Heat Inactivation of Trypsin Inhibitor, Lipoxygenase and Urease in Soybeans: Effect of Acid and Base Additives[1]

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ABSTRACT

Effects of chemical additives on the heat inactivation of trypsin inhibitor (TI), lipoxygenase and urease in soybeans were investigated. The nutritional value of soybeans increases when antigrowth factors, such as TI, are inactivated. Inactivation of lipoxygenase enhances palatability and storage stability. Heat inactivation of antinutritional factors during immersion cooking of dry soymeats was studied without additives. Processing time was varied from 15 min to 2 hr over a temperature range of 120-212 F. The experiments were repeated, with the addition of NaOH or HCl to the cooking water. Without additives, lipoxygenase proved to be the most heat labile and TI, the least. With either acid or base additives, the initial inactivation of urease and lipoxygenase was accelerated significantly; however, while TI inactivation was accelerated by base, it was retarded by acid addition.

INTRODUCTION

Soybean flour, particularly the full-fat type, is gaining considerable importance because of the necessity of providing both calories and protein in feeding hungry people around the world. Development of a simple hand process for villagers, in which whole soybeans were soaked prior to immersion cooking, was reported by Mustakas and coworkers (1,2). Heat treatment is required to improve the nutrition of soybeans and to inactivate several biologically active components (3).

Certain animals cannot utilize raw soybeans, and trypsin inhibitor (TI) is generally implicated. Urease is an enzyme found in soybeans, which catalyzes the conversion of urea (urea amido hydrolase EC 3.5.1.5) into ammonia and carbon dioxide. Its inactivation is important in certain feedstuffs fortified with urea for ruminants (4). Lipoxygenase (linoleate-oxygen oxido reductase EC 1.13.1.13), another enzyme found in soybeans, catalyzes the oxidation of soybean lipids. The enzyme catalyzes the oxidation of unsaturated fatty acids by molecular oxygen, resulting in rancidity, off-flavors and poor storage stability (4).

Inactivation of TI and urease during atmospheric steaming and immersion cooking of presoaked whole soybeans was reported by Albrecht et al. (5). The destruction of crystalline soybean TI activity at acid pH has been studied (6,7). The effect of alkaline pH at elevated temperatures on the destruction of TI activity in soybeans (8,9) and soybean milk (10,11) has been reported. Presoaking soybeans in solutions of various chemicals prior to cooking had little effect on lipoxygenase activity in preparing soy milk (12). However the same studies indicated that lipoxygenase is very sensitive to heat inactivation.

This investigation was made to study the effects of acid and base additives during immersion cooking of dry-dehulled soymeats on inactivation rate of TI, urease and lipoxygenase, and also to determine the effect of the chemicals on the oil and meal fractions of the cooked soymeats.

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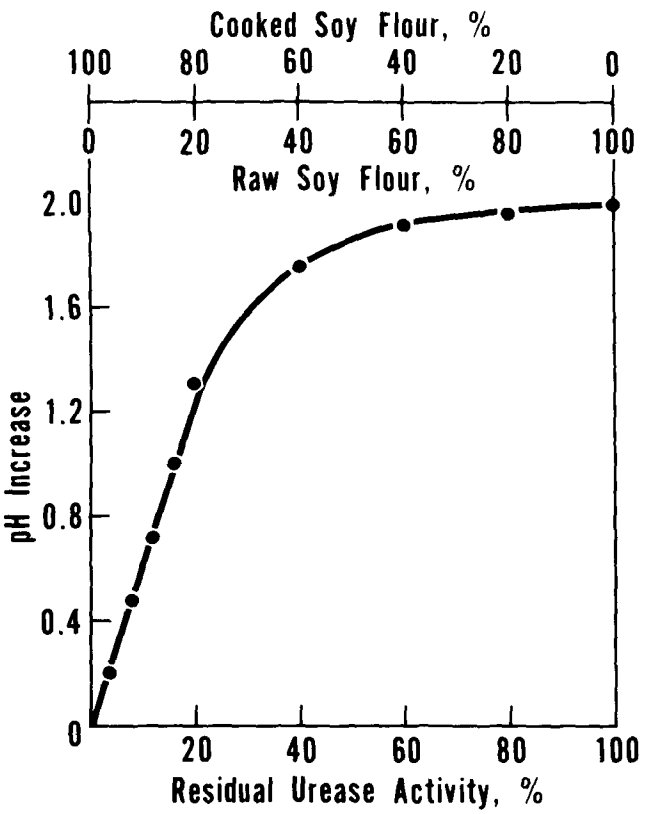

FIG. 1. Conversion of urease test pH increase to per cent residual urease activity.

