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A simple preservation system for extending the shelf-life of tofu

(Sistem pengawetan yang mudah untuk melanjutkan tempoh simpan tauhu)

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Key words: preservation system, shelf-life, tofu

Abstrak

Tauhu yang baru dibuat direndam dalam air yang mengandungi bahan awet dan disimpan pada suhu bilik (25–27 °C). Tauhu yang direndam dalam air yang mengandungi asid asetik pada kadar 1 000 mg/kg dan kalium sorbat 1 000 mg/kg didapati masih boleh dimakan setelah disimpan selama 12 hari. Kajian yang sama juga menunjukkan bahawa tauhu yang direndam dalam larutan yang mengandungi asid asetik 1 000 mg/kg dan kalium sorbat 2 000 mg/kg boleh disimpan sehingga 14 hari. Selepas direndam dalam air yang mengandungi asid asetik 1 000 mg/kg dan kalium sorbat 1 000 mg/kg selama 10 hari, tauhu didapati mengandungi kalium sorbat sebanyak 250 mg/kg.

Abstract

Freshly prepared tofu samples were immersed in aqueous solutions containing preservatives and stored at room temperatures (25–27 °C). Tofu immersed in solutions containing a mixture of acetic acid at 1 000 mg/kg and potassium sorbate at 1 000 mg/kg remained acceptable up to 12 days of storage. Similar tests also showed that tofu immersed in solutions containing a mixture of acetic acid at 1 000 mg/kg and potassium sorbate at 2 000 mg/kg remained acceptable up to 14 days of storage. After immersing in solutions containing a mixture of acetic acid at 1 000 mg/kg and potassium sorbate at 1 000 mg/kg at room temperatures for 10 days, tofu had a sorbic acid content of 250 mg/kg.

Introduction

Tofu is one of the popular non-fermented soybean foods in Malaysia. It plays an important role in nutrition as a source of high-quality protein. According to Tee et al. (1988), fresh tofu has 86.7% water, 7.2% protein, 3.4% fat, 2% carbohydrate and 0.7% ash (inorganic residue). Tofu sold in the retail market is produced mostly by the small food processors. It is normally packed in water to prevent weight loss due to evaporation and breakage during handling.

Under tropical conditions, freshly made tofu can be kept for only 1–2 days before spoilage occurs. Temperature fluctuations during transportation and the initial microbial loads cause variations in the storage life of tofu. This short shelf-life not only result in wastage but also limits the area of distribution. Refrigeration methods of preservation are not only expensive but also not easily available in retail stores in the wet markets. Pontecorvo and Bourne (1978) successfully extended the shelf-life of fresh tofu stored at 24 °C and 37 °C to 10 days by immersion in aqueous solution containing 4% sodium chloride and 10% lemon juice. However, tofu stored in such

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high concentrations of salt and lemon juice could be too salty and sour to our oriental taste. Lim (1984) evaluated the effect of immersion solutions containing different preservatives on the shelf-life of tofu. After 23 days of storage at 10–15 °C, tofu samples immersed in solutions of 5 000 mg acetic acid per kilogram or mixtures of 5 000 mg acetic acid and 1 500 mg potassium sorbate per kilogram were still acceptable. Miskovsky and Stone (1987) also reported that immersion solutions of above concentrations were able to preserve tofu for at least 21 days at 4–7 °C.

Acetic acid is listed as a general preservative permitted in foods by the United States Food and Drug Administration (Desrosier and Desrosier 1982). It is often used as artificial vinegar in foods and is also permitted under the Malaysian Food Regulations (Anon. 1985) as an acidity regulator in foods. Potassium sorbate and sorbic acid are listed as antimycotic preservatives permitted in foods by the United States Food and Drug Administration (Desrosier and Desrosier 1982). Sorbic acid is a polyunsaturated fatty acid which can be metabolised by animals and humans (Deuel, Calbert et al. 1954). It has been found to be effective against the common pathogenic bacteria *Staphylococcus aureus* (Pierson et al. 1979; Robach and Sofos 1982). The use of sorbic acid and its salts as a preservative in foods is regulated by the 6th schedule (Regulation 20) of the Malaysian Food Regulations (Anon. 1985, 1988). However, its usage is not permitted in tofu unless studies can show its effectiveness against spoilage. To date, no such petition has been obtained from its use in tofu.

The objective of this study is to develop a simple, low-cost method of preservation that could increase the shelf-life of tofu to 1 week or longer under room temperatures. The approach is the application of known food-grade chemicals to create an environment hostile to microorganisms.

