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Effect of processing variables on the canned quality of fava beans

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Effect of processing variables on the canned
quality of fava beans

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by

Basima Sa'di Abou-Dheir

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Major: Food Technology

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa

1980

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INTRODUCTION

The fava bean, a legume found mostly in northern Africa and southwest Asia, was eaten in ancient Egypt and was found in the tombs of Pharaohs dating from the fifth and sixth Dynasties 2,000 years B.C. In ancient Greece, fava beans were considered to be an appropriate food for athletes in order to improve their athletic performance. Today it is produced in large amounts with a world production of 5.3 million metric tons. Sinha (1977) reported that Africa produces 778,000 metric tons, North and Central America 43,000 metric tons, South America 134,000 metric tons and Asia 835,000 metric tons. The major areas of production are the Mediterranean basin countries such as Egypt, Greece, Italy, Lebanon and Europe, namely Spain, Austria and the majority of the nine member states of the European Economic Community. According to the same report, in 1972, fava beans contributed 1.33 million tons of protein to the total protein production (183.37 million tons) of the different groups of food crops produced in the world.

The fava bean is classified botanically as a subspecies of the subfamily of vetches (Vicieae), family Papilionaceae, order Leguminosae (pulses). Fava beans are also called broad beans (Vicia faba L.), English beans, tick beans, horse beans, field beans, and Faba vulgaris. They are used in large quantities as a protein supplement for livestock and poultry

feed, as well as for human consumption. In addition, Presber (1972) considered them as a possible new field crop for the prairie region of Canada. The fava bean plays an increasingly important role in the diet of the Egyptian people today. It is eaten in different forms as the first meal of the day. In the green stage, it is used in salads or cooked as a vegetable. In the dry stage, however, the seeds are either baked whole (baked beans or Medamis), fried (bean cakes or Taamia), stewed (besaria), or sprouted and cooked in boiling water as a soup (sprouted bean soup or Nabet). Eskin and Henderson (1974) reported the following average composition for the fava bean, protein 27%, crude fat 1.5%, crude fiber 8%, ash 3.5%, carbohydrates (starch and sugars) 47%, all on a dry weight basis. Analysis for amino acids showed that the fava bean is rich in lysine (6.59 g/16 g N) with limiting quantities of methionine (0.73 g/16 g N) and cystine (0.84 g/16 g N). The starch component, which is comprised of 69.0% amylopectin and 31.0% amylose, gelatinizes at a low temperature and increases slightly in viscosity. Vitamins A, B (B₁ and B₂), and C have been found in fava beans in lower concentrations than in other legumes. In areas where the bean is eaten in large quantities, lemon juice and olive oil are added to help palatability and increase vitamins C and E.

Reports of favism, a disease caused by certain varieties of fava bean, dates back to the fifth century in the

Mediterranean basin. Mager et al. (1969) defined favism as a hemolytic syndrome caused either by inhalation of the pollen from fresh Vicia fava or ingestion of the seeds in any of the following forms: fresh raw bean, fresh cooked bean, dried cooked bean, and through mother's milk in breast-fed children. Luisada (1941) reported that the hemolytic attack varies in intensity from one individual to another and it is followed by hemoglobinuria, jaundice and vascular symptoms. Seasonal incidences of favism occur mostly between the month of May and August when the seeds are fresh. Mager et al. (1969) found that the disease occurs more in males than in females and in children under four years of age. A major decrease in glucose-6-phosphate dehydrogenase was persistent in all individuals exhibiting a history of favism. However, a low level of glucose-6-phosphate dehydrogenase did not necessarily imply a case of favism. Recent evidence reported by Lin and Ling (1962a) suggested that the substances responsible for favism are pyrimidine glucosides naturally occurring in the bean. One major pyrimidine isolated and identified by many workers is vicine which is thought to be responsible for hemolysis and has been implicated as a causative agent of favism.

As we have seen from statistics cited above, the fava bean is a main source of food and protein for humans as well as for animals. Unfortunately, the favism problem associated with the bean is a limiting factor to its production and development as a substitute source for protein in the

diet. Also, a review of the literature showed a deficiency in research done on the physical and chemical properties of the fava bean under various processing techniques. This situation indicates a need for extensive research that could improve the quality of the bean and inhibit its toxic effects.

The purpose of this study is to optimize the effects of soaking and blanching (water and steam) on the beans and to evaluate the effects of processing on the following qualitative and quantitative characteristics of canned beans:

1. Color and texture
2. Vicine concentration
3. Microbial growth of thermophiles.

LITERATURE REVIEW

A number of researchers have worked with several dry fava bean varieties and reported their chemical composition (Table 1).

There were slight variations in the results obtained for the different constituents of the bean. However, the use of different bean varieties, different stages of harvest maturity and/or different techniques of chemical analysis could account for this variation. In the canning of dried beans, the soaking and blanching processes seem to be eminently important.

Processing Effects

In soaking and blanching of the bean, the two major variables that affect the quality of the finished product are temperature and time. Soaking time is a very important aspect of preparing dried beans for further processing.

One important factor that affects the rate of imbibition during soaking in legumes is the hardseededness or what is referred to sometimes as the hardshell condition. Bourne (1967) defined a hardseed as one which fails to imbibe water within a reasonable period of time when it is moistened. In addition, the hardseed condition constituted a problem to seedsmen and to food processors because the seeds would not sprout or soften during cooking.

Shafica et al. (1975) reported a wide variation

Table 1. Chemical constituents of Vicia faba seeds (dry basis)

Material	Reference	Variety	Moisture	Total protein %	Crude fat %	Crude fiber %	Ash %	Total carbohydrate %	Thiamine mg/100 g
Dry broad beans	Tabekhia and Mohammed (1971)	Ribaya	9.2						601
Canned baked beans	Tabekhia and Mohammed (1971)	Ribaya	72.8						274.1
Dry fava bean	Eskin and Henderson (1974)	<u>Vicia faba</u> L. var. Minor		27	1.5	8	3.5	47	
Dry fava bean	Bhatty (1974)	Baladi		30.5		7.4	3.3	57.8	
Dry fava bean	Bhatty (1974)	Erfordia		27		8.2	3.1	60.7	
Dry fava bean	Bhatty (1974)	Afghanistan		28.4		7.8	2.7	60.3	
Dry fava bean	Bhatty (1974)	Egypt		32.8		6.8	3.3	56.1	
Dry broad bean	Ismail (1976)	Giza (Egypt)	10.5	25.8	1.7	5.7	3.2	53.3	
Dry broad bean	Salem and Hegazi (1973)	Giza I	10.35	28.10		5.90	4.22	46.42	
Dry broad bean	Salem and Hegazi (1973)	Giza II	10.30	28.62		6.08	4.35	45.83	

in the rate of imbibition depending on the bean variety when steeping was done at room temperature. However, this variability declined considerably at temperatures of 81.7 to 93°C (190 to 200°F) which were effective in reducing the time of imbibition. Cotyledons imbibed water quicker than the whole seed both at room temperature and 93°C (200°F). This observation indicates the importance of the role played by the seed coat.

Nordstorm and Sistrunk (1977) worked on pinto, kidney and dwarf Horticulture #4 soybeans and studied the effect of the soaking time on some qualitative attributes in the bean. They found no significant difference in shear press values and percent splits with varying soaking time. Normal hydration ratios were found in all bean types. Although vitamin E retention was not affected by soaking time, riboflavin content was higher after six hours than after fourteen hours of soaking. Along the same lines, Rizley and Sistrunk (1979), working with blackeyed peas, found that longer soaking time had no effect on the color, texture or flavor of the peas.

The method used in soaking is another important factor studied by some researchers. Shafica et al. (1975) studied the effect of the steeping solution on the calcium content of the broad beans. They observed a small loss of calcium from bean seeds when steeping in distilled water. In tap water, however, the calcium concentration increased distinctly in the seeds. This increase was higher in

whole seeds than in decorticated ones. Similar, although slightly lower, values were obtained in boiled tap water. When calcium concentrations were increased in the distilled water treatment, both the rate and the concentration of calcium taken up by whole seeds, as well as by decorticated ones, increased. Calcium levels dropped in both whole seeds and cotyledons (decorticated) when ethylene-diamine-tetra-acetic acid (EDTA) was added to tap water used for steeping the seeds for various periods of time.

The same authors followed up their above mentioned studies by observing the effect of the steeping conditions on the baking qualities of beans. They reported that the baking time of the beans was reduced when steeping was done in distilled water and baking in tap water. Increasing calcium concentration up to 20 mg/liter (ppm) in distilled water used for steeping, resulted in an increase in calcium concentration in baked beans, which were judged to be excellent in flavor and texture. However, increasing EDTA concentration and steeping in tap water caused a decrease in the uptake of calcium of baked beans but resulted in an evenly baked bean with good flavor and texture.

Rizley and Sistrunk (1979) found that soaking solutions containing 1% pyrophosphate produced a lighter color in peas than soaking solutions containing 1% bicarbonate at two pH values (6.0 and 8.5). Soaking solutions containing bicarbonate at pH 8.5 resulted in lower shear press values.

In addition to soaking, blanching is the next step used before canning dried beans. The two most common types are steam and water blanching. Nordstorm and Sistrunk (1979) reported that beans blanched in steam rated higher in sensory color, liquor-viscosity, bean wholeness and general appearance. They attributed it to the fact that steam blanching may aid in setting color and facilitate better starch gelatinization without leaching, thus, giving a better overall appearance and a firmer texture. The blanching method, however, did not affect percent splits and vitamin E retention, while riboflavin was higher in steam blanched beans and in 80°C water blanching as compared with 99°C water blanching.

Working with peas, Rizley and Sistrunk (1979) found that boiled peas were lighter in color and less green than steamed cooked samples. Also, boiled peas were lower in shear press values indicating better texture.

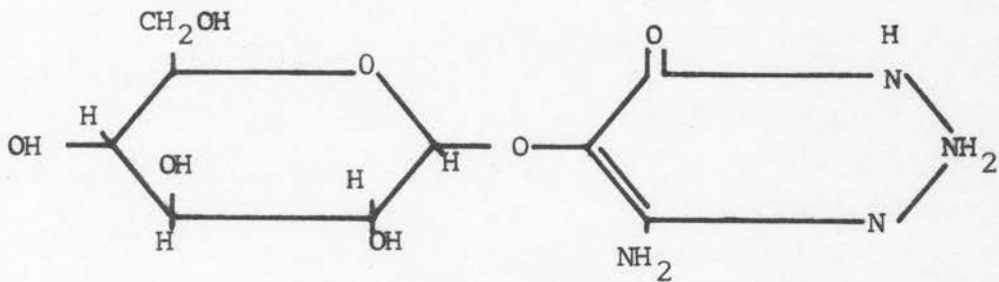
Quast and De Silva (1976) cooked samples of several types of dry legumes at different temperatures and various times using pressure cookers. They concluded that cooking time can be reduced with higher cooking temperatures. Rizley and Sistrunk (1979) working with peas found that a 20-minute cooking time increased discoloration, although texture and flavor were improved as compared with a 10-minute cooking period.

Nordstorm and Sistrunk (1977) compared the effect of

two canning media on the finished product of canned dry beans. They used a tomato sauce versus a water solution and reported significantly lower drained weights and higher shear press when canning with the tomato sauce. However, the beans packed in water had higher pH values, percent splits and vitamin E retention, while higher riboflavin concentrations were found in beans canned with tomato sauce.

Favism

Favism, which is a problem associated with certain varieties of fava beans, could be a limiting factor in production and processing. Studies on favism by Lin and Ling (1962a) showed that fava beans contain organic compounds such as arginine, betaine, creatine, trigonelline, amino-adipic acid, carbamide, convicine and vicine. The latter is of major concern in this study since it is thought to be the main toxic compound causing favism. In the same study, the authors briefly discussed the physical and chemical properties of vicine. Physically, vicine is a uniform, colorless, needle-shaped crystal which melts at 239-241°C. It is soluble in water, as well as in acid and alkali. Vicine has a maximum and a minimum absorption spectra at 274 nm and 248 nm, respectively, when dissolved in water. Chemically, vicine is a glycoside of which d-glucose and aglycone can be obtained after hydrolysis. It has no reducing powers and has the following chemical structure as determined by Bendich and Clements (1953).



2,4-diamino-6-oxypyrimidine-5-(β -D-glucopyranoside)

Bendich and Clements (1953) extracted vicine by using 2% sulfuric acid and recovered 0.14%. Their results showed a maximum absorption of 274 nm in 0.1 N hydrochloric acid.

Lin and Ling (1962a) extracted the glucosides from two kilograms of fava bean by using 54% ethanol followed by precipitation with mercuric sulfates. This method yielded a recovery percentage of 0.5.

More recently, using an ultra-violet spectrophotometer, Collier (1976) found the maximum absorbance of a protein free-extract of fava beans to be at 273.5 nm when dissolved in 0.1 N HCl. Since this absorbance is close enough to that of pure vicine, he postulated that this method yields a fairly accurate estimate of total vicine content. The advantages of this method are its simplicity and rapidity as compared to other methods.

In the same year, Jamalian et al. (1976) described methods to elucidate the nature of the nonprotein nitrogen fraction of the bean, which is thought to be the source of its toxicity.

Extracts from the different parts of the bean were compared to several standards consisting of nucleic acids, nucleosides and nucleotides using thin layer chromatography (TLC) and spectrophotometry. At least five distinct fluorescing components were present in the major bean extracts, none of which corresponded with any of the standards. However, the absorption of one of them compared with that of vicine.

In 1977, Jamalian et al. (1977b) developed a rapid and accurate method to estimate vicine in broad beans. Thin layer chromatography and spectrophotometry were used to isolate and identify vicine from a variety of samples of fresh freeze-dried broad beans. The level of vicine calculated on a percent dry weight ranged from 0.38 to 2.38% depending on the variety. Vicine tended to concentrate in the flesh of the seed rather than in the pods or the flowers.

In 1969, Mager et al. reported that vicine was the compound responsible for favism which is inherited by a sex-linked gene of intermediate dominance. They also found that there is an association between susceptibility to hemolysis and a deficiency of glucose-6-phosphate dehydrogenase in red blood cells. This deficiency in turn causes subnormal levels of reduced glutathion (GSH) in erthrocytes which become susceptible to toxins in Vicia faba. They concluded that any change in glutathion concentration is an index of toxicity. Bowman and Walker (1961) reported a positive action of Vicia faba on the glutathion of erthrocytes when incubating a 1-ml

blood sample from a sensitive subject with 0.1 or 0.2 ml extracts of pistils and pollen of the bean. Ingestion of the bean or inhalation of the pollen might result in an in vivo hemolysis since there is a direct action on the red blood cells in vitro. Jamalian et al. (1977a) reported a reduction in glutathion content of human red blood cells to be an indication of toxicity caused by favism. They used an aqueous extract from fresh, dry, mature broad bean seeds obtained from a favism endemic area in Iran. Those extracts were incubated with blood from control subjects that were sensitive to favism. They found that the seed coats of dry mature seeds and the flesh from fresh seeds had low toxicity. However, the immature whole seeds and coats of fresh mature seeds were toxic. This led them to conclude that the substance which is toxic is water soluble. In vivo experiments conducted by Lin and Ling (1962b) showed that when vicine was added to a rat's basal diet at a rate of 0.06 g/kg, the weight gains over a period of 41 days were lower than those rats fed the basal diet (control group). However, the group of rats tested by these authors consisted only of three males and three females all of considerably different initial weights. Except for "discrete lowering of the food intake and rate of growth," they found that the only toxic effect was a loss of hair in male rats that received the glycosides. In vitro experiments by the same authors (1962c) showed 38% inhibition of glucose-6-phosphate dehydrogenase activity when a homogenate of normal human red blood cells was

incubated with vicine at a concentration of 5×10^{-3} M.
However, this high vicine concentration is not likely to
occur by the intake of a regular portion of beans.

