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Research Article

The Flora Analysis and Control of Spicy Rabbit Meat Package Product

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Abstract: Pre-sterilized by using preservatives on ingredients, combine with air conditioned preservation and radiation sterilization technology to make spicy rabbit Meat packaging products and achieve the purpose of storage at room temperature for a month. By isolation of spoilage bacteria in spicy rabbit meat products and resistance of the corresponding strain experiment, determine the dominant strains and screen out an effective preservation against the dominant strains and find out its sources by micro-ecological research, fabricate the optimal concentration of preservation and immersion time for the raw materials. Isolate *Bacillus* and *Clostridium perfringens* from spicy rabbit meat packaging products, after making bacteria analysis determine dominant strains R003 and P001. Then by making a micro-ecological analysis derive a conclusion that the source of spoilage bacteria is spices, finally after screening out the preservation and observing the effect, derive that the best preservation concentration is 6 g/kg, immersion time is 30 min. As a result, immerse spices for pretreatment in 6 g/kg preservation for 30 min and achieve the purpose that the diced rabbit meat packaging product can be preserved for one month at room temperature.

Keywords: Modified atmosphere, nuclear radiation, package products, preservatives, shelf life, spicy rabbit meat products

INTRODUCTION

Rabbit meat is rich in vitamins and calcium and is easy to digest and absorb, also has the effect of beauty, preventing blood clots and protecting blood vessel wall (Yun, 1999). Rabbit meat has less convective tissue and fiber, is easier to digest than pork, beef and mutton, especially good to the health of the old (Xuedong, 1999). Rabbit meat owns the advantage of animal food and vegetative food, not only can build up one's body and reduce diseases, have action on anti-aging and keep fit, but also keep skin cells alive and maintain the skin flexibility. So the rabbit meat is popular with the young women. This study is aimed at studying on rabbit meat, which used the technology of gas adjustable and irradiation and after placed for a time at room temperature condition, analyze bacteria group of spoilage bacteria and find the sources of spoilage bacteria, according to the features of bacteria have sieved, attempt to find a preservation or compound preservation composed of multiple preservations to achieve the purpose of improving the condition of keeping products in store and preserving products in room temperature by improving storage temperature.

MATERIALS AND METHODS

Food raw materials: Rabbit meat, fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon.

Reagents and culture medium: Nutrient AGAR culture medium; PSA medium; MRS culture medium; VRBGA culture medium; MSA medium; Reagents: Beef extract, peptone, yeast extract, saffron. Make gram staining solution as the literature recorded.

Equipment: XS-18 biological microscope, DT-200 Electronic Balance, pHS-3C pH meter, DFG30/HG101 electric blast drying oven, HG303 type electric drying incubator, Automatic electric pressure LDZX-40B2 vertical steam sterilizer, adjustable million electric furnace, BCD-195WIV refrigerator, HH-8 number substantially constant temperature water bath Electric Appliance, SW-CJ-1F type clean bench, pH 5.5-9.0 Precision dipstick.

Preparation for spicy rabbit meat products: Handle rabbit meat ahead, put into the tumbler, add the preserved marinade, turn the paddle 20 min and mix, mount the specified stainless steel cylinder with lid for marinating rabbit leg marinated does not exceed 50 kg in each cylinder. Add spice bag, boil the quality

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stipulated brine in the cooking pot and then put the quality stipulated rabbit meat into the cooking pot, control temperatures at 90~95°C, cook 18 min in micro-boiling state, ensure products be neither fresh, nor be rot, quickly place them inside the vacuum quenching machine and cool to 18°C or less. Take the rabbit meat, into the tumbler to mix, proportionally, add condiment powder, liquid condiment, seasoning oil in turn. Handle the sample inside the boxes, pull a vacuum and fill with N_2, package on it, regulate packaging parameter as the case, required vacuum time 3-5 sec,vacuum pressure -0.1 MPa, the time of filling N2 1-2 sec, N2 filling pressure 0.3 MPa, concentration more than 99%, sealing temperature 170-180°C, sealing time 3 sec. The samples should be sent to the radiation center for irradiation whose source is 60Co γ-ray, irradiation dose 3 KGy, irradiation time 20 min and then should be frozen in 4° for spare. Rabbit meat is divided into four and respectively preserved at 30°C for 5, 7, 10 and 15 days, respectively for test.

