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Preparation of High-Quality Fermented Fish Product

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Cover letter

Dear Editors and Reviewers,

Thank you for your letter and for all the kindly and detailed editorial and peer review comments concerning our manuscript entitled "Preparation of fermented fish product with high quality" (ID: JoVE60265). Those comments are all valuable and very helpful for revising and improving our paper. We have studied comments carefully and have revised this manuscript according to editorial and review comments and suggestions. Revised portion are marked in red in the paper. The main corrections in the paper and the details of responses to editorial and review comments are addressed below this letter.

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.

Sincerely yours,

Fang Yang, Lulu Zhu

TITLE:

Preparation of High-Quality Fermented Fish Product

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KEYWORDS:

sturgeon meat, marinating, drying, fermentation, sterilization, *Saccharomyces cerevisiae*, taste, flavor, texture, color, sensory properties

SUMMARY:

The goal of the protocol is to provide an industrialized fish fermentation technique based on inoculation of *Saccharomyces cerevisiae*.

ABSTRACT:

This protocol provides a method for preparation of industrialized fermented fish product with sturgeon (*Aquilaria sinensis*) meat product. The procedures were: (1) pretreatment of farmed sturgeon including decapitation, evisceration, skinning-off, cleaning and cutting; (2) marinating fish cubes in 6–12% (w/v) salt solution (1:1, fish cube mass to solution volume); (3) drying fish cubes to a water content of 50–60% by hot air (40–60 °C) or by vacuum; (4) fermentation involving inoculating fish cubes with 0.4–1.6% (w/w) *S. cerevisiae* in flavor solution to fish cubes

and fermenting at 25–35 °C for 6–10 h; (5) sealing fish cubes in vacuum packages with marinating and fermenting solutions; (6) sterilizing at 115–121 °C for 10–20 min. The sturgeon meat product prepared by this method has delicious taste which is mellow and thick, has various types and large amounts of volatile flavor compounds such as alcohols and esters which could mask musty and unpleasant odor from fish, has moderate salt content but good texture properties such as high springiness, gumminess and chewiness, and has bright russet color and attractive appearance. This new technique could also be applied in the processing of other fish to provide convenient fish snack foods which could be stored at room temperature. It is appropriate for both marine and freshwater fish.

INTRODUCTION:

Current commercial marinated fish product in China has the problem of heavy salty taste, insufficient wine aroma, poor elasticity and pale color, which decreases the acceptability to consumers. Therefore, a new technique for a high-quality fish meat product with wine aroma needs to be created and optimized.

In recent years, application of modern fermentation techniques in meat and fish has attracted attention from more and more researchers[1-4]. By inoculation of starter cultures into meat and fish, food safety has been enhanced, the processing time has been shortened; and the product sensory properties have been modified. Saithong et al.[5] isolated lactobacillus bacteria (LAB) from natural plaa-som and used this LAB as a starter culture, which induced high acidity and suppressed pathogenic bacteria. Zeng et al.[6] reported that inoculation with the autochthonous starter cultures reduced fermentation time and improved the sensory properties of samples. Casaburi et al.[7] claimed that the use of microbial starter cultures influence the development of aroma in fermented meats. In these starter cultures, *S. cerevisiae* could produce wine aroma by alcoholic fermentation and could also give the product other improved organoleptic qualities. Therefore, *S. cerevisiae* is a suitable starter culture for wine-aroma products[8-10] and wine-aroma fish product could be made by *S. cerevisiae*.

In the process of making wine-aroma fish product, the texture of meat and fish could be affected by salt content, water content, pH, protein denaturation, etc. Therefore, marinating, drying, fermentation and sterilization could all influence the texture characteristics. The formation of flavor and taste is complicated and is mainly affected by marinating and fermentation, because it is highly related to hydrolysis of carbohydrates, proteins and lipids, and mild lipid oxidation[11,12]. It could also be affected by addition of spices[13]. For development of color, Maillard reaction occurs which is involved in the process of fermentation and sterilization[10].

This article could provide technical support for the industrialization of fermented fish product with wine aroma, which is of great significance to the development of the fish processing industry. This technique could improve taste of product by increased proteolysis (more free amino acids and TCA-soluble peptides), modify flavor mainly by alcohols (ethanol, 1-octen-3-ol, 2-methyl-1-propanol and 3-methyl-1-butanol), esters (ethyl acetate) and aldehydes (nonanal, 3-methylbutanal and benzaldehyde), increase mouthfeel by higher hardness, springiness, gumminess and chewiness, and give more attractive russet color by and a bright surface[14]. It also

gives consumers convenience because the product can be stored at room temperature. As described by other previous studies[15-17], fermentation with *S. cerevisiae* has also been proved to significantly improve organoleptic qualities in other meat or fish products.

