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Heat Sterilization of Bottled Soymilk

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SUMMARY

Putrefactive anaerobe 3679 grew well in fish extract medium, brain-heart infusion, and in liver broth with 1% sodium thioglycolate added but less than 1% of the total cells sporulated. The TSP medium, an activated culture technique of Zoha *et al.* (1958), resulted in 50% sporulation. Using soymilks with 7% total solids, inoculated with 50,000 spores of P.A. 3679/ml, and bottled in 6-ounce soft drink bottles, it was found that a temperature of 121°C for 3 min was adequate to produce sterility. Since a heat treatment of 4 min at 121°C is recommended for development of optimum protein nutritive value in soymilk (Hackler *et al.*, 1965), sterilization of soymilk in 6-ounce soft drink bottles for 4–5 min at 121°C would result in sterility with an additional safety factor and result in retention of high nutritive value in the milk.

INTRODUCTION

Soymilk is an aqueous suspension containing principally proteins, fat, and sugars and is an ideal substrate for microbial growth. Yan (1927) reported that organisms that proliferated in soymilk were acid formers, gas formers, and putrefiers.

Preliminary observations in our laboratory showed that soymilk undergoes two types of spoilage under different conditions. At room temperature, it underwent acid curdling with a rapid drop in pH accompanied by separation of curds and whey, similar to curdling of cow's milk. This type of spoilage usually occurred in soymilk after standing 24 hr at room temperature. Proteolytic spoilage, however, appeared in soymilk stored in the cold. One week storage at 1°C resulted in putrefaction of soymilk.

Tan (1958) studied the effect of pasteurization and heat sterilization of soymilk. He pasteurized soymilk for 30 min at 60°C and reported that the soymilk could be stored for three days with no substantial spoilage. Tan also tested nine temperature-time combinations for the sterilization of soymilk. Five min at 120°C were shown to be adequate heat processing for one year of storage without spoilage. However, this time and temperature combination was not necessarily the minimum heating required to sterilize soymilk. Since the time increments tested by Tan were in multiples of 5 min, shorter heating times at this temperature for the sterilization of soymilk could be more closely estimated through the use of soymilk inoculated with a heat-resistant test organism.

Use of the minimum heat-sterilization process for soymilk is important from the standpoint of flavor, color, and nutritive values. Hackler *et al.* (1965) investigated the effect of heat treatment on the nutritive value of soymilk protein when fed to weanling rats. Their results indicated that the protein efficiency ratio (PER) of heat-processed soymilk depended upon both the time and temperature of the treatment. When soymilk was heated at 121°C for 2, 4, and 32 min, the PER obtained for the soymilks were 1.78, 2.21, and 1.79, respectively. The nutritive value was shown to decline after the initial 4 min of heating. The rise in PER after a 2-min heating was a result of the heat inactivation of the antigrowth factors in the soybean; hence, the wholesomeness of soymilk depended very much upon the optimal heat treatment given to the product.

The over-heating of soymilk would also bring about color darkening and the development of cooked flavor.

Therefore, this portion of the study was undertaken to determine the minimum processing time for the sterilization of soymilk containing the test organism, National Canners Association strain of putrefactive anaerobe (PA) 3679, a resistant strain of *Clostridium*, which is widely used in the canning industry for determining safe sterilizing times and temperatures.

EXPERIMENTAL METHODS

Heat penetration studies. Soymilk samples of 7% solids content were produced from pre-soaked beans. The soymilk was bottled in standard 6-fluid-ounce soft-drink bottles, capped, and heated in an agitating retort with a speed of rotation of 20 rpm. Two hundred ml were filled into each bottle with a uniform headspace of 2 in. The bottles were rotated along their long axis during heating. A thermocouple was inserted into one of the bottles situated in the center of the retort. The internal temperature of the bottle was recorded automatically in °F by a potentiometer as heating progressed.

Two types of heating were tried. Water heating was done by filling the retort with hot water followed by steam cooking. The cooling cycle was carried out by the gradual in-flow of cold water into the retort under pressure. The bottles were rotated continuously until cooling was completed. Steam cooking was done by direct heating with steam under pressure. The bottles were air cooled by the gradual balancing of the pressure in the retort with cold compressed air. Both water and steam cooking schedules were controlled manually.

Spore production. Putrefactive anaerobe 3679 was tested for growth and spore production in various media: fish extract, brain-heart infusion (Difco), and liver extract broth. Anaerobic conditions were provided by growing the organism in liter Erlenmeyer flasks containing 700 ml of media.

