Effect of Cysteine Addition on the Volatiles of Extruded Wheat Flour

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The sulfur-containing amino acid cysteine was added at concentrations ranging from 0% to 1.0% to wheat flour and extruded in a corotating twin screw extruder. A total of 18 sulfur-containing compounds was tentatively identified along with 4 pyrazines and 1 furan. The fact that most of the sulfur-containing compounds were thiazoles indicated that these compounds may be formed due to thermal degradation of cysteine. The increase in concentration of cysteine in the wheat flour did effect the concentration of most volatiles, including non-sulfur-containing volatiles; however, the addition of cysteine over the 0.5% level did not seem to greatly increase volatile production.

Keywords: *Extrusion; flavor; cysteine; wheat flour; volatiles*

INTRODUCTION

Extrusion is a thermal processing technique that involves the application of high heat, high pressure, and shear forces to an uncooked mass, such as wheat flour. These processing conditions result in a number of chemical changes that lead to the final "cooked" product. The chemical changes that occur include gelatinization of starch molecules, crosslinking of proteins, and the generation of flavors. One problem which may occur concerning the generation of flavors is that as the mixture is passed through the extruder die, the sudden drop in pressure results in the flashing off of water when the temperature of the product in the die exceeds 100 °C. This results in the steam stripping of the volatiles and loss of overall flavor. Volatiles may also become bound to proteins or starch during extrusion or may thermally decompose. Palkert and Fagerson (1980) studied the retention of 10 flavor compounds added prior to the extrusion of soy protein. These researchers found that there was between 78% and 96% loss during extrusion. Kim and Maga (1994) found that functional group and chain length played a key role in flavor retention and that under some conditions longer chain length acids, aldehydes, and alcohols could be totally retained during extrusion of high-amylose corn starch. To compensate for this loss, manufacturers may add volatiles near the die of the extruder or postextrusion (Camire, 1991). Another method to enhance flavor may be the addition of reactive flavor precursors to the flour prior to extrusion to increase production of flavor volatiles or change the flavor profile.

There has been a limited amount of research conducted dealing with the addition of reactive precursors to flour during extrusion. Ho et al. (1989) added 0.5% thiamine HCl to corn flour prior to twin screw extrusion at a product temperature of 180 °C and found thiazole compounds from the degradation of thiamine. Maga and Kim (1989) added various amounts of protein

sources, such as soy flour, soy protein concentrate, sodium caseinate, whey protein concentrate, and gluten, to corn starch and analyzed the volatiles produced during extrusion by gas chromatography and sensory evaluation but did not identify the volatiles. These researchers found that protein type and amount, as well as extrusion conditions, did play a role in the formation and retention of volatiles and sensory differences. Bailey et al. (1994) added whey protein concentrate to corn meal at concentrations ranging from 0% to 20% and measured the flavor and color of the extrudate. They found that the production of pyrazines, furans, and other heterocyclics increased as the product temperature and concentration of whey protein concentrate, an excellent source of lysine and sulfur-containing amino acids, was increased. Izzo et al. (1994) added ammonium bicarbonate or ammonium bicarbonate and pyruvaldehyde to the water source during the extrusion of wheat flour. These researchers found that the addition of the nitrogen source (ammonium bicarbonate) somewhat influenced the production of volatiles, specifically pyrazines, and had a great effect on the color of the extrudate. However, the addition of both the ammonium bicarbonate and the pyruvaldehyde resulted in greater production of pyrazines. They concluded that ammonium bicarbonate was too reactive and led to the formation of brown pigments instead of flavor compounds.

Another type of reactive precursor which could be added to the flour during extrusion is amino acids. Amino acids would provide a primary amino group, which is less reactive than free ammonia, and possibly a functional group on the side chain. In this study the amino acid cysteine was used. The amino acid cysteine has been shown to be a very important precursor to a number of food flavors, particularly meat flavor (MacLeod, 1986). There has been a great deal of research that focused on the production of volatiles from the reaction or thermal degradation of cysteine in model systems (Shu et al., 1985a,b; Shu and Ho, 1988; Zhang et al., 1988; Zhang and Ho, 1989, 1991a,b). Although extensive flavor research has been accomplished in this area, the volatiles produced during the extrusion processing of a complex food product with added cysteine

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have not been studied. Therefore, the objectives of this research were to study the volatiles that were produced during the extrusion of wheat flour with various levels of the amino acid cysteine added and determine the concentration of the volatiles retained in the extruded product.