It is worth noting that the introduced protocol could also be applied in other species of fish, such as grass carp, silver carp, black carp, bighead carp, cod, salmon, etc. For high quality of fish products, fish without processing should be used, such as fresh fish, fish in ice or frozen fish stored for less than 1 year. Besides, since mild lipid oxidation could enhance flavor while extensive lipid oxidation brings unpleasant flavor, fish with less fat is preferred or skimmed fish is recommended.

PROTOCOL:

1. Sample preparation

1.1. Thaw frozen sturgeon meat under flowing water below 20 °C. Then, clean, skin off and cut the fish into cubes (2 cm × 2 cm × 1.5 cm).

NOTE: Raw material could be fresh or frozen sturgeon meat.

1.2. Mix fish cubes with 6–12% (w/v) salt solution at a rate of 1:1 (fish cube mass to solution volume), at 10 °C for 1–3 h.

1.3. Put the marinated fish cubes onto a stainless-steel gridding plate and dry fish cubes at 40–60 °C for about 6–10 h by hot air, or dry the fish cubes in vacuum, until the water content decreases to 50–60%.

1.4. Mix dried fish cubes with the flavor solution inoculating 0.4–1.6% (w/w, starter culture mass to fish cube mass) *S. cerevisiae* at a rate of 1:1 (fish cube mass to solution volume). Seal the mixture in a container and ferment at 25–35 °C for 6–10 h.

NOTE: The fermentation must be performed under anaerobic environment.

1.4.1. Prepare the flavor solution to include 25% spices liquid, 40% yellow wine, 7% white wine, 25% cane sugar, 2% monosodium glutamate, and 1% vinegar in mass fraction.

1.4.2. Prepare the spice liquid is as follows. Mix spices including 1.2 units of scallions, 1 unit of ginger, 0.6 units of star anise, 0.6 units of fennel, 0.3 units of green tea and 0.3 units of pepper with 40 units of water and boil for 0.5 h. Collect the filtrate. Mix the residue with another 40 units of water and boil for 0.5 h again. Combine filtrates of the two boils and make up to 100 units using boiled water.

NOTE: It is a must to pause the protocol here until the spice liquid has cooled to the room temperature before the addition of starter culture. This pause could avoid inactivation of yeast

by heat.

1.5. Seal fermented fish cubes in vacuum package with marinating and fermenting solutions at a rate of 11–13:1 (fish cube mass to solution volume). The vacuum strength, sealing time and cooling time are 0.085 MPa, 3.6 s and 5.5 s, respectively.

NOTE: The vacuum package material is polyethylene terephthalate/cast polypropylene (PET/CPP). The package weight is 50–80 g.

1.6. Sterilize packed fish cubes before storage and sale. The sterilization F-value should be more than 4.5 min. The sterilization temperature and time are 115–121 °C and 10–20 min, respectively. Maintain pressure constant at 0.12 MPa for cooling after sterilization.

NOTE: When sterilization F-value is 4.5 min and sterilization temperature is set as 121 °C, the sterilization time is 11.4 min in the equipment conditions of this article. Revise the sterilization time when the type of equipment used is different.

2. Estimation of shelf life of fermented sturgeon meat product

NOTE: The estimation of shelf life of fermented sturgeon meat product uses accelerated shelf life testing (ASLT) method with Arrhenius model according to the method of Wahyuni et al. with some modifications[18].

2.1. Store products at 20 °C, 30 °C, 40 °C.

2.2. Predict the shelf life by the Arrhenius model using rancidity rates. Measure rancidity rates by testing acid values (AV) under different temperatures.

2.3. Measure AV on the 0^{th}, 14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th} days.

3. Chemical analysis

3.1. Determination of salt content

NOTE: We measure salt content according to the method proposed by Zeng et al.[19].

3.1.1. Accurately weigh 1 g of the sample and add 10 mL of 0.1 M $AgNO_3$ + 10 mL of HNO_3.

3.1.2. Heat the mixture gently on an induction-cooker for 10 min.

3.1.3. Cool the mixture with running water, and add distilled water (50 mL) and ferric alum indicator (5 mL).

3.1.4. Titrate the mixture with standard 0.1 M KSCN until the solution turns permanent brownish-

red.

NOTE: Calculate and express the results as % NaCl.

3.2. Determination of moisture content

3.2.1. Determine moisture content according to the method of Zeng et al.[19].

3.3 Determination of pH value

NOTE: We measure pH value according to the method of Zeng et al.[6].

