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Effect of Selected Canning Methods on Trypsin Inhibitor Activity, Sterilization Value, and Firmness of Canned Navy Beans

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The effects of selected canning methods on trypsin inhibitor activity and the relationships among sterilization, firmness, and trypsin inhibitor activity were investigated. Retort-cooking at 115.6 and 121.1 °C for longer than 35 and 20 min, respectively, did not increase trypsin inhibitor inactivation. The presence of Ca^{2+}/EDTA in the brine had a pronounced effect in increasing inactivation of trypsin inhibitor activity. Retort-cooking of navy beans in brine containing EDTA and calcium chloride at 115.6 °C for 35 min or at 121.1 °C for 15 min was optimal in terms of trypsin inhibitor inactivation, sterilization value, and firmness of the canned product.

Dry beans (*Phaseolus vulgaris* L.) are generally recognized as a good source of food protein and dietary fiber. However, most of legume seeds contain antinutritional factors such as trypsin inhibitor (Liener and Kakade, 1980).

Protein quality is a function of essential amino acid content and bioavailability. In food legumes protease inhibitors are important (Liener, 1958). The most studied protease inhibitors are the inhibitors of trypsin and chymotrypsin, which play a key role in the digestion of proteins (Liener and Kakade, 1980).

Active trypsin inhibitors have a double-detrimental effect on the utilization of bean proteins because they inhibit trypsin or chymotrypsin and decrease protein digestibility and they contain relatively high content of cystine (Kakade et al., 1969). Approximately 40% of the growth depression as well as 40% of the pancreatic hypertrophic effect on rats can be attributed to trypsin inhibitors in the unheated soybeans (Kakade et al., 1973; Liener, 1976). Heat treatment methods for inactivating the protease inhibitors of legume seeds include boiling, dry roasting, dielectric heating, microwave radiation, and extrusion cooking (Liener, 1981).

Inactivation of trypsin inhibitors improves the nutritive value of food proteins as demonstrated with rats (Liener, 1979). Different protease inhibitors have different degrees of heat stability (Chang and Satterlee, 1982; Apostolatos, 1984). The Bowman–Birk proteinase inhibitor, which has a molecular weight of 7861 and contains seven disulfide cross-linkages, can inhibit both trypsin and chymotrypsin and is very heat-resistant (Odani and Ikenaka, 1973). The properties of a trypsin inhibitor purified from the seeds of *P. vulgaris* cv. Kintoki are similar to that of the Bowman–Birk proteinase inhibitor (Miyoshi et al., 1978). A trypsin inhibitor with a molecular weight of 10000 purified from kidney beans (*P. vulgaris*) is unaffected by heating (up to 90 °C) or by pepsin and by low pH value (pH 2) (Pusztai, 1968). Autoclaving (wet heat) at 120 °C for 20 min has been found to be more efficient than dry heating at 100 °C for 2 h for the inactivation of trypsin inhibitor activity (Tan and Wong, 1982). The protein efficiency ratio (PER) of navy beans can be improved by inactivating 80% of trypsin inhibitor activity (Kakade and Evans, 1965). Inactivation of trypsin inhibitor activity in crushed field bean flour follows first-order kinetics, and the rate of loss increases with increasing the moisture content from 2% to 55% (Buera et al., 1984).

Pressure cooking (121 °C) over 30 min decreased the nutritive value (decreased methionine, lysine, valine, and available lysine) of the black beans (Bressani et al., 1961). Autoclaving longer than 5 min has an adverse effect on the essential amino acids of navy beans (Kakade and Evans, 1965). Protein efficiency ratio decreases from 1.57 to 0.67 in the navy beans autoclaved from 5 to 60 min.

Cooking temperature and time have a significant effect on the firmness of canned pinto and light red kidney beans (Quast and da Silva, 1977; Wang et al., 1988). Light red kidney and pinto beans processed at 121 °C for 20 min are firmer than those processed at 115.6 °C for 45 min (Davis, 1976). Although trypsin inhibitor activity can be decreased by thermal processing, the relationships among sterilization value (*F* value), firmness, and trypsin inhibitor activity have not been reported.

The purposes of this study were (1) to investigate the effect of packing brine composition and retort-cooking methods on trypsin inhibitor activity in navy (Upland) beans and (2) to investigate the relationship between the textural quality and trypsin inhibitor activity after processing.

MATERIALS AND METHODS

Materials. Navy beans, *P. vulgaris* L., of the Upland cultivar were grown in a test plot of North Dakota State University in Fargo, ND. The beans were stored in plastic bags at 4 °C until use.

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Moisture Content. Moisture content was determined by drying the sample in an air-circulated oven at 110 °C until a constant weight was obtained (AOAC, 1984).