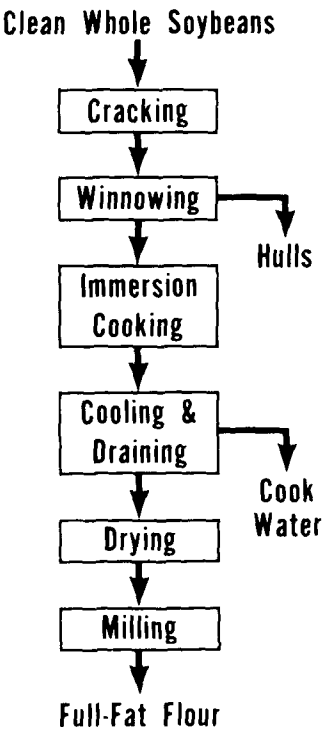

FIG. 2. Immersion cooking procedure for soybeans.

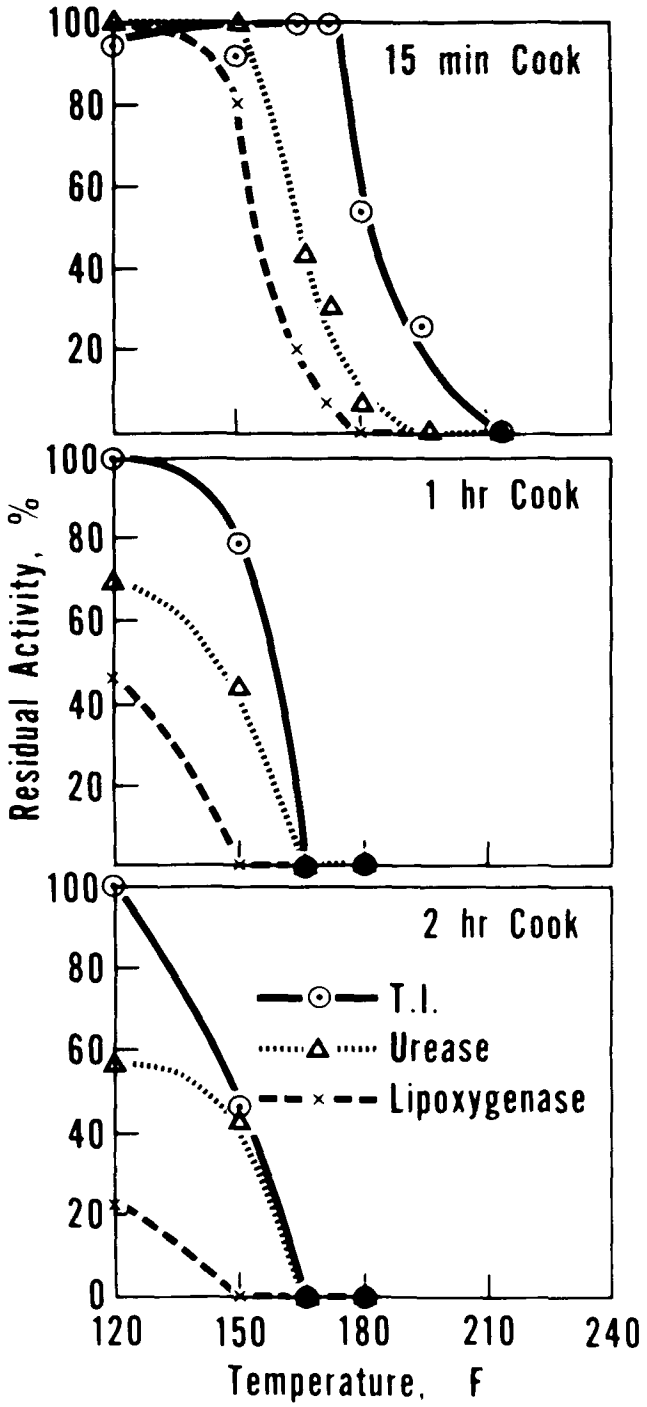

FIG. 3. Heat inactivation during immersion cooking without additives.

