Effect of Autoclaving and Conventional and Microwave Baking on the Ergot Alkaloid and Chlorogenic Acid Contents of Morning Glory (*Ipomoea tricolor* Cav. cv.) Heavenly Blue Seeds

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Ipomoea tricolor Cav. cv. (Heavenly Blue morning glory) seeds contain about 20% high-quality protein, about 16% fat, and 55.5% carbohydrate and inhibited the activity of trypsin to the extent of about 11 mg/g of seed flour. The total ergot alkaloid content of 52 mg/100 g of the seeds was reduced by 8–21% when the seed flour was mixed with a commercial wheat flour preparation (Bisquick flour mix) in a ratio of 1:9 and baked as a muffin in a autoclave at 121 °C for 18 min. Similar losses occurred when pure ergonovine was cobaked with the flour mix. Lysergol was more stable than ergonovine during baking. The corresponding decrease for the same preparation baked in a microwave oven for 90 s was about 30%; after conventional baking at 204 °C for 18 min, 23–31%. Losses in the crust were somewhat higher than in the crumb. Parallel studies on the decrease of chlorogenic acid showed that this compound is more labile than the alkaloids; it decreased about 100% in the crust fraction and 65% in the crumb fraction of the convection-baked muffin. Microwave baking reduced the chlorogenic acid content by 77% of the original. These findings show that varying degrees of thermal destruction of ergot alkaloids and chlorogenic acid occur in a typical flour mix at ordinary baking temperatures.

Contamination of cereal grain with ergot sclerotia formed by *Claviceps purpurea* and related fungal species is a widely recognized phenomenon (Baumann et al., 1985; Schoch and Schlatter, 1985; Scott and Lawrence, 1982; Porter et al., 1981). The sclerotia contain a variety of potentially toxic ergot alkaloids some of which, such as ergonovine (ergometrine), are also used as drugs (Cordell, 1981). Wheat, rye, and related *Gramineaceous* cereal seeds and seed stock also become contaminated by ergot alkaloids from the widely distributed wild-type higher plants of the family Convolvulaceae (La Bonte and Darding, 1988). The most important member of this family is the morning glory weed plant (*Ipomoea*). In a previous paper we reported that the Heavenly Blue variety of this plant contains substantial amounts of ergot alkaloids (Friedman et al., 1989).

Chlorogenic acid, a chromosome-damaging or clastogenic compound, was also found to be present in a series of morning glory varieties (Friedman et al., 1989).

Although harvesting, cleaning, and milling of grain generally remove most of the ergot from contaminated crops, this is not always the case (Shuey et al., 1973). Commercial wheat and rye flours are reported to contain small amounts of ergot alkaloids (Scott and Lawrence, 1980). Lorenz (1979) reports that consumption of ergot-contaminated grain may result in serious illness or even death. Since most grain is subjected to food processing such as baking and cooking (Ziderman et al., 1989), it is of both theoretical and practical interest to find out whether ergot alkaloids survive such processing. Previous studies (Scott and Lawrence, 1980, 1982; Wolff and Ocker, 1985) suggest that *Claviceps*-derived ergot alkaloids in grain are susceptible to thermal destruction during conventional baking. The main objective of this study was to measure the stability of Heavenly Blue morning glory seed derived ergot alkaloids and chlorogenic acid during autoclaving and conventional and microwave baking. A second objective was to measure the protein, amino acid, carbohydrate, fat, mineral, and trypsin inhibitor contents of the Heavenly Blue morning glory seeds.

EXPERIMENTAL SECTION

Baking Experiments. Heavenly Blue morning glory seeds (obtained from a local store) were milled in a Wiley mill to pass a 40-mesh screen. The resulting flour was then added to ergot-free wheat flour (Bisquick baking mix, General Mills, Minneapolis, MN) to product 1:9 mixture of morning glory and wheat seed flours. A 30-g sample of the mixed flour was suspended in 16 mL of distilled water. The dough was then placed into suitable muffin baking aluminum pans (i.d., 2.25 in.; height, 1.25 in.) and subjected to three types of baking. In the first, the muffin pan was placed in a preheated convection oven at 204 °C and baked for 18 min. In the second, the glass muffin pan (i.d., 2.5 in.; height, 1.75 in.) was placed into a microwave oven (J. C. Penney, Model 863-5973) which was then set at the high-temperature setting and left to bake for 90 s. In the third, the glass muffin pan was autoclaved at 121 °C for 18 min. The baked products were then air-dried at room temperature and divided into fractions. The conventionally baked muffin was divided as follows: 2-mm-thick top crust (outside portion not in contact with the baking pan), 2-mm-thick bottom crust, and crumb (middle portion). The autoclaved and microwave-baked muffins were divided into outside (about 2 mm thick) and inside fractions. Each fraction was ground in a mortar before analysis.