MATERIALS AND METHODS

The Egyptian fava beans used in the following experiments were purchased in Kuwait and shipped to the United States where they were inspected by the United States Department of Agriculture. The beans were kept refrigerated at 3°C.

Prior to any experiments, damaged beans with noticeable breaks or blemishes in the seed coat were sorted out and discarded. Only seeds free of foreign material were used. In all experiments, the beans were washed twice with tap water, then drained and blotted dry.

Proximate Analysis of Fava Beans

To determine the chemical composition of the fava beans the following analyses were conducted using standard procedures. The total protein percent was determined using the micro-Kjeldahl procedure (AOAC, 1970). Crude lipid was extracted with hexane in a gold fish apparatus by using method 30-20 of AACC (1969). The ash content was determined by using the AOAC (1970) dry ash method. Moisture content was measured using the air-oven method (AOAC, 1970). Duplicate samples were analyzed in each test and the results are the averages of the two replicates.

Water Imbibition

In all of the following experiments, 10 dry beans were weighed before and after the application of each treatment to measure water uptake. Peroxidase tests were performed on all samples to indicate blanching effectiveness as described by Joslyn (1946).

Soaking experiment

In this experiment, the three soaking temperatures used were 25, 37 and 50°C. The latter temperature was included to assess its effect on the reduction of soaking time. The soaking times applied to the 25°C were 0.5, 1, 2, 3, 4, 6, 8 and 12 hours. The same soaking times were used for the 37°C with the exclusion of the 0.5 hour. Soaking times of 0.5, 1, 1.25, 1.50, 2, 4 and 8 hours were used for the 50° soaking temperature. Each treatment was replicated six times.

Blanching experiment

In this part of the study, three major groups of blanching experiments were investigated. The blanching temperatures and times were selected from standard commercial practices in the United States.

In group I, the beans were soaked at 25, 37 and 50°C for 1, 4, 8 and 12 hours. This was followed by water blanching at 60, 82 and 93°C for 30, 45 and 60 minutes. The treatments

that were subjected to the 82°C temperature were replicated three times.

In group II, the beans were continuously water blanched at 60, 82 and 93°C for 0.75, 1, 1.5, 2, and 2.5 hours. Each treatment was replicated six times.

In group III, the beans were soaked at 25 and 37°C for 2, 4 and 6 hours followed by steam blanching at an average temperature of 95°C for 3 minutes. Each treatment was replicated twice. The length of blanching and soaking periods were determined by the time required to get a negative peroxidase test.

Canning Procedure

Samples containing 4.5 oz of dry beans and 450 ml of tap water were subjected to the 12 different treatments described in Table 2. Two samples were used for each treatment.

After soaking and blanching, the beans were drained and placed in No. 2 (307 x 409) enameled cans (Freund Can Company). A 2% sodium chloride brine was added up to one-fourth of an inch from the rim of the can. The cans were then covered with lids (but not sealed) and placed in an Arnold steamer for a period of five minutes, after which they were immediately sealed. They were then autoclaved at 115.5°C (240°F) for 40 minutes and cooled immediately to 35-37.7°C (95-100°F) with cold water. The 24 cans of beans were stored at room temperature until sensory as well as instrumental evaluations were performed.

Table 2. Soaking and blanching treatments

Treatment number	Soaking temp. (°C)	Soaking time (hr)	Type of blanch ^a	Blanching temp. (°C)	Blanching time (minutes)
1	25	4	WB	82	30.00
2	25	4	WB	82	50.00
3	25	12	WB	82	30.00
4	25	12	WB	82	50.00
5	37	4	WB	82	30.00
6	37	4	WB	82	50.00
7	37	12	WB	82	30.00
8	37	12	WB	82	50.00
9	25	4	SB	95	03.00
10	37	4	SB	95	03.00
11			CWB	82	75.00
12			CWB	82	90.00
13	Commercial can				

^aWB - water blanching; SB - steam blanching; CWB - continuous water blanching.

Quality Evaluation

The quality of the beans submitted to the different treatments in Table 2 was determined via instrumental and sensory procedures after two months of storage.

The sensory evaluation was performed by a panel of five judges who tested the beans using a modified USDA quality grading system for canned dry beans (Figure 1) (USDA, 1975). The modification was introduced in testing the character factor of the beans. Instead of tasting the experimentally packed beans, each panelist randomly selected 10 beans from each treatment and finger felt the beans using their

forefinger and thumb to determine whether the bean was hard or mushy, touch or soft, and slightly firm or slightly soft and graded them using the forms in Figures 1 and 2. The panel was initially trained using different varieties of dried canned beans, pinto, kidney, navy, chili and great northern beans. The panel was further trained using different brands of canned fava beans originating in several Middle Eastern countries. The 12 treatments were evaluated by presenting to the panel five canned samples twice a week. One of the five samples was a commercially canned fava bean (control) produced in the United States. The samples were randomly numbered and presented. In addition, the color and texture of the canned beans were instrumentally tested using a Hunter lab color difference meter D25D2 which was calibrated with a white standard C2-3502 and a Lee Kramer Shear Press (L.E.E. Inc.), respectively.

Isolation of Vicine

Vicine was isolated from three samples of fava beans by modifying the procedure of Brown and Roberts (1972) The first sample which consisted of plain dry beans was used as the control. The other two samples were subjected to soaking at 25°C for four hours or twelve hours. Both samples were water-blanching at 82°C for 30 minutes and were dried at 100°C for 60 minutes. All samples were milled in a "UD CORP CYCLON" sample mill. The ground fava beans were mixed with MeOH-H₂O (1:1) in a blender (5 ml/g of seeds), until well mixed. The

Name _____

Date _____

Product: CANNED DRIED BEANS

			Sample
Code			
Size			
Vacuum			
Net Wt. (oz.)			
Drained Wt. (oz.)			
Quality Factors		Score points	
Color	20	A 18-20 B 16-17** SSTD 0-15	
Absence of defects	40	A 36-40 B 32-35* SSTD 0-31*	
Character	40	A 36-40 slightly firm or slightly soft B 32-35* tough or soft SSTD 0-31* hard or mushy	
TOTAL SCORE	100	A 90* B 80*	
Consistency			
Flavor (Odor)			
Similar Varietal Characteristics			
GRADE			
Comments			

*Limiting Rule

**Partial Limiting rule: A score of 16 shall not be graded higher than U.S. Grade B regardless of the total score.

Figure 1. Modified USDA grading sheet for canned dry beans

SCORE

QUALITY FACTORS	GRADE A					GRADE B				
1. <u>COLOR</u>	20	19	18	17	16 ^a					

Uniformity
(% by net weight
of off-color beans) 3.33 6.67 10.0 15.0 20.0

2. DEFECTS

% of net weight	40	39	38	37	36	35	34	33	32
Loose skins, broken, mashed	1	2	3	4	5	6.25	7.50	8.25	10
Total blemished	0	0.75	1.50	2.25	3	3.75	4.50	5.25	6
Materially blemished	0	0.50	1.00	1.50	2	2.50	3.00	3.50	4
Seriously blemished	0	0.25	0.50	0.75	1	1.25	1.50	1.75	2

3. CHARACTER

% by count	40	39	38	37	36	35	34	33	32
Number of beans based upon 50 beans	1	2	3	4	5	6.25	7.50	8.75	10
Definition:	0.5	1	1.5	2	2.5	3.125	3.75	4.125	5
						Slightly soft or slightly firm; tender skins			
						Presence of hard or mushy beans do not materially affect; slightly tough skins			

^aBeans scoring 16 points cannot be graded higher than U.S. Grade B.

Figure 2. Objective measurement equivalents for quality grade scores

slurry was boiled at 100°C for five minutes in a water bath and then centrifuged at 5,000 RPM for 10 minutes. The supernatant was combined and filtered twice, first using a Buchner Funnel with Whatman No. 41 or No. 42 filter paper, followed by a second filtration with a millipore filter (pore size 0.45 μm). The filtrate was concentrated in a vacuum evaporator at 37°C to dryness. The concentrate was dissolved in distilled water (5 ml/g fr wt extracted) and the pH was adjusted 7.6 with dilute ammonia. A column (1 cm diameter) of Dowex 1 (formate; X8; 200-400 mesh) was used to purify the extract. One ml of resin was used per gram of fresh weight of tissue extracted. The resin was combined with water to form a slurry and was poured into the column. The resin was washed with water (5X bed vol) and it was then activated by washing with 100 ml of distilled water followed by 100 ml of 1 N HCl. The column was rewashed with distilled water until the pH started to increase. An air pump (Beckmann Accu-Flo Pump, 3863) was used to accelerate the percolation process. The sample was concentrated again in a vacuum evaporator at 37°C and weighed. This represented the total weight of crude vicine (W_1). The crude vicine was redissolved in 5 ml of distilled water. Two equal samples were taken from the above solution. The first sample was spotted on a thin layer chromatography (TLC) plate (Randerath, 1962, 1965) using cellulose MN300 (Sigma cell type 100, Sigma Chemical Company). The plates were developed with methanol:HCl:H₂O (70:20:10) as the mobile phase and Adenosine

was used as a standard. The R_f 's were determined and compared to an R_f of .43 (Jamalian et al., 1976) for vicine. The spots corresponding to an R_f of .43 were scrapped off, dissolved in 5 ml of distilled water, filtered using a glass filter, dried and weighed (w_1). The dried sample (w_1) was redissolved in 3 ml distilled water. The clear filtrate containing vicine was scanned in a 1 cm quartz cell between 185-360 nm using a Beckmann DK-IIA to verify the presence of vicine.

The second sample was dried and weighed (w_2). Percent vicine in the latter was obtained using equation 1. The total vicine weight in the crude vicine sample was calculated through equation 2. Hence the total percent vicine in the total sample can be derived from equation 3.

$$\% \text{ pure vicine in sample II} = \frac{w_1}{w_2} \times 100 \quad (1)$$

$$\text{Total vicine weight in crude vicine sample} = W_1 \times \left(\frac{w_1}{w_2} \times 100 \right)$$

$$\text{Total \% vicine in the total sample} = \frac{W_1 \times \left(\frac{w_1}{w_2} \times 100 \right)}{\text{g of ground beans used (dry wt basis)}} \quad (2)$$

$$= \frac{W_1 w_1 \times 100}{w_2 \times \text{g of ground beans (dry wt basis)}} \quad (3)$$

Microbiological Examination of Unprocessed and Processed Fava Beans

The objective of this experiment was to test the efficiency of the different treatments (Table 2) in controlling bacterial growth at different stages of processing. To achieve this goal, spore suspensions of Clostridium sporogenes (PA 3679) and Bacillus stearothermophilus (NCA 1518) cultures were obtained from the Department of Food Technology, Iowa State University, Ames, Iowa.

Production of bacterial spores

A spore suspension of the putrefactive anaerobe Clostridium sporogenes (PA 3679), which is a mesophile, was prepared by inoculating 2.0-5.0% of the vegetative cells into a trypticase soy broth (BBL). The latter was incubated at 37°C for 24-48 hours .

Ten percent of the broth was inoculated into the sporulation medium which contained 3.0% trypticase (BBL), 0.1% yeast extract (Difco) and 1.0% ammonium sulfate. The pH was adjusted to 7.3 using 5 N sodium hydroxide. The inoculated flasks were autoclaved at 121°C for five minutes and incubated at 37°C for approximately 48 hours. When 95% or more of the cells sporulated, as estimated from microscopic examination, the spores were harvested in a refrigerated centrifuge at 7,000 RPM (Sorvall RC2B) for 10 minutes. They were then washed three times in sterilized, deionized distilled water and stored at 5°C for at least 48 hours to allow

for some lysis of remaining vegetative cells and detachment of vegetative materials. The spores were washed again and resuspended in sterilized, deionized distilled water as a concentrated spore suspension, which was used for the production of more spores.

The second spore suspension consisted of the flat sour organism Bacillus stearothermophilus (NCA 1518), which is a thermophile. It was prepared by inoculating 5.0% of the spores or the vegetative cells with trypticase soy broth (BBL) and incubated for 24 hours at 45°C. Meanwhile, tomato juice agar (Difco) was prepared in glass trays (pyrex, 8 in. x 13 in.) covered with aluminum foil. The broth was spread over the surface of the tomato juice agar. It was then incubated at 45°C for 18-28 hours, which was enough time to harvest the spores. The spores were harvested by transferring the broth which was poured on the surface of the tray to a flask. A small amount of sterilized, deionized distilled water was added, mixed with the broth, and centrifuged at 7,000 RPM (Sorvall RC2B) for 15 minutes. The supernatant was removed. Sterilized, deionized distilled water was added again and the centrifugation was repeated for two to three times, each time for five to ten minutes. The supernatant was collected and the precipitate was resuspended in sterilized, deionized distilled water, dispersed in bottles and stored at 5°C.

The number of spores in the final suspensions were estimated by direct microscopic count using the Petrof-Hauser

Bacterial counter.

Inoculation of spores onto the fava beans

Samples to be subjected to each of the 12 treatments described in Table 2 were inoculated with spores of PA 3679 and NCA 1518. The number of spores of the two test organisms inoculated per treatment was 10,000. The usual volume of the inoculum per sample was 1.0 ml. The samples were shaken well to uniformly distribute the spores. The water solutions of the beans were tested after soaking and blanching. However, after canning, samples from each treatment were blended (in a Waring Blender) before testing for bacterial growth. Appropriate dilutions in 0.1% Bacto peptone (Difco) were plated on dextrose tryptone agar (Difco) and thioglycolate media which were used for growing NCA 1518 and PA 3679, respectively, for spore production. PA 3679 plates were incubated at 37°C under anaerobic conditions using GasPak anaerobic system (BBL). As for the NCA 1518 plates, they were incubated at 55°C. The time of incubation for both test organisms was 48 hours (National Canners Association Research Laboratories, 1968).

Heat transfer/thermal death time study

Additional samples were inoculated with 10,000 spores of PA 3679 and NCA 1518. The samples were subjected to treatments 1, 4, 9, and 11 as described in Table 2. The samples were then placed in No. 2 clean cans with holes

punched in the side halfway between center point and 3/4 inch from bottom. The holes were fitted with stoppers. A solution of 2.0% sodium chloride was added and the cans were exhausted and sealed. Calibrated copper constantan thermocouples connected to a potentiometer (Lee and Northrup, 8690-2 millivolt) were inserted in place of the plain stopper. The cans were then placed in the autoclave at 115.5°C (240°F) for 40 minutes and readings of the internal can temperatures were taken every half a minute for the first two minutes and every minute thereafter to determine the heat penetration curve. Come-up times (CUT) were recorded for each can. The sterilizing value (F_0) was calculated as a safety measure prior to opening the processed cans (National Canners Association Research Laboratories, 1968). All aseptic precautions were followed.

Statistical Analyses

Analyses of variance were run to test for statistically significant differences between treatments in the water imbibition experiments. In the quality evaluation experiment, multiple regression and Duncan's multiple range test were used (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Chemical Composition

There was a slight variation in the chemical composition between the variety used in this study (Table 3) and those listed in Table 1. All the varieties in the literature showed adequate protein percentage which ranged from 24.7 to 32.8%. The variety analyzed in this study yielded an average protein percent of 28.6 which falls within the range found by others. Yet, the analysis of the commercial canned bean samples resulted in only 18.02% protein. This difference could be explained by the effects of processing which may have caused protein leaching and degradation. The effect of the storage temperature and the physical condition of the bean on the chemical composition is given in Table 3. At a storage temperature of 25°C the moisture percentage ranged from 6.4 to 9% which compared favorably with the percentages found in the literature. At 3°C, the range was 12.27 to 14% moisture. These results showed that a decrease in storage temperature caused an increase in the moisture content of the beans. This could be explained by a higher humidity in the cooler than that at room temperature. As for the physical condition of the bean, the data showed that there was no significant difference in the moisture content of whole dry beans and that of cracked or finely ground beans. The lipid content of our variety was lower than those reported in the literature. This

Table 3. The effect of the storage temperature on the chemical composition of dry fava beans (dry weight basis)

Material	Storage temp. °C	Moisture	Total protein	Lipid	Ash
		-----%-----			
Whole dry beans	3	12.27	28.7	0.80	2.804
Cracked dry beans	3	14.00		0.82	
Whole dry beans	25	7.00	29.17	0.83	2.82
Cracked dry beans	25	9.00	28.03	0.86	
Finely ground dry beans	25	6.40			
Commercial canned beans		56.69	18.02		

characteristic is beneficial because it decreases the development of rancidity which is a frequent problem in beans. The ash content values which ranged from 2.80 to 2.82% are lower than those cited in Table 1 with the exception of the Afghanistan variety (2.7%).

