Note

High-Pressure Sterilization of Ice Nucleation-active Bacterial Cells

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Ice nucleation active (INA)-bacteria are able to freeze water at a subzero temperature above -5°C .^{1–5)} The ability of INA bacteria may be stronger than that of any other ice nuclei ever known.³⁾ In nature, INA bacterial cells and their fragments cause frost injury to crops and, therefore, most investigations have been directed towards finding methods for lowering or inhibiting their activity.^{6–10)} On the other hand, INA bacterial cells are practically applicable; a commercial product is available as a snow inducer.¹¹⁾ In this case, the cells of *Pseudomonas syringae* are sterilized by beta-irradiation and then freeze-dried.

We have proposed methods for applying INA bacterial cells as effective ice nuclei to the freeze texturing,¹⁰⁾ freeze-drying¹²⁾ and freeze-concentration¹³⁾ of food materials. In these cases, sterilization of the products is necessary if living cells are used. Hayashi *et al.*¹⁴⁾ have proposed that high pressure can be used to kill microorganisms. The application of high pressure to the sterilization of such INA bacteria as *Erwinia ananas* may thus be possible. This paper deals with the high-pressure sterilization of *E. ananas* cells for practical applications.

E. ananas IN-10⁴⁾ was cultured at 20°C for 48 hr in a medium containing bactotrypton (Difco, 10 g), proteose peptone No. 3 (Difco, 10 g), dipotassium phosphate (1.5 g), magnesium sulfate (1.5 g) and glycerol (10 g) in water (1 l), the cells then being collected by centrifugation at $8,000 \times g$ for 20 min. Aliquots of the collected cells were suspended in 10 mM phosphate buffered saline (pH 7), 10 mM acetate buffered saline (pH 3), 10 mM carbonate buffered saline (pH 9) and water at a population density of 10^8 cells/ml in each. Each suspension was packed in a

plastic bottle and then compressed to 2000–5000 kg/cm² by a pressure generator (Hikari Koatsu, KP5B) at 20°C for 10 min. The cell paste obtained by the centrifugation process already mentioned was subjected again to a similar pressure treatment. The pressure-treated cells were diluted with autoclaved physiological saline and then cultured on *Pseudomonas* agar (Difco) at 20°C for 48 hr. Viability is expressed as the living cell number/g of sample.

Table I shows that the cells were sterilized at a

Table I. VIABILITY OF *E. ananas* TREATED BY DIFFERENT PRESSURES AT 20°C

State of cells in the pressure treatment	Living cell number before pressure treating	Pressure (kg/cm ²)			
		2,000	3,000	4,000	5,000
Viability (living cell number/g)					
Suspension					
at pH 3	1 × 10 ⁸	3 × 10 ⁶	< 10	< 10	< 10
at pH 7	1 × 10 ⁸	9 × 10 ⁶	< 10	< 10	< 10
at pH 9	1 × 10 ⁸	8 × 10 ⁶	< 10	< 10	< 10
in water	1 × 10 ⁸	7 × 10 ⁶	< 10	< 10	< 10
in FC-lemon juice*	2 × 10 ⁶	2 × 10 ⁴	< 10	< 10	< 10
(solid: 24%)					
in FC-lemon juice*	3 × 10 ⁶	2 × 10 ⁴	< 10	< 10	< 10
(solid: 36%)					
Paste	> 10 ¹²	6 × 10 ¹⁰	< 10	< 10	< 10

* Freeze-concentrated lemon juice.

Table II. ICE-NUCLEATION ACTIVITY OF *E. ananas* TREATED BY A PRESSURE OF 3,000 kg/cm² FOR 10 MIN AT 20°C

State of cells in the pressure treatment	Degree of supercooling ($^{\circ}\text{C}$)	
	Immediately after treating	24 hr after treating
Suspension		
at pH 3	>5	3.3 ± 1.2
at pH 7	>5	1.6 ± 1.4
at pH 9	>5	2.2 ± 2.0
in water	>5	1.5 ± 1.3
Paste	>5	1.3 ± 1.2
Freeze-dried*	—	1.7 ± 1.6

* The cell paste was compressed at the pressure, allowed to stand at 0°C for 1 day, and then freeze-dried.

hydrostatic pressure of more than 3,000 kg/cm² in all of the media used. The cells were also sterilized in a paste state when they were treated at more than 3,000 kg/cm² for 10 min. The result suggests that the cells can be applied with no hygiene problems to food processes such as freeze texturing, freeze-drying and freeze concentration following a high-pressure sterilization at 3,000 kg/cm².

A water suspension (2 ml) of each sample containing 10⁸ cells was measured for its ice-nucleation activity according to the method in the previous paper.¹⁰⁾ The ice-nucleation activity is expressed as the degree of supercooling (melting temperature minus nucleating temperature).¹⁰⁾ The pressure treatment of the cells under all the environments tested resulted in decreasing their ice-nucleation activity to a degree of supercooling of more than 5°C. However, the cells regained high activity by chilling at 0°C for one day (Table II). Next, the sterilized cell paste was allowed to stand at 0°C for 1 day and then freeze-dried. The freeze-dried product also possessed high ice-nucleation activity (Table II). The result suggests that the product can be supplied as ice nuclei for industrial uses.

An application study was carried out to concentrate fresh lemon juice by the use of INA bacterial cells. Two freeze-concentrated lemon juice samples with solid contents of 24% and 36% were produced, using the bacterial cells according to the method in the previous paper.¹³⁾ Subsequently, each of the samples was treated at 2000–5000 kg/cm² for 10 min at 20°C and then the viability of the cells was determined. As shown in Table I the cells were totally killed at more than 3000 kg/cm². The lemon juice samples treated at 3000 kg/cm² were submitted to gas chromatography to determine the limonen and citral contents according to the method in the previous paper.¹³⁾ An area ratio of limonen to citral (L/C) was used to evaluate the freshness in flavor.¹³⁾ Calculated L/C ratios were 8.63, 8.61 and 8.63 for the raw material, the pressure-treated sample with a solid content of 24%, and that with a solid content of 36%, respectively. A sensory

test by a panel of five trained members showed that the flavor of each pressure-treated sample was not significantly different from that of the original material.

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