JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Influence of Endogenous Ferulic Acid in Whole Wheat Flour on Bread Crust Aroma

Marlene R. Moskowitz,[†,§] Qing Bin,[†] Ryan J. Elias,[§] and Devin G. Peterson*[,†]

[†]Department of Food Science and Nutrition, 145 Food Science and Nutrition Building, 1334 Eckles Avenue, University of Minnesota, St. Paul, Minnesota 55108, United States

[§]Department of Food Science, The Pennsylvania State University, University Park, Pennsylvania 16802, United States

ABSTRACT: The influence of wheat flour type (refined (RWF)/whole (WWF)) on bread crust aroma was investigated. Differences were characterized by aroma extract dilution analysis and quantified utilizing stable isotope surrogate standards. For RWF breads, five aroma compounds were higher in concentration, 2-acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-phenylethanol, 2-acetyl-2-thiazoline, and 2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone, by 4.0-, 3.0-, 2.1-, 1.7-, and 1.5-fold, respectively, whereas three compounds were lower, 2-ethyl-3,5-dimethylpyrazine, (*E*,*E*)-2,4-decadienal, and (*E*)-2-nonenal by 6.1-, 2.1-, and 1.8-fold, respectively. A trained sensory panel reported the perceived aroma intensity of characteristic fresh refined bread crust aroma was significantly higher in RWF compared to WWF crust samples. Addition of 2-acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-phenylethanol, 2-acetyl-2-thiazoline, and 2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone to the WWF crust (at concentrations equivalent to those in the RWF crust) increased the intensity of the fresh refined bread crust aroma attribute; no significant difference was reported when compared to RWF crust. The liberation of ferulic acid from WWF during baking was related to the observed reduction in these five aroma compounds and provides novel insight into the mechanisms of flavor development in WWF bread.

KEYWORDS: *Maillard reaction, bread, whole wheat, refined, ferulic acid, aroma generation, 2-acetyl-1-pyrroline*

INTRODUCTION

With health and nutrition becoming increasingly important to consumers, whole grain foods have been touted as a key component of a healthy diet. Recommendations made by the U.S. Department of Agriculture (USDA) advocate that at least three servings of whole grain foods be consumed daily, which should be substituted for refined grain foods.[1] Whole grain foods provide fiber and phytochemicals, both of which are linked, in an increasing number of epidemiological studies, to the reduced risk of several chronic diseases such as cardiovascular disease, diabetes, cancer, and obesity.[2]

The consumption of whole grain foods, in comparison to refined grain formulated products, can be challenged by observed lower product consumer acceptability ratings.[3] This is, in part, attributable to changes in the flavor profile and intensity of whole grain foods, as well as other color and textural changes. The negative flavor attributes imparted by whole wheat flour (WWF) would be further exacerbated in reduced sugar or salt formulated products, which have their own complications regarding flavor and acceptability by consumers.

Most of the aroma research conducted on bread has focused on either refined wheat flour (RWF)-formulated breads or whole grain rye breads.[4−7] In RWF bread crust, the main compounds reported to contribute to aroma are products of two major sources of aroma generation, the Maillard reaction and lipid oxidation, with a third, albeit minor, pathway resulting from yeast fermentation. Major aroma active products of the Maillard reaction in bread include 6-acetyltetrahydropyridine, 2-acetyl-1-pyrroline (2AP), 3-methylbutanal, methional, 2-ethyl-3,5-dimethylpyrazine (EDMP), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, and 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone, with lipid oxidation resulting in the formation of (*E*)-2-nonenal and (*E*,*E*)-2,4-decadienal.[8] Yeast metabolism has been reported to generate the two odor-active alcohols 2-phenylethanol (2PE) and 3-methylbutanol in yeast-leavened breads.[8]

The compositional differences between RWF and WWF are thought to influence flavor development. The wheat seed is made up of three major components: the starchy endosperm, the phenolic-rich bran, and the lipid-rich germ. RWF consists primarily of only the endosperm, whereas WWF includes all three seed components, which could explain the variation in flavor and aroma between the two types of bread, especially with all other ingredients being equal. For example, the lipid (germ) fraction of WWF is oxidatively labile and, thus, could yield aromas characteristic of lipid oxidation reactions. Jensen et al.[9] reported that the hydroperoxide content in margarine (main lipid source) used in the bread formulation was related to the generation of lipid oxidation products in the bread. Similar results were observed in puff pastry, wherein those made with margarine had a higher degree of lipid oxidation compared to those made with butter, due to a higher level of linoleic acid in margarine.[8] Although these studies investigated only the generation of lipid oxidation products from a major ingredient (margarine), these findings are applicable to the lipid material in WWF.