Extraction Methods. Alkaloids were extracted as described previously (Friedman et al., 1989). All analytical steps were carried out under subdued light at room temperature. Briefly, duplicate samples (5 g) of muffing flours were each extracted three times with 50 mL of acetone–2% aqueous tartaric acid (70:30, v/v). The combined supernatants were then evaporated on a water aspirator to remove acetone. The aqueous residue was washed three times with 50 mL of methylene chloride–isopropyl alcohol (3:1, v/v) and the solution adjusted to pH ≈8 with a saturated sodium bicarbonate solution. Total ergot alkaloids were then again extracted with three 50-mL portions of the methylene chloride–isopropyl alcohol mixture.

Defatted muffin flour was prepared by extracting 5 g of undefatted flour with petroleum ether in a Soxhlet apparatus for 18 h. Chlorogenic acid was then extracted from the defatted flour with 100 mL of 100% ethyl alcohol also for 18 h.

Extracts were analyzed for total ergot alkaloid or chloro-

Table I. Stability of Ergonovine and Lysergol Cobaked with Wheat Flour

sample	% loss (n = 2)	
	ergonovine	lysergol
unbaked flour	0	0
convection oven baked muffin		
crumb	33.4 ± 3.8	27.7 ± 4.2
bottom crust	38.8 ± 3.0	41.2 ± 2.1
top crust	41.9 ± 0.42	36.7 ± 4.2
microwave oven baked muffin		
outside fraction	34.0 ± 4.7	23.1 ± 2.1
inside fraction	34.5 ± 3.2	17.1 ± 2.1
autoclaved mixed flour		
outside fraction	24.7 ± 4.2	20.1 ± 2
inside fraction	17.1 ± 2.1	12.6 ± 4.2

genic acid contents as described previously (Friedman et al., 1989).

Spiking Experiments. Ergot-free wheat flour (30 g, Bisquick baking mix) was cobaked with 1 mg of authentic lysergol and ergonovine-free base.

Thin-Layer Chromatography. Ethanolic extracts of various muffin fractions were evaporated to dryness under a stream of nitrogen, redissolved in 1 mL of methanol, and subjected to thin-layer chromatography as previously described (Friedman et al., 1989). A 15-μL portion of each extract was spotted on a TLC plate along with a chlorogenic acid standard. The plate was then developed with ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:27, v/v/v/v) until the solvent front reached 13 cm. The dried plate was then sprayed with 1% methanolic diphenylboric acid–β-ethylamino ester followed by 5% ethanolic poly(ethylene glycol) 4000. Finally, the plate was observed under ultraviolet light (365 nm) for yellow fluorescence.

Compositional Analyses. Analyses for nitrogen, fat, carbohydrate, moisture, and mineral content and for the presence of trypsin inhibitor and lectins were carried out as previously described for jimson weed seed and soybean flour (Dugan et al., 1989; Friedman and Levin, 1989; Friedman and Gumbmann, 1986).

RESULTS AND DISCUSSION

Stability of Ergot Alkaloids. Table I lists the losses of lysergol and ergonovine that had been added to and cobaked with the wheat flour formula. For baking in a convection oven, the loss of ergonovine from the crumb fraction was lowest (33.4%), compared to 38.8% for the bottom crust and 41.9% for the top crust fractions. For the microwave-baked muffin, the loss for both the inside and outside fractions was the same (34%). After autoclaving, the loss of ergonovine was 17% for inside and 24% for outside fractions. Similar losses occurred when pure lysergol was autoclaved and conventionally baked with flour mix. However, the lysergol was more stable when baked in a microwave oven.

Table II shows that, for mixed Heavenly Blue morning glory–wheat flours, the losses were as follows: 24% for the bottom crust fraction, 25% for the crumb fraction, and 31% for the top crust fraction, with an average value for all three of 26.7 ± 4.1%. For microwave-baked fractions, the inside and outside fractions had similar losses, with an average value of 29.8 ± 1.3% for both fractions. The lowest losses were obtained for autoclaved mixed flour: 8% for the inside and 21% for the outside fractions, respectively.

