amount of pumpkin seed protein isolate had the greatest impact on the sausage quality, followed by the cooking time, and the addition amount of lean meat. The optimal production conditions were pumpkin seed protein isolate of 1.5 g/100 g, lean meat of 80 g/100 g, cooking time of 45 min, and baking time of 2.5 h. Under this condition, the sensory score reached 8.5, and the content of moisture, ash, protein, and fat were 51.16 g/100 g, 2.26 g/100 g, 15.22 g/100 g, and 23.15 g/100 g, respectively. This study can provide a fundamental knowledge for the application of pumpkin seed protein isolate in sausages.

Keywords: *food industry, meat products, sausage, pumpkin seed protein isolate, orthogonal test.*

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1. Introduction

The price of meat products, such as meat patties, sausages, and meatballs, are usually high [1]. In the making process of meat products, the formation of protein three-dimensional mesh structure, and its ability to effectively absorb oil, water, and other food components plays a vital role in the structure and the quality of the final product. The formation process of muscle protein gel is divided into three steps:

1) the dissolution of protein with high salt concentration and depolymerization of myofibrillar protein;

2) the partial folding with temperature;

3) through the combined action of hydrogen bond, disulfide bond, electrostatic interaction forces and hydrophobic interaction forces, the areas that have been folded are further aggregated to form a three-dimensional mesh structure [2].

In order to increase the output of the final product, reduce production costs, and improve the properties related to product quality, meat processing enterprises usually want to add a certain amount of plant protein to the product formula [3]. Plant proteins have a lower price and less fat than animal meat, which is benefit for human health. Soybean protein is the major source used in meat products as a functional ingredient [4]. Besides soybean, the utilization of other plant proteins like pea protein, flaxseed protein, pumpkin seed protein, and sunflower seed protein are increased in food products. Previous work showed that 20 % of pork meat can be replaced by pea products of pea protein isolate (PPI), pea low moisture extrudates (LME), and pea high moisture extrudate (HME) in emulsified cooked

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sausages without influence towards the amino acid profile [5]. Another study showed that using fibrous-like extrudates as meat substitutes which were made from soybean protein and *Coprinus comatus* can improve the meat-like physicochemical and textural properties, taste, and flavor of products [6]. The article presented the technical aspects of the utilization of lentil proteins in the production of raw smoked sausages [7]. The stability, texture, and microstructure of vegetarian sausage prepared with textured fibril soy protein can be effectively improved by pre-emulsified oil [8]. The results showed that substituting pork lean meat with *Lentinula edodes* (LE) increased total dietary fiber, amino acids, and antioxidant activity of the sausage [9]. Pumpkin seed meal, which is a by-product during the oil production of pumpkin seed, contained protein nearly 60 %. Eight essential amino acids of human body and essential amino acid of histidine for children were included in it [10]. Different plant proteins have different functional characteristics, such as oil absorption capacity, water absorption capacity, emulsification capacity, and foaming capacity. These intrinsic physiochemical characteristics can influence the protein performance during food production and storage [11]. Previous study showed that although not all functional properties of pumpkin seed protein isolate were the best compared to soybean proteins, peanut proteins, and sunflower proteins [12]. The shortest wetting time and highest oil-absorbing ability of pumpkin seed protein isolate make it a potential additive in meat products [12].

The object of the research is sausage added with pumpkin seed protein isolate. *The aim of the research* was to study the optimal production condition for pumpkin seed protein sausage.

2. Research methodology

2.1. Materials

Pig hind leg meat, pig backfat, casing, spices, edible salt, sugar, red koji powder, light soy sauce, cooking wine were purchased in local supermarket of Tai Xing in Hezhou city (China). Pumpkin seed protein powder was purchased from Shaanxi Tianzhicheng Biotechnology Co. Ltd. (China).

2.2. Methods of research

2.2.1. The basic recipe of the sausage. The basic formula of meat is 200 g, and other ingredients were added on this basic amount of meat. The edible salt, sugar, light soy sauce, cooking wine, thirteen incense, red coloring agent, modified starch was added with 1 g/100 g, 1 g/100 g, 1.5 g/100 g, 1 g/100 g, 0.1 g/100 g, 0.1 g/100 g and 1 g/100 g, respectively. Besides, ginger and garlic were added with a small amount.

2.2.2. Production process of pumpkin seed protein sausage. First, clean the pig hind leg meat, pig back fat and cut them into small pieces. Then, marinate the meat for more than 30 minutes, put them into the meat grinder, and add pumpkin seed protein isolate into the casing. Later, sausage was air-dried, baked, sterilized, cooled, and packaged.