Received: August 29, 2012
Revised: October 27, 2012
Accepted: October 29, 2012
Published: October 29, 2012

dx.doi.org/10.1021/jf303750y | *J. Agric. Food Chem.* 2012, 60, 11245−11252

An additional distinction between RWF and WWF bread is bran content. Bran is the major source of the phenolic compounds, specifically the hydroxycinnamic acids (HCAs), and the concentrations of these compounds are negligible in RWF in comparison to WWF.[10] HCAs have been reported in model food systems to alter Maillard chemistry and related flavor generation by binding and forming adducts with key transient Maillard intermediates.[11−14] Additionally, other phenolic compounds, such as flavonoids, have been shown to modify aroma development in model systems by also forming adducts with intermediates.[14−19] The mechanism by which this modulation occurs is thought to involve carbonyl trapping, rather than free radical mechanisms, which has been supported by structure identification performed with NMR spectroscopy.[12,20]

With respect to bread flavor, ferulic acid (FA) was shown to suppress the generation of 2AP, a well-known and critical aroma active compound in bread, by reacting with an important precursor of 2AP, methylglyoxal, in a methylglyoxal/proline model system.[11] In wheat, particularly in the bran layer of the seed, the predominant HCA is FA, which is present in three different forms: free, soluble conjugate, and insoluble conjugate.[10] Typically, <1% of the phenolic content in wheat is in the free form, which was considered to be the reactive form related to altering Maillard chemistry and product generation.[11,12] However, little is known about the release of HCAs from the more prevalent bonded form (insoluble conjugate) in products during baking or fermentation and of how the related chemistry affects flavor development in whole wheat bread.

The overall objective of this project was to investigate the influence of wheat flour type (RWF versus WWF) on the aroma profile of wheat bread crust. Focus was placed on defining the reactivity of FA on modulating flavor development in whole wheat bread.

MATERIALS AND METHODS

Chemicals. 2-Acetyl-2-thiazoline (2A2T), butylated hydroxytoluene (BHT), (*E*,*E*)-2,4-decadienal, deuterium oxide, dimethyl fumarate, 2,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine (EDMP), ferulic acid (FA), glucose, hydrochloric acid, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), lithium slivers, magnesium turnings, manganese oxide, methional, methylglyoxal (2-oxopropanal), 2-methyl-3-heptanone, 2-methylpropanal (2MP), (*E*)-2-nonenal, 2-phenylacetic acid chloride, 2-phenylethanol (2PE), phosphoric acid, potassium cyanide, proline, selenium dioxide, sodium bicarbonate, sodium bisulfite, sodium chloride, sodium hydroxide, sodium phosphate, anhydrous sodium sulfate, sulfuric acid, tricaprylin, triethyl orthoformate, 1-vinyl-2-pyrrolidinone, $^{13}C_2$-acetone, $^{2}H_5$-ethyl bromide, $^{2}H_7$-isopropyl bromide, and $LiAl^{2}H_4$ were obtained from the Sigma-Aldrich Co. (St. Louis, MO, USA). 2-Nonyn-1-ol was obtained from Alfa Aesar (Ward Hill, MA, USA). The solvents dichloromethane, diethyl ether, pentane, tetrahydrofuran, and hexane were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Diethyl ether was distilled once before use for synthesis experiments. 2-Acetyl-1-pyroline (2AP)[11] and 2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone (DHDMF)[21] were synthesized as previously described. [$^{2}H_3$]-2-Acetyl-1-pyrroline, [$^{2}H_3$]-methional, [$^{2}H_2$]-(*E*,*E*)-2,4-decadienal, and [$^{2}H_6$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone were gifts from Dr. Gary Reineccius at the University of Minnesota.

Wheat Samples. Milled whole wheat (WWF) and refined wheat (RWF) flour from the same hard red spring wheat kernels were obtained from ConAgra Foods (Omaha, NE, USA). The samples were stored under argon in brown glass vessels with Teflon-lined lids at −20 °C.