Our findings can be compared with the following reported losses of *Claviceps*-derived ergot alkaloids during baking: up to 100% for wheat bread; up to 85% for rye bread, and up to 74% for pancakes (Scott and Lawrence, 1982). Wolff and Ocker (1985) also report that the shape of the bread loaves significantly influenced the extent of ergot alkaloid losses. Thus, for ergometrine added directly to the flour before baking, losses varied from 45 to 60% depending on bread shape.

Table II. Stability of Ergot Alkaloids during Baking of Mixed Heavenly Blue Morning Glory and Wheat Flours (1:9)[a]

sample	% loss (n = 2)
unbaked mixed flour	0
convection oven baked muffin	
bottom crust fraction	23.7 ± 4.4
crumb fraction	25.0 ± 3.8
top crust fraction	31.3 ± 5.2
microwave oven baked muffin	
outside fraction	28.8 ± 1.9
inside fraction	30.8 ± 1.9
autoclaved mixed fluor	
outside fraction	21 ± 4.2
inside fraction	8 ● 2.8

[a] The Heavenly Blue morning glory seeds contained 52 mg of ergot alkaloids/100 g.

Table III. Effect of Heating Time on Stability of Ergonovine, Lysergol, and Ergot Alkaloids of Heavenly Blue Morning Glory Seeds (Heated without Flour)

heating time, min	% loss after autoclaving at 121 °C		
	ergonovine	lysergol	Heavenly Blue morning glory seeds
0	0	0	0
5	12.7 ± 2.1	8.7 ± 1.8	18 ± 2.8
10	16.3 ± 0.3	9.7 ± 1.5	34 ± 2.8
20	19.1 ± 1.2	15.1 ± 3.7	37 ± 1.4
30	31.7 ± 0.6	25.2 ± 0.6	47 ± 4.2
60	40.4 ± 0.07	27.8 ± 1.01	54 ± 2.8

Table IV. Stability of Ergonovine, Lysergol, and Ergot Alkaloids of Heavenly Blue Morning Glory Seeds during Heating (Baked without Flour)

baking procedure	% loss (n = 2)		
	ergonovine	lysergol	Heavenly Blue morning glory seeds
microwave oven	14.5 ± 3.8	13.2 ± 1.2	29 ± 4.2
convection oven	74 ± 1.4	32.6 ± 1.3	71 ± 1.4

Table III shows that the losses of ergonovine, lysergol, and Heavenly Blue morning glory seed derived alkaloids increased with increasing heating time. Lysergol is more stable than ergonovine during heating. The losses were highest for Heavenly Blue morning glory derived alkaloids. Similar results were obtained with convection and microwave oven baked pure alkaloids. Total losses of lysergol were 13% for the microwave oven baked and 32% for convection oven baked compounds, respectively (Table IV).

Our results with ergot alkaloids in Heavenly Blue morning glory seeds and those reported previously for corresponding alkaloids derived from *Claviceps* fungi show that the alkaloids are susceptible to degradation during baking. A variety of factors associated with food processing could influence the extent of such degradations, although the reasons for the wide range in the observed losses (24–100%) are not immediately apparent. Factors that influence this could include the types of baking, the thickness of the dough, influencing the thermal gradient to which the alkaloids are exposed (crumb and crust fractions), the interaction of the alkaloids with nutrients and inert ingredients during baking (with or without flour), and the relative susceptibilities of the individual alkaloids to heat damage (ergonovine vs lysergol). For example, morning glory seeds are reported to contain a

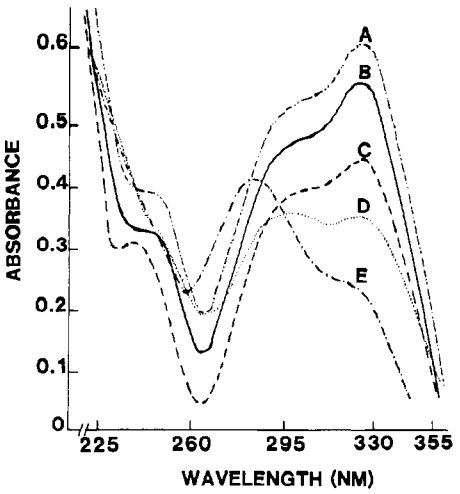

Figure 1. Ultraviolet absorption spectra of chlorogenic acid (A), ethanolic extracts of Heavenly Blue morning glory seeds (B), crumb fraction of convection oven baked muffin (C), microwave oven baked muffin (D), and crust fraction of convection oven baked muffin (E).