EXPERIMENTAL PROCEDURES

Extrusion Conditions. Crystalline L-cysteine (Sigma, St. Louis, MO) was ground in a mill and mixed thoroughly with high-gluten wheat flour (Bouncer, Bay State Milling Company, MA) to the concentrations of 0%, 0.25%, 0.5%, 0.75%, and 1.0% (w/w). The extrusion was carried out on a ZSK-30 corotating twin screw extruder (Werner Pfleiderer Corp., Ramsey, NJ). The unit was equipped with a die having two 3 mm diameter, 5 mm long openings. The length and diameter of each screw were 900 and 30 mm, respectively. The screw configuration used in the experiments consisted of forwarding elements (L/D = 21.9), two mild mixing elements (L/D = 2.7), six kneading elements (L/D = 3.6), and two reverse elements (L/D = 1.1). The barrel has resistance heaters and five independently controlled heating zones. The barrel also has cooling jackets through which cooling water can be circulated at controlled flow rates (solenoids) to prevent overheating of the barrel. The heaters and five solenoids are controlled using a proportional, integral, and derivative (PID) controller. Product temperatures were recorded by a thermocouple inserted at the die plate. Wheat flour was fed into the unit with a K-Tron series 7100 volumetric feeding system (K-Tron Corp., Pitman, NJ). A metering pump (U.S. Electric Motors, Millford, CT) was used to add the water. The extrudate was pelletized by a two blade cutter at the face of the die which was operated at 890, 890, 905, 950, and 900 rpms for the 0%, 0.25%, 0.5%, 0.75%, and 1.0% cysteine added samples, respectively. The cutter was operated at different speeds to compensate for the changes in expansion properties of the extrudate at different levels of added cysteine.

A total of five extrudates was produced at the four concentrations of cysteine added and the control. The following conditions were used: 16% process moisture, 500 rpm screw speed, and 225 g/min mass flow rate. The product temperature measured at the die was 185 °C for all runs. The extrusion conditions were equilibrated by running the extruder with plain wheat flour. Once extrusion conditions were equilibrated, the flour with cysteine was added to the feeder. Samples were taken 10−15 min after the new flour sample containing cysteine was added to the feeder. Samples were run in order of increasing cysteine concentration. The extrusion was run in duplicate.

Volatile Isolation. Each of the extrusion products was ground separately in the presence of dry ice in a Waring blender. The sample was allowed to come to room temperature to sublime all dry ice, and 5 g of each ground sample was weighed into a glass cylinder (0.5 in. o.d., 0.32 in. i.d., 14 in. long). The samples were spiked with internal standard (1 μL of 1 mg/mL toluene-d_8 in methanol) by injecting the internal standard into the sample in the glass tube and connected to a thermal desorption sample collecting system (Scientific Instrument Services, Ringos, NJ). The volatiles were then purged and trapped into thermal desorption tubes containing two equal beds of the resins Tenax TA and Carbotrap. The trapping conditions were as follows: 80 °C oven block temperature, 40 mL/min nitrogen flow, and 1 h trapping time. After trapping, the moisture was removed from the thermal desorption tubes by flowing nitrogen gas through them at 40 mL/min at room temperature for 45 min.

Volatile Analysis. The trapped volatiles were desorbed directly into the GC injection port (220 °C, 5 min) using a Model TD-1 short path thermal desorption apparatus (Scientific Instrument Services, Ringos, NJ). Separation of the volatiles was accomplished using a Varian 3400 gas chromatograph equipped with a nonpolar fused silica capillary column (60 m × 0.32 mm (i.d.), 1 μm film thickness, DB-1; J&W Scientific, Folsom, CA). The GC was operated with an injector

temperature of 270 °C, a helium carrier gas flow rate of 1 mL/min, a split ratio of 10:1, and a detector temperature of 300 °C. During desorption, the column temperature was lowered to −20 °C by putting dry ice in the oven to trap volatiles, the column temperature program was from −20 to 280 °C at 5 °C/min. Quantification determination was accomplished by comparing the peak area of the internal standard to the unknowns. The concentration of each component was converted to parts per billion of the ground extrudate.