Preliminary Studies on Soaking and Blanching

Soaking experiment

The effect of steeping fava beans in tap water at 25, 37 and 50°C on the percent water uptake is shown in Figure 3.

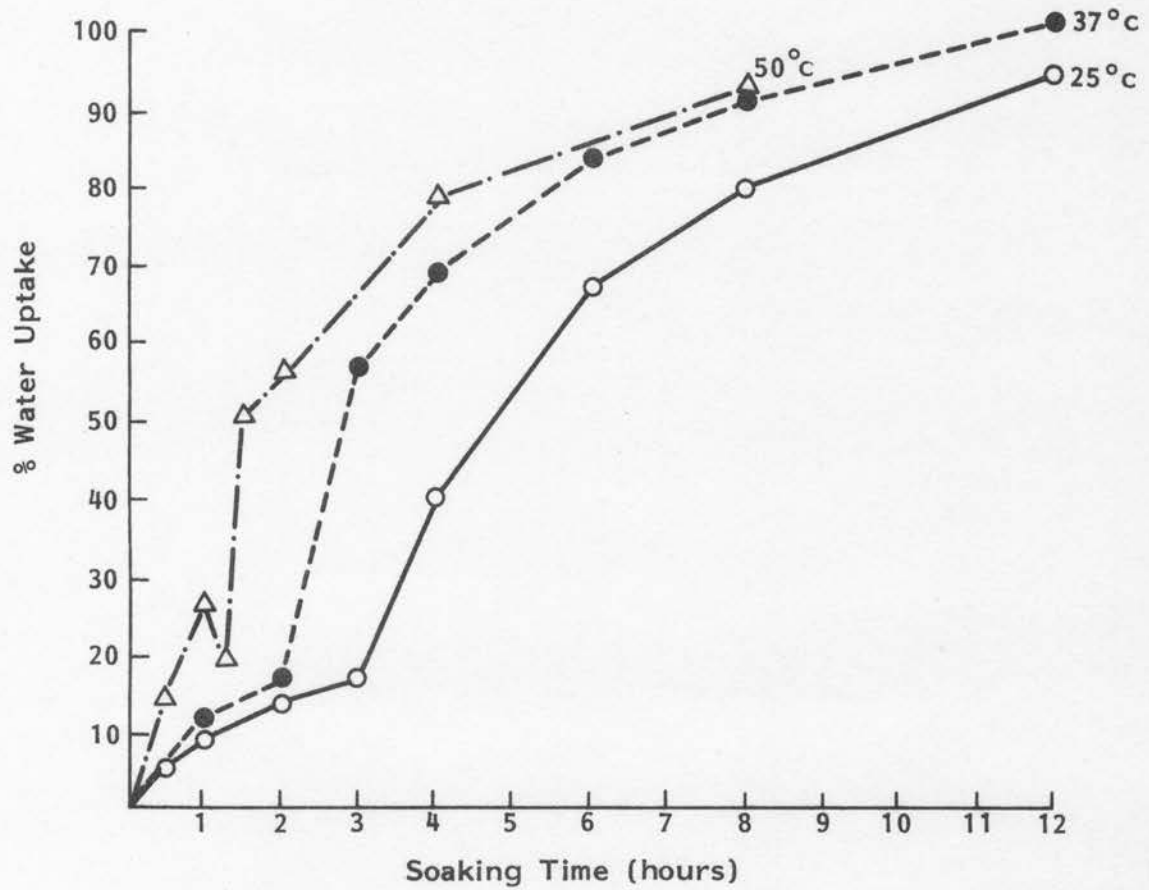


Figure 3. The effect of soaking time and temperature on percent water uptake

Figure 4a illustrates the general appearance of the dry beans. Figures 4b, c, and d are examples of the beans soaked at 25°C for 2, 4, and 8 hours, respectively, while Figures 4e, f, and g show the beans which were soaked at 37°C for 2, 4, and 8 hours, respectively.

The analysis of variance (Appendix A) showed a significant interaction between the effect of time and that of temperature on the beans. At the same soaking temperature, the percent water uptake increased with increasing soaking time. According to the Food and Drug Administration and the National Food Processors Association, dry beans should maintain a moisture level of 55% before canning (National Food Processors Association, Washington, D.C., personal communication, 1979). To reach this level, it was observed that an increase of the soaking temperature reduced the soaking time. For example, increasing the soaking temperature from 25 to 50°C reduced the soaking time required to reach the 55% level from about 5 hours to approximately 2½ hours. The shorter times are preferred to prevent or reduce bacterial growth. In addition, when soaking beans in different kinds of solutions, the decorticated beans resulted in the highest water uptake as compared to whole beans (Abou-Dheir and Wilson, 1979). Our data agree with those of Shafica et al. (1975) who reported the same effects on 4 other fava bean varieties. They also found that cotyledons imbibed water at a faster rate than whole seeds at the same soaking temperature. These results show the importance of the role played

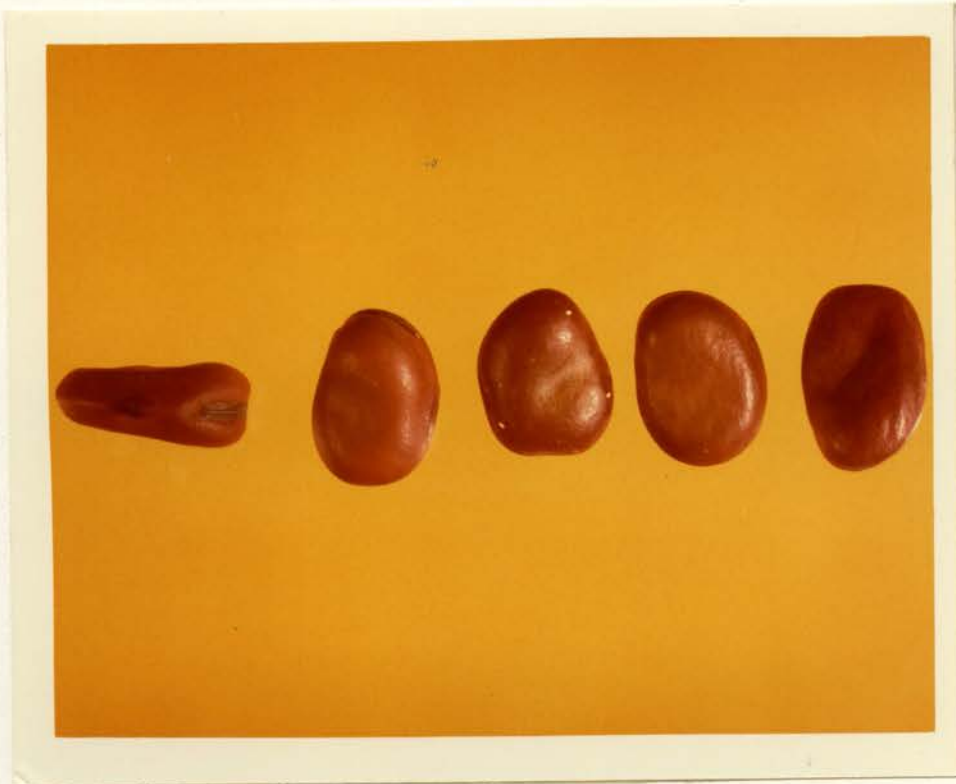


Figure 4a. General appearance of the dry fava beans used in this study



Figures 4b,c,d. Examples of the fava beans soaked at 25°C for 2, 4 and 8 hours, respectively (refer to the slides in Appendix D for proper color representation)



Figures 4e,f,g. Examples of the fava beans soaked at 37°C for 2, 4 and 8 hours, respectively (refer to the slides in Appendix D for proper color representation)

by the seed coat in water imbibition. A common problem encountered in the processing of legumes in general and dried beans in particular is the "hard shell" phenomenon (Hollingsworth, 1972). This problem creates a differential in percent water uptake due to differences in the nature of the bean seed coat. Hard-shelled beans will not imbibe water and will not soften adequately during soaking, blanching and processing.

Water blanching experiment

In this experiment, preliminary observations of the color and texture of the beans led us to eliminate the 60 and 93°C water blanching temperatures because the former temperature resulted in a positive peroxidase test in most samples and the latter caused darkening of the beans, thus lowering the quality. This left us with the water blanching temperature of 82°C which became a constant variable applied to different treatments with varying soaking times and temperatures and different blanching times.

The mean percent water uptake values for the different treatments applied in Group I of the water blanching experiment are presented in Table 4. When soaking for one hour at 25, 37, and 50°C followed by water blanching at 82°C for 45 minutes, the percent water uptake by the beans stayed around the 50 to 55% level, except at 37°C where it was 31%. This is probably due to the fact that the batch of beans was hard-shelled. The data indicate that there is a minimum time

Table 4. The effect of water blanching at 82°C for 30, 45 and 60 minutes on the percent water uptake with different soaking times and temperatures

Soaking temperature °C	Soaking time -----minutes-----	Blanching time	N	Percent water uptake
25	1	30	3	45.36
25	1	45	3	56.48
25	1	60	3	71.30
25	4	30	3	45.23
25	4	45	3	59.05
25	4	60	3	69.22
25	8	30	3	83.03
25	8	45	3	90.22
25	8	60	3	94.69
25	12	30	3	105.59
25	12	45	3	88.97
25	12	60	3	120.04
37	1	30	3	55.96
37	1	45	3	31.57
37	1	60	3	56.28
37	4	30	3	61.59
37	4	45	3	77.67
37	4	60	3	86.63
37	8	30	3	99.26
37	8	45	3	98.41
37	8	60	3	103.24
37	12	30	3	101.54
37	12	45	3	106.24
37	12	60	3	102.32
50	1	30	3	40.16
50	1	45	3	50.98
50	1	60	3	67.47
50	4	30	3	84.00
50	4	45	3	88.75
50	4	60	3	96.93
50	8	30	3	95.66
50	8	45	3	90.87
50	8	60	3	91.39
50	12	30	3	91.36
50	12	45	3	104.16
50	12	60	3	92.07

requirement in soaking of the beans independent of the soaking temperature. This is illustrated by the fact that as soaking time is increased to 4, 8, or 12 hours, other variables being constant, the percent water uptake remained much higher than the 55% level.

High soaking temperatures in turn could be a limiting factor which could affect quality. For example, soaking at 50°C produced lower quality beans due to the rupture of the seed coat and thus would result in a mushy product after canning. At this stage, all variables which resulted in a negative percent water uptake were excluded from subsequent experiments.

All of these conclusions are verified by the analysis of variance which showed that the single most important factor was soaking time (SKTI) (Appendix B). Soaking temperature had little influence while blanching time had some effect. As was found in the soaking experiment, there was a significant interaction between the soaking time and soaking temperature. Yet, there was no interaction between blanching and either soaking time or soaking temperature.

Steam blanching experiment

The effect of steam blanching at 95°C for three minutes on the percent water uptake with different soaking times and temperatures is shown in Figure 5. There was no significant difference between the percent water uptake of steam blanching at 25°C and that at 37°C for those samples subjected to two

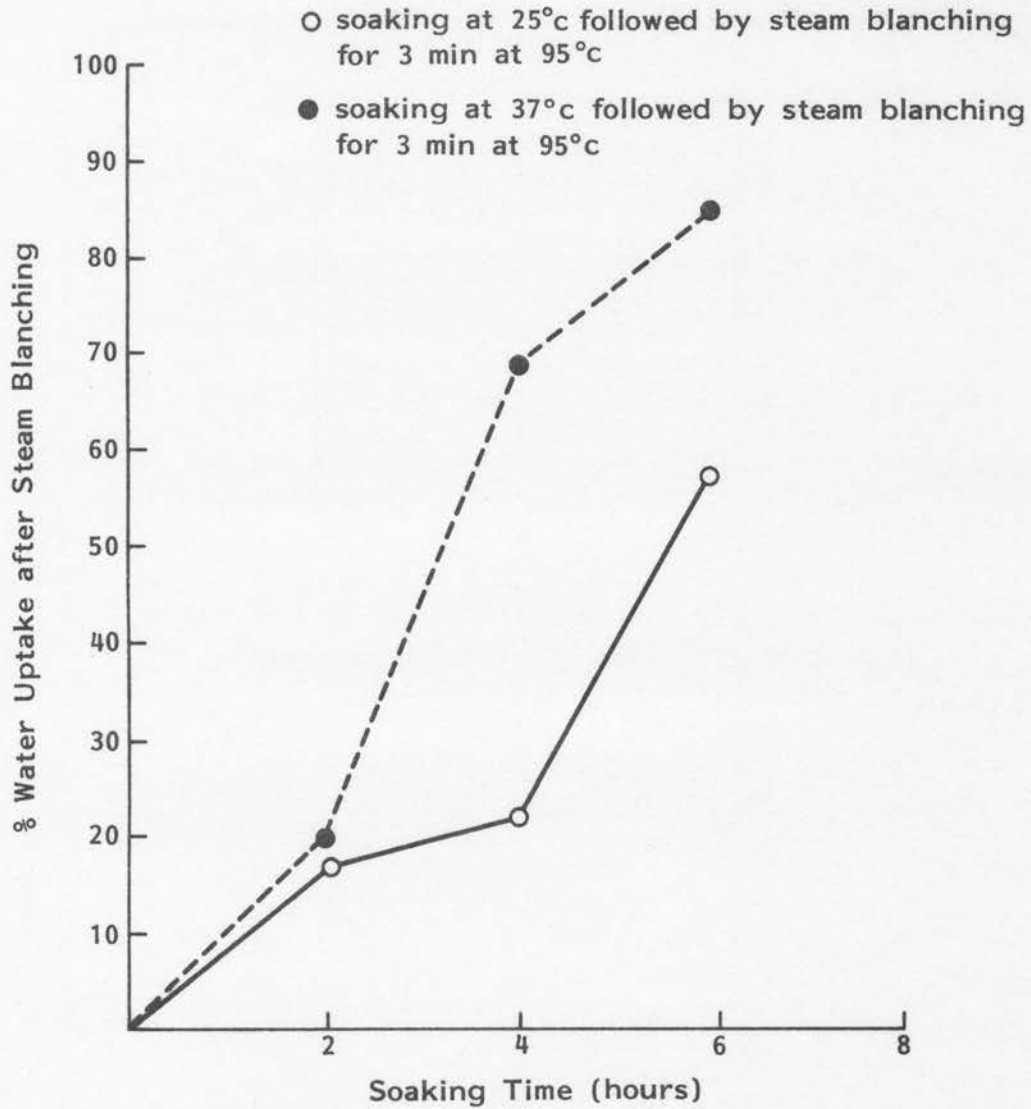


Figure 5. The effect of steam blanching at 95°C for 3 minutes on the percent water uptake with different soaking times and temperatures

hours or less of soaking. However, with longer soaking times a sharp difference in percent water uptake was observed between the two soaking temperatures. The analysis of variance (Appendix A) showed a significant interaction between the soaking time and the soaking temperature on the percent water uptake of the steam blanched beans. For example, if the soaking temperature is increased from 25 to 37°C, the soaking time to reach the 55% level of water uptake is reduced from about five hours to approximately three hours. The quality of the beans under both temperature regimes was acceptable. The advantage of steam blanching for three minutes at 95°C is to inactivate the enzymes and to preserve the firmness of the beans.