The trypticase-sodium chloride-peptone (TSP) medium of Zoha *et al.* (1958) and their activated culture technique were used to achieve spore production for the organism. The organism was first grown in a test tube culture for 12 hr before being inoculated into a 250-ml Erlenmeyer flask culture. The culture was successively transferred three times after 6-hr intervals into new flasks. The last transfer was made into a 3-l sporulation flask, in which the medium was constantly stirred. Anaerobic conditions were established by flushing the flask with natural gas before incubation. An incubation temperature of 37°C was used for the vegetative growth phase. Sporulation occurred during incubation at 35°C for 44 hr.

The spores were harvested by centrifuging the medium in a Sharples supercentrifuge. Spores were washed three times with distilled water and stored at 2°C.

Inoculated test batch. The standardized spore suspension contained 10^7 spores per ml as shown by direct microscopic count.

One ml of spore suspension was pipetted into each bottle containing 200 ml of soymilk yielding a spore count of 50,000 spores/ml of soymilk. The inoculated milk samples were divided into three batches: (1) control batch, which was not heat-processed but warmed to 80°C for 20 min to destroy any vegetative cells present; (2) a second batch was processed for 3 min at 121°C; and (3) a third batch was processed for 4 min at 121°C.

The surviving spores from the three batches were recovered by inoculation of the treated milk into five tubes of thioglycolate broth per each serial dilution. Positive growth tubes were determined after 4 days' incubation at 37°C by their appearance and by microscopic examinations. The most probable number method was used to estimate the number of viable spores in each sample (Standard Methods of Water Analysis, 1936).

RESULTS AND DISCUSSION

Sporulation of PA 3679 in various media. Putrefactive anaerobe 3679 exhibited good growth in fish extract medium, brain-heart infusion, and in liver broth with 1% sodium thioglycolate, but less than 1% of the total cells sporulated.

The TSP medium and the activated culture technique recommended by Zoha *et al.* (1958) were found to be satisfactory, resulting in 50% sporulation.

Heat penetration data. Heat penetration curves of the water-cooking and the steam-cooking systems are shown in Fig. 1. The come-up and cooling times of the two systems were different. Water cooking had a slow come-up but rapid cooling time; steam cooking had just the opposite pattern. Steam was found to be better adapted for commercial sterilization.

Calculation of a minimum processing time for PA 3679 in bottled soymilk. The graphic method illustrated by Ball *et al.* (1957) and by Frazier (1958) was used. The z and F values that characterize the thermal-death-time curve of the organism PA 3679 are given in the Manual for the Canning Industry (Townsend *et al.*, 1965). The z and F values were reported to be 18 and 7.5 when the pH of the heating medium was 6.2 (canned beans). Since the pH in soymilk was close to 6.2, these values were used to estimate the minimum processing time required for sterilization of soymilk. The thermal-death-time curve for the organism was plotted in Fig. 2.

The heat penetration data for bottles of soymilk when steam heated in an agitating retort are given in Table 1,

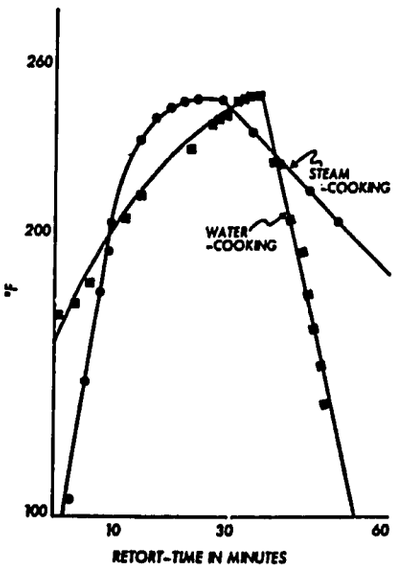

Fig. 1. Heat penetration curves of bottled soymilk in an agitating retort at 20 rpm.

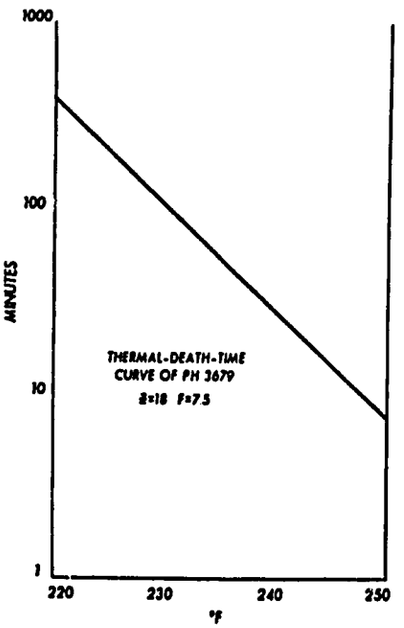

Fig. 2. Thermal-death-time curve of PA 3679.