GC/MS Analysis. Tentative identification of the volatiles was accomplished by GC/MS using a Varian 3400 gas chromatograph coupled to a Finnigan MAT 8230 high-resolution mass spectrometer, using the same GC program as for the separation. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA, and spectra were recorded on a Finnigan MAT SS 300 data system.

RESULTS AND DISCUSSION

Table 1 lists the volatile compounds tentatively identified in the solid extrudate for all five concentrations of cysteine added. Due to the variability between extrusion runs, the range of concentration is presented in Table 1. In general, the amount of flavor volatiles including pyrazines, furans, and sulfur-containing compounds increased as the concentration of the added cysteine was increased from 0% to 0.5%. At levels higher than 0.5%, the quantity of the flavor volatiles did not seem to increase greatly. Of the sulfur-containing volatiles, thiophene, thiazole, and 2,4-dimethylthiazole reached the highest concentrations. Methylpyrazine and 2,5-dimethylpyrazine were non-sulfur-containing flavor compounds which reached high levels and were also affected by the increase in cysteine concentration.

Due to the presence of both a sulfur and nitrogen atom on cysteine, one would expect that these different classes of heterocyclic compounds would be produced. These compounds are typically nonenzymatic browning products but may also be formed through the thermal degradation of cysteine. Pyrazines are important nitrogen-containing flavor compounds which are generally formed in the reaction of an amino group with a carbonyl group in the Maillard browning reaction. Pyrazines provide a roasted or toasted flavor note (Maga, 1992). There were four pyrazines identified from the extrusion of wheat flour with added cysteine. Methylpyrazine was found at similar concentrations for all levels of added cysteine and the control. Furans are oxygen-containing heterocyclics that provide a sweet or caramel-like aroma and may be formed from pyrolysis of sugars (Fors, 1983). In these experiments, 2-acetylfuran was the only furan identified and the addition of cysteine did not seem to effect the production of this sugar degradation product. Both pyrazines and furans have been identified as products from the model system reaction of cysteine with glucose (Zhang and Ho, 1991a).

Thiophenes are sulfur-containing compounds which have been previously reported in cooked meat and are generally responsible for a mild sulfurous odor (Shibamoto, 1980). Sakaguchi and Shibamoto (1978) concluded that thiophenes were formed from the reaction of a sugar or carbohydrate with hydrogen sulfide or an amino acid. Shibamoto (1977) found that 2-formylthiophene may be produced from the reaction of furfural, hydrogen sulfide, and ammonia. This may have been the case in this extrusion. Six thiophenes were identified in the extrusion of wheat flour with added cysteine. Unsubstituted thiophene reached the highest concentration. The flavor characteristics of thiazoles have

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Table 1. Volatile Compounds Identified from the Extrusion of Wheat Flour with Various Levels of Cysteine Added[a]

	concentration (ng/g of reaction mixture)				
compounds	0%[b]	0.25%	0.5%	0.75%	1.0%
pyrazines					
methylpyrazine	+++	+++	++++	+++	+++
2,5-dimethylpyrazine	−	−	+++	+++	++++
ethylpyrazine	−	−	++	++	++
3-ethyl-2,5-dimethylpyrazine	−	−	++	++	++
furans					
2-acetylfuran	+++	+++	+++	+++	+++
thiophenes					
thiophene	++	++	+++	+++	+++
2,5-dimethylthiophene	−	++	++	++	++
vinylthiophene	−	++	++	++	++
2-formylthiophene	−	++	++	++	++
2-acetylthiophene	++	−	+	++	++
2-formyl-5-methylthiophene	−	−	++	++	++
thiazoles					
thiazole	+++	+++	+++	+++	+++
2-methylthiazole	−	++	+++	++	++
4-methylthiazole	−	++	++	++	++
2,4-dimethylthiazole	−	++	+++	+++	+++
4-ethyl-2-ethylthiazole	−	++	++	++	++
2-acetylthiazole	−	++	+++	+++	+++
trimethylthiazole	++	++	++	++	++
2,5-dimethyl-4-ethylthiazole	−	−	++	++	++
5-methyl-2-propylthiazole	−	−	++	++	++
thiazolines and thiazolidine					
2-methyl-3-thiazoline	++	++	+++	++	++
2-methylthiazolidine	++	++	+++	++	+++
2,4-dimethyl-3-thiazoline	−	++	++	++	++
others					
isoprepenylbenzene	−	+++	+++	+++	+++
2-ethyl-1-hexanol	−	++	++	++	++
acetophenone	++	++	++	++	++

[a] + = 1 to 9 ng/g. ++ = 10 to 99 ng/g. +++ = 100 to 499 ng/g. ++++ = 500 to 999 ng/g. [b] Percent cysteine added to wheat flour (weight basis).