Continuous water blanching experiment

The effect of continuous water blanching time and temperature on percent water uptake is described in Figure 6. According to the analysis of variance (Appendix A) there was a significant interaction between the effects of time and temperature on the percent water uptake. In addition, as time and temperature of blanching increased there was a significant increase in the percent water uptake. The samples that were blanched at 82°C for different times yielded the best results as far as color and texture. Moreover, the samples subjected to 82 and 93°C needed approximately the same time (60 minutes) to reach the 55% water uptake level. Hence, 82°C was considered optimum temperature to conserve energy and to maintain good quality

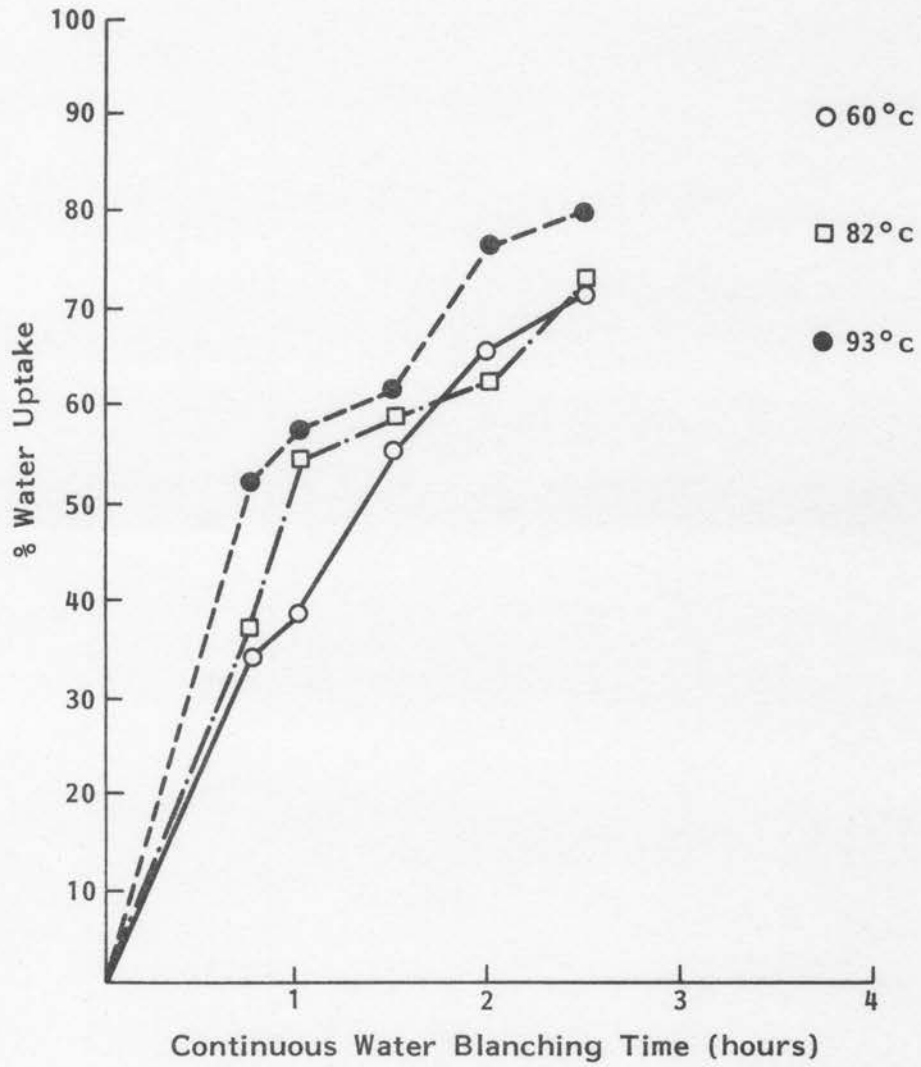


Figure 6. The effect of continuous water blanching time and temperature on percent water uptake

of the beans. The beans that were water blanched at 60°C were eliminated due to their inability to imbibe water, soften and inactivate the enzyme peroxidase.

Quality Evaluation of the Canned Beans

Sensory evaluation

The canned samples that were subjected to the 12 treatments (Table 2) were visually inspected by the five trained members of the panel. The wholeness of the canned beans was rated highly by the panel. Since the rupture of the seed coat across the hilum occurs normally in the processing of dried beans (Figure 7), and as long as the beans retained their shape and content they were judged acceptable. Figure 8 shows that some of the broken beans started to germinate when subjected to long soaking times (12 hours). The consistency of the canned beans was graded "A" by the panel for all treatments, except for treatment 12 which resulted in grade "B". Grade A was an indication of a slightly viscous and clear fluid which separated and drained readily from the bean. This means that the starch-protein matrix of the cotyledons did not rupture and release starch granules into the external fluid. Grade B meant that the brine was reasonably smooth but might have been slightly grainy or slightly lumpy (USDA, 1975). Treatment 12 probably received a "B" grade due to the beans being continuously water blanched for one and one-half hours prior to canning. This blanching time



Figure 7. Examples illustrating the rupture of the seed coat across the hilum under the effect of processing (refer to the slides in Appendix D for proper color representation)



Figure 8. Sprouting in broken beans under the effect of long soaking time (refer to the slides in Appendix D for proper color representation)

period could have caused a slight rupture in the starch-protein matrix. The panel scored all the treatments as having good flavor (Grade A) except for treatment 8 which resulted in an undesirable flavor. This could be accounted for by the long soaking time (12 hours) before canning.

Upon analyzing the data of the quality evaluation, it was found that only the mean color score and the character A of the beans had an effect on treatment (Table 6). However, the visual examination showed a treatment effect on other attributes like loose skin and texture. The following discussion will only include those effects between which significant correlations existed. Correlations between the different processing variables are outlined in Table 5. There was a strongly positive interaction ($r = +0.95$) at the 1% level between soaking time and soaking temperature, while that between soaking time and blanching time was moderately positive ($r = +0.43$). However, soaking time and blanching temperature were negatively correlated ($r = -0.49$) indicating an inverse relationship between them. In addition, blanch time correlated negatively with blanching temperature ($r = -0.73$) at the 1% level. Finally, there were positive significant interactions between the overall treatment effect and the soaking temperature, soaking time and the blanching time. There was no correlation between the blanching temperature and the treatment effect which could be attributed to the fact that the former was constant (82°C) for most of

Table 5. Correlations between the different processing variables

Processing variables	Soaking temperature	Soaking time	Blanching temperature	Blanching time	Treatment
Soaking temperature	---	+0.95981**	-0.42697*	NC ^a	+0.72743**
Soaking time	+0.95981**	---	-0.49269*	+0.43535*	+0.74550**
Blanching temperature	-0.42597*	-0.49269*	---	-0.73339**	NC
Blanching time	NC	+0.43535*	-0.73339*	---	+0.46339*
Treatment	+0.72743**	+0.7455**	NC	+0.46339*	---

^aNC = no correlation.

*Significance at the 5% level.

**Significance at the 1% level.

Table 6. Correlations between the processing variables and the sensory quality attributes

Quality attributes	Processing variables				Treatment
	Soaking temperature	Soaking time	Blanching temperature	Blanching time	
Net weight	NC ^a	NC	NC	NC	NC
Drain weight	NC	NC	NC	NC	-0.44185*
Loose skin score	NC	NC	NC	NC	NC
Character A	NC	NC	NC	NC	+0.43428*
Character B	NC	NC	NC	NC	NC
Character C	NC	NC	NC	NC	NC
Character score	NC	NC	NC	NC	NC
Color score	-0.53730**	-0.56160**	+0.60921**	-0.913494**	-0.53405**
Total score	NC	NC	NC	NC	NC

^aNC = no correlation.

*Significance at the 5% level.

**Significance at the 1% level.

the treatments.

Correlations between the processing variables used and the sensory quality attributes are presented in Table 6. There was no correlation between the soaking temperature and either of the net weight, the drain weight, the loose skin score, the character score, or the total score. Yet, there was a significantly negative correlation ($r = -0.53$) at the 1% level between the color score and the soaking temperature. The same results and effects were found between soaking time, blanching time and the quality attributes. As for the blanching temperature, there was a positive correlation ($r = +0.60$) with the color score. This indicates that the blanching temperature is important to yield a good bean color.

The mean values for the color score from the Duncan multiple range test (Table 7) showed that treatment 8 resulted in the best color (color score = 20.00). Yet, the latter was not significantly different from treatments 5 and 7 (color score = 19.00). In addition, treatment 13 which consisted of the commercial canned beans was lighter in color than our variety. It yielded the lowest score (10.70) which could explain the negative correlation ($r = -0.534$) between the effect of treatments and the color score. In conclusion, the total scores (Table 8) of the quality evaluation of the canned beans indicated that three treatments yielded the best results:

Table 7. Treatment effects on visual color scores, shear press values and Hunter color difference meter readings¹

Treatment number	Mean color score	Mean shear press lb/100 g	Color difference meter	
			Mean L ²	Mean b ³
1	17.50bcd	76.50abc	19.80cd	6.40cd
2	17.20cd	69.50abc	20.05cd	6.20cd
3	18.35bc	85.50abc	20.05cd	6.55bcd
4	18.00bcd	70.00abc	21.65cd	7.10bc
5	19.00ab	76.50abc	21.20bc	7.00bcd
6	18.00bcd	76.50abc	21.50bc	6.10cd
7	19.00ab	58.00abc	22.80b	7.60bc
8	20.00a	54.00abc	23.00b	8.00b
9	17.30cd	77.50abc	20.60bc	6.90bcd
10	17.30cd	110.75a	18.30d	5.65d
11	17.00cd	58.25bc	22.65b	7.20bc
12	16.50d	92.50ab	19.65cd	6.75bcd
13	10.70e	41.60c	29.95a	10.20a

¹Different letters denote significance at the 5% level by the Duncan's multiple range test.

²Lightness (white - 100) and darkness (black - 0).

³Blueness (-) and yellowness (+).

Table 8. Grading of canned dried fava beans subjected to different soaking and blanching treatments

Treat- ment no.	Repli- cate no.	Soak temp. (°C)	Soak time (hr)	Blanch type	Blanch temp. (°C)	Blanch time (min)	Absence of			Total score	Grade
							defect score	Character score	Color score		
1	2	25	4	WB	82	30.00	28.0	26	17.50	71.50	sstd
2	2	25	4	WB	82	50.00	28.5	34	18.35	80.85	sstd
3	2	25	12	WB	82	30.00	31.0	35	19.00	85.00	B
4	2	25	12	WB	82	50.00	27.0	31	19.00	77.00	sstd
5	2	37	4	WB	82	30.00	26.5	25	17.20	68.70	sstd
6	2	37	4	WB	82	50.00	24.5	33	18.00	75.50	sstd
7	2	37	12	WB	82	30.00	30.0	35	18.00	83.00	sstd
8	2	37	12	WB	82	50.00	21.0	35	20.0	76.00	sstd
9	2	25	4	SB	95	03.00	18.0	33	17.30	68.30	sstd
10	2	37	4	SB	95	03.00	26.0	35	17.30	78.30	sstd
11	2			CWB	82	75.00	23.5	35	17.00	75.50	sstd
12	2			CWB	82	90.00	37.0	26	16.50	79.50	sstd
13	6	Commercial can					33.0	32.50	10.50	76.20	sstd

^aWB - water blanching, SB - steam blanching, CWB - continuous water blanching.

Treatment 2: soaking at 25°C for 4 hours + water blanching at 82°C for 50 minutes

Treatment 3: soaking at 25°C for 12 hours + water blanching at 82°C for 30 minutes

Treatment 7: soaking at 37°C for 12 hours + water blanching at 82°C for 30 minutes

The main reason the panel downgraded treatment two and seven is due to loose, mushy and broken skin (Table 8). However, the absence of defect scores proved to be insignificant when analyzing the data statistically. Moreover, the treatment, percent water uptake (Table 4), and the quality of the beans (Table 8) were found to be interrelated. For example, treatments three and seven imbibed a 100% water uptake before canning and resulted in a good quality bean while treatment two had a 60% water uptake before canning and was only five points less in total score. Therefore, treatment two should be subject to more research because it could minimize time, energy and water usage.

Instrumental evaluation

The instrumental quality measurements were tested for any correlation with the processing variables (Table 9). There was no correlation between the soaking time and temperature and the texture of the beans as measured by the Lee Kramer shear press. However, texture was positively correlated ($r = +0.60$) with blanching temperature at the 1% level and negatively correlated with blanching time ($r = -0.49$) at the 5% level. These results imply that the 82°C water blanch-

Table 9. Correlations between the processing variables and the instrumental quality measurements

Quality measurement	Processing variables				Treatment
	Soaking temperature	Soaking time	Blanching temperature	Blanching time	
Texture ^a	NC ^b	NC	+0.60733**	-0.49950*	NC
Color ^c					
L	NC	+0.47342*	-0.78343**	+0.88460**	+0.43836*
a	NC	NC	NC	NC	NC
b	NC	+0.48440*	-0.73828**	+0.84494**	+0.46550*

^a Shear press in lb/100 g.

^b NC = no correlation.

^c Hunter color difference meter.

*Significance at the 5% level.

**Significance at the 1% level.

ing temperature and the 95°C steam blanching temperatures were undesirable to preserve a good texture. Moreover, the implication was that increasing blanching time within the range used in our treatments improved texture. This can be explained by the fact that softer beans are ascribed to lower shear press values. There was no correlation between treatment effect and texture of the bean. This was also evident in Table 7, where the treatments had no significant effect on mean shear press values. Treatment 10 and 13 had the worst textures and were significantly different from the other treatments. Hunter color difference meter (CMD) "L" and "b" readings showed no correlation between soaking temperature and color (Table 9). Yet, soaking and blanching time were positively correlated with CDM "L" and "b" readings. This indicates that the time of blanch is more important than the blanch temperature in relation to the instrumental evaluation of lightness (L) and Yellowness (b). There was an inverse relationship between the blanching temperature and the color of the beans. This is in contradiction with the results obtained in the sensory quality evaluation but could be ascribed to the mean "L" and "b" values of treatment 13 which were significantly different than those of the other treatments (Table 7). Finally, the treatment effect and color were positively correlated.

Correlation between sensory and instrumental evaluation

The color score judged by the panel correlated negatively with the Hunter color difference meter "L" and "b" values (-0.73 and -0.68, respectively) at the 1% level (Table 10). This indicates that as the sensory color score increased (implying a more desirable uniform and dark bean), the instrumental color score values decreased, resulting in a darker bean. A major correlation was found between the Hunter color difference meter "L" and "b" readings (+0.947) at the 1% level. This could be attributed to the breaking of the beans during processing which could have resulted in an increase in lightness and yellowness of the beans. In addition, the "L" and "b" readings were inversely correlated with texture (-0.66 and -0.611, respectively). This is expected in fava beans because dark beans (low "L" and "b" values) were found to be very hard (high spear press values) due to low water imbibition. In contrast, the sensory color score was slightly correlated (+0.374) with texture. The statistical significance of this interaction may be due to the grading panel's preference for a darker colored bean. Thus, the panel was inadvertently selecting firmer beans. The "L" and "b" readings were slightly correlated (+0.457 and +0.397, respectively) with the character A of the beans, indicating a low relationship between instrumental color readings and character A. Therefore, as the beans lightened during processing they softened enough to become grade A quality.

Table 10. Correlation between sensory and instrumental quality measurements

Quality attributes	Character A	Character B	Character C	Color score	Texture (Lee Kramer)	Hunter color difference meter	
						b	a
Character A	-	NC ^a	-0.76**	NC	NC	NC	+0.397*
Character B	NC	-	-0.365*	NC	NC	NC	NC
Character C	-0.76**	-0.365**	-	NC	NC	NC	NC
Color score	NC	NC	NC	-	+0.374*	-	-0.68**
Texture (Lee Kramer)	NC	NC	NC	NC	-	-	-0.611**
Hunter color difference meter							
L	+0.457*	NC	NC	-0.73**	-0.66**	-0.66**	+0.947**
a	NC	NC	NC	NC	NC	NC	NC
b	+0.397*	NC	NC	-0.68**	-0.611**	-0.611**	NC

^aNC = no correlation.