Separation of spoilage microorganisms in rabbit meat: With using the method of aseptic manipulation, get 10 g rabbit meat samples which has been kept 7 days at 30°C, make bacterium colony computation. Pick typical colonies, from different media, for isolation, purification, characterization and strains preservation. With using the method of aseptic manipulation, pick the moderate sample into cooked meat medium culturing for 24 h and then make bacterium colony computation. From different media pick out typical colonies for isolation and get purified colonies, observe and record colony morphology, seed into medium slant, preserve the strain (Chengfeng *et al*., 2001).

Flora analysis of spoilage bacteria in rabbit meat packaging products: Makes bacterium colony computation on the rabbit samples which has been preserved for 5, 10 and 15 day, respectively separately do statistical analysis on the source of spoilage bacteria with the weighted average method.

Source analysis of spoilage bacteria in rabbit meat packaging products: According to the study of Hesham (2004) on the use of irradiation to control food borne pathogens and extend the refrigerated market life of rabbit meat, has draw a conclusion that the rabbit meat contains higher quantity of Aerobic mesophilic micro-organisms, cold-loving Microbe, intestinal bacteria and yeast, mold, respectively amount to 10^6, 10^5, 10^4 and 10^4 CFU/g, respectively. In the experiment the major spoilage bacteria isolated from rabbit meat are bacilli and capsulate bacteris, so the spoilage bacteria in the rabbit meat may not come from rabbit meat, but from spices and seasonings. Aseptically add Nutmegs, star anise, fennel, chilli into the medium and mix uniformly, store them upside-down in the anaerobic glove box at 30°C for 48 h. From different medium pick typical colonies for isolation and get purified colonies, observe and record colony morphology, after gram staining seed into medium slant, preserve the strain (Chengfeng *et al*., 2001). Aseptically add fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon into the media and fix, store them upside-down in the anaerobic glove box at 30°C for 48 h. From different media pick typical colonies for isolation and get purified colonies, observe and record the colony morphology, seed the colony into medium slant after doing a gram staining test, preserve the strain (Chengfeng *et al*., 2001). Aseptically add fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon which are sterilized into the medium and mix, store them upside-down in the anaerobic glove box at 30°C for 48 h. From different media pick typical colonies for isolation and get purified colonies, observe and record colony morphology, after doing a gram staining test seed these colonies into medium slant, preserve the strains (Chengfeng *et al*., 2001).

Resistance determination of spoilage bacteria in rabbit meat packaging products: Seed the strains came from the isolated experiment of spoilage bacteria in rabbit meat into cooked meat medium, after cultured for 24 h at 30°C, place them in 100°C water baths and heat for 20 min. Then separately take 0.1 mL sample into the medium and an aerobically culture for 48 h at 30°C and observe whether there has spoilage bacteria grow or not.

Seed the test strains into cooked meat medium, cultured for 24 h at 30°C, place them in 100°C water baths and heat, when heated to 30, 45, 60, 75 and 90 min, respectively separately take 0.5 mL sample into the medium and an aerobically culture for 48 h at 30°C and observe whether there has spoilage bacteria or not.

Growth inhibition test on typical bacterial strain: Seed the test strains into cooked meat medium, cultured for 24 h at 30°C. Separately take 0.2 mL sample into the medium and coat uniformly, use the hole punch already sterilized whose pore size is 5-6 mm to hole four holes equally. Pick out the agar and drop a drop of molten agar on the bottom, drop 80 μL $NaN0_2$, cultured for 48 h at 30°C, measure accurately the diameter of inhibition zone, record the result in the excel and compute the average result of inhibition zone of $NaN0_2$ (Shiqiang, 2005).

The bacteriostasis experiment of Sodium Sorbate, Nisin, Natamycin, Tea polyphenol is done as the above method.