Table 2. Quantification of Classes of Compounds from Model Systems and Extrusion (Percent of Total Volatiles)

compound class	cysteine[a]	cysteine + glucose[b]	extrusion with cysteine[c]
pyrazines		12.1	25.7
furans		9.1	10.4
thiophenes	1.3	41.4	8.7
thiazoles	12.9	13.0	32.9

[a] Cysteine = cysteine alone at 180 °C for 1 h (Zhang et al., 1988). [b] Cysteine + glucose = Cys and glucose at 180 °C for 1 h (Zhang and Ho, 1991). [c] Extrusion with cysteine = extrusion of wheat flour with 0.5% Cys added.

been previously reviewed by Maga (1975). Thiazoles generally have a meaty flavor and are used extensively in producing meat flavorings. The mechanistic pathway for the production of thiazoles is not well-known. Thiazoles may be formed through the decomposition of thiamine in cooked foods and during extrusion (Ho et al., 1989). The model system of glucose and cysteine revealed very few thiazoles (Zhang and Ho, 1991a); however, many thiazoles were produced from the thermal degradation of cysteine in water (Shu et al., 1985a; Zhang et al., 1988) indicating that thiazoles are mainly produced via the degradation of cysteine. One exception is 2-acetylthiazole which may be formed from the reaction of the Strecker aldehyde of cysteine with pyruvaldehyde (Mulders, 1973). 2-Acetylfuran was identified at relatively high concentrations in these experiments, along with thiazole, which remained at high levels at all added cysteine levels, and 2,4-dimethylthiazole.

Table 2 presents the percent of total volatiles for various classes of compounds found from the thermal degradation of cysteine (Zhang et al., 1988), the reaction of cysteine with glucose (Zhang and Ho, 1991a), and the extrusion of wheat flour with 0.5% added cysteine. The concentration of thiazole and thiazole derivatives found in the extrudate was much higher than that of the thiophenes indicating that the primary route of production for sulfur-containing volatiles may be through the thermal degradation of cysteine. Another explanation for the higher quantity of thiazoles is that the nitrogen-containing heterocyclics, such as thiazoles and pyrazines, have been shown to be retained more efficiently than thiophenes (Palkert and Fagerson, 1980). The large amount of 2-formyl-5-methylthiophene may be formed from the reaction of a sugar degradation product with hydrogen sulfide similar to the way 2-formylthiophene was formed from the reaction of furfural and hydrogen sulfide and ammonia (Shibamoto, 1977).

The fact that there was not a great increase in the production of volatiles above the 0.5% level was surprising. The addition of more cysteine was expected to result in the formation of higher levels of volatiles. This result may suggest that the additional volatiles produced are lost at the die of the extruder. The volatiles measured in this study would have to be condensed on the surface of the extrudate or physically bound in air pockets in the extrudate. The addition of cysteine had other implications on the final extruded product including a visible change in texture and color. For example, the specific mechanical energy (SME) calculated for the five samples was changed due to the addition of cysteine while the processing conditions were held fairly constant. The SME calculated was 452, 704, 776, 764, and 752 kJ/kg for the 0%, 0.25%, 0.5%, 0.75%, and 1.0% added cysteine, respectively. These changes in the

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mechanical energy may have influenced starch and protein breakdown and ultimately flavor production.

The addition of cysteine as a flavor precursor may be used to increase and alter the flavor characteristics of an extruded product. To fully evaluate the volatiles produced during extrusion, a collection system similar to the one described in Nair et al. (1994) must be used to trap volatiles which are released at the extruder die. Although this would better indicate the reactions taking place in the extruder, it would not be a good representation of the flavor profile of the extruded product.

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