Canning Procedure. Beans equivalent to 100 g solid (111.62 g) of Upland were soaked in distilled water for 16 h at room temperature. The soaked beans were blanched in steam at 100 °C for 10 min in a still retort. The blanched beans were filled into 303 × 406 cans (National Can Co., Chicago, IL). Hot brine medium (95–98 °C) was poured into the can. Two commonly used packing brine solutions were used to test their effect on trypsin inhibitor activity. The composition of brine for blank treatment contained 1.25% NaCl. The brine for the other treatments contained 1.25% NaCl and 2.50 mM $CaCl_2$ and 1.34 mM disodium ethylenediaminetetraacetate (EDTA) (Wang et al., 1988). Calcium chloride and EDTA were used to improve firmness and color of canned beans. The headspace of the cans was 1 cm below the can lid. After filling, the cans were sealed (Dixie Model 24 Can Sealer, Athens, GA) and processed in a still retort (Reid Boiler Works, Bellingham, WA) at 121.1 °C for 5–25 min or at 115.6 °C for 5–50 min. The retorted beans were cooled by running tap water to 35 °C and stored at room temperature.

Sterilization Value Determination. Cold-point temperatures of the bean cans during retorting were monitored by thermocouples inserted 3.6 cm from the bottom of the can and recorded by a Speedomax Multipoint recorder (Model 2500, Leeds and Northrup Co., North Wales, PA) at 30-s intervals. The retort temperature (121 or 115.6 °C) was regulated by a temperature and pressure controller (Model 250Q, Taylor Instrument Co., Rochester, NY).

Lethal rates for the retorting process were computed from the following equation: lethal rate = $10^{(T-121.1)/Z}$, Z = 10 °C. The sterilization values (F_0) of the process were calculated from the area under the lethal rate curve. The F_0 value is equivalent to the number of minutes required to destroy the spores of *Clostridium botulinum* at 121.1 °C when Z is 10 °C. F_0 is equal to 1 when the cold-point temperature of canned products is held at 121.1 °C for 1 min (Toledo, 1980).

Trypsin Inhibitor Activity. The canned beans were homogenized in a blender to a fine slurry at 4 °C to avoid the denaturation of proteins. One gram of bean-solid equivalent was extracted with 15–50 mL of 0.01 N NaOH for 3 h. The trypsin inhibitor activity in the suspension was determined by the method of Kakade et al. (1974). A trypsin inhibitor unit (TIU) was defined as an increase of 0.01 absorbance unit at 410 nm. Two samples at each processing condition were analyzed. The suspension were diluted to the point where 1 mL produces 40–60% trypsin inhibition.

Texture Determination. The firmness of canned navy beans (Upland) was measured on an Instron universal testing instrument (Model 1000, Instron Inc., Canton, MA) equipped with a 500-kg weight-beam and an extrusion cup. A 20-kg load cell was used with crosshead control at 20 mm/min. A 100-g portion of the canned beans was measured each time. The kilogram-force at the onset of extrusion was taken to indicate the firmness of beans (Instron, 1972).

RESULTS AND DISCUSSION

Effect of Canning on Trypsin Inhibitor Activity. Trypsin inhibitor activities of the raw and blanched beans were 18650 and 2700 TIU/g of dry solid, respectively, indicating only 14% of the initial trypsin inhibitor was still active after blanching. Blanching (wet heat) had been found to be much more effective in destroying the trypsin inhibitor activity than with dry-heat treatment (Tan and Wong, 1982). Buera et al. (1984) found that heat processing at 30% moisture content was most destructive at trypsin inhibitor activity among the moisture contents tested from 2% to 55%.

Table I. Effect of Retort-Cooking Time and Temperature on the Trypsin Inhibitor Activity in the Blank Treatment (1.25% NaCl Brine) of Canned Navy (Upland) Beans[a]

retort-cooking time, min	tryspin inhib act (TIU)/g beans			
	115.6 °C	% resid	121.1 °C	% resid
5	1583 a	8.5	1013 a	5.4
10	1388 b	7.4	870 b	4.7
15	1005 c	5.4	795 c	4.3
20	985 c	5.3	515 d	2.8
25	970 c	5.2	490 d	2.6
30	888 d	4.8		
35	778 e	4.2		
40	558 f	3.0		
45	522 f	2.8		
50	475 f	2.5		

[a] Means within the same column followed by a different letter were significantly different at $p < 0.05$.