Experiment of marinating spices in preservatives: According to the results of the bacteriostasis experiment, the effect of Nisin and Tea polyphenol is better, so choose the maximum level of concentration of the two preservatives, twice concentration, three times concentration, four times concentration, six times concentration and eight times concentration (and so on, until get the expected result: when seeded spices into medium, there is no bacterium) to immerse fennel, bay

leaves, star anise, pepper, chili, nutmegs., cinnamon for 30 min. With used the method of aseptic manipulation, put the fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon immersed in different preservatives and different concentration into the medium, culture for 48 h at 30°C, observe the growth of bacterium around the spices.

Selection of the optimal proportion of preservative: Choose the optimal proportion of the two preservatives in the above experiment and design 1:0, 3/4:1/4, 2/1:1/2, 1/4:3/4, 0:1 groups, separately immerse fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon for 30 min, With used the method of aseptic manipulation, put the fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon immersed in different preservatives and different concentration into the medium and hold, culture for 48 h at 30°C, observe the growth of bacterium around the spices.

Choice of the optimal immersed time: Choose the best preservative groups to separately soak fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon for 5, 10 and 20 min, respectively. With used the method of aseptic manipulation, put the fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon immersed in different preservatives and different concentration into the medium and hold, culture for 48 h at 30°C, observe the growth of bacterium around the spices.

Observe the effect of the combination of air conditioned preservation, irradiated sterilized technology and preservatives to extend the shelf life of the rabbit meat products: Use 6 g/kg Nisin to separately immerse fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon for 30 min, produce a group of rabbit meat products in the initial method, preserve for 30 d at 25°C, observe whether appear sack expands or not and then assess the effect of preservations to extend the shelf life of the rabbit meat products.

RESULTS AND DISCUSSION

Isolation of spoilage bacteria in rabbit meat: Isolate spoilage bacteria after hyperplasia culture, recorded as R003 and P001 (Fig. 1).

When the rabbit meat products placed for 5 day, the dominant species is R003, accounted for 74.5%, P001 just account for 15.69%; when the rabbit meat products placed for 10d, the dominant species is P001, accounted for 78.1%, the percentage of R003 drops substantially, just accounted for 9.82%; when the rabbit meat products placed for 15 day, the dominant species is P001 and the number of P001 rise, accounted for 83.3%, the percentage of R003 drop, just account for 4.8%. Consequently, in the preserving process of rabbit meat products, the bacterial flora consist of R003 and P001, the dominant species is P001.

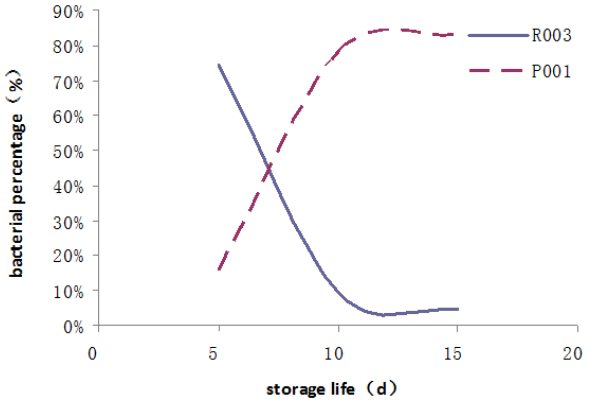

Fig. 1: Change of microflora in the preservation of rabbit meat products

Table 1: The result of boiling experiment and 121°C 20 min sterilized experiment

NAME	R003	P001
Boiling	+	-
121°C	-	-

+: As bacterial growth; -: As no bacterial growth

Table 2: Comparison of the heat resistance of R003

Strain number	30 min	45 min	60 min	75 min	90 min
R003	+	+	+	-	-

+: As bacterial growth; -: As no bacterial growth

Table 3: Bacteriostatic effect of preservation on predominant bacteria

	The size of inhibition zone (mm)				
Strain code	Tea polyphenols	Nisin	Sodium sorbate	$NaN0_2$	Natamycin
R003	0.367	0.384	0	0	0
P001	0	0	0	0	0

Analysis of the source of the spoilage bacteria in rabbit meat: According to the result of colonial morphology and microscope examination of spoilage bacteria, determine that the residual bacteria in the rabbit meat packaging products comes from nutmegs, star anise, fennel, bay leaves and chili.

Resistance determination of the spoilage bacteria in rabbit meat: The result of boiling experiment and sterilized experiment on typical bacterial as Table 1 follow.

