Sulfur Compounds Reduce Potato Toxins during Extrusion Cooking

Iwan Surjawan,[†] Michael P. Dougherty, Rodney J. Bushway, Alfred A. Bushway, Jack L. Briggs,[‡] and Mary E. Camire*

Department of Food Science and Human Nutrition, University of Maine, 5736 Holmes Hall, Orono, Maine 04469-5736, Department of Food Science, University of Kentucky, 216 D W.P. Garrigus Building, Lexington, Kentucky 40546-0215, and Natick Soldier Center, 15 Kansas Street, Natick, Massachusetts 01760

Free sulfhydryl groups in sulfur compounds have been reported to act directly on natural toxins to reduce toxicity. The objective of this study was to reduce protease inhibitors and glycoalkaloids in simulated snack foods by the addition of sulfur-containing compounds prior to extrusion. Thiamine, methionine, and benzyl disulfide were added to potato flakes at levels of 0.5% or 1.0% prior to twin-screw extrusion. Total and free thiols and protease inhibitors were monitored before and after extrusion by colorimetric assays. Potato glycoalkaloids were analyzed by HPLC and by immunoassay. Extrusion reduced potato flake disulfide bonds; disulfide bonds were higher in samples containing added sulfur compounds. Trypsin inhibitor activity was reduced by as much as 79% by extrusion plus methionine. Extrusion significantly reduced carboxypeptidase inhibitor, but only when benzyl disulfide and 0.5% methionine were not added. One percent methionine and thiamine resulted in 60% reductions in glycoalkaloids.

Keywords: *Potato; extrusion; glycoalkaloid; sulfur*

INTRODUCTION

Extrusion cooking is used to process a variety of foods for humans and animals. Unique chemical reactions occur within the extruder barrel, and are due to the combined effects of heat, pressure, and shear. Many studies have shown that natural toxins in foodstuffs may be reduced by manipulation of extrusion operating conditions (*1*). Research has focused on reactive extrusion to enhance the effects of extrusion on toxins. Adding ammonia reduced aflatoxins in peanut meal (*2*) and total glucosinolates in extruded canola (*3*), but this remedy may not be suitable for human foods. Selection of an effective chemical agent with low toxicity and possible nutritional benefits is desirable.

Many sulfur compounds are highly chemically reactive and may offer protection against harmful food components. In other forms of processing, heating in conjunction with thiol addition inactivated trypsin inhibitors in soy (*4*). Friedman (*5*) proposed that reactivity of thiol compounds may be greater than would be expected from their basicity presumably because of: (a) polarization of outer shell sulfur electrons; (b) the availability of d-orbitals in the electronic sulfur; and (c) the ability of sulfur to act as a free-radical trap. Cysteine may inhibit the ability of disulfide-containing enzyme inhibitors to complex with active sites on trypsin and related enzymes by formation of sulfhydryl−disulfide interchange (*6*). The effects of sulfur compounds on texture and protein in extruded foods have been evaluated (*7*). Accerbi et al. (*8*) found that soaking deoxynivalenol-contaminated wheat prior to extrusion did not significantly reduce levels of the mycotoxin.

The objective of this study was to reduce the natural toxins trypsin and carboxypeptidase inhibitors and glycoalkaloids in extruded potato-based snacks by the addition of sulfur-containing food chemicals. The vitamin thiamin, the amino acid methionine, and the flavoring agent benzyl disulfide were selected because of their diverse chemical structures. Although the natural toxins in potatoes may not pose a significant public health problem, the variety of such compounds in potatoes affords the opportunity to simultaneously study extrusion effects on toxins of differing chemical structures.

MATERIALS AND METHODS

Sample Preparation. McCain Foods Ltd. (Florenceville, NB) donated the dehydrated potato flakes (instant mashed potatoes) used in this study. Sacks of potato flakes were not co-mingled prior to addition of sulfur compounds. Batches (5.4 kg) of flakes were mixed with sulfur compounds for 5 min in a Hobart mixer (model VCM-25, Troy, OH). Two levels (0.5% and 1.0%, w/w) of each of three chemicals (benzyl disulfide (BD), methionine (ME), and thiamin hydrochloride (TH)) (Aldrich Chemical Co., Milwaukee, WI) were evaluated. The levels used were selected on the basis of trial studies. Mixtures were stored in plastic bags at room temperature until extruded.