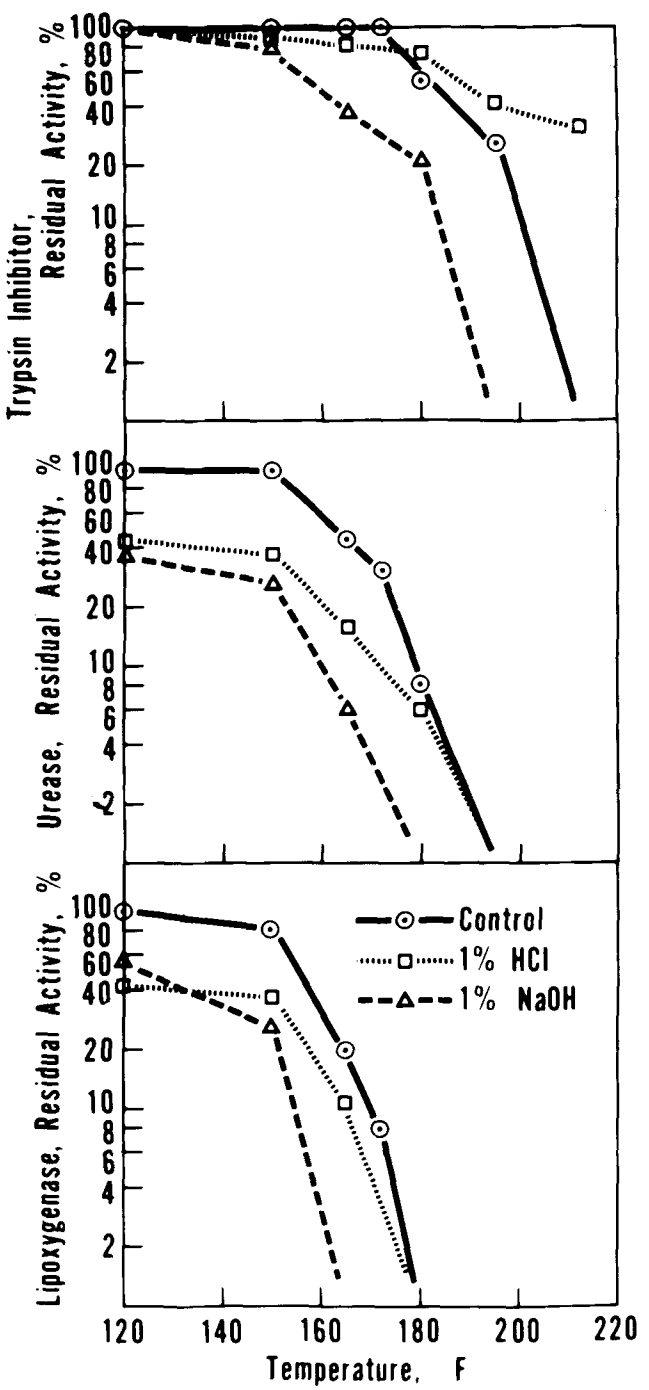

FIG. 4. Effect of acid and base additives on heat inactivation of antinutritional factors during 15 min cooks.

METHODS

Analysis

For analysis, the beans were pin-milled to flour and hexane defatted. Nitrogen solubility index (NSI) for the measurement of water-soluble protein was run by a modified method (pH 7.2) of Smith et al. (13). Urease was determined by the official AACC method (14). For the purpose of providing a common basis for comparison, urease values reported as pH units increase were converted to per cent residual urease activity as follows: Raw soy flour in which the urease was intact was blended in varying proportions with an extrusion-cooked soy flour in which the urease was totally destroyed. The blends were then tested for urease activity, and the pH increase was plotted against per cent of the original urease in the blend to give the curve shown in Figure 1 (P.G. Banks, private communication). TI activity was measured by a procedure obtained from Central Soya Co., Inc. (1957, private communication). By this procedure, trypsin is added to a buffer solution of gelatin in the presence of TI. The reaction is stopped with neutral formaldehyde solution, and the free carboxyl groups are titrated with 0.1 N sodium hydroxide solution. Lipoxygenase was measured by standard AACC method (14). Available lysine was run by a modification of Rao et al. (15). Peroxide values, free fatty acids and ash were run by the standard AOCS procedures (16). Water absorption was run by the method described in the Subcommittee report on the determination of water absorption of soy flour (17).

