

**Studies on the Production of "Tukemono" Containing a Low Concentration of Salt
Using the Nitrogen Gas Packing System: Application for Takuan-zuke**

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In the conventional manufacture of "Tukemono"(Japanese pickles) in Japan, high concentration of salt have traditionally been employed. Therefore, ecological control of microorganisms was performed and "Tukemono" were not spoiled by harmful microorganisms. Recently, reducing the salt content in food has been required for health reasons so the salt concentration of "Takuan-zuke"(Japanese pickled radish) is reduced to 4 - 5% which is less than half of the amount used twenty years ago. In the recent production of "Takuan-zuke", it is often necessary to store "Daikon"(Rahanus sativus) for a long period of time before the final commercial products are produced. It is not easy especially when low concentration of salt(5 - 6%) is employed in response to the recent trend of reducing salt in food products. Therefore, some factories store salted "Daikon" in a refrigeration room. But, storage in a refrigerator is expensive and "Daikon" do not yellow in color which is desirable for "Takuan-zuke". Now most "Takuan-zuke" are produced after "Daikon" are stored in high concentrations of salt,desalted by water and pressed. This process is also expensive. The quality of "Takuan-zuke" is deteriorates in terms of color, texture aroma etc., because of the introduction of harmful bacteria during the desalting and pressing process. In addition wastewater disposal plants are troubled with the disposal of concentrated brine.

Therefore, the development of a method, with which salted "Daikon" can be stored in a low concentration of salt (5 -6%), has been desired. This is not only a problem to be solved for "Takuan-zuke" but also for all "Tukemono". With these points in mind, the nitrogen gas packing system for the storage of salted "Daikon" was developed.

In this study, the apparatus and flow sheet, tests on an experimental scale and on a practical scale, microbiological flora in storage of salted "Daikon" with nitrogen gas packing system and seasoning technique for "Takuan-zuke" containing a low concentration of salt will be discussed.

- 1) The Apparatus and Flow Sheet of the Nitrogen Gas Packing System for the Storage of Salted "Daikon"

Tow apparatus were developed; one was a concrete tank lined with an air-tight bag; the other consisted of a concrete tank with a U-shaped groove on the brim so that an air-tight seal

could be made with film and a rubber band. Both designs were fitted with nozzle for degasing and filling the head space of the tank with nitrogen gas. In the former method, salted "Daikon" were put into the concrete tanks lined with air-tight bags. After that, they were fitted with weighted wooden boards and stones. Air-tight bags were sealed using heat, deaerated and filled with nitrogen gas (about 98% nitrogen gas). The latter method was conducted in the same manner as the former except for the apparatus.

2) Storage of Salted "Daikon" under a Nitrogen Gas Packing system on experimental scale

30Kg of salted "Daikon" containing 5.5% salt were pickled in air-tight bag which had been placed in a rigid container. Weight stones for pressing down were put on the salted "Daikon". The air-tight bag containing the "Daikon" was closed, deaerated and filled with nitrogen gas. It was stored at room temperature. Other experiments were carried at the same time as follow: carbon dioxide gas in the place of the nitrogen gas, deaerated only, sealed only and the ordinary open system. Only under the nitrogen gas packing system, salted "Daikon" could be stored without any deterioration from Dec. 3rd to Jul. 6th(215 days). This means the period of storage could be prolonged over 2 months compared with the ordinary open system.

Besides, the qualities of salted "Daikon" under the nitrogen gas packing system were the best in color, texture and aroma. At the final stage of the nitrogen gas packing storage, about 2% alcohol was produced in the cover brine, malic acid was found in over one half of those in first stage and formation of lactic acid was suppressed. After 215 days, yeasts and lactic acid bacteria coexisted constantly in the cover brine.

The effect of NaCl concentration on storage of salted "Daikon" was also studied. Employing 6, 7 and 8% NaCl concentration, the effect of NaCl on the storage of nitrogen gas packaged salted "Daikon" was investigated.

NaCl concentration (6 - 8%) influenced pH, microbial flora, the amounts of ethanol and organic acids. In 6 and 7% NaCl concentration, yeasts increased along with the formation of ethanol, lactic acid bacteria increased along with the formation of lactic and acetic acid, and salted "Daikon" was acidified at the late stages(in May to early in Jun.). After the growth of yeast had entered the death phase, lactic acid bacteria which appeared during the late stage began to grow.

In 8% NaCl concentration, growth rate of the yeast was delayed, lactic acid bacteria were suppressed, the formation of lactic and acetic acids inhibited, malic acid which is the main organic acid in fresh "Daikon" was not decreased in the final stage, and the salted "Daikon" were stored without any deterioration until early in Jul..

3) Storage of salted "Daikon" under a nitrogen gas packing system on practical scale

7 -20 ton of salted Daikon" containing 6 - 8% salt were pickled in air-tight bag which had been packed closely in concrete tanks which were used for a storage of salted "Daikon" with ordinary open method. Weight stones were put on the salted "Daikon" and the air-tight bags were sealed, deaerated, and filled with nitrogen gas in the same way as the storage on experimental scale. They were stored at room temperature for about 6 months.