Table II. Effect of Retort-Cooking Time and Temperature on the Trypsin Inhibitor Activity in the Ca^{2+}/EDTA Treatment of Canned Navy (Upland) Beans[a]

retort-cooking time, min	tryspin inhib act. (TIU)/g beans			
	115.6 °C[b]	% resid	121.1 °C	% resid
5			668 a	3.6
10			525 b	2.8
15			440 c	2.4
20			432 c	2.3
25			415 c	2.2
30	418 a	2.2		
35	297 b	1.6		
40	284 b	1.5		
45	276 b	1.5		
50	257 b	1.4		

[a] Means within the same column followed by a different letter were significantly different at $p < 0.05$. [b] Processing at 115.6 °C for a time shorter than 25 min was not conducted due to the low sterilization values produced: processing at 121.1 °C for a time longer than 25 min was not conducted due to the high sterilization values produced.

In this study, two retort-cooking methods were used, i.e., 115.6 °C/5–50 min and 121.1 °C/5–25 min. The results (Tables I and II) showed that increasing either temperature or time could further destroy trypsin inhibitor activity in the beans. Increasing temperature of processing decreased the time needed to inactivate similar amounts of trypsin inhibitor activity. Johnson et al. (1980) reported that inactivation of trypsin inhibitor of soy flour slurry heated at 99 °C for 60 min had the same residual trypsin inhibitor activity as those processed at 121 °C for 4 min and 40 s. There were not significant differences when the processing time extended over 20 min at 121.1 °C or over 40 min at 115.6 °C. Canned beans processed at 121.1 °C for 20 min or 115.6 °C for 40 min retained 3% (or less) of the initial trypsin inhibitor activity.

Rackis and McGhee (1975) reported that the maximum body weight, PER, and protein digestibility were obtained in the rats fed soy beans in which only 79–87% of trypsin inhibitors had been destroyed. Meanwhile, they also found that soy beans with 31–45% residual trypsin inhibitor activity could not cause pancreatic hypertrophy in a 28-day feeding. However, the long-term effect of low trypsin inhibitor activity on the physiological function of pancreas is unknown.

Under the same temperature–time processing conditions, the Ca^{2+}/EDTA treatment generally resulted in lower trypsin inhibitor activity than the blank treatment

Table III. Effect of Retort-Cooking and Temperature on the Firmness of Canned Beans[a]

retort-cooking, min	firmness, kg-force/100 g canned beans			
	115.6 °C		121.1 °C	
	blank[b]	Ca^{2+}/EDTA	blank[b]	Ca^{2+}/EDTA
5			46.3 a	113.5 a
10			21.8 b	65.6 b
15			20.7 b	40.1 c
20			18.6 c	38.9 c
25			18.1 c	37.7 d
30	18.2 d	36.9 a		
35	18.4 d	36.8 a		
40	18.9 d	37.6 a		
45	18.6 d	34.8 a		
50	17.4 d	30.6 b		

[a] Means within the same column followed by a different letter were significantly at $p < 0.05$. [b] Blank medium contained 1.25% NaCl.

(1.25% NaCl only) (Tables I and II). The effect of Ca^{2+}/EDTA in the brine of canned beans on the trypsin inhibitor activity might be due to the changes in the pH values. Initial pHs of the blank brine and the calcium chloride (2.5 mM)/EDTA (1.34 mM) containing brine were 6.12 and 7.25, respectively. After retort-processing, the pHs of the liquor in the blank treatment and Ca^{2+}/EDTA treatment were 5.85 and 6.15, respectively. Johnson et al. (1980) reported that increasing the pH value of slurry from 6.7 to 9.5 substantially increased the rate of trypsin inhibitor inactivation. Therefore, increasing pH from 6.12 to 7.25 by Ca^{2+}/EDTA addition reduced the cooking time needed to give the same residual trypsin inhibitor activity.

Relationships among Firmness, Sterilization Value, and Trypsin Inhibitor Activity. The firmness of canned beans decreased as processing time increased in both the blank and Ca^{2+}/EDTA treatment (Table III). Addition of calcium chloride to the soaking or canning medium had been shown to increase the firmness of canned beans (Luh et al., 1975; Uebersax, 1985; Wang et al., 1988). Quast and da Silva (1977) reported that, at a given temperature, the firmness of black beans decreased as a function of time. Increasing temperature could shorten the processing time of black beans (Quast and da Silva, 1977) and could result in energy saving. Canned Upland beans with firmness of 35–118 kg-force/100 g of canned beans as measured by the back-extrusion method described in this study was acceptable by the consumer test (Wang et al., 1987). With the exception of the 115.6 °C/50 min treatment, the firmness of the canned beans with the addition of Ca^{2+}/EDTA was in the acceptable range (Table III). Beans canned without the addition of calcium tended to have high degrees of splitting and clumping.