Materials and methods

Preparation of tofu

A single batch of soybean was purchased locally from wholesaler and kept at 10 °C until use. Soybeans (150 g) were soaked overnight in 500 mL distilled water at room temperatures. The soaked beans were drained, rinsed and blended with 1 L of distilled water in a Waring blender for 5 min at high speed. The resultant slurry was strained through two layers of filter cloth. The soymilk produced had a total solid content of 9.6%. To make tofu, the soymilk was heated on a hot-plate to boiling with constant stirring. A suspension of 4.5 g of calcium sulphate in 20 mL of distilled water as coagulant was then stirred into the hot soymilk and left to coagulate. After 5 min, the coagulated curd was scooped carefully into a rectangular plastic mould (15 cm x 12 cm x 6.5 cm, perforated with holes at all sides) lined with cheese-cloth. The top of the curd was then covered with cheese-cloth and a weight (900 g) was applied to press out the soybean whey. The block of tofu formed (15 cm x 12 cm x 2.5 cm) was then cut into four equal pieces (7.5 cm x 6 cm x 2.5 cm) of 130 g each.

Preservation of tofu

Various aqueous solutions containing preservatives were used as immersion solutions. Tofu sample was placed in a transparent plastic container containing 500 mL of the immersion solution. The immersion solutions of different concentrations prepared in sterilized distilled water were:

- sterilized, distilled water (control),
- potassium sorbate at 5 000 mg/kg,
- sodium acetate at 5 000 mg/kg,
- calcium propionate at 6 000 mg/kg,
- acetic acid at 5 000 and 6 000 mg/kg,
- citric acid at 1 200 mg/kg,
- lactic acid at 1 200 mg/kg,
- acetic acid (1 000 mg/kg) + calcium propionate (6 000 mg/kg),
- acetic acid (2 000 mg/kg) + calcium propionate (6 000 mg/kg),

- acetic acid (1 000 mg/kg) + sodium benzoate (1 600 mg/kg),
- acetic acid (2 000 mg/kg) + sodium benzoate (800 mg/kg), and
- acetic acid (1 000 mg/kg) + potassium sorbate (250–2 000 mg/kg).

The samples were placed at room temperatures (25–27 °C). All tests were done in triplicates.

Visual observation

The conditions of tofu and immersion solutions, i.e. colour, odour, air bubble formation and cloudiness were visually observed on each working day.

pH

A Corning Model 7 pH meter with a glass electrode was used for measuring the pH of the immersion solutions and the different tofu samples. The pH meter was calibrated with standard buffer solutions of pH 4.0 and 7.0.

Microbiological analysis

A piece of tofu sample (10 g) was blended with 90 mL of quarter-strength Ringer's solution as diluent to provide a 1:10 dilution. One mL of the 1:10 homogenate was then added to 9 mL of diluent to give a 1:100 dilution. This process was repeated to obtain further 10-fold dilutions. For total plate count (TPC), the ICMSF (International Commission on Microbiological Safety for Food) procedure (ICMSF 1978) for Pour Plate Method using commercially prepared plate count agar (Oxoid) was used. All inoculated plates were incubated at 37 °C for 48 ± 2 h. The TPC was computed according to ICMSF standard plate count. TPC for the duplicate samples were then averaged to obtain the colony forming units per gram (cfu/g) of sample.

The yeast and mould counts were made by using the ICMSF procedure (ICMSF 1978) for Pour Plate Method with commercially prepared potato dextrose agar (Oxoid) acidified to pH 3.5 with sterile 1% tartaric acid. All inoculated plates were incubated at 32 °C for 72 ± 2 h. The presence of yeast colonies was confirmed by examination with a microscope (Leitz Laborlux S). The yeast and mould counts were computed according to ICMSF standard plate count.

Diffusion of preservative

A study to determine the amount of sorbic acid that diffused from the immersion solution (1 000 mg acetic acid/kg + 1 000 mg potassium sorbate/kg) to tofu was carried out. In this study, eight pieces of tofu were immersed individually in 500 mL of the soaking solution. After 1, 2, 3, 4, 5, 8, 9 and 10 days of storage at room temperatures, a piece of tofu was weighed and blended with 150 mL of distilled water and centrifuged, and the supernatant analysed for sorbic acid by spectrophotometric method (Anon. 1986). All analyses were carried out in duplicate, from which the mean was obtained.

Results and discussion

Tofu samples were considered to be still in good condition when there were no change in odour, colour and texture, and while the immersion solutions remained clear without air bubble formation. There is no microbiological standard for tofu. However, as most food products begin to deteriorate rapidly when containing more than 10^6 microorganisms per gram (ICMSF 1978), a maximum total plate count of 10^6 was taken as the guideline for tofu acceptability.

