

Corn Gluten Meal Odorants and Volatiles After Treatment to Improve Flavor

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ABSTRACT

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Production of corn gluten meal (CGM), a high-protein coproduct from wet milling of corn, is increasing as production of fuel ethanol from corn increases. Unpleasant taste and odor have limited the use of CGM in human food. Adjustment of pH and extraction with water have been reported to reduce the off-flavor of CGM but the improvement is not enough for substantial addition of CGM to the human diet. More study of CGM is needed. In this study, volatile compounds released under different conditions of pH, water extraction, and temperature were identified and compared using solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). The water-extractable portion, which im-

proves the taste of CGM by its absence, was dried and analyzed by SPME-GC-MS. In addition, materials extractable from CGM with methylene chloride were identified by gas chromatography-mass spectrometry (GC-MS). Further, the spontaneous generation of a CGM-like odor accompanied by a change in physical appearance of the CGM sample was described. Flavors and odors known to be associated with the identified CGM compounds were listed. Some possible origins of the volatiles, from degradation of corn constituents or as fermentation products of the corn steeping process, were noted.

Corn gluten meal (CGM) is the high-protein coproduct of the corn wet-milling process (May 1987). Production of CGM is increasing as the production of fuel ethanol from corn increases. CGM protein is high in the amino acids cystine and methionine, while soy and some other plant proteins are deficient in these amino acids. A plant-based product providing complete protein for human nutrition could be produced by combining CGM with soy or other plant proteins. Unfortunately, the taste and odor of CGM are not appealing to most people and the 1.7 million metric tons of corn gluten meal currently produced annually in the United States (CRA 2004; RFA 2004) goes primarily to animal feed. Wu et al (1994, 2001, 2002) have improved the taste of CGM with pH adjustment and water extraction, but off-flavors and off-odors that remain or return have prevented CGM from being widely accepted as a human food.

This study contributes valuable information about CGM that is not available elsewhere and is of interest to those studying or utilizing CGM. Others may report further information about CGM and, by building upon additional layers of knowledge, lead to a final resolution of the problem of off-odor and off-flavor in CGM. The findings reported here serve to suggest further areas of inquiry on CGM.

In this study, SPME-GC-MS was used to identify volatile components of CGM at pH 4, at pH 7, and after extraction with water. The SPME was conducted at 25°C and 100°C to compare CGM room temperature volatiles with those released during cooking when CGM products are boiled in water.

Further information about CGM was obtained from additional analyses and observations. The water-extractable portion, which improves the taste of CGM by its absence, was dried and analyzed by SPME-GC-MS. A methylene chloride extract of untreated flash-dried CGM was partitioned, concentrated, and analyzed by direct injection into the GC-MS. This extraction technique permitted identification of less polar materials in CGM that were either not volatilized or not absorbed on the SPME fiber. In addition, spon-

aneous generation of CGM off-odor from a previously odorless CGM sample was observed. This event was strongly suggestive of possible future areas of research.

Overall, this report is confined to identification of components in volatiles and extracts as well as identifying those components with known odors. The data does not address the relative contribution of the odor components to the off-odor of CGM nor does it quantify the amounts of components present in original material. Some possible origins of the CGM compounds, from degradation of corn constituents or as fermentation products of the corn steeping process, are noted.

MATERIALS AND METHODS

CGM and CGM wet cake (WC) were obtained from Williams Bio-Energy, Pekin, IL, from different batches. WC is the material remaining at the end of the corn wet-milling process after the water has been removed mechanically. The WC is then flash-dried by brief heating to 400°C to produce CGM (May 1987).

A portion of the fresh wet cake was stored in a cooler and analyzed by SPME-GC-MS (Pawliszyn 2000) the day after collection. Other portions were treated as described below. Lyophilization caused fresh wet cake to lose 58% of its weight when completely dry, presumably due to moisture loss. Lyophilized WC consisted of 71.3% protein, 2.8% moisture, and 4.4% fat by weight. CGM had 66.5% protein ($N \times 6.25$), 8.3% moisture, and 2.0% fat by weight. Nitrogen was measured using the Kjeldahl method, and moisture was measured by weight loss after oven drying at 130°C for 1 hr (AACC International 2000). Fat was determined by petroleum ether extraction (AOAC International 1998).