Breadmaking. Bread samples were prepared according to AACC method 10-10B.[22] Briefly, two liquid solutions were prepared, one consisting of salt (24 g) and sugar (96 g) and the other consisting of yeast (32 g; Fleischmann's Active Dry, ConAgra Foods), with each dissolved in 200 g of distilled water. Flour samples were removed from the freezer 1 h prior to mixing to bring to room temperature. Flour (1600 g; RWF or WWF) was added to a mixing bowl, and a well was made in the center for the addition of all liquids, including bringing the total amount of distilled water used in the recipe to 1032 or 1164.8 g for RWF and WWF breads, respectively. A farinograph was used to determine the amount of water and time needed to form each dough at optimal mixture (peak gluten formation). Vegetable shortening (48 g; Crisco, The J. M. Smucker Co., Orrville, OH, USA) was melted prior to addition at 60 °C and added to the liquids. The dough was mixed for 2 min on the lowest speed, followed by 2 min each at the next two highest speeds, and finished with 1 min at the next highest speed in a standing 10-speed mixer (KitchenAid, KSM75W, St. Joseph, MI, USA). Batches of dough were taken on the basis of 100 g of flour (177 g dough for RWF, 185.3 g for WWF) and were placed in a proofing oven (National MFG Co., Lincoln, NE, USA) held at 30 °C and 85% relative humidity. First punch occurred at 52 min, a second punch 25 min after that, and the final punch 13 min after that. The dough was then panned and replaced in the proofing cabinet for a final 33 min. The loaves were placed in a rotary oven (Despatch Oven Co., Minneapolis, MN, USA), preheated to 215 °C, primed with a beaker filled with 1 L of water, and baked for 17 min. The loaves were removed from the pans and immediately prepped for extraction as outlined in the following section.

Preparation of Bread Crust Extracts. The crust was separated from the bread immediately after baking, frozen in liquid nitrogen, and ground to a powder using a mortar and pestle. The crust (1250 g) was extracted with 2.5 L of dichloromethane spiked with 2-methyl-3-heptanone (8 μg) as an internal standard and BHT (8 μg) as an antioxidant. The extraction was conducted under a blanket of argon gas and agitation overnight, at room temperature, for 15 h. The solvent was removed, and the residue was extracted again with another 2.5 L of the dichloromethane solution, with agitation under argon for an additional 3 h. The solvent was removed, pooled together, and dried over anhydrous sodium sulfate. The extract was filtered and concentrated to ca. 100 mL using a Vigreux distillation column (60 cm) and further fractionated by solvent-assisted flavor evaporation (SAFE).[23] The volatile isolate was neutralized by washing three times with a 1:1 volume of 0.5 M sodium bicarbonate and once with a half-volume equivalent of saturated sodium chloride.[15] Anhydrous sodium sulfate was added, and the solution was frozen prior to a final distillation to 1 mL. The isolate was frozen (−20 °C) prior to further analysis.

Gas Chromatography−Olfactometry−Mass Spectrometry (GC-O-MS): Aroma Extract Dilution Analysis (AEDA). GC-O analysis was performed on a GC (Agilent Technologies, model 6890N, Santa Clara, CA, USA) equipped with a DB-5 ms column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific, Folsom, CA. USA) coupled with a MS (Agilent Technologies, model 5973, operated in EI mode) and a Gerstel sniffing port (Gerstel, Inc., model ODP 2, Linthicum, MD, USA). The effluent was split 1:1 after separation, between the MS and ODP 2 port. Purified air was bubbled through distilled water and purged the end of the sniffing arm at a rate of 20 mL/min. The GC conditions were as follows: the sample (0.5 μL) was injected using a cold on-column injection technique, with the helium carrier gas set to a constant flow of 1 mL/min. The oven program was 40 °C for 2 min, and then the temperature was increased at a rate of 3 °C/min until 150 °C and then raised by 30 °C/min to 250 °C and held for 5 min. Each sample was serially diluted by half-volume in dichloromethane until no further aromas were detected. The largest dilution at which each aroma was detected was defined as the flavor dilution (FD) value. Each dilution was analyzed in duplicate by two panelists (one female, one male).