2.2.3. Single factor test. In the present study, the single factors of added amount of the pumpkin seed protein isolate (0.75 g/100 g, 1.5 g/100 g, 2.25 g/100 g, 3.0 g/100 g, and 3.75 g/100 g), lean meat (50 g/100 g, 60 g/100 g, 70 g/100 g, 80 g/100 g, and 90 g/100 g), cooking time (30 min, 35 min, 40 min, 45 min, and 50 min), and baking time (1 h, 1.5 h, 2.0 h, 2.5 h, and 3 h) on the quality of pumpkin seed protein isolate were determined. When exploring the effect of each single factor, the addition amount of pumpkin seed protein isolate, lean meat addition, cooking time and baking time were fixed as 2.25 g/100 g, 60 g/100 g, 40 min, and 2.5 h, respectively. The sensory evaluation and textural analysis of hardness were taken as the evaluation indicators.

2.2.4. Orthogonal test. After single factor test of each factor, the orthogonal test was adopted to determine the optimal formula. Table 1 showed the design of factors and levels of orthogonal test.

Table 1

The design of factors and levels

Levels	Factors		
	A (g/100 g)	B (g/100 g)	C (min)
1	1.5	60	35
2	2.25	70	40
3	3.0	80	45

2.2.5. Nutritional analysis and textural analysis. The content of protein, fat, water, and textural analysis of the pumpkin seed protein isolate sausage were conducted.

3. Research results and discussion

3.1. The effect of pumpkin seed protein isolates on the quality of sausagec

In the practical application of plant proteins in food industry, the high proportion of plant protein used in meat products will cause the decline of water-absorbing ability, soft quality structure, poor cohesion, and other problems. Therefore, the addition amount of pumpkin seed protein isolate in this study was set in a small amount. As shown in Fig. 1, the sausage hardness showed the highest level when the addition amount pumpkin seed protein isolate was 0.75 g/100 g and showed the lowest level when the addition amount pumpkin seed protein isolate was 3.75 g/100 g.

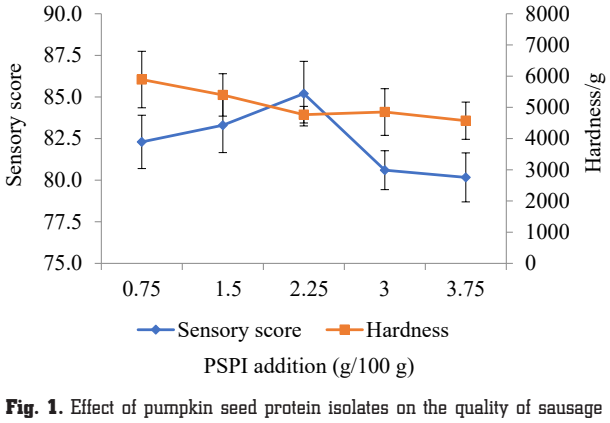

Fig. 1. Effect of pumpkin seed protein isolates on the quality of sausage

The sensory evaluation showed increased value when the addition value of pumpkin seed protein isolates increased from 0.75 g/100 g to 2.25 g/100 g. As the existence of bitter peptides, the protein powder showed bitter taste. At the protein isolate addition range from 0.75 g/100 g to 2.25 g/100 g, the sausage tasted no bitter taste. With the

increased amount of pumpkin seed protein isolates from 2.25 g/100 g to 3.75 g/100 g, the sausages showed bitter taste, as a result, the sensory value decreased. When the addition amount of the pumpkin seed protein isolates was 2.25 g/100 g, the average sensory evaluation reached a maximum value. After considering the sensory evaluation and the textural analysis of hardness, the three levels of pumpkin seed protein isolate of 1.5 g/100 g, 2.25 g/100 g and 3.0 g/100 g were selected for orthogonal test.

3.2. The effect of lean meat on the quality of sausage

As shown in Fig. 2, the sensory evaluation showed increased value when the addition amount of lean meat from 50 g/100 g to 70 g/100 g. In this range, the increased amount of lean meat makes the sausage structure became tighter, and the sausage hardness showed increased value and the meat flavor also increased.