GC-MS standards were 2,3 dihydrobenzofuran, farnesyl acetone, geranyl acetone, ethyl linoleate, stigmaterol, linoleic acid, skatole from Sigma-Aldrich (St. Louis, MO), and 1,3 butanediol, 1,4 butanediol, benzophenone, and myristic acid from Fisher Chemicals (Fairlawn, NJ). CGM spectra were also compared with mass spectral standards from the computer library of Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer 1989).

Preparation for WC Analysis

The wet cake (WC) treatments are summarized in Table I. The fresh WC sample was refrigerated overnight without any treatment and analyzed by SPME-GC-MS the following day. All other samples were treated on the day of collection and stored lyophilized until analysis.

The lyophilized WC pH 4 was wet cake taken from the factory directly to the laboratory and lyophilized without further treatment. The WC pH 7 dried all sample was prepared by adding 250 mL of double distilled (DD) water to 50 g of fresh WC. The sample was

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adjusted to pH 7 with KOH and the slurry was stirred at room temperature for 30 min, then refrigerated and lyophilized.

The WC pH 7 1:10 dilution 2× was prepared by addition of 500 mL of DD water to 50 g of fresh WC, the sample was adjusted to pH 7 with KOH, and the slurry was stirred at room temperature for 30 min. The slurry was divided into four glass centrifuge bottles, each holding ≈130 mL of slurry and then centrifuged at 624 × g for 10 min at room temperature (Sorvall RC-5B refrigerated superspeed centrifuge) to separate the liquid from the solids. The liquid was decanted, frozen, and lyophilized for later analysis as pH 7 water-extractable soluble solids. An additional 500 mL of DD water was added to the remaining solid portion and the sample was again adjusted to pH 7 with KOH. The slurry was stirred for an additional 30 min at room temperature, centrifuged as before, and separated into liquid and solid fractions. The liquid from this second treatment was discarded and the remaining solid portion was lyophilized for later analyses as WC pH 7 1:10 dilution 2×.

TABLE I
Treatments of Corn Gluten Wet Cake Meals

Sample	pH Adjustment	Extraction	Drying Method
Fresh WC pH 4	None	None	None
WC pH 4	None	None	Lyophilized
WC pH 7 DA ^a	pH 7	None	Lyophilized
WC pH 7 EM ^b	pH 7	Extracted–solid fraction	Lyophilized
WC pH 7 SS ^c	pH 7	Extracted–liquid fraction	Lyophilized

^a DA, dried all. Solid and liquid portions were not separated before lyophilization.

^b EM, extracted meal. After 30 min of stirring at room temperature the liquid portion was removed before lyophilization of the meal.

^c SS, soluble solids. After 30 min of stirring at room temperature the liquid portion was removed and lyophilized.

Analysis of WC Volatiles

Gas chromatography-mass spectrometry (GC-MS) was performed using a GC system (HP 6890) attached to a mass selective detector (HP 5972A). The column was fused silica HP-5MS capillary (0.25 μm film thickness, 30 m × 0.25 mm i.d.). A solid-phase

TABLE III
Volatiles of Soluble Solids Extracted from Wet Cake During Treatments that Improve Flavor

Retention Time (min)	Name	Area Counts
13.1	2-Pentyl-furan	<1
22.1	Safranal	<1
28.6	α-Ionone	10
28.9	Dihydro-β-ionone	7
29.1	Geranyl Acetone	19
30.1	β-Ionone	7
30.4	Benzeneacetaldehyde	8
32.1	Nerolidol	11
32.7	Ethyl laurate	10
37.1	Ethyl tridecanoate	9
39.7	Methyl palmitate	5
41.1	Palmitic Acid	6
40.6	E-11- Hexadecenoic acid ethyl ester	17
40.9	Ethyl palmitate	712
41.8	Geranyl linalool isomer	4
42.3	Thiosulfuric acid, S-(2-aminoethyl) ester	4
43.3	Linoleic acid	11
44.0	Ethyl linoleate	591
44.8	9,17-Octadecadienal	3
44.2	Ethyl oleate	194
44.7	Ethyl stearate	25
48.1	Erucic acid	6
54.1	Squalene	95

^a GC-MS TIC area counts × 10⁻⁶.