According to Table 1, the bacterium (can't sterilized by boiling) which have the better heat resistance are R001, R003 and R007, the bacterium (can sterilized by boiling) which have the worse heat resistance are R002, P001, R008, R009, R004, R005 and R008. The heat resistance of P001, P003 and L001 as Table 2 follows.

According to the result of Table 1 and 2, the most heat resistant bacterial is R003, inactivated until boiling at 100°C for 75 min.

Tablet 4: Bacterium picking out rate after immersing spices in different concentration nisin

	Bacterium picking out rate of sample (%)							
Name	0.5 g/kg	1 g/kg	2 g/kg	3 g/kg	4 g/kg	5 g/kg	6 g/kg	Sterile water
Star anise	0	0	0	0	0	0	0	33.3
Fennel	100	100	100	100	33.3	0	0	100
Nutmegs	100	100	100	100	100	33.3	0	100
Chili	100	100	100	16.7	0	0	0	100
Pepper	50	33.3	16.7	16.7	0	0	0	100
Bay leaves	100	100	100	100	16.7	0	0	100
Cinnamon	33.3	16.7	16.7	16.7	0	0	0	33.3

Table 5: Bacterium picking out rate after immersing spices in different concentration tea polyphenols

	Bacterium picking out rate of sample (%)								
Name	0.5 g/kg	1 g/kg	2 g/kg	3 g/kg	4 g/kg	5 g/kg	6 g/kg	7 g/kg	Sterile water
Star anise	0	0	0	0	0	0	0	0	16.7
Fennel	100	100	100	100	0	0	0	0	100
Nutmeg	100	100	100	83.3	66.7	33.3	16.7	0	100
Chili	100	100	100	66.7	0	0	0	0	100
Pepper	66.7	50	33.3	0	0	0	0	0	100
Bay leaves	100	100	66.7	50	0	0	0	0	100
Cinnamon	33.3	16.7	16.7	16.7	0	0	0	0	33.3

Growth inhibition experiment of dominant strain: So choose P001 and R003 as test strains in this experiment. The bacteriostatic effect of Sodium sorbate, Nisin, Natamycin, Tea Polyphenols and $NaNO_2$ on P001 and R003 as Table 3 follows.

Experiment of spices immersed in preservation: The result of immersing fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon in different concentration Nisin for 30 min, as Table 4 follows.

From Table 4, 0.5 g/kg Nisin can fully inhibit bacterial growth in Star anise and inhibit bacterial growth in Star anise in a degree but not all, but can hardly inhibit bacterial growth in fennel, nutmegs, chili, bay leaves and cinnamon; 1 g/kg Nisin can fully inhibit bacterial growth in Star anise and have better ability to inhibit bacterial growth in pepper than 0.5 g/kg Nisin. One g/kg Nisin can also inhibit bacterial growth in cinnamon, but can hardly inhibit bacterial growth in Fennel, nutmeg, chili and bay leaves; 2 g/kg Nisin can fully inhibit bacterial growth in Star anise and have better ability to inhibit bacterial growth in pepper and cinnamon than 1 g/kg Nisin, but can hardly inhibit bacterial growth in fennel, nutmeg, chili and bay leaves; 3 g/kg Nisin can fully inhibit bacterial growth in Star anise and have better ability to inhibit bacterial growth in pepper and cinnamon than 2 g/kg Nisin and have strong ability to inhibit bacterial growth in chili, but can hardly inhibit bacterial growth in fennel, nutmeg and bay leaves; 4 g/kg Nisin can fully inhibit bacterial growth in Star anise, pepper, chili and cinnamon and have strong ability to inhibit bacterial growth in fennel and bay leaves; 5 g/kg Nisin can fully inhibit bacterial growth in Star anise, pepper, chili, Cinnamon, bay leaves and fennel and have strong ability to inhibit bacterial growth in nutmegs; 6 g/kg Nisin can fully inhibit bacterial growth in Star anise, pepper, chili, Cinnamon, bay leaves, fennel and nutmegs. According to the results, 6 g/kg Nisin can fully inhibit bacterial growth in all spices, so the optimal concentration of Nisin is 6 g/kg.