Extrusion. Four control samples without added chemicals were extruded at different times to provide an estimate of extruder variability over time. A Werner & Pfleiderer model ZSK 30 twin-screw extruder (Ramsey, NJ) was used to process the potato flake mixtures. A constant set of extruder operating conditions was used: feed moisture, 15%; mass flow rate using an AccuRate feeder (Whitewater, WI), 10.5 kg/hr; screw speed, 200 rpm; barrel temperature profile, 38−38−116−116−138 °C from feed end to die; and a single die (9.7 mm long × 4 mm diam). The length and diameter of each screw were 963 mm and 30 mm, respectively. The high-shear screw configuration used in the experiment consisted of a total of 504 mm of 42°

* To whom correspondence should be addressed. Phone: 207-581-1627. Fax: 207-581-1636. E-mail: camire@maine.edu.
[†] University of Kentucky.
[‡] U.S. Army Natick Soldier Center.

10.1021/jf010087n CCC: $20.00
Published on Web 05/30/2001

forward conveying elements; 56 mm of 28° conveying elements; 14 mm of 28° narrow-pitch conveying element; 260 mm of 20° conveying elements; 7 mm of 41° Igel; 14 mm of 45/5 kneading block elements at 671 mm; 20 mm of 45/5 kneading block elements at 739, 813, and 883 mm; 14 mm of 45/5 left-handed kneading block elements at 685 and 759 mm; and 20 mm of 20° reverse elements. Extrudates were cut with a motorized single-blade cutter at 592 rpm, and were collected on stainless steel screens and then allowed to air-cool at ambient temperature for approximately 15 min. When the extrudates were cool, they were immediately packaged in #10 tin cans by semiautomatic can sealers (Rooney Machine Co., Washington, DC) at 27 mmHg and stored at room temperature until analyzed. Duplicate extrusion runs were made for each treatment.

Moisture Content. Extruded samples were milled in a Thomas-Wiley laboratory mill (model 4, Arthur H. Thomas Co., Philadelphia, PA) to allow passage through a 2-mm screen. Moisture was determined in triplicate as loss in weight of 1 g samples after 16 h at 105 °C.

Sulfur Analyses. Total thiols were measured using the 2-nitro-5-thiosulfobenzoate (NTSB) method (*9*), and free thiols were measured by the 10 mM 5,5′-dithiobios 2-nitrobenzoic acid (DTNB) method (*9*). Disulfide bonds were calculated as the difference between total thiols and free thiols. Approximately 30 mg of the ground sample (≥ 40 mesh), which had been dried overnight under vacuum at 70 °C and 25 mmHg, was used for each assay. Each extrusion run and unextruded potato flakes were assayed 5 times.

Protease Inhibitors. AACC Method 71-10 (as modified by Zhao and Camire (*10*)) was employed for detecting trypsin inhibitor (TI). Carboxypeptidase inhibitor (CPI) was analyzed by the method of Dao & Friedman (*11*). Duplicate analyses of each extrusion run and potato flakes were made.

***Trypsin Inhibitor* (*TI*).** Approximately 1.5 g of ground sample (≥ 40 mesh) was dissolved in 50 mL of the extraction solution (0.015 N NaOH; pH 12) for 3 h at room temperature with magnetic stirring at low speed. TI activity assay was performed in a set of 5 screw-cap glass 20-mL test tubes. Different portions of the extract (0, 1.0, 1.5, and 2.0 mL) from each sample were pipetted into the glass test tubes. An additional tube (the last of the group) contained 2.0 mL of extract and was used for the blank. The volume of the first 3 tubes was proportionally adjusted to 2 mL with distilled water. Trypsin enzyme solution (2 mL) was added into each tube, followed by vortex mixing and incubation in a water bath at 37 °C for 5 min. At timed intervals, 5 mL of prewarmed (37 °C) substrate solution was added to each tube (not blank) and incubated in the water bath for exactly 10 min. The incubation was terminated by adding 1 mL of 30% acetic acid into each tube. The blank was prepared by adding acetic acid prior to the addition of the substrate. Then the solution was mixed and filtered to pass Whatman no. 3 filter paper. The absorbance of filtrates was measured at 410 nm with Beckman spectrophotometer. The final unit of trypsin inhibitor activity was determined as TIU/g of sample on a dry weight basis.