TABLE II
Solid-Phase Microextraction GC-MS Results for Treated Wet Cake Meals

Retention Time (min)	Name	pH 4				pH 7			
		Fresh		Dried All		Dried All		Water-Extracted	
		25°C	100°C	25°C	100°C	25°C	100°C	25°C	100°C
3.7	Acetic acid				13		17	11	
5.5	2,3 Butanediol		7		37		13	13	
8.0	1,3 Butanediol	580	340	227	152	159	118	136	106
8.9	Butyrolactone	136	131	92	78	67	60	48	39
12.4	1,4- Butanediol		12			7	5		
14.6	2-Butanol	94		31	24	20	16	19	16
18.8	Phenylethyl alcohol	12	46	46	124	32	137	6	
22.5	Benzothiazole					3	7	2	
22.6	2,3-Dihydro-benzofuran			14	26	7	9		
23.5	E,E-2,4-Decadienal			3					
25.3	4-Vinyl-2methoxy-phenol		40	11	87	15	51	3	11
27.2	4 Methyl, 1,3-benzenediamine					18	24	16	33
27.7	1,3-Dihydro 2 H benzimidazol-2-one	200		64		12	22	114	17
29.1	Geranyl acetone						8		8
33.2	Diphenylmethanone	141	23	19	6	7	5	5	
36.1	Myristic acid		17		16		9		12
36.4	Phenanthrene								8
39.3	Farnesyl acetone						23		85
40.3	Palmitic acid		3,481	17	1,344	102	999	31	4,752
40.9	Ethyl palmitate			10	220				
41.8	Phenothiazine		2						
43.3	Linoleic acid		4,740	362	3,088	405	2,203	279	4,767
44.0	Ethyl linoleate		122	26	28				
44.8	9,17-Octadecadienal			24					
54.0	Squalene		30		15	5	6		27
59.4	Ergost-5-en-3-ol			8		2			
60.5	Stigmast-5-en-3-ol			25	20	10	15	8	

^a Values reported are GC-MS TIC area counts × 10⁻⁶.

^b Temperatures at which volatiles were collected on the SPME fiber.

microextraction device (Supelco, Bellefonte, PA) with an 85- μm polyacrylate or polydimethylsiloxane (PDMS) coating was used for collection of volatiles. The data in Tables II and III were obtained with the PDMS fiber. For collection of volatiles, 5 g of WC sample were placed in a 600-mL beaker, 50 mL of DD water, and a Teflon stir bar were added, and the beaker was covered tightly with four layers of aluminum foil. The mixture was allowed to equilibrate for 1 hr (25°C) or was allowed to boil. At this point, the SPME device was inserted through the aluminum foil and allowed to absorb volatiles for 1 hr. The absorbed compounds were desorbed at 250°C onto the inlet injector fitted with a 78.5 mm \times 6.5 mm, o.d., tapered wool-packed splitless injection sleeve for 0.5 min on the gas chromatograph. The carrier gas was helium maintained at 40 psi, and the temperature was initially held at 50°C for 10 min, after which the temperature ramping was increased at 5°C/min to a final temperature of 315°C. All mass spectra were acquired in the electron impact (EI) mode at 70 eV. The electron multiplier was set at 1,680V.

Identification of Compounds

Compounds were identified by a combination of mass spectral data and GC retention time. Spectra were compared with known standards or by computer with the Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer 1989). This software permits the comparison of the mass spectrum of the analyte peak with the mass spectra of known standards that are recorded in the Wiley mass spectral library program.

These can be displayed side by side for individual evaluation. Additionally, the Wiley library software is able to compare the similarity of the analyte spectrum with the spectra of known standards in the computer library. The software assigns a rating of the spectral match between the analyte peak and the library standard, which it lists as a similarity index value (SI). The SI rating scale is 1–100, with 100 being a perfect match. Identical compounds may have an SI of 96–99 due to small differences in instrumental parameters and chromatographic background. In this study, compounds were identified first by the library SI, and then by a visual comparison of the analyte spectra to the library spectra of known standards. In the case of overlapping peaks, a spectral extraction was performed and carefully evaluated before identification of the component was made. Analyte GC retention times were compared with either the retention of a pure standard under identical GC-MS conditions or published GC retention data utilizing Korvats and ethyl ester retention indices (IUPAC 1997; Acree and Arn 2004). No attempt at quantitation was made other than recording the area counts of GC peaks as reported in the MS total ion count (TIC) mode. In cases where an analyte peak could not be clearly identified, it is not reported.