The experiment of immersing pepper, star anise, fennel, cinnamon, chili, nutmegs, bay leaves in tea polyphenols for 30 min, as Table 5 follows.

From Table 5, 0.5 g/kg tea polyphenols can fully inhibit bacterial growth in Star anise and inhibit bacterial growth in Star anise in a degree but not all, but can hardly inhibit bacterial growth in fennel, nutmegs, chili, bay leaves and cinnamon; 1 g/kg tea polyphenols can fully inhibit bacterial growth in Star anise and have better ability to inhibit bacterial growth in pepper than 0.5 g/kg tea polyphenols. 1 g/kg tea polyphenols can also inhibit bacterial growth in cinnamon, but can hardly inhibit bacterial growth in Fennel, nutmeg, chili and bay leaves; 2 g/kg tea polyphenols can fully inhibit bacterial growth in Star anise and have better ability to inhibit bacterial growth in pepper than 1 g/kg tea polyphenols, but can hardly inhibit bacterial growth in fennel, nutmeg and chili; 3 g/kg tea polyphenols can fully inhibit bacterial growth in Star anise, pepper and cinnamon and have certain ability to inhibit bacterial growth in nutmeg and chili. Three g/kg tea polyphenols have better ability to inhibit bacterial growth in bay leaves than 2 g/kg tea polyphenols, but can hardly inhibit bacterial growth in fennel; 4 g/kg tea polyphenols can fully inhibit bacterial growth in Star anise, pepper, fennel, chili, cinnamon and bay leaves. and have stronger ability to inhibit bacterial growth in nutmeg than 3 g/kg tea polyphenols; 5 g/kg tea polyphenols can fully inhibit bacterial growth in Star anise, pepper, chili, Cinnamon, bay leaves and fennel and have stronger ability to inhibit bacterial growth in nutmegs than 4 g/kg tea polyphenols; 6 g/kg tea polyphenols can fully inhibit bacterial growth in Star anise, pepper, chili, Cinnamon, bay leaves, fennel and nutmegs.

Table 6: Bacteriostatic effect of immersing spices in 6 g/kg nisin for different time

	Bacterium picking out rate of immersing samples in nisin for different time (min)			
Name	5	10	20	30
Star anise	0	0	0	0
Fennel	16.7	0	0	0
Nutmeg	100	100	83.7	0
Chili	0	0	0	0
Pepper	0	0	0	0
Bay leave	33.3	0	0	0
Cinnamon	0	0	0	0

According to the results, 7 g/kg tea polyphenols can fully inhibit bacterial growth in all spices, so the optimal concentration of tea polyphenols is 7 g/kg.

The optimal choice of immersion time: Immerse fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon in 6 g/kg Nisin for 5, 10 and 20 min, respectively. The result of experiment as Table 6 follows.

According to Table 6, Immerse star anise, chili, pepper and cinnamon in 6 g/kg Nisin for 5 min and achieve the desired effect, but the bacteriostatic effect on star anise, nutmeg and bay leaves can't achieve the desired effect; Immerse star anise, chili, pepper and cinnamon in 6 g/kg Nisin for 10 min and achieve the desired effect, but the bacteriostatic effect on nutmeg can't achieved the desired effect; Immerse star anise, chili, pepper, fennel, bay leaves and cinnamon in 6 g/kg Nisin for 20 min and achieve the desired effect, but the bacteriostatic effect on nutmeg can't achieved the desired effect.

Focused on the above findings, Immerse star anise, chili, pepper and cinnamon in 6 g/kg Nisin for 5 min, can efficiently inhibit bacterial growth in them; immerse star anise, chili, pepper and cinnamon in 6 g/kg Nisin for 10 min, can efficiently inhibit bacterial growth of fennel, bay leaves; immerse nutmeg in 6 g/kg Nisin for 30 min, can fully inhibit bacterial growth of it.

Observe the effect of the combination of air conditioned preservation, irradiated sterilized technology and preservatives to extend the shelf life of the rabbit meat products: Use 6 g/kg Nisin to immerse fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon for 30 min, produce a group of rabbit meat products in the initial method and preserve at 25°C. As a result, not appear gassy pouch and keep consistent quality.