For low-acid foods, adequate heat must be provided to meet the 12 *D* values for the destruction of *Clostridium botulinum* spores (NCA, 1968). The sterilization value (F_0 value) for *C. botulinum* is 2.45. However, in commercial processing, thermal processes are usually performed at higher F_0 values than 3 to provide additional safety. The American Can Co. (1981) reported that F_0 values for commercial processes of peas in brine in no. 2 (307 × 409) and no. 10 (603 × 700) cans were 7 and 11, respectively. Therefore, retort-processing of navy beans in brine containing calcium chloride and EDTA at 115.6 °C for 35 min and at 121.1 °C for 15 min were optimal for canning beans in terms of sterilization value, firmness, and trypsin inhibitor activity (Table IV). Processing times longer than 35 min at 115.6 °C or 20 min at 121.1 °C have no remarkable benefit in terms of trypsin inhibitor inactivation and could decrease firmness and nutritional values of canned beans and increase processing energy or production costs.

Table IV. Sterilization Value (F_0 Value) of Varying Retort-Cooking Processes

retort-cooking time, min	F_0 value[a]	
	115.6 °C	121.1 °C
5	0.48 ± 0.01	2.44 ± 0.12
10	1.45 ± 0.04	5.84 ± 0.25
15	2.51 ± 0.07	9.53 ± 0.46
20	3.60 ± 0.11	13.40 ± 0.67
25	4.74 ± 0.15	17.50 ± 0.89
30	5.89 ± 0.19	
35	7.06 ± 0.23	
40	8.24 ± 0.27	
45	9.50 ± 0.40	
50	10.71 ± 0.43	

[a] Means of six runs ± SD.

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Registry No. EDTA, 60-00-4; $CaCl_2$, 10043-52-4; trypsin inhibitor, 9035-81-8.

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Texture Characteristics, Protein Solubility, and Sulfhydryl Group/Disulfide Bond Contents of Heat-Induced Gels of Whey Protein Isolate

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The total sulfhydryl (SH) group and half-cystine contents and various properties (water-holding capacity, transmittance, firmness, elasticity, protein solubility) of gels of whey protein isolate (WPI) prepared at different protein concentrations, pHs, and temperatures have been investigated. Assays were also carried out in the presence of *N*-ethylmaleimide or of sodium chloride. Gel texture (firmness, elasticity) is not strictly related to the total number of disulfide (S–S) bonds. At neutral and alkaline pHs, gel firmness and total SH groups decreased with increasing pH (6.5–9.5), while gel elasticity, transmittance, and protein solubility (in buffers without S–S bond-reducing agent) increased. Hydrophobic interactions and intermolecular S–S bonds caused by SH/S–S interchange reactions predominate in the WPI gel network and are probably responsible for (1) the firmer gel structure at increasing protein concentration and (2) the heat resistance at high temperature (ca. 135 °C). The high elasticity of gels at neutral and alkaline pHs may result from intermolecular S–S bonds. The gels formed at acid pHs are nonelastic and markedly solubilized in denaturant-containing buffers. Hydrogen bonds may represent the main molecular bonds in the network of such acid gels.

Whey protein concentrates (WPCs) and whey protein isolates (WPIs) containing from 25 to 95% protein in the dry state are produced from cheese whey by several techniques including gel filtration, ion exchange, ultrafiltration, adsorption, polyphosphate precipitation (Cheftel and Lorient, 1982; Modler, 1985). The functional properties of whey proteins are affected not only by the processing history of WPCs and WPIs but also by the nature and the amount of both the protein and the nonprotein components (de Wit et al., 1983, 1986). One of these functional properties is the ability to form heat-induced gels under appropriate conditions. The gelation of WPC and of its constitutive proteins has been investigated under various experimental conditions (de Wit and de Boer, 1975; Hermansson, 1975; Hermansson and Åkesson, 1975; Hegg, 1982; Harwalkar and Kalab, 1985 a,b; Paulsson et al., 1986; Dumay, 1987). The dependence of gelation on disulfide (S–S) bonds has been demonstrated by the addition of reducing agents (Dunkerley and Zadow, 1984; To et al., 1985). Addition of cysteine at low concentration enhanced gel firmness, while higher concentrations reduced it (Schmidt et al., 1978, 1979). Gel transparency was related to the total sulfhydryl (SH) group content in WPC (Hillier et al., 1980). Voutsinas et al. (1983) observed that the thermal functional properties of WPC (thickening, coagulation, gelation) were significantly correlated with a composite of the content of SH group before heating and of protein hydrophobicity. In contrast, Kohnhorst and Mangino (1985) found that the SH group content in WPC powder was not a good indicator for gel strength.

Little is known about SH group and S–S bond contents in gels obtained from WPC or from the individual whey proteins (Li-Chan, 1983; Beveridge et al., 1984). In the

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