CGM Extraction with Methylene Chloride

CGM (250.0 g) was exhaustively extracted with 1.0 L of CH_2Cl_2 using a Soxhlet apparatus for 24 hr. The extracted CGM after CH_2Cl_2 evaporation did not possess the distinctive odor present in the unextracted CGM, indicating that the odor compounds were present in the CH_2Cl_2 extract. The extract volume was then reduced slowly to ≈ 10 mL by rotoevaporation at a cool (20°C) temperature to prevent loss of volatile constituents. Some of the extract was immediately examined by GC-MS. The remaining extract was transferred to a 250-mL Erlenmeyer flask and placed in an airhood to allow for complete volatilization of CH_2Cl_2 .

The resultant extract possessed an intense odor of unextracted CGM. The extract was then partitioned between 25 mL of hexane and 50 mL of DD water. The hexane fraction was examined directly by GC-MS, while the water fraction was re-extracted in a separatory funnel with 5 mL of CH_2Cl_2 , after which the CH_2Cl_2 fraction was examined by direct injection into the GC-MS under the parameters described above.

Spectra were compared with known standards or by computer with the Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer 1989) as described above.

RESULTS AND DISCUSSION

Wet-Milling and Off-Flavor in CGM

Corn wet-milling involves steeping corn in water in the presence of sulfur dioxide and lactobacilli bacteria to soften the kernel. The corn is then milled to separate germ, fiber, starch, and protein. The protein (corn gluten) is dewatered mechanically and then flash-dried at 400°C (May 1987). CGM has a distinctive off-odor and an off-flavor described by taste panelists as strongly fermented and somewhat bitter (Wu et al 1994). The off-odor may be removed or reduced (Wu et al 1994, 2001) but it returns on standing at room temperature in a sealed vessel. The characteristic off-odor is generated intensely when CGM is heated in boiling water.

Given the method by which CGM is produced, it was expected that the off-odors and off-flavors would be due to 1) sulfur compounds either from reactions with the added SO_2 or from degradation of sulfur-containing proteins, 2) by-products of lactobacillus fermentation, and 3) products produced by the high heat of the flash-drying process. Due to the presence of 2–4% of lipid material in CGM, low molecular weight lipids and lipid oxidation products were also expected to contribute to the off-flavor and off-odor of CGM.

Products from the fermentation of proteins by lactobacilli during the steeping process were considered to be potential contributors to the off-odor and off-flavor in CGM because lactobacillic fermentation of proteins was known to cause off-flavors and off-odors. For instance, lactobacillus fermentation of proteins at low pH in the anaerobic conditions of pig gut produces skatole (3-methyl-1H-indole), a strongly offensive odor and a component of the off-flavor boar taint in pork (Jensen et al 1995). Corn wet-milling involves similar fermentation conditions, that is, lactobacillus growth in the presence of high protein concentration at approximately pH 4 (May 1987).

Wu et al (2003) previously reported the results of SPME-GC-MS of untreated CGM volatiles and identified a number of odor/flavor compounds among the volatiles. Interestingly, with the exception of small amounts of sulfur dioxide, acetic acid, and phenothiazine, sulfur-containing compounds and low molecular weight lipid products were not detected in the untreated CGM volatiles reported by Wu et al (2003).

Selection of Treatment for WC

Corn wet-milling produces both CGM and WC at approximately pH 4. WC has a uniform light yellow color, while flash-dried CGM has a darker golden yellow color with granules of brown color presumably generated from superheating during the flash-drying process. Taste panels have found the most improved taste in treated WC rather than treated CGM (Wu 2001). Because most of the distinctive CGM odor is present in fresh wet cake, WC was chosen as the starting material for treatment to eliminate possible variations due to flash-drying conditions. Because adjustment of WC to pH 7 and extraction with water has been shown previously to improve the flavor of WC (Wu 2001), volatiles were collected from WC under conditions representative of the stages of this treatment to monitor the effect of each step. In Table II, volatiles from WC collected fresh from the factory were compared with those of WC meals that were lyophilized only, adjusted to pH 7 and lyophilized, and finally adjusted to pH 7, extracted twice with water, and lyophilized. In Table III, the volatiles of soluble solid materials removed by water from WC at pH 7 are presented. Collection of volatiles was conducted at 25°C and at 100°C for comparison between room temperature and cooking temperature because intense CGM odor has been observed during cooking of food products containing $\leq 10\%$ of CGM. The areas

reported in Tables II and III were ordered by GC retention time for ease of comparison, so that each column presented the chromatographic areas of the identified volatile components from the stages of treatment and at the temperatures indicated.

