

Study on the Effect of Pickled Cabbage using Freeze-drying Protective Agent

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Abstract: The protective effect of pickled cabbage using biological material was researched in the study. Firstly, the research determined the centrifugal condition of bacterial sludge and then detected the influence of various protective agents on the survival rate of lactobacillus. The final test result can show the influence degree of various protective agents on the survival rate of the freeze-drying lactobacillus was sodium glutamate>skim milk>mannitol>sucrose>glycerol>maltose. The best formula of the protective agent was: the skim milk, sucrose, glycerol, maltose and sodium glutamate shall be 6% and the mannitol shall be 4%. The survival rate of the lactobacillus after using the sodium glutamate with the best protection effect can reach 65.5%. The experience proved that these protective agents and ratio can guarantee the nutrients in the pickled cabbage not destroyed.

Keywords: Freeze-drying protective agent, lactobacillus, pickled cabbage

INTRODUCTION

Pickled cabbage generates a large amount of lactic acid mainly by the fermentation of lactobacillus rather than the osmotic pressure of the salt to inhibit the corruption of microorganism. The pickled cabbage uses the saltwater at low concentration or small amount of salt to preserve various fresh vegetables. After fermented by the lactobacillus, it can be made into a kind of sour salted product. As long as the lactate content reaches a certain concentration and makes the product isolated from the air, it can reach the objective of long storage. The salt content of the pickled cabbage is 2 to 4% and it is a kind of low-salt food. Generally speaking, the fiber-rich vegetables or fruits can be made into the pickled cabbage, like cabbage, Chinese cabbage, carrot, white radish, garlic, verdant, cuke, onion, cabbage, etc., (Lim-Chi *et al.*, 2009). After the pickling and seasonings, the vegetable can have the special flavor and many people regard as a kind of common edible garnish. Therefore, living in the environment with guaranteed food, the modern people are still able to make the pickles. The pickle shadow scatters at all over the world and the flavor varies for different regional variations. The pickled vegetable is rich in the lactobacillus to help digestion. However, making the pickled cabbage has a certain rules, just as it cannot touch the raw water or oil; otherwise it is easy to be corrupted. If eating the contaminated pickled cabbage, it is easy to have diarrhea or food poisoning (Feng-Di *et al.*, 2007).

Fermented foods have long history in many cultures. Today two most well known instances of

traditional fermented cabbage side dishes are sauerkraut and Korean kimchi. The Roman writers Cato (in his *De Agri Cultura*) and Columella (in his *De re Rustica*), mentioned preserving cabbages and turnips with salt. It is believed to have been introduced to Europe in its present form 1,000 years later by Genghis Khan after invading China. The Tartars took it in their saddlebags to Europe. There it took root mostly in Eastern European and Germanic cuisines, but also in other countries including France, where the name became choucroute.

The vacuum freeze-drying technique is now widely used in the food industry and it is suitable for the drying of heat-sensitive and easily-oxidized substances. The dried material can maintain the original shape and it is easy to restore the original feature after the rehydration. De Giulio *et al.* (2005) the lactobacillus dry goods prepared in the method is featured with the advantages of easy storage, easy carry and good rehydration. However, preparing the high concentrated lactobacillus leavening agent powdery product with high viable count content per unit weight and strong vigor after the rehydration must reduce the injury on the lactobacillus cell membrane in the freeze-drying process. According to the report, after directly freeze-drying the lactobacillus culture solution, the bacterial survival is only about 10-30% (Chen *et al.*, 2013). If the lactobacillus cell is suspended in the suitable medium for freeze-drying, the survival rate will reach over 70%. With the test bacterium of lactobacillus acidophilus in the white screen pickle juice, the research determined the suitable centrifugation condition and freeze-drying medium to reduce the injury on the thallus in the freeze-drying process.

Freeze-drying is used to preserve food, the resulting product being very lightweight. The process has been popularized in the forms of freeze-dried ice cream, an example of astronaut food. It is also widely used to produce essences or flavourings to add to food (Santivarangkna *et al.*, 2007). Because of its light weight per volume of reconstituted food, freeze dried product is also popular and convenient for hikers. More dried food can be carried per the same weight of wet food and has the benefit of "long life" compared to wet food that tends to spoil quickly. The hikers then reconstitute the food with water available at point of use. Instant coffee is sometimes freeze-dried, despite the high costs of the freeze-driers used. The coffee is often dried by vaporization in a hot air flow, or by projection onto hot metallic plates. Freeze-dried fruits are used in some breakfast cereal or sold as a snack and are an especially popular snack choice among toddlers, preschoolers and dieters, as well as being used by some pet owners as a treat for pet birds. Culinary herbs are also freeze-dried, although air-dried herbs are far more common and less expensive (Kandyliis *et al.*, 2010).