The results obtained were almost in agreement with the results on the experimental scale and were as follows. Under the nitrogen gas packing system, the quality of the salted "Daikon" was superior in color, texture and aroma to those from ordinary open method. Furthermore, the period of storage could be prolonged about 2 months. In 6 - 8% NaCl concentration, salted "Daikon" was acidified at the late stage (late in May to early in Jun). However, in 8 -9% NaCl concentration, they were not acidified at the late stages (early in Jul.). The pH and the amount of the alcohol present during the storage of salted "Daikon" in concrete tanks was different in the cover brine and the lower salted "Daikon". The qualities of the "Daikon" also changed with the depth of storage in the concrete tank. The temperature change of the cover brine was close to the atmospheric temperature while the temperature of salted "Daikon" in lower parts(2m below the top) was maintained at 10°C.

The conditions of the upper part in practical scale almost the same as those throughout of the experimental scale. The limit (immediately before deterioration) of the storage of salted "Daikon" can be predicted by pH, color, and turbidity of cover brine. The variation of oxygen (2% - 4%) in air-tight bag did not affect the quality of salted "Daikon". It proved adequately fit for practical use as the result of experiments.

4) Studies of Yeasts and Lactobacilli on the Storage of Salted "Daikon" under the Nitrogen Gas-Packing System(Experimenting to Establish the Technical System)

A comparative study was carried out on the storage of constituents and microbial flora in "Tuke-eki"(soaked fluid of salted "Daikon") in both the nitrogen gas-packing system and the conventional open-system from Dec. 19, 1982 to Jul. 2, 1983. Yeast flora in "Tuke-eki" was studied first.

Until early April, *Saccharomyces servazzii* was the dominant yeast species in both nitrogen gas-packing system and open system. Later, it was replaced by *Debaryomyces hansenii* in open system, while *Sacch. servazzii* was maintained as the dominant yeast in nitrogen gas-packing system. The growth of harmful microorganisms for "Daikon", such as *Hansenula anomara*, *Picha membranaefaciens*, *Candida* sp. 2, *Rhodotorularubra* were suppressed by keeping the

"Tuke-eki" at pH 4.3 and 2% ethanol concentration due to the presence of this yeast.

The main characteristic of the nitrogen gas-packing system is growth inhibition of *Deb. hansenii*. This growth inhibition is considered to be caused by the low concentration of oxygen and low temperature in the early stage of storage, by carbon dioxide gas which was released by *Sacch.servazzii* in the middle to late stage of storage. As a consequence, the "Daikon" can be maintained in good condition for a long period of storage in the nitrogen gas-packing system. However, it began to putrefy with the accumulation of an excessive amount of lactic acid causing the acidification when the atmospheric temperature rose over 20°C.

The changes in flora of lactic acid bacteria were studied in both the nitrogen gas-packing system and conventional open system since the growth of these bacteria were thought to be cause of acidification. In the early stages of storage, about 10^5 cells /ml of unidentified lactobacilli were found in both system, then, after about 40 days, they decreased to below 1×10^3 cells /ml probably due to the lowering of pH and the atmospheric temperature. From the middle to late stages, *Lactobacillus bavaricus*, *Lactobacillus curvatus*, *Lactobacillus coryniformis* subsp. *torquens*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus coryniformis* subsp. *coryniformis*, and some unidentified lactobacilli were found in both systems. The growth of *L. bavaricus*, *L. brevis*, and *L. coryniformis* which are regarded as undesirable lactobacilli producing hydrogen sulfide, ammonia and slimes were fairly well suppressed in the nitrogen gas-packing system as compared with the conventional open system. Specifically, in the nitrogen gas-packing system, the number of those lactobacilli was 1 /100 of the open system and their growth delayed about one month. This explains why salted "Daikon" can be stored for a long period of time in the nitrogen gas-packing system.

In the late stages, however, salted "Daikon" started to putrefy even in the nitrogen gas-packing system, causing acidification accompanied with the increase of *L. brevis*, *L. coryniformis* and *L. lantarum*, which reached a level of 10^5 – 10^6 /ml.

It is considered that suppressing the growth of those bacteria is the most important for further improvement of the nitrogen gas-packing system.

Sacch. servazzii was the first yeast identified in Japan. It was considered to be one of the major yeasts in the storage of salted "Daikon" containing a low concentration of salt. Reduced salt usage and disposal problems, improved and more uniform product quality, and improved sanitation are some of the potential benefits that the tukemono industry could derive from the use of a closed brining tank (N₂ gas packing system). This nitrogen gas-packing system has now been adopted in practice for the storage of low-salted "Daikon". Now, over 20,000 tons are stored using this method in Japan. "Takuan-zuke" produced by this method are superior to those made by the ordinary process. This nitrogen gas-packing system is expected to be widely applicable to other "Tukemono".