Table V. Stability of Chlorogenic Acid during Baking of Mixed Heavenly Blue Morning Glory and Wheat Flour[a]

sample	% loss (n = 2)
unbaked mixed flour	0
convection oven baked muffin	
crust fraction	100
crumb fraction	65.1 ± 1.9
microwave oven baked muffin	77.0 ± 0.92

[a] The Heavenly Blue morning glory seeds contain 1.11 g of chlorogenic acid/100 g.

large number of structurally related ergoline-type alkaloids (Genest et al., 1965; Der Marderosian, 1967; Wilkinson et al., 1987). These include agroclavine, chanoclavine, elymoclavine, ergometrine (ergonovine), ergonovinine, ergosine, ergosinine, ergine (lysergic acid amide), isoergine (isolysergic acid amide), lysergic acid α-(hydroxyethyl)amide, penniclavine, and setoclavine. Individual alkaloids in morning glory seeds may vary in their susceptibility to destruction by heat and other food processing conditions. In addition, the pattern of the individual alkaloids in Heavenly Blue morning glory seeds also differs significantly from that in sclerotia of the *Claviceps* fungi (Porter et al., 1974; Scott and Lawrence, 1982). Either or both of these factors could account for variations in the rate of destruction of the alkaloids originating from different natural sources. Additional studies are needed to clarify this possibility.

Stability of Chlorogenic Acid. Figure 1 depicts the ultraviolet spectra of authentic chlorogenic acid, ethanolic extracts of Heavenly Blue morning glory seeds, and several fractions of baked muffins. The absorption maximum at 328 nm, characteristic of chlorogenic acid, was exhibited only by the ethanolic extract of the crumb fraction of the oven-baked and microwave-baked fractions. The crust fractions lacked the absorbance, thus demonstrating the absence of chlorogenic acid, as confirmed by TLC analysis.

Table V lists the chlorogenic acid content of the various fractions calculated from the absorption spectra. The results show that losses of chlorogenic acid ranged from 65 to 100%. This conclusion is also supported by data from thin-layer chromatography. On the TLC plates, only the ethanolic extracts of the crumb fraction and the microwave-baked muffin exhibited a yellow fluorescence with

Table VI. Trypsin Inhibitor Content of Heavenly Blue Morning Glory Seed Flour and of Heavenly Blue Morning Glory Seed Flour Cobaked with Wheat Flour (n = 2)

treatment	units of trypsin (TIU) inhib/g sample	mg trypsin inhib/g sample
none[a] (native seeds)	1712 ± 29	11.0 ± 0.2
microwave baked (outside fraction)[b]	43.0 ± 5.6	0.27 ± 0.05
microwave baked (inside fraction)	44.0 ± 7.8	0.28 ± 0.07
convection oven baked (crumb)	25.0 ● 4.2	0.15 ± 0.02
convection oven baked (top crust)	55.0 ± 7.8	0.34 ● 0.03
convection oven baked (bottom crust)	21.0 ± 1.4	0.122 ± 0.01

[a] 100% Heavenly Blue morning glory seeds. [b] Baked samples contained 10% Heavenly Blue morning glory seed and 90% wheat flour.

R_f 0.64, identical with that obtained with authentic chlorogenic acid.

The cited observations suggest that heat during baking destroys chlorogenic acid more effectively than ergot alkaloids from Heavenly Blue morning glory seeds and that thermal heat produced in a convection oven generally appears more effective than microwave radiation. Since chlorogenic acid is widely distributed in plant foods (Hurrell and Finot, 1984), it would be worthwhile to find out whether the susceptibility of chlorogenic acid to destruction by thermal heat and microwave radiation is influenced by the type of food in which it is present.

Trypsin Inhibitor Content. Table VI shows that Heavenly Blue morning glory seeds contained significant amounts of a trypsin inhibitor. Additional studies showed the absence of any chymotrypsin inhibitors. Since the seed inhibitor inactivates only trypsin but not chymotrypsin, it is probably of the Kunitz-type, which inhibits only trypsin, rather than of the Bowman–Birk-type, which inhibits both trypsin and chymotrypsin (Friedman and Gumbmann, 1986). The table also shows that both microwave and conventional baking inactivate approximately 60–80% of the inhibitor content. This is similar to that reported for trypsin inhibitors in soybean seeds.