Similar to cryoprotectants, some molecules protect freeze-dried material. Known as lyoprotectants, these molecules are typically polyhydroxy compounds such as sugars (mono-, di- and polysaccharides), polyalcohols and their derivatives. Trehalose and sucrose are natural lyoprotectants. Trehalose is produced by a variety of plant (for example selaginella and arabidopsis thaliana), fungi and invertebrate animals that remain in a state of suspended animation during periods of drought (also known as anhydrobiosis).

MATERIALS AND METHODS

Material:

Lactobacillus species: Laboratory dedicated lactobacillus reagent produced in Beijing Jiuzhou Tianrui Science and Technology Co., Ltd.

MRS agar count culture medium: Hangzhou Baisi Biotechnology Co., Ltd. BS1136

Anti-freeze compound: Skim milk, sucrose, mannitol, glycerol, maltose, sodium glutamate

Instrument and equipment:

Centrifuge: Model, Sigma1-14; Maximum speed 14,800 rpm; Relative centrifugal force 16.163* g. Ultra-low temperature freezer: produced in Sanyo company. Temperature range: -15~-136°C. Freeze dryer LyoQuest-85 type, upright AISI 316L cold trap, ice volume 8. Portable high-voltage steam sterilizer, electronic scale ACS-6C type.

Method: It firstly conducted the proliferation culture on the lactobacillus with MRS culture medium. The culture environment is 37°C after 24 h the culture bacterium

amount can reach 109/mL. In order to avoid the influence of harmful metabolite, the thallus and culture solution were separated to conduct the centrifugation separation of the culture solution. Six groups of centrifugation conditions were selected to conduct the control test, measure the bacterial sludge and make the reasonable choice (Asha and Gayathri, 2012). The prepared and sterilized protective agent was added, respectively in the bacterial sludge at the volume ratio of 1:4, after placing for 10 min, counted the viable organism. Four mL bacterial suspension was taken to add in the small dried bottle, then covered with the rubber plug, placed in the ultra-low temperature freezer below -40°C and pre-froze for 24 h. At -70°C, 0.001 mbar, freeze-dried for 24 h (Kuratsu *et al.*, 2010).

Cleaned the pickled cabbage and cut into slice, after pulping, heated to 120°C and cooled for reservation. 1ml microbial was taken to the pickled cabbage juice, kept 37°C and cultured for 24 h, after taking out, the acidity of pickled cabbage juice was measured with the titration.

RESULTS AND ANALYSIS

Selection of centrifugation condition: According to the research data of the dairy lactobacillus, 6 kinds of centrifugation conditions were selected to be examined and tested the bacterial sludge's quality after the centrifugation, the viable organism amount in the bacterial sludge and determined the optimum centrifugation condition. Seen from the bacterial sludge's quality after the centrifugation, the total number of bacterial colony in Program 4 was the most.

However, it cannot determine the mechanical damage of the thallus under the centrifugation condition, so the viable organism was counted after diluting the bacterial sludge in the above centrifugal tube and the result can show that the viable organism amount in the centrifugation condition in 4 was the most, which was consistent with the centrifuge bacterial sludge amount. Under the condition, the centrifugal bacterial sludge amount was the most and the centrifugation had little damage to the cell and the viable organism was also the most, thus determining the optimal centrifugal condition was 24 min, 3000 r/min.

Selection of protective agent: The skim milk, sucrose, mannitol, glycerol, maltose and sodium glutamate were added in the bacterial sludge to detect the influence of these protective agents on the survival rate of the lactobacillus. The experiment result can be seen in Table 1 (Juyang *et al.*, 2013). Seen from the experimental data in Table 2, among various protective agents, in addition to the mannitol, when the content was 6%, the protective effect on the pickled cabbage was the best and when the mannitol content was 4%, the protective effect was the best. The protective effect

Table 1: Centrifuge experiment result

No.	Rotating speed (r/min)	Time (min)	Bacterial sludge's quality after the centrifugation (g)	Total sum of bacterial colony (CFU/mL)
1	3000	12	0.381	0.7×10 ¹⁰
2	4000	12	0.764	1.4×10 ¹⁰
3	5000	12	0.823	1.9×10 ¹⁰
4	3000	24	0.981	2.3×10 ¹⁰
5	4000	24	1.969	2.1×10 ¹⁰
6	5000	24	2.564	1.8×10 ¹⁰