Volatiles from Treated CGM Wet Cake

Results from analysis of headspace volatiles above WC/water slurries of the treated samples are shown in Table II. The term "dried all" identifies treated meals in which the solid and liquid portions were not separated before lyophilization, while the term "extracted" identifies the treated meal from which the liquid portion had been removed before lyophilization. This distinction was made to permit comparison of changes in the volatiles as a result of pH adjustment alone versus changes that resulted from the removal, by extraction with water, of compounds that were released from WC by pH adjustment. Although the objectives of this study were identification rather than quantitation, the GC-MS conditions were held constant during analyses. This allowed the areas of peaks in the GC-MS total ion count (TIC) chromatogram data (Table II) to provide the reader with a sense of the relative quantity of each component, not only within a treatment stage (columns) but between treatment conditions (rows). The areas of peaks in the GC-MS TIC chromatogram data are reported as area counts $\times 10^{-6}$. The purpose of the analyses was to look for trends in the volatiles under progressive treatment conditions and not to precisely quantitate specific components.

The data in Table II demonstrate differences in CGM volatiles collected at room temperature versus cooking (boiling) temperature. Higher temperatures lead to increased volatilization of higher MW compounds, the release of CGM heat decomposition products, and the possible displacement of smaller molecules from the SPME fiber by the higher MW volatiles (Niedziella 2000). Lower temperatures detect heat-labile compounds as well as those otherwise subject to displacement from the fiber by higher MW compounds. Both sets of information are useful in exploring mechanisms causing off-odor and off-flavor in CGM. In Table II, palmitic and linoleic acids show the effect of collection temperature on volatiles detected in dramatically higher quantities at 100°C compared with 25°C across all CGM treatment conditions. Also notable were the increases in phenylethyl alcohol and 4-vinyl methoxyphenol at 100°C.

Compounds collected at 25°C but none or lesser amounts at 100°C included 1, 3 butanediol, 1,3 dihydro-2H-benzimidazol-2-one, diphenylmethanone, and ergost-5-en-3ol (Table II). Compounds collected at 100°C but none or lesser amounts at 25°C were phenylethyl alcohol, 4-vinyl-2-methoxy-phenol, myristic acid, farnesyl acetone, palmitic acid, ethyl palmitate, linoleic acid, and squalene (Table II). These compounds may contribute to the distinctive odor of heated CGM.

The effect of pH on CGM volatiles is seen in Table II by comparing the pH 4 and pH 7 columns. Compounds detected only at pH 7 were benzothiazole, 4 methyl 1, 3 benzenediamine, and farnesyl acetone. Ethyl palmitate and ethyl linoleate were detected at low levels in CGM volatiles at pH 4 but were not detected in volatiles of CGM at pH 7 (Table II). However, ethyl palmitate and ethyl linoleate were major components detected in the volatiles of the soluble solids extracted from CGM at pH 7 (Table III). The changes in distribution of these compounds may be related to the mechanism by which pH adjustment improves flavor. Compounds reduced by water extraction at pH 7 included acetic acid, 2,3 butanediol, 1,4 butanediol, phenylethyl alcohol, and 4-vinyl-2-methoxyphenol (Table II). Because water extraction at pH 7 is known to improve flavor, these compounds are included among the compounds that may improve the taste of CGM by their absence.

Compounds present under all four treatment conditions were 1,3 butanediol, butyrolactone, 2-butanol, phenylethyl alcohol, 4-vinyl-2-methoxyphenol, 1,3 dihydro-2H-benzimidazol-2-one, diphenylmethanone, myristic acid, palmitic acid, linoleic acid, and

squalene (Table II). Additionally, the gradual loss of 1,3 butanediol and butyrolactone across treatment conditions are noted.

The role of lipids or compounds with similar solubilities merits further examination. Although lipids comprised only 4.4% of the WC, long chain fatty acids were major peaks detected in the WC volatiles as seen in Table II. Fatty acid ethyl esters (FAEE) were present in small amounts in WC volatiles. FAEE were not detected in volatiles of WC meal after treatment with water extraction at pH 7 (Table II). However, FAEE were major components detected in volatiles of the soluble solids extracted from WC by water at pH 7 (Table III).