Experimental Procedure

The procedure is outlined in Figure 2. Whole soybeans (Hawkeye, 1970 crop) were cracked in a hand-operated

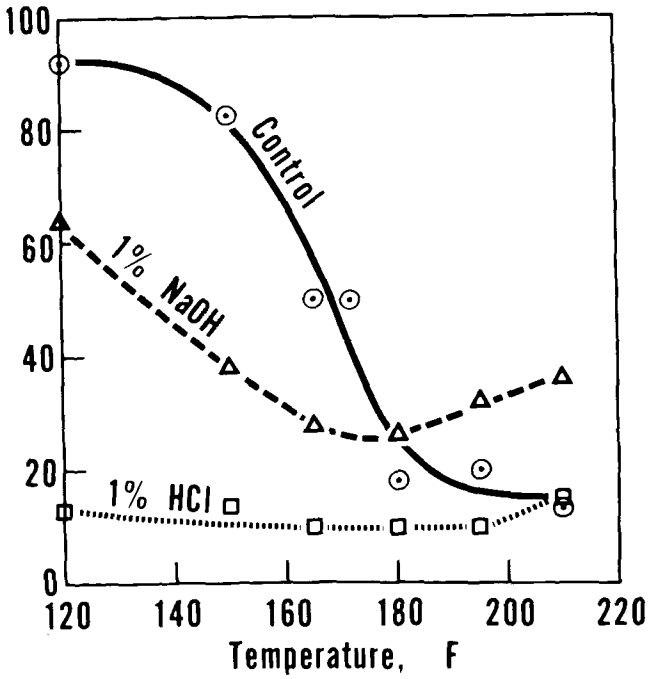

FIG. 5. Effect of additives on heat denaturation of protein in 15 min immersion cooks.

corn cracking mill (Cecoco, Central Commercial Co., Osaka, Japan). Clearance was adjusted to just crack the beans, yielding mostly halves and quarters. These larger particles were used to minimize losses to the cooking water. Hulls were separated in a hand-operated winnower (Cecoco).

Six liters distilled water (with additives, if any) were heated to slightly above the experimental temperature, then added to 2 kg cracked meats in a pail. Beans were not presoaked prior to immersion cooking and had an initial moisture of 6%. The following amounts of additives were added to 6 liters water to give a 1% w/w concentration: hydrochloric acid (37.5%), ACS: 159 g; sodium hydroxide (97%), ACS: 62 g; sulfuric acid (96%), ACS: 62.5 g; phosphoric acid (85%), ACS: 71 g; sodium bicarbonate (100%): 60 g; and ammonium hydroxide (58%), ACS: 103 g. The bean slurry was placed in a hot water bath, and the experimental temperature was generally achieved within 1-2 min. After the prescribed holding time, the acid or base, if any, was neutralized and the slurry was cooled to less than 100 F in an ice bath. The bean slurry was held an additional 30 min to allow for the diffusion of the neutralizing agent into the beans. Beans were drained and squeezed in cheesecloth to remove excess water, then tray-dried overnight at 120 F in a forced air dryer (Hurricane). The pH of the drained cook water was 6.5 ± 0.8. The pH of a 10% slurry of the defatted flours was in the range 5.0-5.9 when hydrochloric acid was used and 6.6-7.6 when sodium hydroxide was used. The dried meats were pin-milled to full-fat flour.

RESULTS AND DISCUSSION

Heat Inactivation

Lipoxygenase was shown to be the most heat labile of the three factors studied (lipoxygenase, urease and TI) (Fig. 3). In the 15 min cooks, decrease in activity is first observed at 150 F for lipoxygenase, compared to 165 F for urease and 180 F for TI. Total inactivation is achieved in 15 min at 180 F for lipoxygenase, at 195 F for urease and at 212 F for TI. The inactivation curves are reasonably parallel, showing a uniform response with increasing temperature.