***Carboxypeptidase Inhibitor* (*CI*).** A 300-mg portion of the ground sample was dissolved in 10 mL of Tris-HCl buffer solution (pH 7.5) with magnetic stirring at low speed for 1 h at room temperature. The suspension was centrifuged at 6842*g* for 10 min in a Beckman tabletop centrifuge (model TJ-6R, Palo Alto, CA). The supernatant was saved for the inhibition assay. Aliquots (100 μL) of the supernatant extract were pipetted into glass tubes, followed by the addition of 2.8 mL of substrate reagent (0.025 M Tris-HCl buffer containing 0.5 M NaCl (pH 7.5), 1 mM hippuryl-L-phenylalanine in 0.025 M Tris-HCl buffer, and 10% LiCl). The suspensions were incubated at 25 °C for 4 min to reach temperature equilibrium. Then, the suspensions were transferred into cuvettes, followed by placing each cuvette into the cell of the Beckman spectrophotometer. A 100- μL portion of the diluted enzyme (2.5 U/mL) was then added; and the absorbance at 254 nm was read immediately and at 1-min intervals from 0 to 5 min. The change in absorbance (ΔA_{254}) was calculated from the initial portion of the curve. The activity of the sample was initially estimated on the basis of the known activity (CPU/mg protein) of the blank. The carboxypeptidase inhibitor unit (CPIU/g) was defined as the difference of the activity (CPU/g) between the blank and the sample.

Glycoalkaloids. Samples were extracted with 5:3:2 v/v THF/water/acetonitrile (*11*, *12*), then glycoalkaloids were precipitated with ammonium hydroxide and detected by HPLC with the UV detector at 200 nm. The standard was a mixture of 0.888 mg/mL α-solanine and 0.976 mg/mL α-chaconine with 98% purity; standards were purchased from Sigma Chemical Co., St. Louis, MO. The concentrations of α-solanine, α-chaconine, and TGA were reported in mg/100 g of sample (dry weight basis). A competitive glycoalkaloid enzyme-linked immunosorbent assay (ELISA) kit (AP 002, Envirologix, Portland, ME) was also used to quantitate changes in glycoalkaloids due to extrusion. A Hyperion Inc. (Miami, FL) MicroReader I, version 3.0, was used to measure absorbance at 450 nm. The assay is calibrated with α-solanine, but results are calculated as mg total glycoalkaloids per 100 g sample, dry basis. Each extrusion run and unextruded flakes were evaluated in triplicate.

Statistical Analyses. The statistical analyses were performed with Systat software version 6.0.1 (SYSTAT, Evanston, IL). The General Linear Program program was used to evaluate the results as a one-way analysis of variance. Differences among treatments were determined with Tukey's honest significant different (HSD) test ($p \leq 0.05$). The data were not analyzed as a factorial experiment so that data from the control with no added compounds and the potato flakes used as the base for the snacks could be compared with data from the sulfur-treated samples.

RESULTS AND DISCUSSION

Extrusion Operation. The extrusion runs in this study were considered stable. There was no significant change in mass temperature among runs; mean mass temperature was 171 °C. The torque (73% and 71%) and the calculated SME values (361 and 351 kJ/kg) for ME (1.0%) and TH (1.0%), respectively, were significantly higher than those of other treatments. The pressure built up at the die during extrusion of the potato flakes containing 1.0% ME was the highest: 254 psi. Moisture content after extrusion ranged from 4.93 to 9.04% (Table 1). The high pressure that developed during the extrusion of the 1.0% ME samples produced a significantly lower moisture.

Sulfur Distribution. Total thiols and disulfide bonds were significantly higher in samples containing 1% sulfur compounds, compared with the those of control product (Table 1), with the exception of total thiols in the 0.5% BD treatment. Free thiols were higher in all extruded samples than in the nonextruded potato flakes. Extrusion may free some sulfur groups involved with protein stabilization.

Protease Inhibitors. Sulfur treatments in combination with extrusion, except for 0.5% benzyl disulfide, significantly reduced trypsin inhibitor (Table 2). Zhao and Camire (*10*) reported up to 85% TI reduction in abrasion potato peels. However, those peels had a relatively high level of 45 TIU/g; peels obtained by steam-peeling had lower TI (8.06 TIU/g), and no further reductions occurred with extrusion.