*Significance at the 5% level.

**Significance at the 1% level.

Finally, there was no correlation between texture and the character score under these conditions (Cussler et al., 1977) because no power functions were used in this evaluation. However, since the "L" value was positively correlated to character A and negatively correlated to texture one might expect an existing correlation between character A and texture (Table 10). Further transformation of the data is necessary to define this correlation if it exists.

Isolation of Vicine

Vicine, which is the compound thought to be the main causal agent of favism in fava bean was isolated using a modified version of Brown and Roberts' (1972) method. Even though this method was found to be the most rapid and accurate of the procedures evaluated, it still took from one to two weeks for each analysis. Moreover, the procedure of Lin and Ling (1962a) using HgSO_4 and H_2SO_4 was evaluated but was found to be hazardous, time-consuming and had low yields of vicine. Thus, further work is necessary to develop a more rapid technique. The use of High Performance Liquid Chromatography (HPLC) may help to eliminate this problem. In studying its physical characteristics, vicine was found in the form of uniform needle-shaped colorless crystals (Figure 9). It was also soluble in water, in acid and in alkali compounds. These results agree with those of Lin and Ling (1962a). Physically, vicine can be identified by using spectrophotometry. The



Figure 9. Uniform needle-shaped colorless vicine crystals .

selection of vicine from the whole dry bean sample (control) had a maximum absorption of 275 nm and a minimum of 220 nm when dissolved in water (Figure 10).

Treatments 1 and 2, which consisted of soaking the beans at 25°C for 4 and 12 hours, respectively, followed by water blanching at 82°C for 30 minutes, showed a slightly lower maximum absorption of 270 nm. These discrepancies may be due to the heat treatment the beans received. Yet, the minimum absorption was noticeably longer with a wavelength of 240 nm (Figures 11 and 12).

The maximum absorption spectrum of the control beans compared with Lin and Ling's (1962a) but the minimum wavelength did not. Bendich and Clements (1953) reported a maximum wavelength of 274 nm when dissolved in 0.1 N HCl and a maximum of 269 nm when dissolved in 0.1 N NaOH. The same results were reported by Jamalian et al. (1976).

Thin layer R_f values are normally used to identify unknown compounds by comparing that of the unknown to those of known standards. The standard used in this experiment was Adenosine which has an R_f value of .49. Our measurements showed that increasing the soaking time from 4 to 12 hours slightly decreased the R_f value of vicine from .43 to .40. The reference value for vicine was .43 which was found by Jamalian et al. (1976). The vicine that was isolated from the dry bean sample (control) yielded an R_f value of .44 which was very close to the 4-hour soaking treatment but

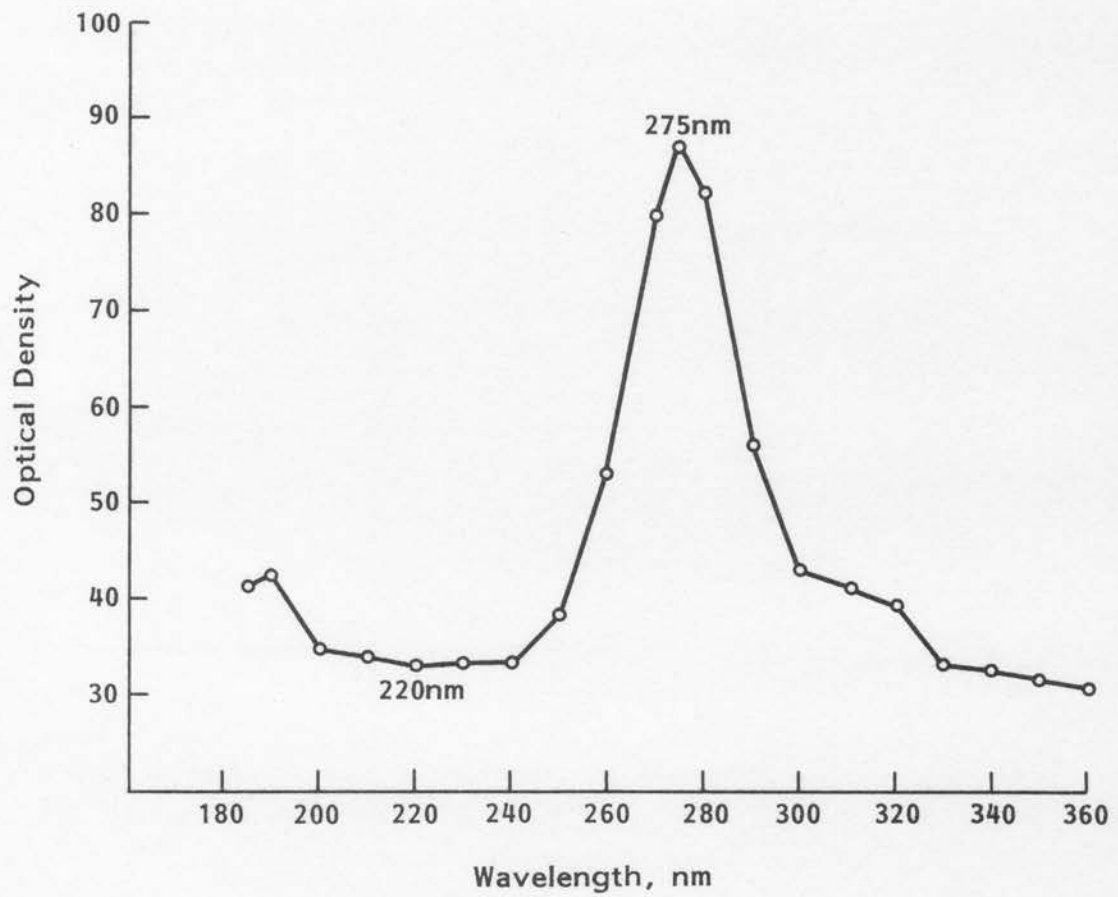


Figure 10. Ultraviolet absorption spectra of vicine for a sample of ground dry beans

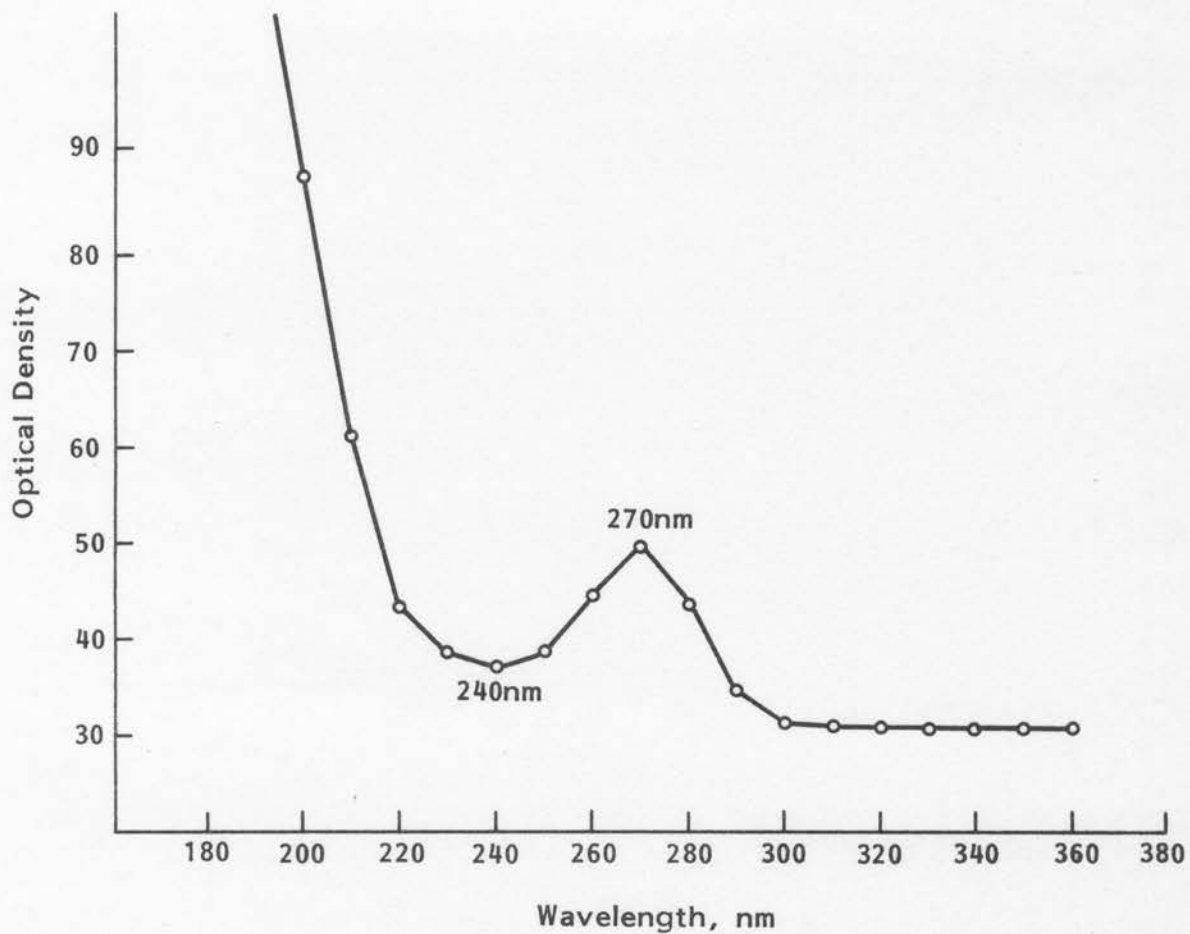


Figure 11. Ultraviolet absorption spectra of vicine for a sample of ground beans subjected to 4 hours of soaking at 25°C and 30 minutes of blanching at 82°C

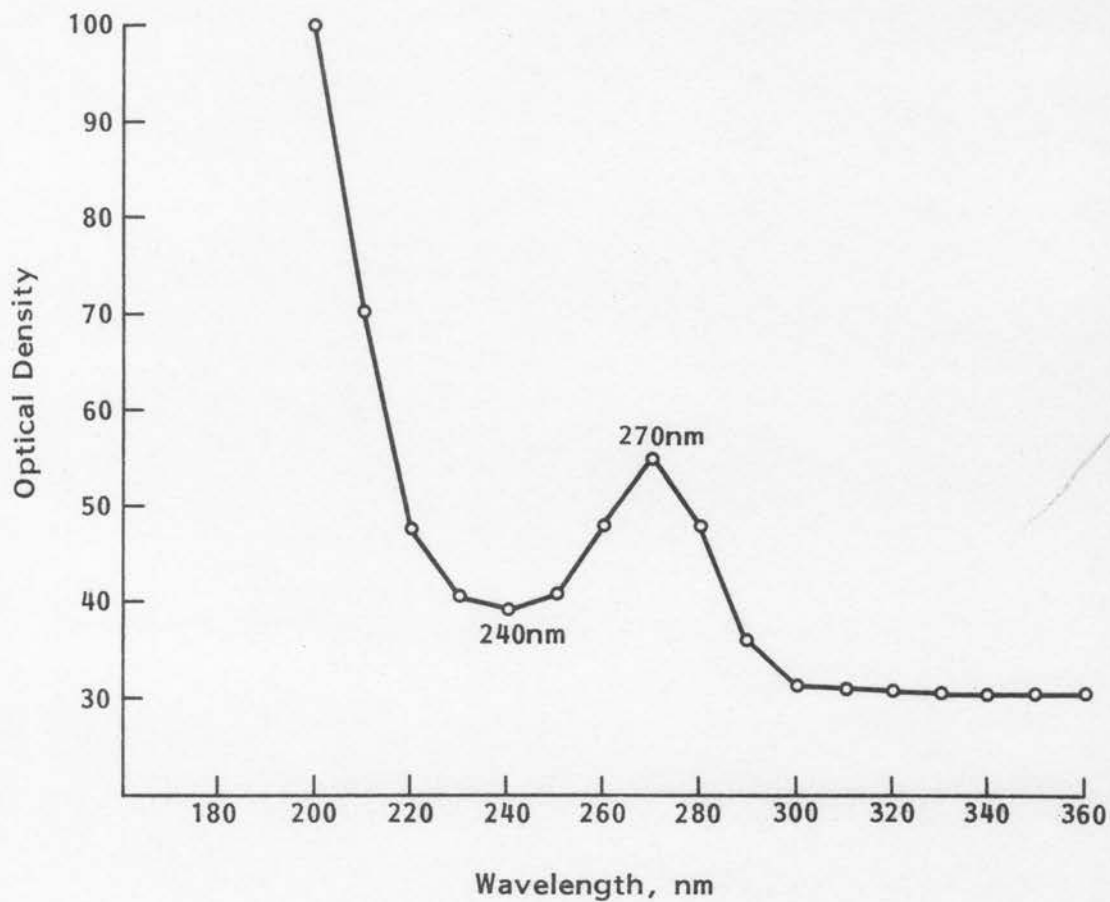


Figure 12. Ultra violet absorption spectra of vicine for a sample of ground beans subjected to 12 hours of soaking at 25°C and 30 minutes of blanching at 82°C

but higher than that of the 12-hour soaking treatment (Table 11). The slight variation in R_f values for vicine and Adenosine in our study compared to the literature value is probably due to temperature, humidity and thin layer chromatography (TLC) plate differences. Using the previous tests as a presumptive identification of the vicine, Figure 13 showed that the percent vicine in the control was significantly higher (fivefold) than that of the treated samples. The difference could be attributed to the processing of the beans (soaking and blanching) which might have caused the leaching of vicine out of the bean and not its destruction. Leaching is more likely since vicine is soluble in water, acid and alkali. Our results agree with those of Lin and Ling (1962a) who detected 2.5% vicine in whole dry beans. Moreover, the percent vicine in our control was close to that reported by Jamalain et al. (1977b) who conceded that differences in percent vicine do exist among known cultivars.

Table 11. Average R_f values obtained from TLC for the vicine and the Adenosine (standard) under different soaking and blanching treatments

Treatment (hours)	R_f Adenosine (standard)	R_f vicine
0	0.48	0.44
4	0.48	0.43
12	0.46	0.40
Literature value ^a	0.49	0.43

^aRanderath (1962); Jamalain et al. (1976).

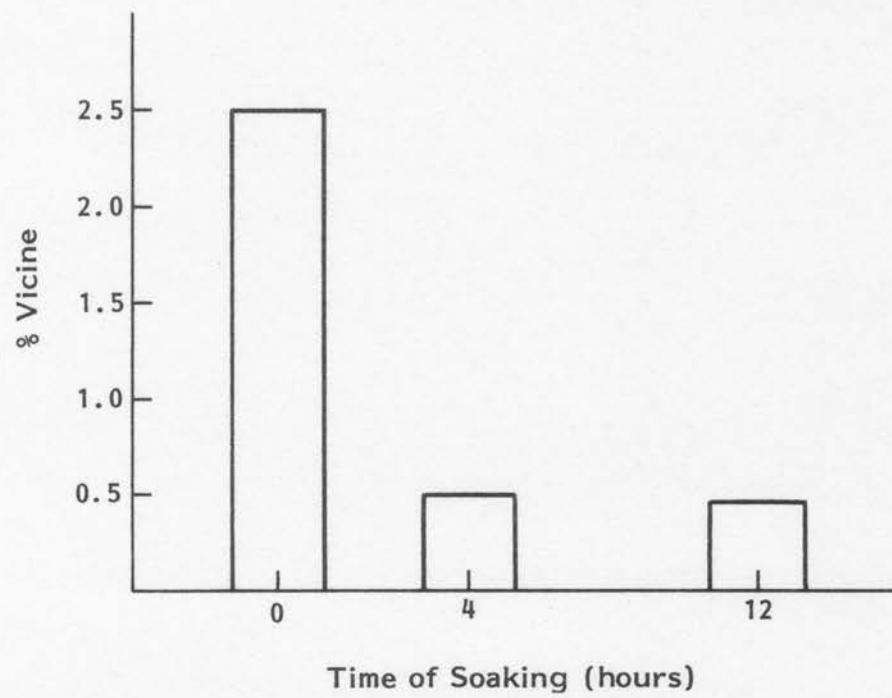


Figure 13. The change in percent vicine with different soaking times at 25°C followed by water blanching at 82°C for 30 minutes

Survival of Thermophillic Organisms

To study the efficiency of the different treatments in controlling bacterial growth at different stages of processing, 10,000 spores of Clostridium sporogenes (PA 3679) and Bacillus stearothermophilus (NCA 1518) were inoculated before soaking or blanching. Figure 14 shows the effect of soaking time and temperature on the number of survivors of NCA 1518 and PA 3679. There was not any significant growth of the two test organisms at the 4-hour soaking time. Yet, there was a noticeable increase in the number of survivors of NCA 1518 at a soaking temperature of 25°C and a soaking time of 12 hours, although NCA 1518 is not expected to grow at a temperature of 25°C. The optimum temperatures for the growth of NCA 1518 is between 45 and 55°C.