In the 1 hr cooks, TI again displayed more initial

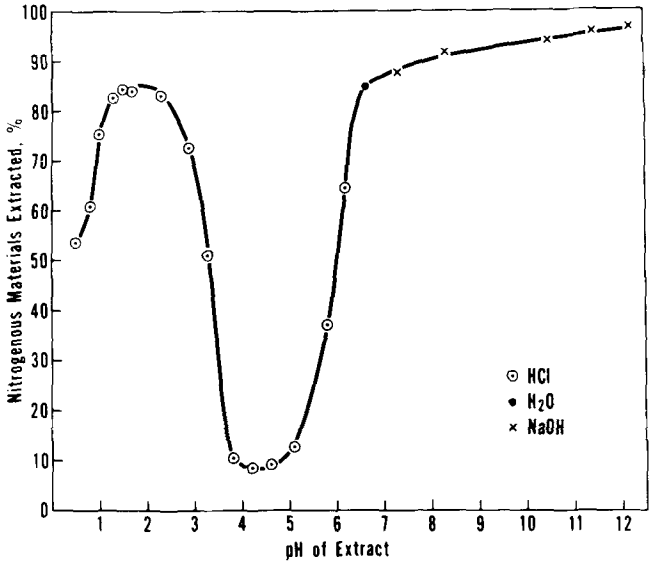

FIG. 6. Extractability of proteins in defatted soybean meal as a function of pH. Taken from Smith and Circle (18).

resistance to inactivation than urease; however total inactivation was achieved for each at 165 F. TI inactivation appears more time dependent than urease, and when that time requirement is met, the inactivation rates for TI and urease become the same. This is also demonstrated in the 2 hr cooks, where the curves become almost identical at temperatures above 150 F. The test for urease is often used as an index for adequate heat treatment. When the time of heat treatment is sufficiently long, the rates of inactivation of urease and TI are shown to be about equal. However, when high temperature, short time cooking processes such as extruder cooking are used, the inactivation rates may vary enough that urease could be completely inactivated while considerable TI is still present.

Total inactivation of the three factors can be achieved equally well in 1 hr at 165 F or 15 min at 212 F. Rackis (18) has shown that TI was inactivated in full-fat or defatted flakes in 15 min of steaming at 100 C. Albrecht et al. (5) have shown that, with overnight soaking of whole soybeans, boiling for only 5 min was sufficient to inactivate the inhibitor.

Higher NSI values were obtained in 1 hr at 165 F (NSI 28%) than in 15 min at 212 F (NSI 13%). The choice of time and temperature conditions for inactivation of the factors would be influenced by amount of protein denaturation to be tolerated.

Inactivation with Heat Plus Additives

The effect of the addition of 1% NaOH or HCl to the cooking water on the heat inactivation of urease, lipoxygenase and TI was examined. Since measurable inactivation was obtained for all three factors in the 15 min cooks, the 2 hr cooks were eliminated as a variable in the additive experiments.

Inactivation of lipoxygenase: Without the additives, no inactivation of lipoxygenase occurred in 15 min at 120 F (Fig. 4). Addition of either the acid or the base to the cooking water caused inactivation of about half the enzyme in 15 min at 120 F. As the temperature was increased, the effect of the acid additive diminished until its accelerating effect was cancelled out at 180 F. In the case of caustic addition, total inactivation is achieved at 165 F, compared to 180 F without additive.

Inactivation of urease: No decrease in urease activity was obtained at 120 and 150 F with the control (Fig. 4). The use of either additive caused inactivation of more than half the enzymes in 15 min at 120 F. As the temperature was increased above 150 F, the effect of both additives on