CONCLUSION

In western developed countries, the technology of gas adjustable and irradiation has been widely used in food preservation field, but in our country the technology is still in its infancy and immaturity. Besides, the most food handled with the technology of gas adjustable and irradiation should be a need for refrigeration, this defect obviously limits the sales of air-conditioned and irradiational food. So the experiment aims to find what resources carry spoilage bacteria into products by microflora analysis of spoilage bacteria in rabbit meat packaging products, according to the characterize of the screened bacterial, attempt to find a compound preservative consists of a preservation or a few preservations to improve the preservation condition of production, to achieve the purpose of preserving for more than one month at normal temperature.

According to the spectrum of Nisin, it has obvious effect on gram-positive microorganisms, but can't curb the growth of Gram-negative bacteria, yeasts and molds. The mechanism of Nisin meets the theory Pore Formation which Engelke *et al*. (1992) proposed. In the special membrane potential, Nisin is adsorbed in cell membrane of sensitive organism, C-terminal of Nisin invades into cell membrane and forms transparent channel which allows hydrophilic molecules whose molecular weight less than 0.5 kDa flows, lead to membrane depolarization and lose of ATP, resulting in the leakage of protoplasm and then death. Due to the difference of Gram's positive and negative on Cell-wall structure. Cell-wall structure of Gram's positive bacterium has efficient peptidoglycan and high cross-linking degree; Cell-wall structure of Gram's negative bacterium has less peptidoglycan and complex composition, which consists of phosphatide, protein and LPS, compactly, just allow hydrophilic molecules whose molecular weight less than 600 Da flows. Because of the difference of cell-wall, Gram's positive and negative has differences on toxicity, stain-ability, drug susceptibility (Zengli, 2004), the molecular weight of Nisin is 3500 Da, so Nisin can't go through cell-wall of Gram's negative bacterium and arrive at cell membrane. Conclusively, The bacteriostatic effect of Gram's positive bacterium is better than Gram's negative bacterium.

In this experiment, the process of cooking and seasoning rabbit meat is separate. Keep rabbit meat and other ingredients under strict control of avoiding bacterial contamination, especially the processing of rabbit cook, cooling, excarnation. In addition, introduce the most advanced technology of gas adjustable and irradiation whose irradiation dose is 1-8 KGy. In initial experiment, we guess that the processing could bring spoilage bacteria into rabbit meat products is the seasoning process and then compare the result of the isolation of spoilage bacteria in rabbit meat with the isolation of spoilage bacteria in spices, finally, the suspicion is confirmed.

Besides the study of Hesham (2004) on the effect of the technology of irradiation on borne pathogen, he found salmonella has stronger resistance to irradiation. In this experiment we found gram-positive bacillus and

capsulate bacteris which has thick capsule also have strong resistance to irradiation. When the irradiation dose is below the limit standard of the state food allows, simply depending on irradiation technology is difficult to completely inactivate.

According to the theory Pore Formation, the binding site of Nisin is cellular membranes to destroy the integrity of cellular membranes. The binding site of tea polyphenols is cell wall to destroy the integrity of cell wall and then destroy the integrity of cellular membranes (Jingxin and Wenjuan, 2010). From the perspective of the bacteriostatic mechanism, the effect of two kinds of preservations should be mutually reinforcing. But in this experiment, we find that the mix of two kinds of preservations will produce flocculation and in subsequent experiments the effect of the mix of two kinds of preservations is less than one preservation.

In this study, isolate bacillus and perfringens, after microflora analysis of spoilage bacteria and resistance determination of spoilage bacteria, determine the superior bacterium are R003 and P001. Then after making analysis of microbial ecology, arrive at the conclusion that the source of spoilage bacteria is spices, finally after Selecting preservation and observing the effect of preservation, get that the optimal concentration of preservation is 6 g/kg; the optimal immersed tine is 30 min.

The experimental results proved that immerse fennel, bay leaves, star anise, pepper, chili, nutmegs, cinnamon in 6 g/L Nisin for 30 min can preserve for a month at 25°C and keep bacterial total below the state standards, have no apparent effect on original flavor, so this has great significance to prolong the shelf life of these products.

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