3.3.1. Homogenize 10 g of the sample with 90 mL of deionized water.

3.3.2. Measure the pH value with a digital pH meter.

4. Headspace solid-phase microextraction followed by gas chromatography-mass spectrometry (SPME-GC/MS) analysis

NOTE: We measure flavor according to the method of Gao et al. with some modification[17].

4.1. Extraction of volatile flavors

4.1.1. Weigh 2 g of the sample accurately, and then put it into the sample vial.

4.1.2. Add 2.5 mL of saturated sodium chloride solution and 0.5 M 2,4,6-trimethylpyridine into the sample vial with a rotor, and then close the vial cap.

4.1.3. Put the sample vial on the magnetic stirrer and stir for 10 min to mix the sample homogenously.

4.1.4. Insert the fiber into the headspace of the sample vial.

4.1.5. Adsorb at 60 °C for 30 min, and then desorb at 250 °C for 3 min in the injection port.

4.2. Analysis of flavor by GC-MS

4.2.1. Refer to the **Table of Materials** for details of the instrument used.

4.2.2. Use Helium (purity > 99.995%) as the carrier gas, and set the flow rate to 0.9 mL/min.

4.2.3. Set the column temperature to 40 °C for 3 min initially, and then increase to 90 °C at a rate of 5 °C/min. Subsequently, increase to 230 °C at a rate of 10 °C/min.

4.2.4. Run MS in electron ionization (EI)+ mode, and set electron energy as 70 eV. Set scan range from 30 m/z to 500 m/z. Set emission current to 80 μA. Use interface and source temperatures of 250 and 200 °C, respectively.

4.2.5. Identify the volatile flavor compounds by NIST2005 and Willey 7 standard libraries. Semi-quantify the retention index (RI) of volatile compounds using an internal standard (2,4,6-trimethylpyridine).

4.2.6. Calculate the concentration of volatile flavor compounds by comparing the peak area of each flavor compound with that of the internal standard. Express the results as μg/kg.

5. Texture profile analysis

NOTE: Analyze texture profile by following a previous study[20].

5.1. Perform texture profile analysis (TPA) with a texture analyzer equipped with a cylindrical probe (P/36R).

5.2. Apply two consecutive cycles. Deformation degree of TPA is 50% and the trigger force is 5 x *g*.

5.3. The speed of cylindrical probe for pre-, in- and pro-test, are 2, 1 and 5 mm/s, respectively. Calculate the texture parameters by its inbuilt software.

6. Color measurement

NOTE: Measure color according to the method of Czerner et al. with some modifications[21].

6.1. Measure samples by using colorimeter. Record the lightness value (L∗), greenness/redness value (a∗) and blueness/yellowness value (b∗) of the samples.

6.2. Measure five replicates for each sample.

7. Sensory evaluation

7.1. Conduct sensory analysis of samples by at least 20 trained panelists (23 panelists as an example, 12 man and 11 women, ages 20–40) using a previously method with some modifications[20].

7.2. Score the samples from 0 to 10 for taste, flavor, color and texture. Score 0 represents “dislike extremely” and score 10 represents for “like extremely”.

NOTE: Scoring criteria are shown in **Table 1**. The overall score is composed of a sum of different contributors (30% taste score + 30% flavor score + 20% color score + 20% texture score). An

overall score of 6 is viewed as the borderline of acceptable quality.

[Place Table 1 here]

REPRESENTATIVE RESULTS:

The suitable salt concentration, marinating time and temperature make the product's textural quality better. The best marinating conditions were as follows: the salt concentration of 8% in the marinating solution; marinating time of 2 h; and marinating temperature of 10 °C. See **Figure 1**.

In drying process, the final moisture content and drying temperature could influence the texture and sensory quality. The best drying conditions were as follows: moisture content of the dried fish cubes of 55% and drying temperature of 50 °C. See **Figure 2**.

In the fermentation process, the addition of *S. cerevisiae*, the temperature and the time all affect the taste, flavor, color and texture properties of fish. The failure fermented fish and successful fermented fish are compared. The successful fermented fish has high scores for taste, flavor, color and texture resulting in harmonious taste, fermentative fragrance, winey, russet color, bright surface, caramel and al dente texture. The failed fish product has the low scores for taste, flavor, color and texture resulting the poor taste, bitterness, sourness, fishiness, pale color, rough surface, coarse mouthfeel and deteriorated texture. The reason for failed fish product could be failure of anaerobic conditions in the fermentation. The addition of 0.8% *S. cerevisiae* and fermentation at 28 °C for 6 h resulted in the best sensory quality. See **Table 2**.