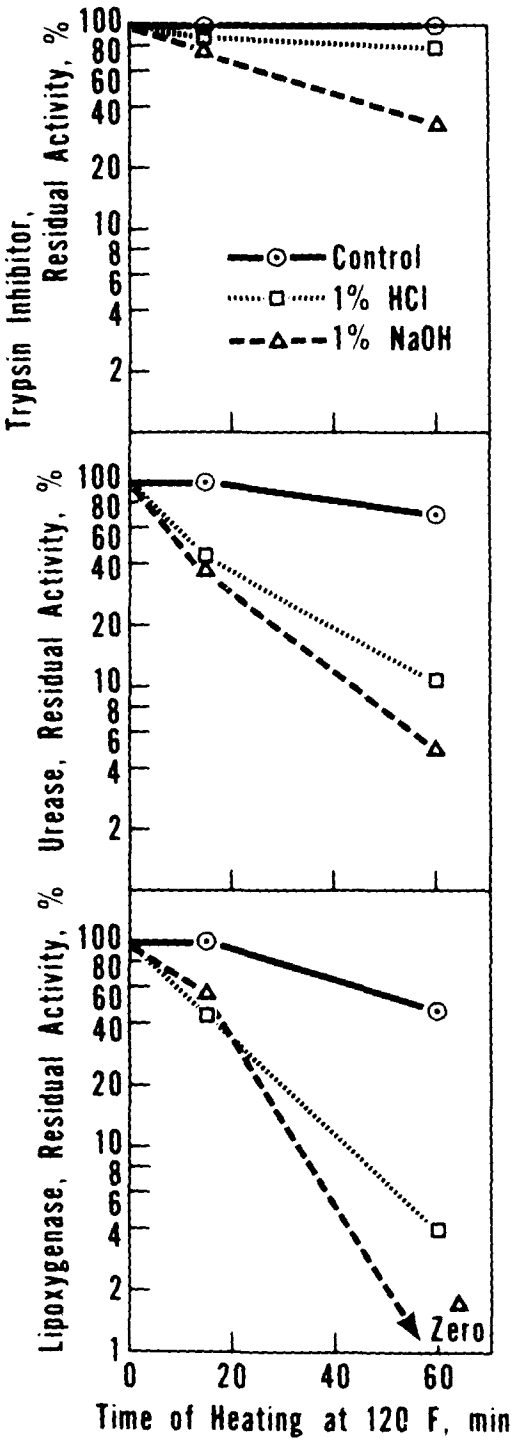

FIG. 7. Effect of heating time on inactivation with additives.

inactivation was diminished. At 195 F, the effect of acid was erased, total inactivation being achieved under the same conditions by the control. However, with NaOH, total inactivation was achieved in 15 min at 180 F, compared to 195 F for the control.

Inactivation of TI: Neither additive caused significant inactivation at 120 and 150 F (Fig. 4). At 165 F, the use of acid was associated with reduced TI activity, but at 180 F and above the TI activity was stabilized by the additive. Obara and Watanabe (7) and Rambaud (8) also reported that TI inactivation with heat is retarded at low pH values. The effect of NaOH is undiminished at the higher temperature, total inactivation being achieved in 15 min at 195 F, compared to 212 F for the control.

Effect on NSI: The use of either additive resulted in lower NSI values at temperatures ranging from 120 to 165 F (Fig. 5). At 180 F and above, the use of base resulted in slightly higher NSI values than the control. The effect of base was considerably less than the effect of acid. The use of acid resulted in very low NSI values at all temperatures investigated. This appears to be heat rather than chemical denaturation, since the pH of the cook waters was in the range 1.8-2.3. The proteins should be quite soluble at these pH values (if unheated) as shown in Figure 6, from the data of Smith and Circle (19).

Effect of Heating Time on Inactivation

The effect of HCl and NaOH in reducing the activity of lipoxygenase, urease and TI was much more pronounced when the heating time was extended from 15 to 60 min at 120 F (Fig. 7). Residual lipoxygenase activity was zero with caustic and 4% with acid, compared to 47% for the control. Residual urease activity was 11% with acid and 5% with base, compared to 70% for the control. Residual TI activity was 79% for acid and 33% for base, compared to 100% for the control.

Effect of Additive on Oil Fraction

This type of cooking of dry soymeats was shown to be quite effective in preventing the oxidation of soybean lipids by lipoxygenase, as evidenced by the low peroxide values of the oil (Table I). Cooking without additive, the values never exceeded 2.4 meq/kg. Cooking with HCl, the highest value obtained was 2.6 meq/kg. Cooking with NaOH, the peroxide values did not exceed 3.2 meq/kg, except at 212 F when the value was 7.8 meq/kg. Mustakas et al. (4) have shown that samples of full-fat flours with peroxide values of 3.9 or less were free of rancid odor and taste after 2 years of storage at room temperature.