Only the thiamin treatments and 1% methionine reduced carboxypeptidase inhibitor (Table 2). Because the carboxypeptidase inhibitor is more thermally stable, the conditions used here should be modified to produce greater destruction. To our knowledge, no previous studies have examined carboxypeptidase inhibitor (CPI) in extruded potato. As a natural toxin, CPI is equally important with other proteinase inhibitors such as those

Table 1. Moisture and Sulfur Content of Extruded Sulfur-added Potato Flakes and Nonextruded Potato Flakes

treatment	level of addition (%)	moisture (% d.b.)	total thiol[a] (μmol/g)	free thiol[a] (μmol/g)	disulfide bond[a,b] (μmol/g)
dl-methionine	0.5	6.24 ± 1.80 b	9.16 ± 0.36 d	2.46 ± 0.40 ab	6.70 ± 0.01 c
dl-methionine	1.0	4.93 ± 0.52 a	12.08 ± 0.64 b	2.95 ± 0.61 a	9.14 ± 0.26 b
thiamin-HCl	0.5	9.04 ± 2.50 b	8.69 ± 0.51 de	1.74 ± 0.28 b	6.95 ± 0.10 c
thiamin-HCl	1.0	6.78 ± 0.40 b	10.49 ± 0.48 c	1.85 ± 0.24 b	8.64 ± 0.39 b
benzyl disulfide	0.5	5.79 ± 0.57 b	8.54 ± 0.57 de	1.75 ± 0.28 b	6.79 ± 0.45 c
benzyl disulfide	1.0	7.35 ± 1.39 b	14.21 ± 1.09 a	2.18 ± 0.45 ab	12.02 ± 0.47 a
control[c]	0	7.32 ± 1.89 b	8.00 ± 0.52 e	2.87 ± 1.10 a	5.14 ± 0.40 d
nonextruded potato flakes[d]		7.31 ± 0.66 b	7.03 ± 0.40 f	0.57 ± 0.16 c	6.46 ± 0.06 c

[a] Means ± standard deviations within columns followed by different letters are significantly different ($p \leq 0.05$, Tukey's HSD test). Calculated as the difference of total thiol and free thiol. [b] Mean values ($N = 10$) on a dry weight basis. [c] $N = 20$. $N = 15$.

Table 2. Trypsin and Carboxypeptidase Inhibitor Activity and Percent Reduction by Addition of Sulfur Compounds to Potato Flakes Prior to Extrusion

treatment	trypsin inhibitor (TI)[a] (TIU/g)	% TI reduction[b]	carboxypeptidase inhibitor (CPI)[a] (CPIU/g)	% CPI reduction[b]
dl-methionine (0.5%)	4.13 ± 0.29 c	47.2	31.43 ± 0.98 bc	2.8
dl-methionine (1.0%)	1.64 ± 0.63 d	79.0	28.00 ± 3.07 c	13.5
thiamin-HCl (0.5%)	5.87 ± 1.51 bc	24.9	28.69 ± 1.71 bc	11.3
thiamin-HCl (1.0%)	2.77 ± 0.88 d	64.6	27.69 ± 2.11 c	14.4
benzyl disulfide (0.5%)	6.26 ± 0.41 bc	19.9	31.40 ± 2.45 bc	3.0
benzyl disulfide (1.0%)	4.68 ± 0.44 c	40.1	30.31 ± 0.72 bc	6.3
control (0%)	7.82 ± 1.97 a		32.36 ± 1.49 b	
nonextruded potato flakes	6.40 ± 2.44 ab		40.87 ± 2.04 a	

[a] Means ± standard deviations ($n = 6$) within columns followed by different letters are significantly different ($p \leq 0.05$, Tukey's HSD test). [b] Percent reduction as compared with control potato flakes extruded without added sulfur compounds.

Table 3. Individual and Total Glycoalkaloid Reduction by Addition of Sulfur Compounds to Potato Flakes Prior to Extrusion[a]

	HPLC detection			
treatment	α-solanine (mg/100 g)	α-chaconine (mg/100 g)	TGA (mg/100 g)	TGA by immunoassay (mg/100 g)
dl-methionine (0.5%)	0.99 ± 0.03 bc	1.05 ± 0.02 b	2.04 ± 0.01 bc	9.80 ± 3.68
dl-methionine (1.0%)	0.36 ± 0.02 e	0.35 ± 0.04 c	0.71 ± 0.04 d	9.55 ± 0.64
thiamin-HCl (0.5%)	1.12 ± 0.04 b	1.16 ± 0.08 b	2.28 ± 0.12 b	7.35 ± 4.03
thiamin-HCl (1.0%)	0.33 ± 0.01 e	0.33 ± 0.02 c	0.65 ± 0.03 d	5.85 ± 1.06
benzyl disulfide (0.5%)	2.05 ± 0.14 a	2.21 ± 0.07 a	4.26 ± 0.21 a	14.45 ± 1.63
benzyl disulfide (1.0%)	1.02 ± 0.06 b	1.14 ± 0.09 b	2.15 ± 0.14 b	14.55 ± 5.16
control	0.85 ± 0.03 cd	0.92 ± 0.01 b	1.77 ± 0.04 c	9.68 ± 4.78
nonextruded potato flakes	0.76 ± 0.09 d	0.93 ± 0.22 b	1.77 ± 0.32 c	Not determined