Possible effects on flavor were predicted from the compositional changes after pH adjustment and water extraction. Reduction of acetic acid, 2, 3 butanediol, 1, 4 butanediol, phenylethyl alcohol, butyrolactone, and 4-vinyl-2-methoxyphenol should reduce flavors associated with these compounds. These are sour (acetic acid), fruity (2, 3 butanediol), honey (phenylethyl alcohol), caramel (butyrolactone), and clove (4-vinyl-2-methoxyphenol) (Acree and Arn 2004). Additionally, a strong unpleasant odor that was not associated with honey was present in the water-soluble portion of the methylene chloride extract of CGM. This may be a concentration effect from the significant phenylethyl alcohol peak or from an associated trace component.

The soluble solids of the extracted liquid fraction (Table III) were those compounds that were extracted by water from WC at pH 7, not lost to volatilization during lyophilization and detectable in headspace at 100°C. There were no detectable volatiles in headspace above the soluble solids at 25°C. Values for soluble solids volatiles in Table III cannot be compared quantitatively to the volatiles from the treated meals in Table II because the soluble solids data (Table III) measure the volatiles from 5 g of a concentrated extract from a starting amount (50 g) of WC, while the meal volatiles (Table II) represent volatiles obtained from 5 g of the treated WC meals. The TIC areas in Table III are presented only for comparison with the other components in Table III.

Following removal from WC of the extracted liquid fraction (Table III), taste panelists have reported improvement in flavor of the remaining meal (Wu et al 2001). In Table III, ethyl esters of fatty acids were predominant in the soluble solids, while fatty acids were present in only small amounts. This is in contrast to the volatiles above the treated meals seen in Table II, where fatty acids are major components and fatty acid esters are minor components or not detected at all. Ionone isomers, safranal and 2-pentylfuran, were also detected among the volatiles from the soluble solids shown in Table III. Their contribution to flavor is discussed below.

Benzothiazole was detectable in volatiles of CGM after the pH was adjusted from acid to neutral (Table II). Benzothiazole is a known off-flavor product of the nonenzymatic browning pathway that occurs at typical storage temperatures and contributes stale, sour-green sulfury flavor characteristics (Sucas 2002, 2004). This odor has also been described as sulfuric and quinoline-like (Shiratuchi 2003). The role of benzothiazole in CGM odor merits further exploration.

Methylene Chloride Extract of CGM

Untreated flash-dried CGM was extracted with methylene chloride and partitioned into hexane and water-soluble fractions. Both fractions possessed odors reminiscent of the unextracted CGM, with the hexane fraction having more of a rancid oil odor, while the water fraction possessed a distinctive phenolic odor. The components identified by GC-MS analyses of the methylene chloride fractions are shown in Table IV. The hexane fraction contained pentylfuran, phenylethyl alcohol, decadienal isomers, ionenes and ionones, alkanes, alkenes, long chain fatty acids and their esters (C14-C18), and farnesyl acetone. The water fraction contained phenylethyl alcohol, 4-vinyl-2-methoxy phenol, lolilide isomers and trace amounts of linoleic acid. Of these, pentylfuran,

TABLE IV
Components Identified by GC-MS of Hexane and Water Partition of a CGM Methylene Chloride Extract

Retention Time (min)	Name	Hexane Fraction	Water Fraction
13.7	Pentylfuran	Present	
16.4	4-Vinyl-2-methoxy-phenol		Present
19.5	Phenylethyl alcohol	Present	Present
25.4	2,4 Decadienal	Present	
26.7	Dehydro-ar-ionene	Present	
28.7	Trans- α -ionone	Present	
29.1	Vanillin		Present
29.3	Geranyl acetone	Present	
30.1	β -Ionone	Present	
33.6	2,6-Bis (1,1-dimethylethyl)- 4-ethylidene -2,5- cyclohexadien-1-one	Present	
34.4	6(E), 8(E) - Heptadecadiene	Present	
36.8	Myristic acid	Present	
37.1	Octadecane	Present	
37.3	(-)- Loliolide		Present
38.8	Pentadecanoic acid	Present	
38.9	1-Nonadecene	Present	
39.6	Farnesyl Acetone	Present	
39.7	Palmitic acid methyl ester	Present	
41.1	Palmitic acid ethyl ester	Present	
42.3	Palmitic acid	Present	
45.3	Linoleic acid	Present	Present