In the sterilization process, the suitable sterilization intensity and sterilization temperature was 4.5 min and 121 °C. The sterilization technique can guarantee the safety of the product stored at room temperature, and a study of minimal sterilization could reduce the damage to textural quality. See **Table 3** and **Figure 3**.

For storage, Arrhenius equation of AV changing rate variation with temperature is lnk = -2337.97/T + 2.98913. The fat oxidation kinetics model equation of fermented wine-aroma sturgeon meat product is $A = 0.38 \cdot e^{0.0078t}$ at 25 °C. The fat oxidation kinetics model equation of fermented wine-aroma sturgeon meat product is $A = 0.38 \cdot e^{0.0100t}$ at 35 °C. The prediction of the shelf life is 264 days and 205 days at 25 °C and 35 °C, respectively. See **Figure 4**.

FIGURE AND TABLE LEGENDS:

Figure 1: Effect of salt concentration and marinating parameters on the textural properties of the final product. (**a**) Salt concentration; (**b**) Marinating time; (**c**) Marinating temperature. Values and error bars are defined as means ± standard deviation (SD).

Figure 2: Effect of moisture content and drying temperature on textural properties and sensory evaluation of the final product. (**a**) The effect of moisture content on the textural properties of the final product; (**b**) The effect of moisture content on sensory evaluation of the final product;

(**c**) The effect of different drying temperatures on the textural properties of the final product. Values and error bars are defined as means ± SD.

Figure 3: Effect of sterilization temperature on textural properties and sensory evaluation of the products. (**a**) Textural properties; (**b**) Sensory evaluation. Values and error bars are defined as means ± SD. Means in the same indicator with different letters are significantly different ($P < 0.05$).

Figure 4: Arrhenius curve of AV changes of the products.

Table 1: Scoring criteria for sensory quality of fermented wine-aroma sturgeon meat product.

Table 2: Sensory properties (taste, flavor, color and texture) of successful fermented fish and failed fish product. Values are expressed as means ± SD. Means in the same row with different superscript letters are significantly different ($P < 0.05$).

Table 3: Effect of sterilization conditions on the safety of the products. Number of samples = 20.

DISCUSSION:

In this study, a new technique for the production of high-quality fermented fish product with wine aroma and tests for sensory properties are provided. The key processes of this technique are marinating, drying, fermentation and sterilization. In the marinating process, the salt concentration, the temperature and the time all affect the textural properties of the fish. The hardness and chewiness of the product increase gradually with the increase of salt concentration (0–12%, w/v) and the prolongation of marinating time (0–2.5 h); but the increase after 2 h is not significant. Meanwhile, chewiness increases with increased marinating temperature while hardness decreases when temperature is higher than 10 °C. In the drying process, the final moisture content and drying temperature could influence the state of protein, and thus result in different texture characteristics. With the decrease of final moisture content (75%–45%) in fish pieces, the hardness and chewiness of the product gradually increase. When the final moisture content of the drying end reduces from about 75% to about 55%, the change of elasticity of the product is not significant ($P > 0.05$). When the final moisture content reduces to less than 55%, the elasticity of the product decreases significantly. The reason could be that unbound water has little influence on texture characteristics while bound water has significant impact. Drying temperature could also affect textural quality. When the final moisture content is fixed at 55%, drying products at 40 and 50 °C has no significant difference in hardness, chewiness and elasticity ($P > 0.05$). In contrast, product dried at 60 °C is much higher in hardness and chewiness; and significantly lower in elasticity. In fermentation process, the addition of *S. cerevisiae*, the temperature and the time all affect the flavor property of fish. The suitable addition of *S. cerevisiae* (0.8%), fermentation temperature (28 °C) and fermentation time (6 h) could improve sensory receptivity for harmonious taste, fermentative fragrance, winey, russet color, bright surface, caramel and al dente texture. In the sterilization process, the sterilization intensity and temperature both affect the safety and texture property of fish. The safety of the product gradually increases with the increase of sterilization intensity. When the sterilization duration is

higher than 4.5 min, the product can meet the commercial sterility requirements. The product sterilized at a higher temperature at the same sterilization intensity (duration) has a better textural quality.

For the improvement of this technique, it is noteworthy that lipid oxidation should be considered and controlled, since extensive lipid oxidation may bring unpleasant flavor, resulting in reduced overall organoleptic quality. The solution could be using fish with less fat or addition of food antioxidants.

Among the limitations of this study, it should be mentioned that only sensory properties and microbial safety have been examined. The nutritional value has not been investigated which could be performed in the future to provide a comprehensive evaluation of this product and technique.