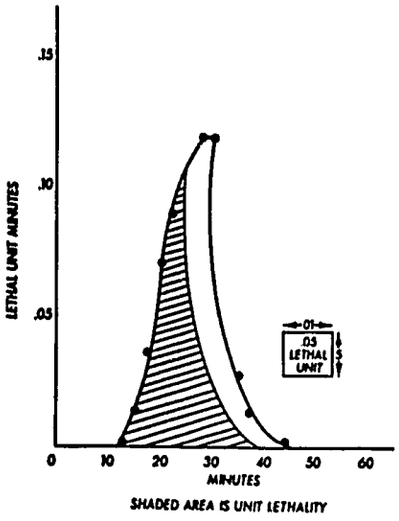

Fig. 3. Lethality curve of PA 3679 in the thermal processing of soymilk.

Table 1. Relationship between heating time and units of sterility (steam heat at 121°C).

Heating time Min	Internal temperature of bottle °F	Thermal death time Min	Unit of sterility per min $\frac{1}{\text{T.D.T. Min}}$
0	84	>650	<0.0015
2.5	106	>650	<0.0015
5	148	>650	<0.0015
7.5	180	>650	<0.0015
10	204	>650	<0.0015
12.5	216	650	0.0015
15	232	80	0.014
17.5	242	24	0.042
20	246	13	0.077
22.5	248	10	0.1
25	248	10	0.1
27.5	249	8.6	0.12
30	249	8.6	0.12
32.5	244	24.0	0.048
35	238	36	0.028
40	226	80	0.013
42.5	220	180	0.006
45	210	390	0.0026
47.5	216	650	0.0015
50	206	>650	<0.0015

Table 2. Incidence of viable spores in soymilk inoculated with PA 3679 before and after heat treatment at 121°C for 3 and 4 min.

Sample	Numbers of Positive Tubes/Serial Dilution [1]			
	0.01	0.001	0.0001	MPN of Organisms per Sample
Control 1 [2]	1	1	0	4×10^4
Control 2	2	0	0	4.5×10^4
A1 (3 min)	0	0	0	0
A2 (3 min)	0	0	0	0
B1 (4 min)	0	0	0	0
B2 (4 min)	0	0	0	0

[1] Five tubes used per serial dilution.
[2] Control samples were heated to 80°C for 20 minutes to destroy all vegetative cells.

columns 1 and 2.

The thermal-death-time in minutes for each temperature increment in the bottle as heating progressed is presented in Table 1, column 3. The data were derived from the thermal-death-time curve of Fig. 2.

The death rate of the organism during the heating is the reciprocal of the thermal death time or

$$\frac{1}{\text{T.D.T. in min.}}$$

for each increment of heating. The death rate of an organism during sterilization is also expressed as the unit of sterility per unit time. The relation of the heating time and the units of sterility per minute for the entire period of 50 min of the retort operation are shown in Fig. 3. The area under the curve of the figure yields the units of sterility. The curve was derived from data in Table 1, columns 1 and 4.

The minimum sterilization process of a system is defined as the total heating required for "one" unit of sterility.

The total area under the curve (Fig. 3) was 1.48 units of sterility for a 4-min holding at 121°C. The shaded area under the curve was exactly 1 unit of sterility, but, in order to insure safety from spoilage, a 10% margin was added. Therefore, 1.1 units of sterility were used to estimate the holding time at 121°C required to sterilize soymilk:

$$\frac{1.48}{4 \text{ min}} = \frac{1.1}{X \text{ min}}$$

$$X = 2.9 \text{ or } 3 \text{ min}$$

Therefore, the minimum processing time for sterility of soymilk using PA 3679 as the test organism was estimated to be 3 min at 121°C.

Inoculated test batch. The number of viable spores in each heat-treated sample is given in Table 2. Only 10% of the inoculated spores were recovered in the control samples. No viable spores were found in the soymilk heated for 3 or 4 min at 121°C.

Therefore, the estimated minimum processing time of 3 min at 121°C appears to be adequate to sterilize soymilk in 6-oz. soft-drink bottles with a total solids content of 7% or lower.

Hackler *et al.* (1965) have shown that the nutritive value of soymilk protein reached an optimum level when soymilk had been heated at 121°C for 4 min. In view of their results and the findings in this section, the trypsin inhibitor retention index described by Van Buren *et al.* (1964), which was recommended by Hackler for assessing nutritionally underheated soymilk, could also be used as a guide for determining sufficiency of heat treatment for sterilization of soymilk under ordinary conditions.

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