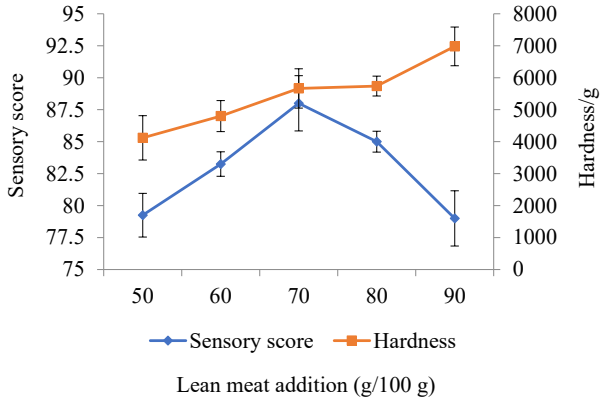

Fig. 2. Effect of lean meat on the quality of sausage

When the addition amount of lean meat increased from 70 g/100 g to 90 g/100 g, the hardness of the sausage increased and the sausage structure became hard, as a result, the sensory score showed decreased value. After considering the sensory evaluation indicator, the three levels of lean meat of 60 g/100 g, 70 g/100 g and 80 g/100 g were selected for orthogonal test.

3.3. The effect of cooking time on the quality of sausage

As shown in Fig. 3, the sensory score increased when the cooking time increased from 30 min to 40 min and then decreased when the cooking time increased from 40 min to 50 min. With the increased cooking time from 30 min to 40 min, the meat fragrance increased and the sausage structure became tighter.

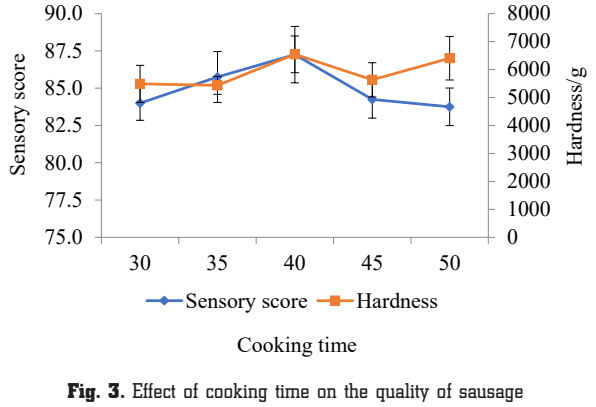

Fig. 3. Effect of cooking time on the quality of sausage

When the cooking time increased from 40 min to 50 min, the fat contained in sausage was constantly flowed away, and the structure of the sausage was too tight, resulting in a phenomenon of casing cracked. Therefore, after considering the sensory evaluation indicator, the three levels of cooking time of 35 min, 40 min, and 45 min were selected for orthogonal test.

3.4. The effect of baking time on the quality of sausage

As shown in Fig. 4, the sensory score increased when the baking time increased from 1 h to 2.5 h, because the casing of the sausage became tighter, and its chewiness was increased.

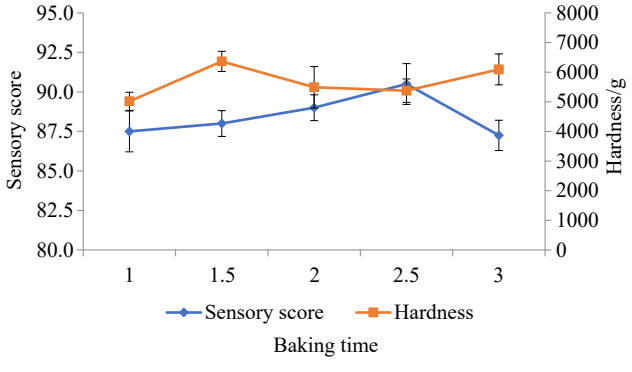

Fig. 4. Effect of baking time on the quality of sausage

When the baking time was 2.5 h, the sausage has the highest sensory evaluation. After cooking time of 2.5 h, the water-holding capacity of the sausage decreased, the sausage surface showed uneven appearance and its hardness increased. According to the sensory evaluation score of these five levels, baking time of 2.5 h was selected as the fixed value of the orthogonal test.

3.5. Results of orthogonal test

The range (R) indicates the extent to which the factors influence the indicators. The larger the range, the greater the influence of the factor on the sausage quality. According to the analysis in Table 2, the amount of pumpkin seed protein had the greatest impact on the sausage, followed by the cooking time, and the amount of lean meat.