After soaking at different times and temperatures, the effect of water blanching at 82°C for 30 minutes on the number of survivors of Bacillus stearothermophilus (NCA 1518) and Clostridium sporogenes (PA 3679) were tested (Figure 15). Compared to the initial inoculation (control), a significant survival in the number of NCA 1518 could be observed after both 4 and 12 hours of soaking at 37°C. This implies that the water blanching temperature of 82°C was high enough to cause some heat activation of the spores resulting in their germination and limited growth.

Studies on the effect of water blanching at 82°C for

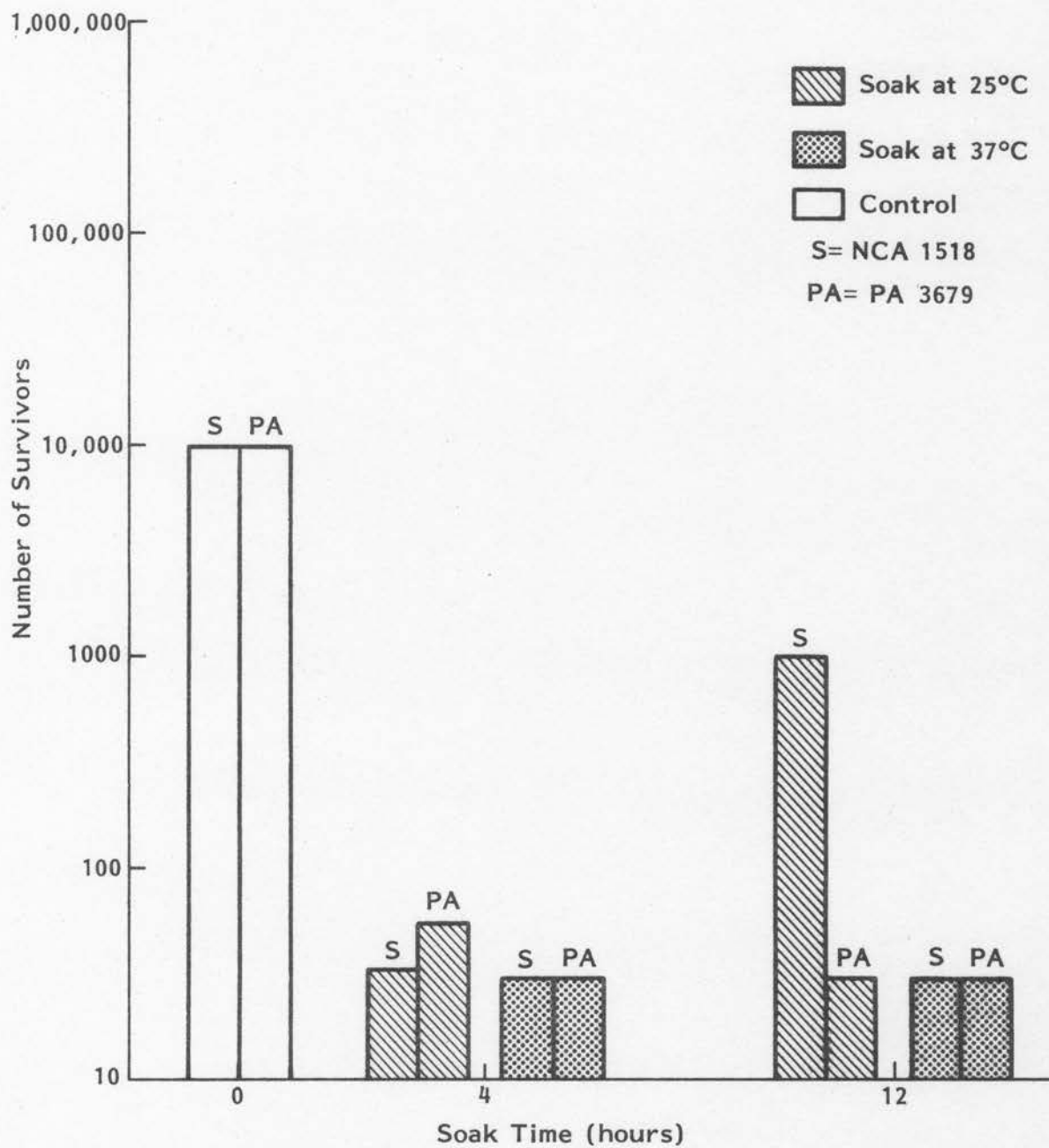


Figure 14. The effect of soaking time and temperature on the number of survivors of *Bacillus stearothermophilus* (NCA 1518) and *Clostridium sporogenes* (PA 3679)

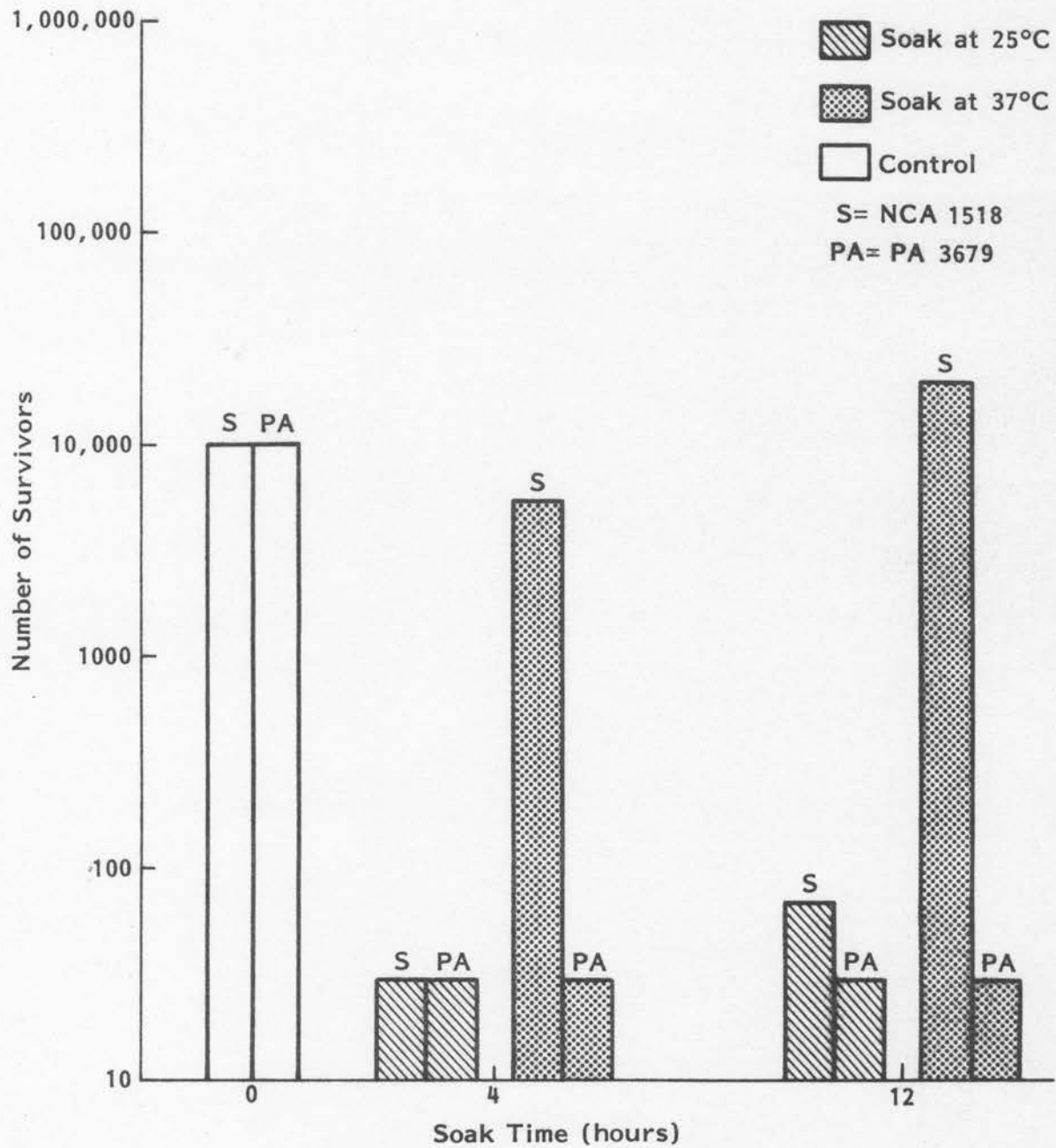


Figure 15. The effect of water blanching at 82°C for 30 minutes on the number of survivors of Bacillus stearothermophilus (NCA 1518) and Clostridium sporogenes (PA 3679) with different soaking times and temperatures

50 minutes on the number of survivors of the two test organisms with different soaking times and temperatures are presented in Figure 16. No significant growth was detected with 4 hours of soaking at both 25 and 37°C. However, there was a tremendous increase in the number of NCA 1518 and PA 3679 after 12 hours of soaking at 37°C compared to the beans soaked at 25 and 37°C for 4 hours. This could be explained by the fact that the test organisms have produced spores in the soaking solution and after exposure to a blanching temperature of 82°C for 50 minutes they were heat activated and subsequently germinated. This is further confirmed by the sensory evaluation results where the sensory panel detected off odors.

The number of survivors of the two test organisms was also determined in beans soaked at different times and temperatures after steam blanching at 95°C for 3 minutes (Figure 17). No growth was obtained for both test organisms as compared to the initial inoculation (control). Although PA 3679 exhibited a slight ability to grow, it was insignificant and could be considered within the experimental error range. Hence, steam blanching at 95°C for 3 minutes seems to be a safe procedure in controlling heat resistant bacteria.

The effect of continuous water blanching at 82°C on the number of survivors of NCA 1518 and PA 3679 (Figure 18) produced increased growth of the two test organisms with a blanching time of 75 minutes. Yet, after 90 minutes, growth of NCA 1518 was insignificant, while that of PA 3679 was detectable.

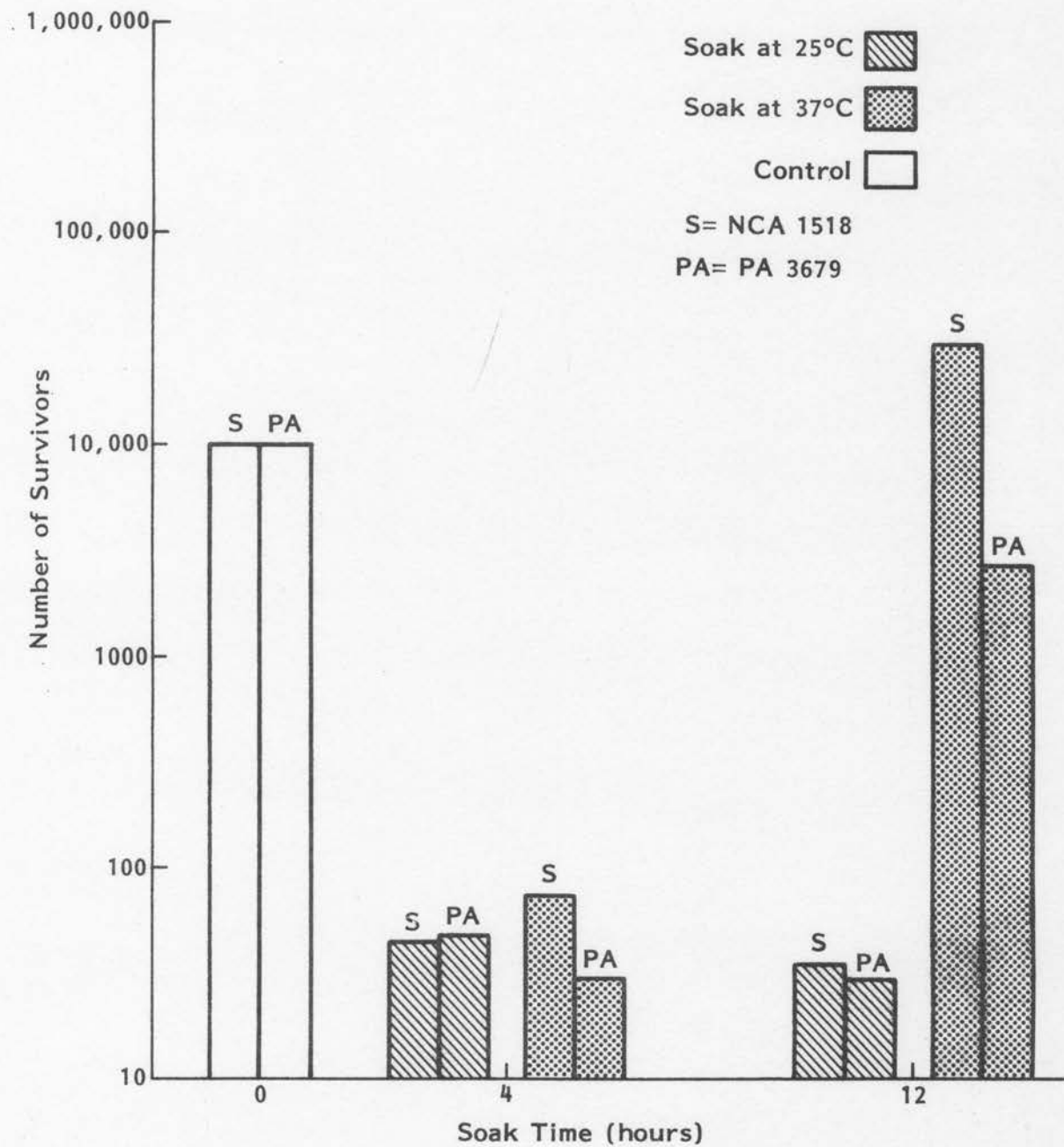


Figure 16. The effect of water blanching at 82°C for 50 minutes on the number of survivors of *Bacillus stearothermophilus* (NCA 1518) and *Clostridium sporogenes* (PA 3679) with different soaking times and temperatures