The fermented fish product prepared by this technique has delicious taste which is mellow and thick and has various types of and large amounts of volatile flavor compounds such as alcohols and esters which could mask musty and unpleasant odors from fish. It has moderate salt content but good textural properties such as high springiness, gumminess and chewiness and has a bright russet color and attractive appearance. This new technique could also be applied in processing of other fish to provide convenient fish snack foods which could be stored at room temperature. It is of great significance to the development of fish processing industry.

In the future, more investigation of this technique could be carried out. For example, only one strain of *S. cerevisiae* was used as starter culture for development of wine aroma in this study, different strains could be used to develop unique complex and rich flavor. Therefore, it is necessary to further study the effect of fermentation process and mixing ratio of different strains on the product quality in the subsequent research, in order to optimize the mixing ratio and process parameters which can further improve the quality of fermented fish product.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure

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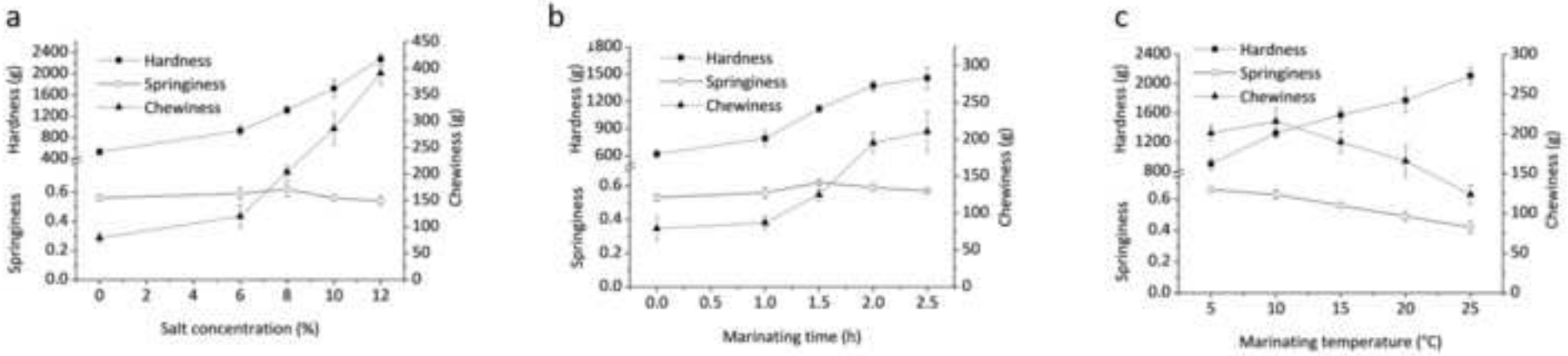

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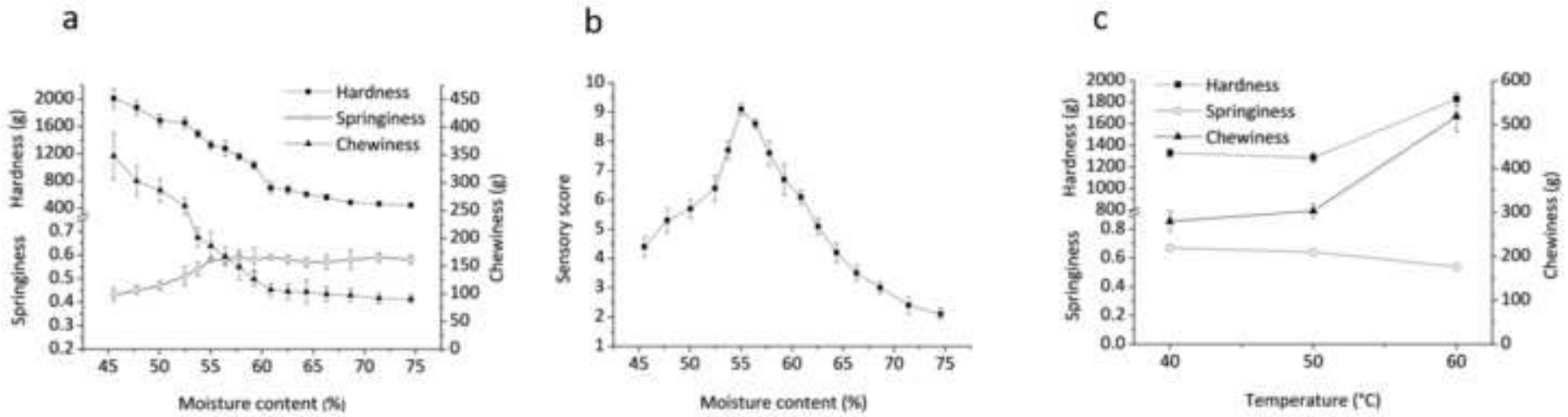