Table 2

Sensory evaluation of pumpkin seed protein isolate sausage

Items (10 points)	Requirements
Color (2 points)	The transverse section has bright pink color
Flavor (3 points)	Its salty is moderate salty and meat aroma is strong and prominent
Appearance (2 points)	The sausage is full of mixtures with uniform thickness, no damage, clean surface, firm ligation, and no content in the ligation site of the casing
Structure (3 points)	The tissue was tight, elastic, well sectioned, free of cartilage and other impurities and astomatous

The best combination of pumpkin seed protein isolate sausage formula conditions was $A_1C_3B_3$, which is showed in Table 3. The optimal condition was pumpkin protein isolate of 1.5 g/100 g, lean meat of 80 g/100 g, and cooking time of 45 min.

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Table 3

Results of orthogonal test

No.	PSPI addition	Blank	Lean meat addition	Cooking time	Sensory score
1	1	1	1	1	7.5
2	1	2	2	2	6.6
3	1	3	3	3	8.6
4	2	1	2	3	7.8
5	2	2	3	1	6.6
6	2	3	1	2	7.8
7	3	1	3	2	6.8
8	3	2	1	3	6.4
9	3	3	2	1	6.0
$K1$	22.70	22.10	21.70	20.10	–
$K2$	22.20	19.60	20.40	21.20	–
$K3$	19.20	22.40	22.00	22.80	–
$k1$	7.57	7.37	7.23	6.70	–
$k2$	7.40	6.53	6.80	7.07	–
$k3$	6.40	7.47	7.33	7.60	–
R	1.17	0.93	0.53	0.90	–
Factor Priority	ACB				
Optimal combination	$A_1C_3B_3$				

Orthogonal test results were verified in triplicate and the obtained sausage under the optimal condition were showed in Fig. 5.

Fig. 5. Final product of the sausage

The verified experiments showed that the sensory score was 8.5, which is in coincidence with the result of orthogonal test (8.6).

3.6. Nutrient and cooking loss of pumpkin seed protein isolate sausage

It has been demonstrated that natural protein and hydrolyzed proteins of potato tend to reduce meat cooking loss [13] and the cooking loss of the final sausage was 6.98 % (Table 4).

Pumpkin seed protein sausages contained water, ash, protein, fat of 51.16 g/100 g, 2.26 g/100 g, 15.22 g/100 g, and 23.15 g/100 g, respectively.

As showed in Table 5, its hardness of 6372.2 g, springiness of 0.852, cohesiveness of 0.43, gumminess of 2720.7, chewiness of 2318.8, resilience of 0.171 were determined by textural analysis.

At present, the results of texture determination are mainly reflected in the actual utility of texture analyzer and the evaluation of the texture properties of a certain food, and are not applied in new product development, product quality control and quality evaluation. This is mainly due to the lack of corresponding standard data for product sensory indicators and quality indicators and parameters of textural analyzer. Therefore, the textural analysis determined in this study can only use as a reference indicator instead of determined indicator of the sausage quality.

Table 4

Determination results of pumpkin seed protein isolates

Indicators	Result
Cooking loss	6.98 %
Water	51.16 g/100 g
Ash	2.26 g/100 g
Protein	15.22 g/100 g
Fat	23.15 g/100 g

Table 5

The textural analysis of pumpkin seed protein isolate

Indicators	Results
Hardness	6372.2 g
Springiness	0.852
Cohesiveness	0.43
Gumminess	2720.7
Chewiness	2318.8
Resilience	0.171

This study provided an optimal production condition for pumpkin seed protein isolate sausage. It is noteworthy that bitter peptides are widely present in plant protein isolate. The undertaken study showed when the pumpkin seed protein isolate addition higher than 2.25 g/100 g, the sausages showed obvious bitter taste. The optimal addition was only 1.5 g/100 g which is much less compared the lean meat of 80 g/100 g. Thus, it is necessary to eliminate the bitter taste of pumpkin seed protein isolate in order to expand the utilization of it in the future study.

4. Conclusions

According to the single factor test and orthogonal test, under the basic pork meat amount of 200 g, the addition amount of lean meat of 160 g, pumpkin seed protein isolates of 3 g, edible salt of 2 g, sugar of 2 g, light soy sauce of 3 g, cooking wine of 2 g, thirteen incense of 0.2 g, red coloring agent of 0.2 g, modified starch of 2 g, cooking time of 45 min, baking time of 2.5 h at 60 °C were determined. Under such condition, the sausage has a sensory score of 8.5. The products contained moisture of 51.16 g/100 g, ash of 2.26 g/100 g, protein of 15.22 g/100 g and fat of 23.15 g/100 g. Besides, its cooking loss was 6.98 %.

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