Table 2: Influence of various protective agents on the survival rate of lactobacillus

Protective agent	Survival rate					
	Protective agent content 0%	Protective agent content 2%	Protective agent content 4%	Protective agent content 6%	Protective agent content 8%	Protective agent content 10%
Skim milk	32.4	57.4	64.2	65.0	66.3	64.2
Sucrose	31.9	53.6	56.9	64.1	62.5	61.0
Mannitol	32.2	59.2	64.6	63.8	60.7	59.5
Glycerol	31.7	55.4	57.8	61.4	58.2	57.2
Maltose	32.3	51.9	52.4	59.6	59.5	56.8
Sodium glutamate	31.9	52.0	64.1	65.5	60.7	59.1

on the pickled cabbage was different for various freeze-drying protective agents and the sequence was sodium glutamate>skimmilk>mannitol>maltose. The protective effect of sodium glutamate was the most significant, the survival rate of the dried viable organism amount can reach 65.5% or more, therefore, with the method of bacteria counting, the sodium glutamate can be selected as the frozen protective agent of the lactobacillus.

CONCLUSION

In the vacuum freeze-drying process, main factors influencing the lactobacillus bacteria ferment survival rate, fermentation activity and storage stability include culture medium, culture condition, harvest period, initial cell concentration, drying protective agent medium, frozen speed, drying process, water activity, packaging, preservation temperature and rehydration condition, wherein, the drying protective agent is the most critical factor. The test firstly chose the centrifugation condition of the bacterial sludge and then evaluated the protective effect of various protective agents.

The research result can show the total sum of living organism bacterial colony in the bacterial sludge was the most when the pickled cabbage was in the centrifugation condition of 24 min and 3000 r/min. Skim milk, sucrose, mannitol, glycerol, maltose and sodium glutamate can be acted as the drying protective agent of pickled cabbage. When the sodium glutamate content was 6%, the drying protective effect on the pickled cabbage was the best and then was the skim milk, mannitol, sucrose, glycerol and maltose.

REFERENCES

Asha and D. Gayathri, 2012. Antagonistic potential of lactobacillus spp against enteropathogenic bacteria: Purification and characterization of their bacteriocins. *Adv. J. Food Sci. Technol.*, 4(5): 265-269.

Chen, H., Q. Zhang, Q. Luo and G. Shu, 2013. Effect of five materials including sucrose, lactose, skim milk, yeast, vitamin B2 on survival of *Lactobacillus bulgaricus* during freeze-drying. *Adv. Mat. Res.*, 700: 255-258.

De Giulio, B., P. Orlando, G. Barba, R. Coppola, M. De Rosa, A. Sada, P.P. De Prisco and F. Nazzaro, 2005. Use of alginate and cryo-protective sugars to improve the viability of lactic acid bacteria after freezing and freeze-drying. *World J. Microb. Biot.*, 21(05): 739-746.

Feng-Di, J., J. Bao-Ping, L. Bo and H. Bei-Zhong, 2007. Microbial changes during the salting process of traditional pickled Chinese cabbage. *Food Sci. Technol. Int.*, 13(01): 11-16.

Juyang, Z., P. Li, B. Kong, M. Zhang and M. Liu, 2013. Oxidation level of vacuum-packaged shredded meat treated with different concentrations of potassium sorbate, sodium diacetate and nisin. *Adv. J. Food Sci. Technol.*, 5(01): 24-28.

Kandyliis, P., M.E. Manousi, A. Bekatorou and A.A. Koutinas, 2010. Freeze-dried wheat supported biocatalyst for low temperature wine making. *LWT-Food Sci. Technol.*, 43(10): 1485-1493.

Kuratsu, M., H. Yoshimitsu and D. Tohru, 2010. Analysis of the lactobacillus metabolic pathway. *Appl. Environ. Microb.*, 76(21): 7299-7301.

Lim-Chi, M., R.A. Rahim, H.Y. Wan and A.B. Ariff, 2009. Formulation of protective agents for improvement of *Lactobacillus salivarius* I 24 survival rate subjected to freeze drying for production of live cells in powdered form. *Food Bioprocess Tech.*, 2(4): 431-436.

Santivarangkna, C., K. Ulrich and F. Petra, 2007. Alternative drying processes for the industrial preservation of lactic acid starter cultures. *Biotechnol. Progr.*, 23(2): 302-315.