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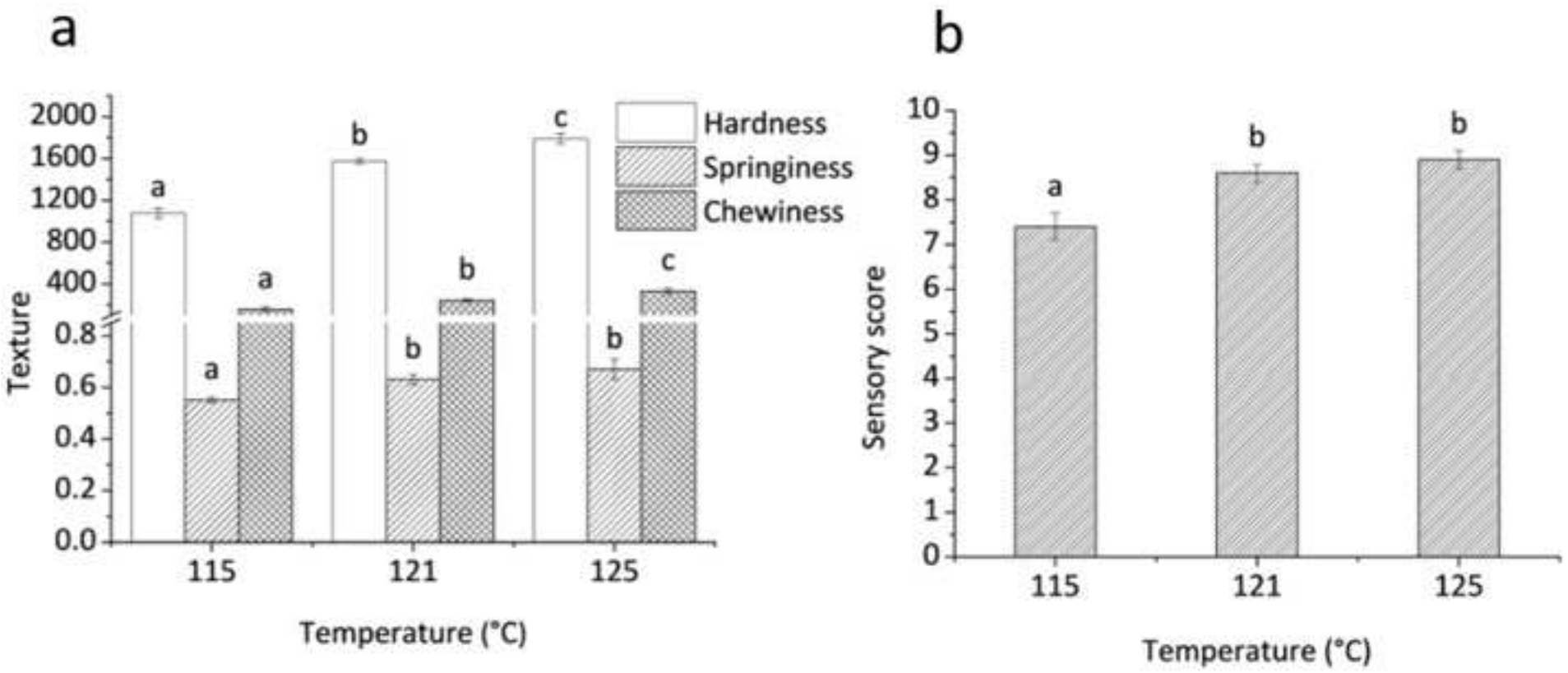