TABLE V
Flavor of Wet Cake and Corn Gluten Meal Volatiles

Compound	Flavor	Reference
Acetaldehyde ^a	Pungent	Acree and Arn (2004)
Acetic acid	Sour	Acree and Arn (2004)
2,3-Butanediol	Fruity	Acree and Arn (2004)
Butyrolactone	Caramel	Acree and Arn (2004)
2-Butanol	Vinous	Acree and Arn (2004)
Benzeneacetaldehyde ^a	Hawthorne	Acree and Arn (2004)
Pentylfuran	Green bean, butter	Acree and Arn (2004)
Phenylethyl alcohol	Honey	Acree and Arn (2004)
α -ionone	Wood, violet	Acree and Arn (2004)
Benzothiazole	Sour-green sulfur	Sucan (2002, 2004)
4-Vinyl-2-methoxyphenol	Clove	Acree and Arn (2004)
(E,E)-2,4-decadienal	Fatty, deep fried	Buttery and Ling (1998)
Dehydro-ar-ionene	Licorice	Acree and Arn (2004)
Saffranal	Herb, bitter	Windholz (1976)
Ethyl decanoate ^a	Grape	Acree and Arn (2004)
Geranyl acetone	Magnolia	Acree and Arn (2004)
β -Ionone	Seaweed, violet	^a _b
Loliolide	Tea, tobacco	^a _c
Ethyl laurate	Leaf	Acree and Arn (2004)
Ethyl palmitate	Waxy	Acree and Arn (2004)
Skatole	Fecal, pungent	Windholz (1976)
Squalene	Faint, agreeable odor	Windholz (1976)
Vanillin	Vanilla	Windholz (1976)

^a Identified in CGM volatiles by Wu et al (2003).

^b Shiratuchi (2003), Acree and Arn (2004).

^c Damste and Koopmans (1977), Klok et al 1984, Kodama and Fujimori (1982).

dehydro-ar-ionene, trans- α -ionone, and long chain hydrocarbons are odorants not previously identified in CGM (Wu 2003) and contribute odors of green bean, butter, licorice, wood, violet, and alkane. In addition, the first CH₂Cl₂ extract of CGM contained traces of safranal that were not detected in the subsequent partitioning into hexane and water-soluble fractions.

Pentylfuran was not detected in headspace volatiles of treated or untreated WC or CGM (Table II), but it was detected in the hexane fraction of the methylene chloride extract of CGM (Table IV) and also in the headspace volatiles of soluble solids extracted from WC at pH 7 (Table III). Both the methylene chloride extraction and the water extraction at pH 7 improved the odor of CGM. Compounds such as pentylfuran that are present in both extracts may be associated with the improvement in taste or odor reported by these treatments. Pentylfuran contributes percepts of green bean and butter (Acree and Arn 2004).

Phenylethyl alcohol was detected in both the water and hexane extractable fractions of a methylene chloride extract of CGM (Table IV). Phenylethyl alcohol was also present in the CGM volatiles at all stages of treatment (Table II). Phenylethyl alcohol contributes percepts of honey, spice, rose, and lilac (Acree and Arn 2004). Clearly, phenylethyl alcohol contributes to CGM odor. It is not known whether phenylethyl alcohol plays a role in CGM off-odor.

Carotenoid Degradation Products

Corn is rich in carotenoid pigments, primarily carotenes and xanthophylls (Weber 1987). Three classes of carotenoid degradation products identified in this study are aglycones of glycosides known to impart flavor.

Loliolides are carotenoid degradation products which, when combined with glycosides, are flavor components in tea and tobacco (Kodama and Fujimori 1982; Klok et al 1984; Damste and Koopmans 1997). These were identified in the water fraction of the CH₂Cl₂ extract of CGM (Table IV).

Ionine derivatives are carotenoid degradation products that are moieties of flavor components when combined with glycosides (Kodama et al 1981). Ionones and ionenes were detected in the volatiles of the pH 7 water-soluble solids extracted from WC (Table III) and also in the hexane fraction of the methylene chloride extract of CGM (Table IV). Safranal was detected as a trace component in the CH₂Cl₂ extract of CGM before partitioning into hexane and water fractions. It was also detected as a trace component in the headspace volatiles of water soluble solids extracted from WC at pH 7 (Table III). Safranal is a carotenoid degradation product and, when linked with glucose to form picrocrocin, it has a bitter taste (Windholz 1976). Taste panelists have reported a bitter flavor note in CGM (Wu 1994). Because safranal was found in both the pH 7 water extract and the methylene chloride extract, it is associated with the compounds that improve the taste of CGM by their removal from CGM.

The degradation of corn carotenoids during wet-milling and the subsequent formation of picrocrocin or related compounds may be a source of the bitter aspect of CGM flavor. Further study of this process is recommended.