Tofu immersed in sterile distilled water (control) and stored for 1 day at room temperatures (25–27 °C) developed an objectionable putrid odour with a TPC exceeding 1.5×10^9 cfu/g. The surface became soft and slimy while the immersion solution turned cloudy with air bubble formation. Therefore, the rate of spoilage for tofu immersed in water at room temperatures without preservative was rapid. The tofu retailers often change the immersion water hoping to slow down the

rate of spoilage, but this may not be practical especially during transportation.

Immersion solutions containing a higher regime of preservatives were used in this study as preliminary studies carried out with lower preservative concentrations were ineffective in extending the shelf-life of tofu. Potassium sorbate, sodium acetate, calcium propionate, citric acid and lactic acid when used singly under the conditions of this study were not able to prolong the shelf-life of tofu (*Table 1*). Sodium benzoate and calcium propionate when used in combination with acetic acid were also unable to prolong the shelf-life of tofu to more than 3 days. However, at room temperatures (25–27 °C), tofu immersed in solution containing acetic acid at 1 000 mg/kg and potassium sorbate at 1 000 mg/kg remained acceptable up to 12 days of storage, whereas tofu immersed in acetic acid at 1 000 mg/kg and potassium sorbate at 2 000 mg/kg remained acceptable up to 14 days of storage *(Table 1* and *Table 2)*. Lowering the concentrations of sorbate to 500 mg/kg or less were unable to extend the shelf-life of tofu to more than 3 days. As sorbic acid itself is relatively tasteless and odourless, tofu samples immersed in solutions containing either acetic acid at 1 000 mg/kg + potassium sorbate at 1 000 mg/kg or acetic acid at 1 000 mg/kg + potassium sorbate at 2 000 mg/kg had no objectionable taste or odour. However, the immersion solution containing acetic acid at 1 000 mg/kg + potassium sorbate at 1 000 mg/kg was more acceptable than that of solution containing acetic acid at 1 000 mg/kg + potassium sorbate at 2 000 mg/kg because the later had twice the strength of sorbate but could only extend the shelf-life by another 2 days. Even though acetic acid at concentration of 6 000 mg/kg could prolong the shelf-life of tofu to 7 days, the strong smell of acetic acid imparted to the tofu samples was undesirable.

The pH of tofu samples immersed in aqueous solution containing acetic acid at 1 000 mg/kg and potassium sorbate at 1 000 mg/kg at room temperatures for 10 days decreased from the initial pH of 5.7 to 4.9, whereas that of the immersion solution increased from 4.2 to 4.8 (*Figure 1*). The shape of the pH curves indicated a near pH equilibrium (4.8–4.9) for both tofu and its immersion solution after 10 days of storage.

Table 1. Effect of immersion in aqueous solutions on the keeping quality of tofu stored at room temperatures (25–27 °C)

Immersion solution	Storage life (days)
Sterilized distilled water (control)	<1
Potassium sorbate (5 000 mg/kg)	<1
Sodium acetate (5 000 mg/kg)	<1
Calcium propionate (6 000 mg/kg)	<1
Citric acid (1 200 mg/kg)	<1
Lactic acid (1 200 mg/kg)	<1
Acetic acid (5 000 mg/kg)	2
Acetic acid (6 000 mg/kg)	7
Acetic acid (1 000 mg/kg) + calcium propionate (6 000 mg/kg)	2
Acetic acid (2 000 mg/kg) + calcium propionate (6 000 mg/kg)	2
Acetic acid (1 000 mg/kg) + sodium benzoate (1 600 mg/kg)	3
Acetic acid (2 000 mg/kg) + sodium benzoate (800 mg/kg)	2
Acetic acid (1 000 mg/kg) + potassium sorbate (250 mg/kg)	2
Acetic acid (1 000 mg/kg) + potassium sorbate (500 mg/kg)	3
Acetic acid (1 000 mg/kg) + potassium sorbate (1 000 mg/kg)	12
Acetic acid (1 000 mg/kg) + potassium sorbate (2 000 mg/kg)	14

Table 2. Effect of immersion in aqueous solutions containing potassium sorbate and acetic acid on the microbial counts of tofu stored at 25 °C

Duration of storage (days)	Total plate counts (cfu/g)			Yeast and mould counts (cfu/g)		
	Control	0.1% PS + 0.1% AA	0.2% PS + 0.1% AA	Control	0.1% PS + 0.1% AA	0.2% PS + 0.1% AA
0	6.9×10^2	4.2×10^2	1.6×10^2	$<3 \times 10^2$	$<1 \times 10$	$<1 \times 10$
4	$>3.0 \times 10^9$	8.5×10^4	4.7×10^3	$>3 \times 10^5$	$<1 \times 10$	$<1 \times 10$
8	–	1.5×10^5	5.1×10^3	–	$<1 \times 10$	$<1 \times 10$
12	–	7.7×10^5	5.4×10^3	–	$<1 \times 10$	$<1 \times 10$
14	–	5.8×10^6	5.5×10^4	–	$<1 \times 10$	$<1 \times 10$