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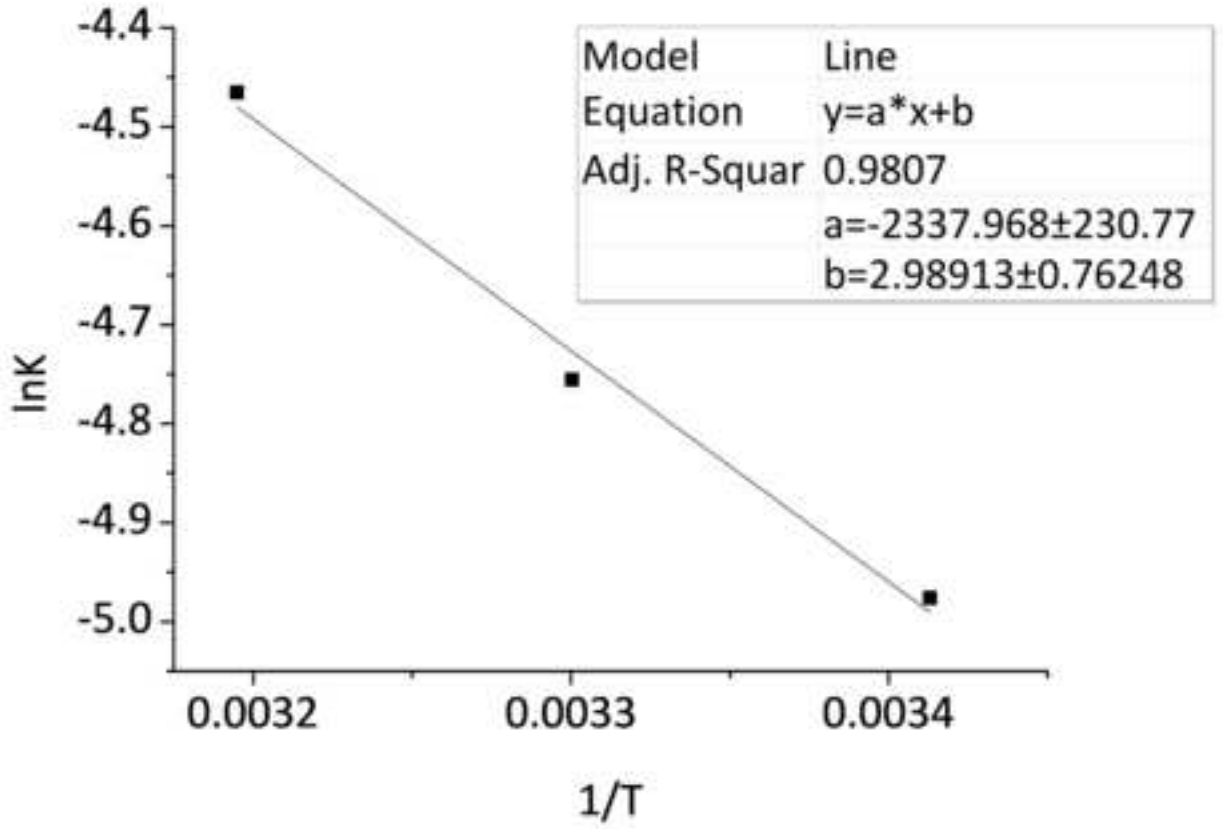

Table 1. Scc

Quality parameter
Taste
Flavor
Appearance
Texture

›ring criteria for sensory quality of fermented wine-aroma sturgeon meat product.

Description	Score
Acceptable sweet and salty; harmonious taste; wine taste; not astringent	8-10
Too heavy or light (sweet or salty); harmonious taste; wine taste; not astringent	6-8
Too heavy or light (sweet or salty); light wine- and after- taste; astringent	3-6
Too light wine- and after- taste; obviously astringent	0-3
Mellow wine flavor; rich fermentation aroma; no weird smell	8-10
Mellow wine flavor; light fermentation aroma; no weird smell	6-8
Light wine flavor; light fermentation aroma; light weird smell	3-6
No wine aroma; light fermentation aroma; obviously weird smell	0-3
Russet color; glossy appearance; bright surface	8-10
Russet color; bright surface	6-8
Yellow color; nonuniform	3-6
Pale color; rough surface	0-3
Acceptable chewiness	8-10
Hard	6-8
Light springiness	3-6
Soft texture; coarse mouth-feel	0-3

Table 2. Sensory properties (taste, flavor, color and texture) of successful fermented fi
expressed as means ± SD. Means in the same row with different superscript letters a

	Taste	Flavor	Color	Texture	Overall
successful fermented fish	8.7±1.3[a]	8.7±0.8[a]	8.9±0.8[a]	8.8±0.6[a]	8.8±0.1[a]
failed fermented fish	4.5±0.5[b]	5.2±0.4[b]	5.9±0.5[b]	3.8±0.4[b]	4.9±0.2[b]

sh and failed fish product. Values are
are significantly different (P <0.05).

Sensory descriptions
harmonious taste, fermentative fragrance, winey, russet color, bright surface, caramel and al dente
poor taste, bitter, sour, fishy, pale color, rough surface, coarse mouthfeel and deteriorated texture

Table 3 Effect of sterilization conditions on the safety of the products

Sterilization intensity (min)	Temperature (°C)	Time (min)
	115	15.1
3	121	8.7
	125	2.1
	115	18.8
3.9	121	10.4
	125	3.6
	115	21.2
4.5	121	11.4
	125	4.2
	115	23.5
5.1	121	12.4
	125	4.8
	115	26.9
6	121	13.6
	125	5.6

. Number of samples: 20.