Table V lists flavors and odors associated with volatiles identified from CGM. Although some of these compounds are reported to have pleasant odors, they may represent decomposition products from other offensive compounds. Examples would be the aglycones of glycosides described above. In addition, because odor per-

ception is concentration-dependent, a compound with a positive food-related aroma at low concentration can be very disagreeable and not have a food-like odor at higher concentrations. This study did not address concentration effects.

Generation of CGM Off-Odor

Two similar samples of WC pH 7 water-extracted soluble solids were each analyzed by SPME-GC-MS using polyacrylate (PA) fibers for SPME. Both samples were yellow powders that had been lyophilized and stored at 0°C in 16-oz clear glass jars with a relatively small amount of sample (<math><5\text{ g}</math>). The jars were brought to room temperature and briefly opened and resealed. No CGM odor was present in either sample. The volatiles of sample 1 were analyzed within 12 hr. Sample 2 was allowed to stand at room temperature for 48 hr. During this time, the appearance of sample 2 gradually changed. The fine yellow powder darkened to a brown color, and the powder drew together into clumps defined by the cracks appearing between them. This occurred at room temperature in a sealed vessel. This did not happen to treated WC samples that were subjected to the same storage and rewarming conditions. When sample 2 was opened after 48 hr, the characteristic odor of stale CGM was strongly present. By the time the analysis was completed, sample 2 had turned completely brown. This is the first reported observation of odorless WC extractables releasing the characteristic CGM odor while undergoing a change in physical appearance.

A small amount of skatole was detected in the volatiles of sample 2 when analyzed by PA fiber. Skatole was not detected in volatiles of sample 1. Because skatole is a known product of lactobacillus fermentation of proteins at low pH, and because corn wet-milling involves similar conditions during the steeping process, the presence of skatole in the headspace volatiles of sample 2 suggests that bacterial action during and after the steeping process may play a significant role in the production of off-odor and off-flavor in CGM and in the increase in off-odor during storage. Further study of the role of steepwater bacteria in creation of CGM odors is recommended.

CONCLUSIONS

Solid phase micro extraction (SPME) and solvent extraction followed by GC-MS have provided new information about CGM during treatment to improve flavor. These are effective methods for monitoring the results of treatments to improve the flavor and odor of CGM and provide more rapid assays than the traditional method of taste panel evaluation. They do not replace the need for taste analysis of final products but should greatly accelerate progress in bringing CGM into the human food market by providing laboratory methods to assess treatment effects.

SPME GC-MS headspace analysis of WC treated to improve flavor found changes in volatiles after pH adjustment and water extraction. Knowledge of these changes may contribute to identification of the mechanisms by which flavor is improved. Exploration of the roles of benzothiazole, pentylfuran, phenylethyl alcohol, and related compounds in creation of CGM taste and odor may be helpful.

Twenty-two components were identified by GC-MS in CH_2Cl_2 extracts of CGM that had been partitioned into water and hexane fractions. Of these, pentylfuran, dehydro-ar-ionene, trans- α -ionone, and long chain hydrocarbons are odorants not previously reported in headspace volatiles of CGM and contributed odors of green bean, butter, licorice, wood, violet, and alkane.

Three classes of carotenoid degradation products, whose glycosides are known to have flavors of tea, tobacco, and bitter, were identified in WC and CGM. The processes of carotenoid degradation coupled with glycoside formation may be areas worthy of exploration in the study of CGM off-odor and off-flavor.

The volatiles released during a spontaneous generation of CGM off-odor in a previously odor-free CGM extract included skatole, a strongly offensive smelling compound. It is a product of bacterial action under conditions similar to corn steeping. Further study of the contribution of steepwater bacteria in CGM off-odor creation is recommended.

Additional studies that build on the information provided here are needed to ultimately resolve the problem of off-odor and off-flavor in CGM. Techniques such as Fourier transform infrared (FTIR) spectroscopy and purge and trap (dynamic headspace) sampling could provide additional useful information in the study of CGM. Further study of the compositional and conformational differences in CGM after treatments that improve flavor may be helpful. The generation of CGM off-odor and off-flavor during storage and cooking needs to be further explored.

Increased value added utilization of CGM is especially important because CGM is a coproduct of the production of fuel alcohol from wet-milled corn. Any increase in utilization of CGM will improve the economics of fuel alcohol production.

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