Note: 0.1% = 1 000 mg/kg PS = potassium sorbate
0.2% = 2 000 mg/kg AA = acetic acid

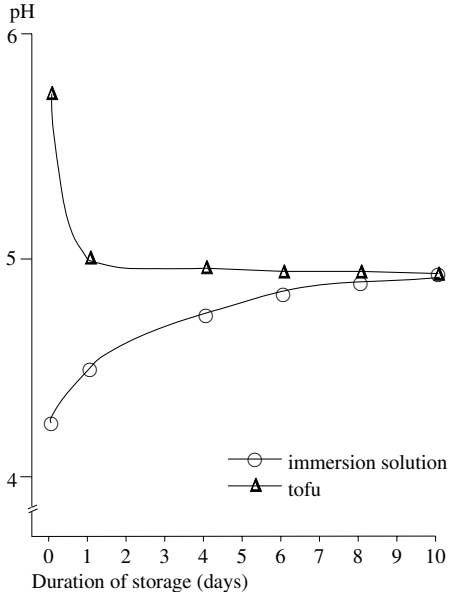

Figure 1. pH of tofu and its immersion solution (acetic acid at 1 000 mg/kg + potassium sorbate at 1 000 mg/kg) stored at room temperatures for different days

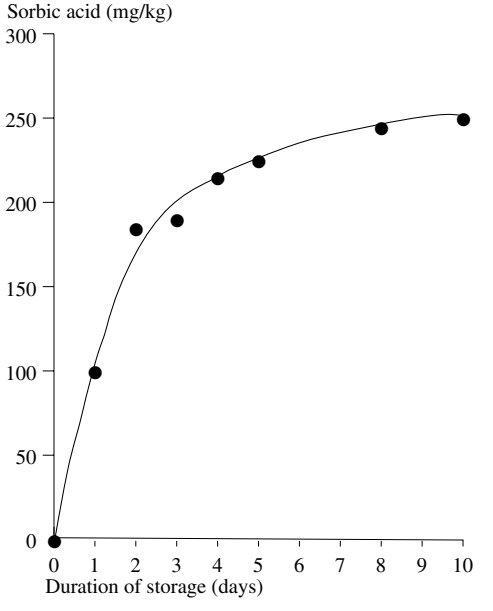

Figure 2. Sorbic acid content of tofu immersed in aqueous solution containing acetic acid at 1 000 mg/kg and potassium sorbate at 1 000 mg/kg stored at room temperatures for different days

There was, therefore, an active diffusion between the soluble contents of tofu and its immersion solution. The tofu probably started to deteriorate when the immersion solution has reached a pH of 4.8 which ultimately resulted in spoilage in another 2 days.

Immersion solutions containing only potassium sorbate at concentrations 1 500–5 000 mg/kg have pH values ranging from 7.0 to 7.5. Isao (1982) has shown that the preservative action of potassium sorbate is pH-dependent. It is more effective at lower pH values of close to 4.0. This probably explains why a combination of potassium sorbate and acetic acid as a preservative system was more effective in tofu preservation.

Results of diffusion studies (*Figure 2*) showed that tofu immersed in solution containing acetic acid at 1 000 mg/kg and potassium sorbate at 1 000 mg/kg at 25-27 °C for 10 days contained 250 mg sorbic acid/kg. Previous studies (Lim 1989)

using the scanning electron microscope on the microstructure of tofu showed the presence of a three-dimensional interconnecting honeycomb network structure in tofu. Therefore, sorbic acid has migrated into tofu by diffusion into the three-dimensional honeycomb network structure. Considering the levels of sorbic acid permitted in various food products to be in the range of 200–1 000 mg/kg (Anon. 1985), the levels of sorbic acid diffused into the tofu samples were on the lower limit.

The Malaysian Food Regulations (Anon. 1985, 1988) have no specifications for tofu. Under the circumstances, tofu is classified as 'food not elsewhere standardized' for which the use of listed food preservatives is forbidden unless petition is made to the Director of Health based on an acceptable effective dosage. However, sorbic acid is known to be one of the most harmless preservatives which is non-toxic to humans (Deuel, Alfin-Slater et al. 1954). Its metabolism is considered to be similar to that of fatty acids (Deuel, Calbert et al. 1954). Under the provisions of the Malaysian Food Regulations on 'food not elsewhere standardized', tofu manufacturers can apply to the Ministry of Health for permissison to use potassium sorbate in tofu as a preservative. The effective levels of sorbate can also be reduced to that necessary to achieve the desired shelf-life (for example 7 days).

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