[a] Means ± standard deviations ($n = 6$) within columns followed by different letters are significantly different ($p \leq 0.05$, Tukey's HSD test).

for trypsin and chymotrypsin. In contrast to trypsin and chymotrypsin inhibitors, CPI is highly stable during normal cooking methods such as boiling, oven baking, and oven microwave baking (*13*). CPI has the lowest molecular weight (4200) among potato proteinase inhibitors (*14*). The stability of CPI during normal cooking methods may be related to its small molecular weight. Such a relatively small molecule should have a rather compact three-dimensional structure with relatively little conformational freedom. CPI contains 39 amino acid residues (*15*) and three intramolecular disulfide bonds. Like TI, CPI was expected to alter its structure by cleaving its disulfide bonds during extrusion cooking and rendering them inactive through manipulation of thiol groups.

Glycoalkaloids. Methionine and thiamin treatments (ea. 1%) caused significant decreases in total glycoalkaloids. We had theorized that the reactivity of thiols may disturb the conformation of both α-solanine and α-chaconine by cleaving one or more attached sugar molecules (rhamnose, glucose, and galactose) from the aglycon (i.e., solanidine). The removal of one or more of the sugar molecules from the aglycon should have altered the polarity of the TGA.

Previous studies have reported that TGA in potatoes are relatively stable under regular cooking or processing (*16* − *19*). Extrusion alone under varying conditions was insufficient to reduce glycoalkaloids in potato flakes and peels, respectively (*20*, *21*).

The TGA content in potato flakes (NPF) was compared with that of the control to determine the stability of TGA under extrusion alone. The result confirmed the finding of Zhao et al. (*21*) that both α-solanine and α-chaconine were stable with extrusion alone. Both the flakes and the control had similar TGA contents (1.77 mg/100 g dry weight basis), suggesting that TGA are highly resistant not only to temperature (138 °C) but also to the intensive mixing and high pressure that occur during extrusion.

The TGA contents of the benzyl disulfide and 0.5% methionine and thiamin samples were significantly higher than that of the control. We attribute these increases to (1) enhanced extractability of glycoalkaloids from samples containing added sulfur compounds, and (2) differences among sacks of potato flakes used to prepare individual treatments. Immunoassays for TGA found no significant differences among samples (Table

3), but values were considerable higher than those obtained by HPLC.

CONCLUSIONS

Our data indicate that thiols played a significant role in reducing protease inhibitor levels and glycoalkaloids (as determined by HPLC method) in the potato flakes during twin-screw extrusion cooking. Thiamin and methionine, when added at the 1% level, reduced protease inhibitors and glycoalkaloids in potato flakes extruded under the conditions used in this study. During twin-screw extrusion cooking, disulfide bonds in the potato flakes were partially detached to form free thiols. Reduction in potato toxins may have been influenced by the presence of native free thiols and of added sulfur-containing chemicals. The reactivity of thiols with the natural toxic compounds during extrusion suggested preliminary theories for their mechanism of reaction. The structural integrity of the trypsin inhibitor in the potato flakes was prone to destruction through cleavage of its disulfide bonds. For TGA, the reactivity of thiols may disturb the conformation of both α-solanine and α-chaconine which, in turn, alters their polarity. Despite the effectiveness of the thiols in reducing natural toxins in potato during extrusion cooking, the strong sulfur smell of benzyl disulfide, and to some extent the other chemicals, may limit their use for human foods. Flavor and aroma masking may be necessary. Animal feeding studies should be performed to confirm that reduction in natural toxins increases nutritional quality.

ACKNOWLEDGMENT

The U.S. Army provided the use of their facilities for extrusion.

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Received for review January 19, 2001. Revised manuscript received April 17, 2001. Accepted April 17, 2001. Maine Agricultural and Forest Experiment Station external publication #2487.

JF010087N