Lectin Content. Duplicate analyses revealed the presence of 0.586 ± 0.09 μg/50 μL of lectins, a value similar to that found for jimson weed seeds (Friedman and Levin, 1989).

Amino Acid Composition. Three analyses with samples containing about 5 mg of protein (N × 6.25) were used to establish the amino acid composition of Heavenly Blue morning glory seeds: (a) standard hydrolysis with 6 N HCl for 25 h in evacuated sealed tubes (Friedman et al., 1979); (b) hydrolysis with 6 N HCl after performic acid oxidation to measure the half-cystine and methionine content as cysteic acid and methionine sulfone, respectively (Menefee and Friedman, 1985); (c) basic hydrolysis by barium hydroxide to measure tryptophan (Friedman and Cuq, 1988).

Tables VII and VIII list the proximate analyses and amino acid composition of the protein (plus any free amino acids) of Heavenly Blue morning glory seed flour. The values of the amino acid scoring pattern of the essential amino acids for an ideal protein, as defined by the Agricultural Organization of the United Nations (Mercer et al., 1989), are also shown for comparison (Table VIII). Although the glutamic acid residues comprise about one-fifth of the total, the essential amino acid pattern of the seed flour exceeds that of high-quality protein (Mercer

Table VII. Proximate Composition of Heavenly Blue Morning Glory Seeds

material	content
carbohydrate	55.5%
protein (N × 6.25)	20.1%
fat	16.3%
H_2O	9.0%
calcium	0.215%
magnesium	0.347%
sodium	0.064%
potassium	1.68%
copper	24 ppm
manganese	45 ppm
iron	768 ppm
zinc	82 ppm

Table VIII. Amino Acid Content of Heavenly Blue Morning Glory Seed Flour (Duplicate Values)[a]

amino acid	g/100 g		g/100 g protein		FAO[e] (g/100 g protein)
Asp	2.8	2.7	13.8	13.4	4.0
Thr	0.96	1.0	4.8	5.4	
Ser	1.3	1.3	6.2	6.2	
Glu	4.2	4.1	20.7	20.2	
Pro	1.0	0.99	5.0	4.9	
Gly	1.3	1.3	6.5	6.5	
Ala	1.3	1.3	6.5	6.5	
Val	1.2	1.2	6.0	6.0	5.0
Cys[b]	0.41	0.42	2.1	2.2	3.5[f]
Met[c]	0.30	0.30	1.5	1.5	
Ile	1.0	1.0	5.1	5.1	4.0
Leu	1.9	1.9	9.7	9.7	7.0
Tyr	0.67	0.67	3.3	3.3	6.0[g]
Phe	1.1	1.1	5.7	5.7	
His	0.69	0.67	3.4	3.3	
Lys	1.4	1.4	7.1	7.1	5.5
Arg	1.6	1.6	8.0	8.1	
Trp[d]	0.13	0.13	0.64	0.64	

[a] % N = 3.22. [b] Determined as cysteic acid after performic acid oxidation. [c] Determined as methionine sulfone after performic acid oxidation. [d] Determined in a separate analysis after hydrolysis by barium hydroxide. [e] Provisional Amino Acid Scoring Pattern for an ideal protein (Mercer et al., 1989). [f] Cys + Met. [g] Tyr + Phe.

et al., 1989). If it were possible to eliminate the ergot alkaloids from the seeds through food processing or through molecular biology–plant genetic techniques, morning glory seeds could serve as a source of high-quality protein equivalent to that of casein.

In conclusion, ergot alkaloids present in Heavenly Blue morning glory seeds appear to have a greater resistance to destruction during autoclaving and conventional and microwave baking than the reported stabilities during conventional baking of related alkaloids produced by fungi. Microwave baking was somewhat less effective than heating in a convection oven in reducing the ergot alkaloid and chlorogenic acid content of Heavenly Blue morning glory seed flour cobaked with wheat flour. Both conventional and microwave baking procedures inactivated most of the trypsin inhibitors present in the seed flour. Possible nutritional and toxicological consequences of these observations await further study.

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Registry No. Chlorogenic acid, 327-97-9; trypsin inhibitor, 9035-81-8; ergonovine, 60-79-7; lysergol, 602-85-7.