The free fatty acid values did not vary significantly, except when NaOH was used. The free fatty acids increased with the cooking temperature; it appears that the cooking temperature should not exceed 165 F when caustic is used, if the formation of free fatty acids is to be avoided. These additional free fatty acids would be expected to have detrimental effect on the stability of the oil.

Effect of Additives on Meal Fraction

The most frequently employed chemical test for measuring the effect of processing conditions on the nutritional properties of soybean protein is "available lysine." There was no change in available lysine with processing temperature for the control (Table I). Slightly lower available lysine values were obtained with caustic at 212 F. A significantly lower value was obtained with acid at 212 F.

Water absorption values with acid were much lower than the control at 195 and 212 F. Slightly higher values were obtained with caustic, except at 212 F.

Effect of Additive Concentration

The effect of additive concentration was studied by repeating the 15 min cooks at 165 F at reduced additive concentrations. The concentrations of acid and base were reduced from 1.0% to 0.25% and 0.10%. With the base additive, the effect on inactivation of TI, urease and lipoxygenase is substantially reduced at 0.25% and at 0.10% the effect is not significant (Table II). The same is true for NSI values.

With the acid additive, 0.25% is about equal to 1.0% in the inactivation of TI and lipoxygenase but considerably less effective in the inactivation of urease. A higher NSI value is obtained at 0.25%. At 0.10%, the effect of the acid additive all but disappears.

Comparison of Other Acids and Bases

The experiments were repeated, substituting 1% phosphoric or sulfuric acid for HCl in 15 min cooks at 165 F.

TABLE I

Effect of Additives on Oil and Meal Fractions during 15 Min Cooks

Additive	Processing temperature, F	Oil		Meal	
		Peroxide value, meq/kg	Free fatty acid, %	Water[a] absorption, %	Available lysine, % protein
None	120	1.3	0.17	163	6.4
	150	1.3	0.16	145	6.2
	165	0.6	0.15	190	6.3
	172	1.9	0.17	200	---
	180	0.8	0.16	180	6.3
	195	0.1	0.12	200	6.3
	212	2.4	0.30	225	6.4
1% HCl	120	1.3	0.17	180	6.3
	150	1.1	0.23	188	6.0
	165	1.9	0.41	175	5.8
	180	0.8	0.16	208	5.9
	195	1.3	0.23	155	5.5
	212	2.6	0.33	136	5.3
1% NaOH	120	0.9	0.14	220	6.2
	150	2.4	0.24	200	6.1
	165	3.0	0.30	223	6.0
	180	1.3	0.73	210	5.8
	195	3.2	1.03	210	---
	212	7.8	1.43	165	5.9

[a]Full-fat flour, basis.

Neither sulfuric nor phosphoric acid was as effective as HCl in promoting the thermal inactivation of TI and urease, although both showed a slight advantage over HCl in inactivation of lipoxygenase (Table II). NSI values were considerably higher with both substitute acids.

The experiments were repeated substituting 1% ammonium hydroxide or sodium bicarbonate for sodium hydroxide. TI is shown to be more heat labile in the presence of NH_4OH than when NaOH is used; sodium bicarbonate has the least effect. Lipoxygenase is readily destroyed by each of the three bases. Urease is destroyed about equally well with NaOH or NH_4OH, but considerably less with $NaHCO_3$.

TABLE II

Effect of Various Acid and Alkaline Additives and Additive Concentration on Inactivation in 15 Min Cooks at 165F

Additive	NSI,[b] %	Residual activity, %		
		Lipoxygenase	TI[a]	Urease
1% H_3PO_4	34	5	100	36
1% H_2SO_4	46	5	100	24
1% HCl	10	11	82	6
0.25% HCl	24	16	86	21
0.10% HCl	47	16	100	35
None	50	20	100	44
1% NH_4OH	14	0	0	16
1% $NaHCO_3$	36	4	88	36
1% NaOH	28	0	37	6
0.25% NaOH	39	14	83	30
0.10% NaOH	52	11	100	40

[a]TI = trypsin inhibitor.

[b]NSI = nitrogen solubility index.

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