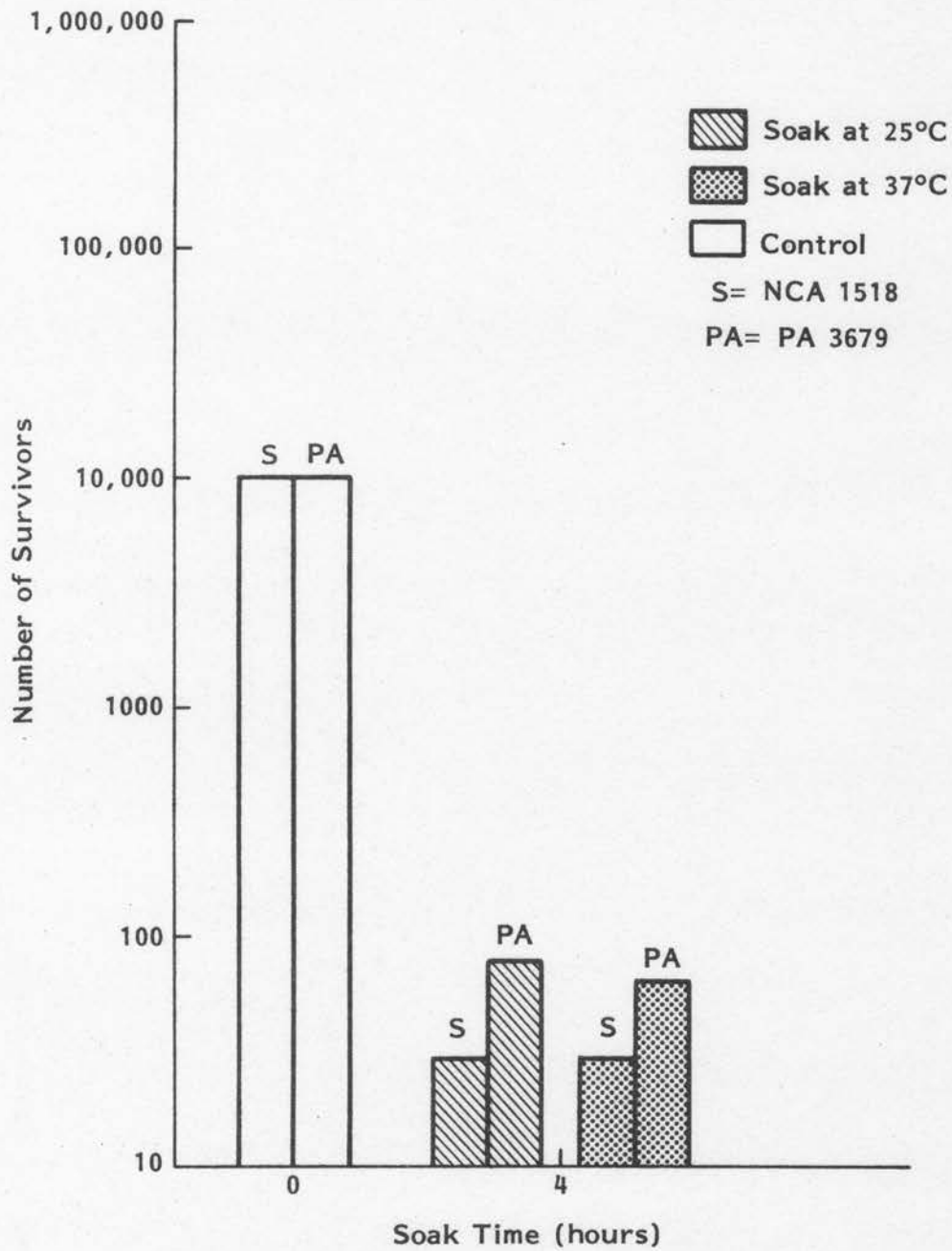


Figure 17. The effect of steam blanching at 95°C for 3 minutes on the number of survivors of *Bacillus stearothermophilus* (NCA 1518) and *Clostridium sporogenes* (PA 3679) with different soaking times and temperatures

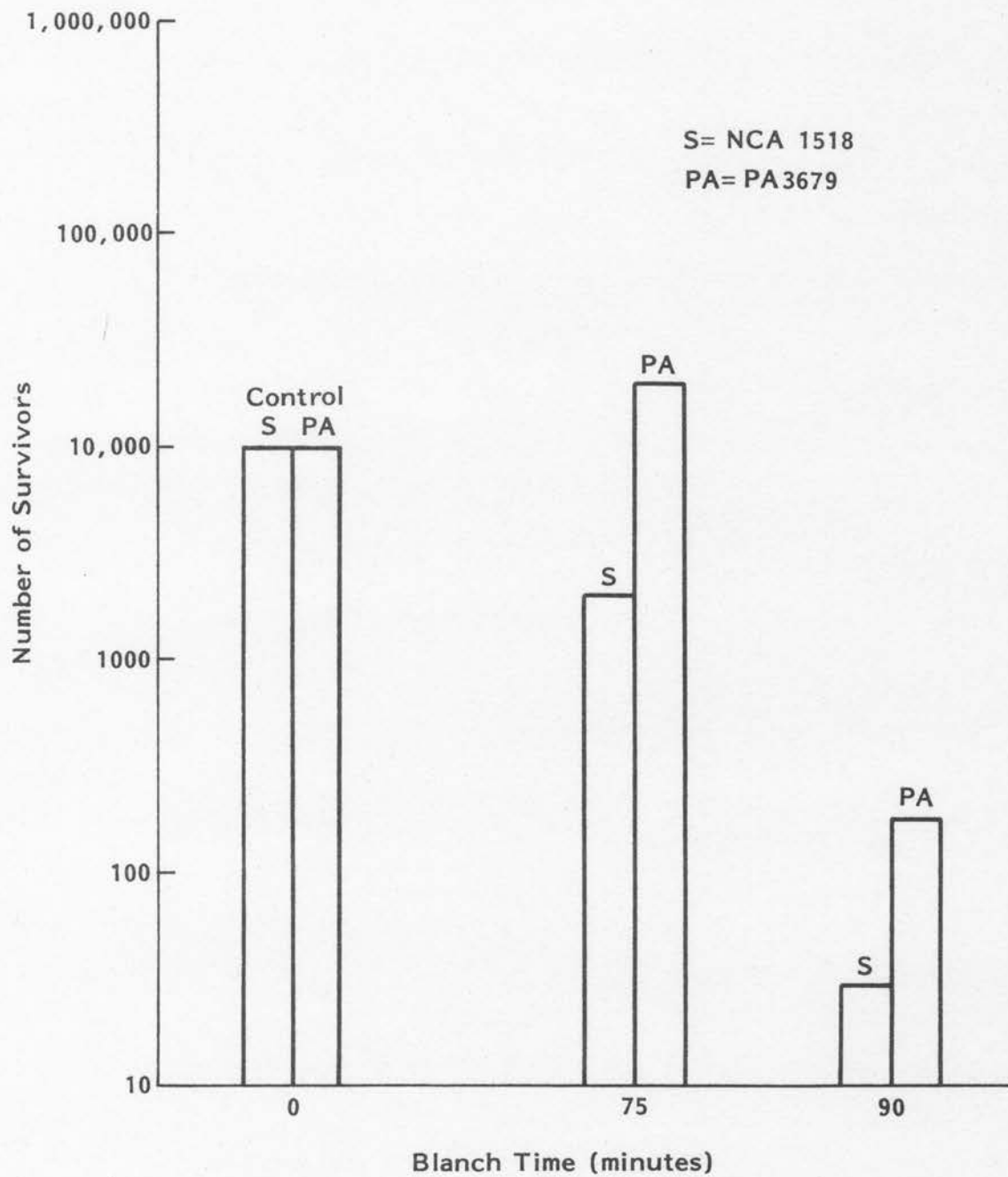


Figure 18. The effect of continuous water blanching at 82°C on the number of survivors of Bacillus stearothermophilus (NCA 1518) and Clostridium sporogenes (PA 3679)

A possible explanation would be that the organisms sporulated and produced some growth before the samples reached the temperature of the water bath (82°C). The 82°C is too high for the continued growth of either organism.

After canning, none of the treated samples showed any significant growth of thermophillic bacteria except for those canned samples subjected to treatment 3 (Table 2). This is illustrated in Figure 19, where NCA 1518 flourished after 12 hours of soaking at 25°C . The large number of survivors could be ascribed to slow cooling of the can after processing and to the fact that the internal temperature of the can stayed within a range favorable for the growth of NCA 1518.

As a safety control measure, heat penetration curves were determined for treatments 1, 4, 9, and 11. Several processing runs were performed before time and temperature characteristics were recorded for each treatment. Then, using the formula method (National Cannery Association Research Laboratories, 1968), heat penetration curves were drawn for each of the individual treatments. Symbols used in conjunction with each curve are defined in Appendix C. The retort temperature and time used in all the treatments were 115.5°C (240°F) and 40 minutes, respectively. Figure 20 outlines the heat penetration curve of canned fava beans subjected to soaking for 12 hours at 25°C followed by water blanching for 50 minutes at 82°C . The curve depicts a lag in the heat penetration during the initial heating period. This lag can be attributed to the time

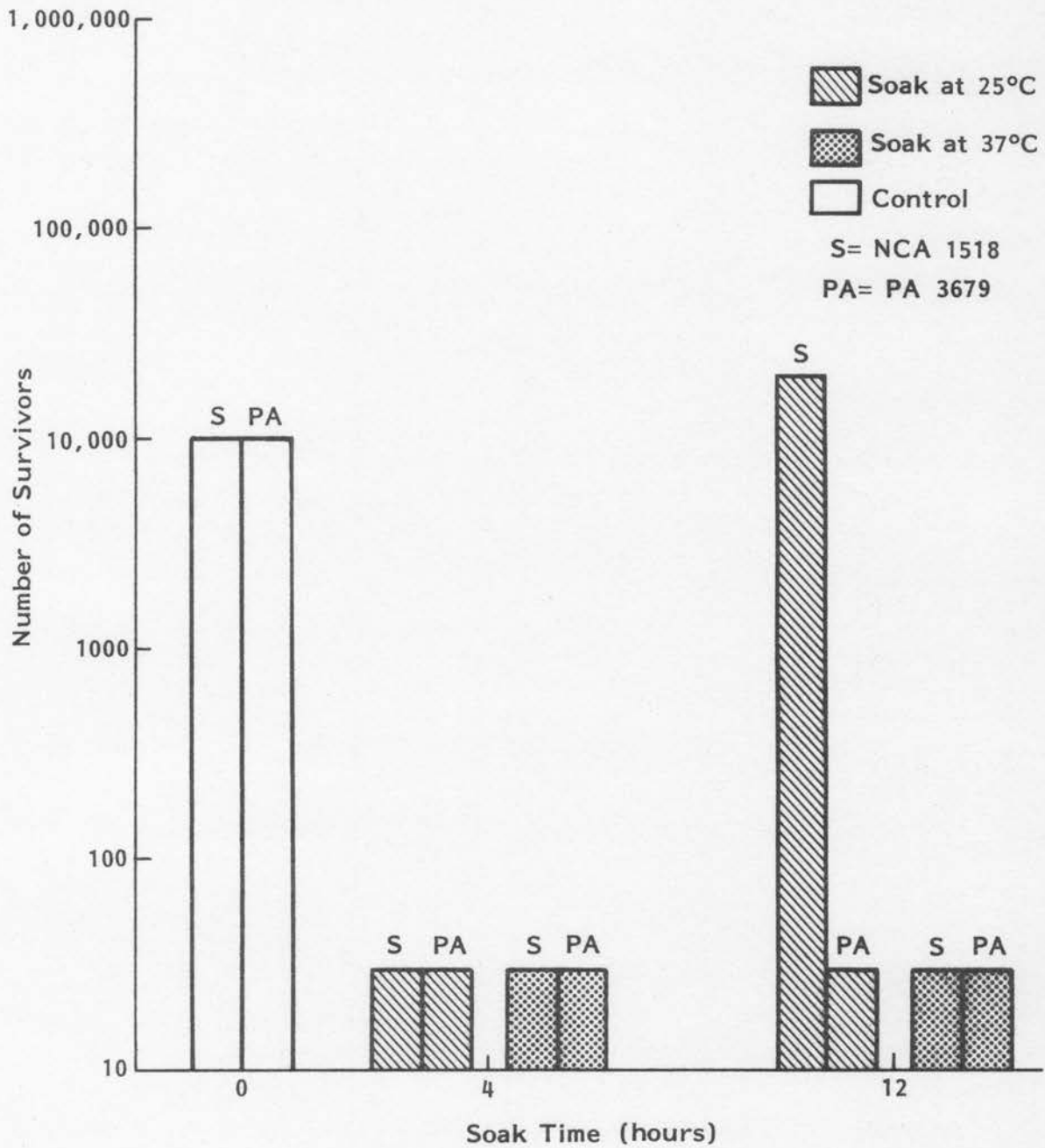


Figure 19. The change in the number of survivors of *Bacillus stearothermophilus* (NCA 1518) and *Clostridium sporogenes* (PA 3679) in canned fava beans subjected to different soaking times and temperatures followed by water blanching at 82°C for 30 minutes

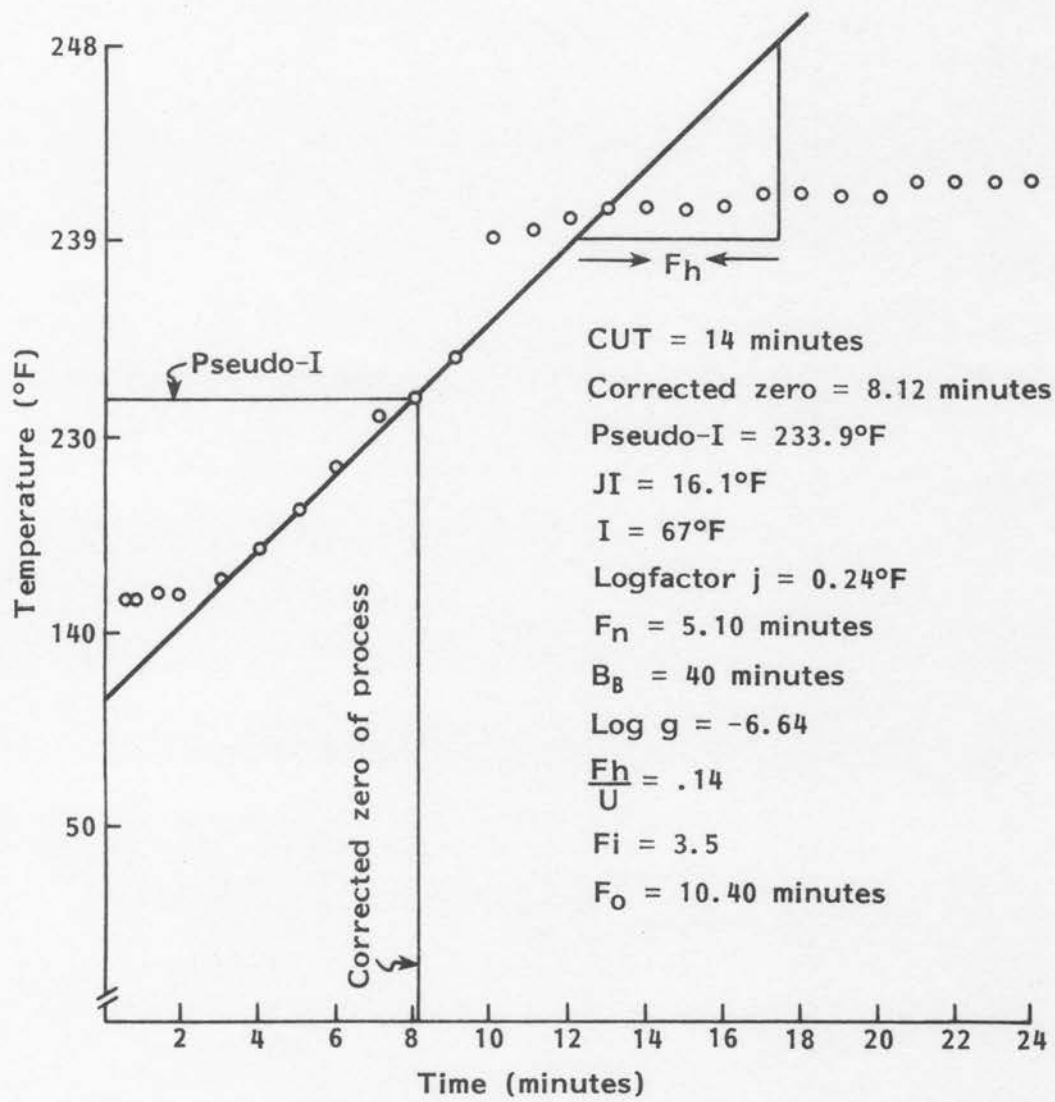


Figure 20. The heat penetration curve of canned fava beans subjected to soaking for 12 hours at 25°C followed by water blanching for 50 minutes at 82°C

required for the heat to penetrate the "cold point" of the can which is usually the most difficult zone to sterilize in a given container. This curve indicates a convection type of heat transfer which pertains to discrete food particles suspended in liquid. Heat transfer by convection could have been accompanied by conduction heating, which is characteristic of viscous liquid-solid food materials, especially as the temperature approached 240°F. This could be associated with more water imbibition and breaking of the beans at this stage and thus a leaching of solids into the solution. The calculated sterilization value F_0 was 10.14 minutes which is sufficient to kill the spores of Clostridium botulinum ($F_0 = 2.78$ minutes). The F_0 is defined as the number of minutes required to destroy a given number of spores at 250°F when $Z = 18$. A Z value of 18 is usually assumed for Clostridium botulinum when the thermal death time has not been determined in the product under consideration (Esty and Meyer, 1922). The 2% salt brine that was added to the can may have helped to lower the heat resistance of the bacteria (National Cannery Association Research Laboratories, 1968).