Bulge bag numbers
1
2
4
0
1
1
0
0
0
0
0
0
0
0
0

Name	Company
2,4,6-trimethylpyridine	Tokyo Chemical Industry Co., Ltd.
Colorimeter	Hunterlab
DB-WAX column	Agilent
Digital pH meter	Mettler toledo Instrument (Shanghai) Co., Ltd.
Drying oven	Shanghai Yiheng Scientific Instrument Co., Ltd.
Frozen sturgeon	Huada Marine Industry Group Co., Ltd
Gas chromatograph-mass spectrometer	Thermo Fisher Scientific
Humidities incubator	Shanghai Yiheng Scientific Instrument Co., Ltd.
Saccharomyces cerevisiae	Angel Yeast Co., Ltd
Spices	Auchan Supermarket
Sterilization pot	Longqiang Machinery Technology Co., Ltd.
Supelco	Sigma
Texture analyzer	Stable Micro Systems, Ltd.
Vacuum package machine	Quanzhou Yiminxin Electromechanical Co., Ltd.

Catalog Number
Purity 98%
UltraScan Pro1166
30 m × 0.25 mm × 0.25µm
DELTA-320
DHG-9070A
-
TSQ Quantum XLS
LHS-250HC-II
-
-
RHS-03-700
65µm, PDMS/DVB
TA-XT2i
YMX-958-10L

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Title of Article: Preparation of fermented fish product with high quality

Author(s): Fang Yang, Lu-Lu Zhu, Pei Gao, Pa-Wei Yu, Pei-Pei Yu, Qi-Xing Jiang, Yan-Shun Xu, Wen-Shui Xia, Xiao-Bei Zhan

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Date:	May 15th, 2019

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Dear Editors and Reviewers,

Thank you for your letter and for all the kindly and detailed editorial and peer review comments concerning our manuscript entitled "Preparation of fermented fish product with high quality" (ID: JoVE60265). Those comments are all valuable and very helpful for revising and improving our paper. We have studied comments carefully and have revised this manuscript according to editorial and review comments and suggestions. Revised portion are marked in red in the paper. The main corrections in the paper and the details of responses to editorial and review comments are addressed below this letter.

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.

Sincerely yours,

Fang Yang, Lulu Zhu

Response to editors' comments:

Remark 1: Submit each figure as a vector image file to ensure high resolution.

Response: We have turned figures into .tif files with 1920 x 1080 pixels.

Remark 2: Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Response: We have checked through the whole manuscript.

Remark 3: Protocol Language: Please ensure that ALL text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique.

Response: Protocol language has been corrected.

Remark 4: Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Response: Discussion has been modified.

Remark 5: Figures: 1) Define error bars in Fig 1, 3.

Response: Values and error bars in Figure 1, 2 and 3 are defined as means ± standard deviation (SD) which have been added in the manuscript.

Remark 6: Tables: Please split tables into individual excel files and labels them Table 1 and Table 2. Please add legends for each table and ensure that each has been called out in the manuscript text.

Response: Table 1, 2 and 3 have been split and legends have been given. They have been called out each in the manuscript.

Remark 7: Commercial Language:JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent.

Response: Commercial sounding language has been deleted in the revised version and correspondingly, commercial products have been guaranteed in table of materials.

Remark 8: If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment.

Response: Figures and tables are original.

Response to reviewers' comments:

Reviewer #1:

Remark 1-10:

1. Line 32: -> drying fish cubes to a water content of ……

2. Line 34: -> inoculating fish cubes with 0.4~1.6% (w/w) S. cerevisiae ……

3. Line 35: -> sealing fish cubes in ……

4. Line 48: "Therefore, a new ……" needs revision.

5. Line 58: ->claimed.

6. Line 66: "dehydration" or "drying"? They should be uniformed in the whole manuscript.

7. Line 92: Why to highlight these sentences?

8. Line 120: The note should be more cautious by explaining how to pause and how to prevent the yeast from being inactivated.

9. Line 315-317: "The hardness ……" and "The hardness ……" should be combined to one sentence.

10. Line 323: ->(P>0.05), no space.

Responses (Except for Q7): All the sentences have been corrected according to the reviewer's suggestion.

Response to Q7: This is required from the journal.

Reviewer #2:

Remark 1: Some information lacking in materials and Methods with regard to spice liquid.

Response: We have clarified the method of making spice liquid in 1.4.2.

Remark 2: Language (English) needs to be revised especially in the discussion part.

Response: Discussion has been modified.