The heat penetration curve of canned fava beans subjected to soaking for four hours at 25°C followed by steam blanching for three minutes at 95°C is shown in Figure 21. A longer lag period for heat penetration was depicted for this treatment. This lag is probably due to a longer time required for the heat to be transferred to the "cold point" in the can

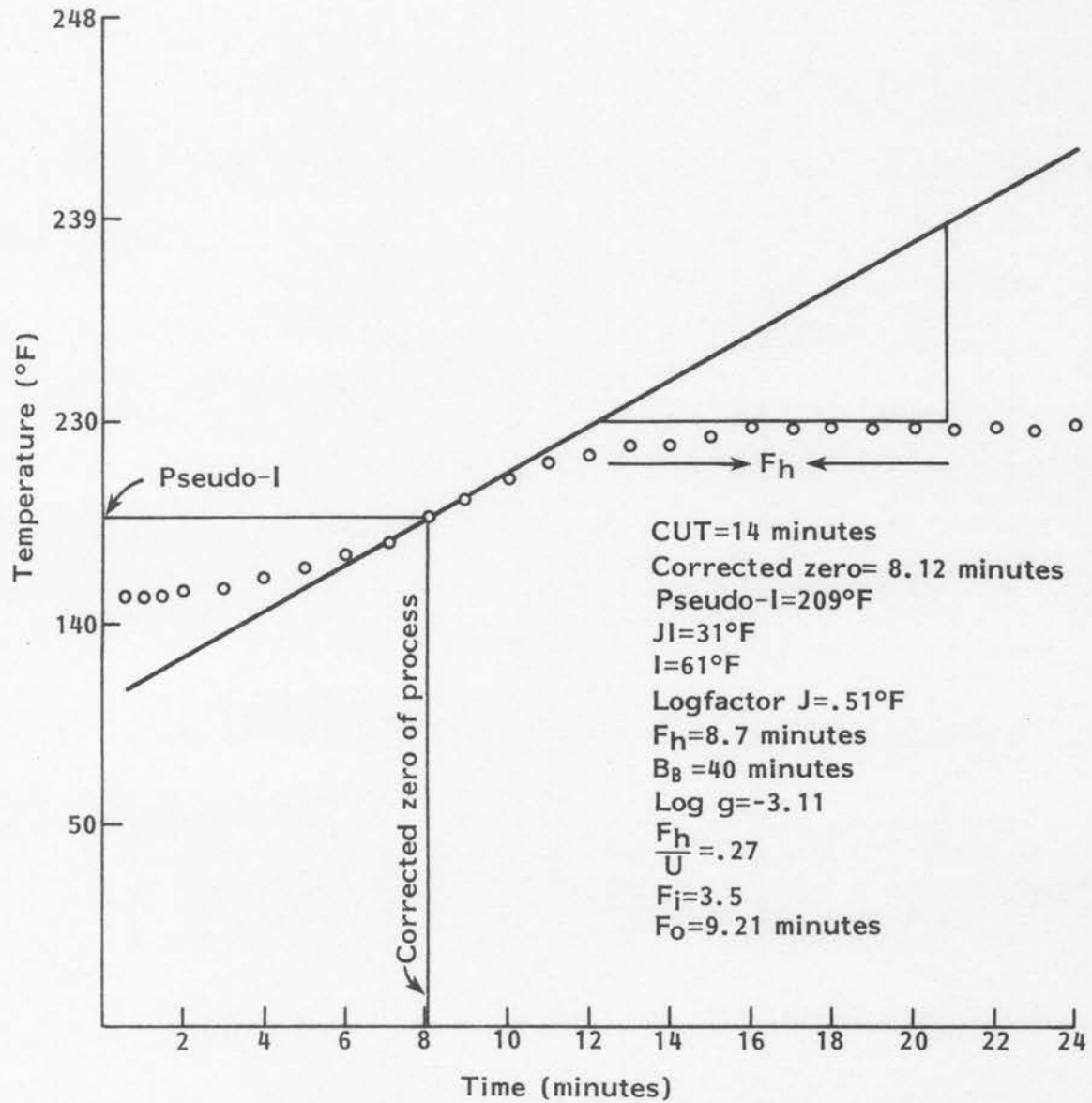


Figure 21. The heat penetration curve of canned fava beans subjected to soaking for 4 hours at 25°C followed by steam blanching for 3 minutes at a temperature of 95°C

which in turn resulted in a larger number of survivors of the two test organisms. Moreover, the internal temperature of the can increased at a slower rate and reached a maximum of 110°C (230°F) only. This could be an indication of the consistency of the heat transfer to the can. However, a longer processing time could elevate the maximum temperature to 115.5°C (240°F). The calculated F_0 was 9.21 minutes which was enough to destroy the spores of the two test organisms.

Figure 22 shows the heat penetration curve of canned fava beans subjected to direct water blanching for 45 minutes at a temperature of 82°C . In this figure, there was an obvious change in the slope of the heat penetration curve with no lag period observed. This implies that the "cold point" in the can heated very rapidly. In this situation, a broken heating curve could be drawn using the same steps of the formula method. The broken heating curve indicates a change in the characteristics of the contents of the can representing a definite shift from convection to conduction heating during the process. The sterilization value for this curve was 10.42 minutes which is beyond the level necessary for Clostridium botulinum to be killed.

The F_0 value for the canned fava beans subjected to 25°C for 4 hours followed by water blanching at 82°C for 30 minutes was found to be approximately 10.7. Additional heat penetration curves are necessary to further characterize this treatment.

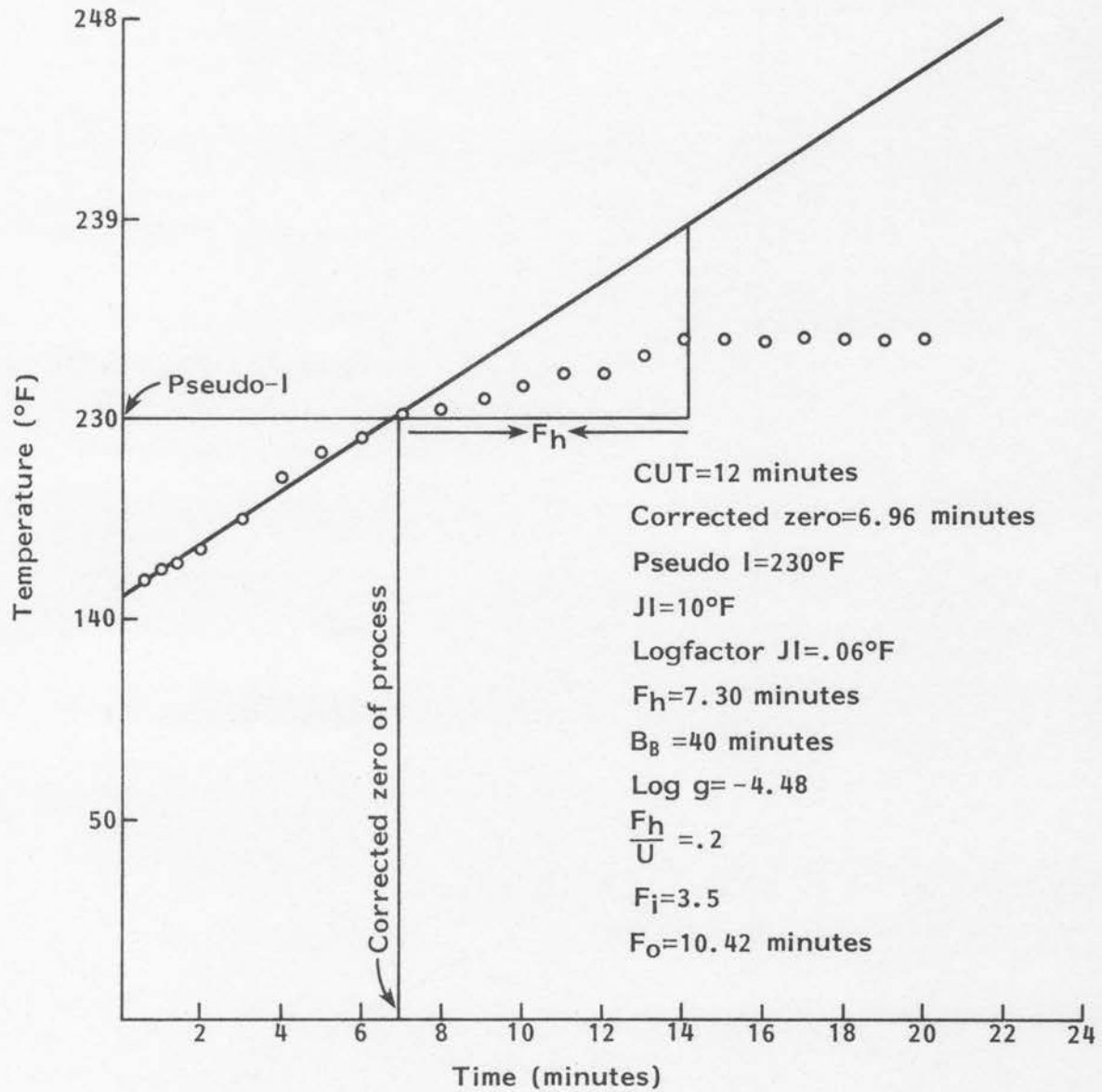


Figure 22. The heat penetration curve of canned fava beans subjected to direct blanching for 45 minutes at a temperature of 82°C

SUMMARY AND CONCLUSIONS

The preliminary studies showed that the "hardshell" condition which is characteristic of legumes is an obstacle in the processing of fava beans. This explains why longer blanching times were required in our studies. Based on the soaking and blanching study, it was found that the soaking time was highly significant. Additional observations indicate that as the soaking temperature was increased the soaking time required to reach the 55% level of water uptake decreased. The peroxidase test was another factor considered in the selection of our treatments. A negative peroxidase test is desirable to inactivate the enzymes particularly lipoxygenase which causes off flavors.

After canning the beans, sensory and instrumental quality evaluations resulted in certain interactions and conclusions. First, the sensory evaluation indicated that the wholeness, the consistency and the odor of the beans were acceptable for most of the 12 treatments selected. Moreover, data analyses produced certain correlations between the processing variables used and the sensory quality attributes evaluated. The only two attributes that were correlated (negatively or positively) with the processing variables were the color score and the character A of the beans (Table 6). The instrumental quality measurements showed no correlation between the soaking time and temperature and the texture of the beans.

In addition, these measurements implied that the blanching temperatures used were undesirable because they resulted in a tougher texture. However, increasing the blanching time improved the texture of the beans. Thus, for the blanching of fava beans, it is recommended that if the blanching temperature is increased, blanching time should also be increased to offset the toughening of the beans and to obtain a desirable texture. The color readings using the Hunter color difference meter were in contradiction with those obtained in the sensory evaluation. This could be attributed to the mean "L" and "b" values of treatment 13 (commercial beans) which were found to be lighter and yellower in color than our variety (Table 7). The total scores of the quality evaluation of the canned beans indicated that three treatments yielded the best results:

Treatment 2: soaking at 25°C for 4 hours + water blanching at 82°C for 50 minutes.

Treatment 3: soaking at 25°C for 12 hours + water blanching at 82°C for 30 minutes.

Treatment 7: soaking at 37°C for 12 hours + water blanching at 82°C for 30 minutes.

We would recommend that further work be done on treatment 2 which might help save time, energy and water.

The growth of thermophiles was not a problem in canned fava beans. Yet, long soaking (12 hours) prior to canning could result in undesirable flavors due to possible growth of bacteria and or germination of the beans during processing. In hot weather countries where the spores of Bacillus

stearothermophilus could grow and reproduce, the cans should be stored in areas below 28°C.

In the isolation of vicine experiment, the physical as well as the chemical characteristics of vicine in the dry bean sample were similar if not identical to that found by other researchers. Our results show that soaking at 25°C for 4 and 12 hours followed by water blanching at 82°C for 30 minutes drastically decreased the vicine concentration in the beans. This could be explained by the water solubility of vicine which could have been leached out by the soaking solution. This implies that the problem of vicine could be eliminated with the proper processing techniques which in turn could increase the market for the consumption of fava beans in the United States. Yet, more research is needed to establish conclusively that vicine is the principal causative agent of favism. Likewise, better analytical techniques need to be developed for more rapid quantitative tests. The use of HPLC (high performance liquid chromatography) might aid in the isolation and quantification of vicine.

Water soluble vitamins are expected to be lost by the soaking and blanching processes, but further research is needed before one can make definite conclusions about the nutritional changes during these processes.

In general, these results reinforce our belief that higher quality commercial fava beans can be produced for consumer use.

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APPENDIX A

Test of hypothesis using the ANOVA mean square for soaking, soaking followed by steam blanching and continuous water blanching:

Source	DF	ANOVA SS	F value	PR > F
Soaking experiment				
Temperature	2	9563.92	72.74	0.0001
Time	9	119413.71	201.83	0.0001
Temperature*time	10	9804.71	14.91	0.0001
Steam blanching experiment				
Treatment	10	10077.37	15.30	0.0091
Continuous water blanching experiment				
Temperature	2	2160.04	53.77	0.0001
Time	4	12893.30	160.46	0.0001
Temperature*time	8	1215.48	7.56	0.0001

APPENDIX B

Test of hypothesis using the ANOVA mean square for soak temperature*soak time*blanch time:

Source	DF	ANOVA SS	F value	PR > F
Soak time	3	38248.39	57.75	0.0001
Soak temperature	2	564.29	1.28	0.31
Soak temperature* soak time	6	5524.01	4.17	0.02
Blanch time	2	2746.83	6.22	0.01
Soak time* blanch time	6	1389.54	1.05	0.44
Soak temperature* blanch time	4	678.49	0.77	0.56

APPENDIX C

Definition of the symbols used in the calculation of the heat penetration data:

- IT The initial temperature of the canned food (the average temperature of the container contents at the time steam is turned on in the retort)
- RT Retort temperature
- I RT - IT
- CUT Come up time (time from steam-on until the retort temperature is reached)
- jI To obtain this value, determine the point on the linear scale corresponding to the CUT multiplied by 0.58. Draw a vertical line through this point to intersect the extension of the straight line portion of the heating curve. Draw a horizontal line through this point of intersection to the temperature scale (pseudo-I)
- JI Pseudo-I - RT
- j The value represents the time lag before the heating curve assumes a straight line on semi-log paper ($j = jI/I$)
- f_h The time in minutes required for the straight line of the curve to traverse one logarithmic cycle
- U The lethality of a process in terms of minutes at retort temperature
- g RT minus product temperature (on the heating curve) at the end of the process
- f_h/U Factor related to the value of g
- B The process time in minutes (this corresponds to Ball's symbol B_B)
- $B_B = f_h (\log jI - \log g)$

Z value Represents the number of °F required for the curve to traverse one logarithmic cycle, and measures the change in thermal death time or death rate with change in temperature

APPENDIX D