Positive compound identifications were based on comparison with mass spectra, odor, and LRI of authentic compound. LRI values were calculated using an *n*-alkane ladder. Nine of the 10 aroma compounds

(d-1, d-3−10; Table 1) were positively identified on the same GC-MS system described above with the slight modification that the effluent

Table 1. GC-MS-CI Quantification Parameters for Select Aroma Compounds

			CI ion[a]		
compound[b]	code	mol wt	unlabeled	labeled	calibration factor
2-methylpropanal	d-1	72	73	80	1.00
2-acetyl-1-pyrroline	d-2	111	112	115	0.95
2-ethyl-3,5-dimethylpyrazine	d-3	136	137	142	0.87
methional	d-4	104	105	108	0.96
(*E*)-2-nonenal	d-5	140	141	143	0.84
2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone	c-6	144	145	149	0.69
2-acetyl-2-thiazoline	d-7	129	130	134	1.00
(*E*,*E*)-2,4-decadienal	d-8	152	153	155	0.87
2-phenylethanol	d-9	122	105	107	1.00
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	d-10	128	129	135	1.00

[a]Select ion monitored. [b]Compound positively identified (LRI, MS, and authentic compound).

flow was stopped to the olfactometry port. All samples were run on two columns of different polarities, a DB-5 ms (30 m × 0.25 mm × 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) and a DB-Wax (30 m × 0.25 mm × 0.25 μm film thickness; J&W Scientific). GC conditions for DB-5 ms were the same as above, whereas those for DB-Wax were as follows: 40 °C for 2 min, ramped at 5 °C/min to 230 °C, and held for 5 min. The MSD conditions were as follows: capillary direct interface temperature, 250 °C; source temperature, 150 °C; mass range, 35−250 amu at 6.35 cycles/min. The positive identification of the remaining compound, 2AP (d-2, Table 1), was conducted on a GC (Agilent Technologies, model 6890) coupled to a Varian MS-Ion Trap (Saturn 3, Agilent Technologies) equipped with a DB-5 ms column (30 m × 0.25 mm × 0.5 μm film thickness; J&W Scientific). The GC conditions were the same as above with the exception that 1 μL was injected in splitless mode at 200 °C. The MSD conditions were as follows: transfer line temperature, 250 °C; ion trap temperature, 150 °C mass range, 35−150 amu.

Hydroxycinnamic Acid Analysis. Phenolic acids were characterized on the basis of previous methods reported by Krygier et al.[24] and Sosulski et al.[25] Briefly, 10 g of the starting material (RWF or WWF) was rinsed twice with hexane (25 mL). The solid material was washed six times (50 mL) with a solution of acetone/methanol/water (35:35:30). The extract was pooled and solvent removed in vacuo. The pH was adjusted to 2 (HCl) and then extracted with hexane (1:1; 5 times) to ensure removal of all lipids. This resulting aqueous layer containing free and soluble conjugate phenolics was then extracted six times, with a 1:1 volume of diethyl ether/ethyl acetate, to separate the free phenolic acids. The remaining aqueous material was digested for 4 h with 2 N sodium hydroxide (200 mL), with stirring, under an atmosphere of argon. The pH of the resulting digest was decreased to pH 5 and was then extracted six times with a 1:1 volume of diethyl ether/ethyl acetate. The original meal was also digested for 4 h with 2 N sodium hydroxide (200 mL), under a blanket of argon with stirring. Following digestion, the meal was defatted with hexane (1:1; 5 times), and the phenolics were extracted six times with a 1:1 volume of diethyl ether/ethyl acetate. All diethyl ether/ethyl acetate extracts were evaporated in vacuo to dryness and then redissolved in methanol. Extracts were frozen (−20 °C) prior to LC-MS analysis.

Quantification of Ferulic Acid (FA) Liberated in Whole Wheat Bread Crust during Manufacture. The release of FA from its bound form during bread production was quantified by utilizing carbon-13 labeled FA as an internal standard that was applied on the top of the dough (crust region) prior to baking. $^{13}C_6$-Benzene ring labeled FA was synthesized according to the method in ref 26. Immediately prior to baking, a 2.5 mL aqueous solution (606 mg labeled FA/L) was sprayed evenly onto the surface of the bread dough. Immediately after baking, the bread was allowed to cool to room temperature, the top portion of bread crust was removed and ground, and a 5 g subsample was extracted with 20% ethanol aqueous solution (50 mL) for 16 h. The extract was then centrifuged at 4000 rpm at 5 °C for 20 min (Beckman Coulter, model Allegra X-30, Brea, CA, USA) and filtered (cellulose fiber papers, grade P5, Fisher Scientific), and the permeate was pooled. The ethanol was removed under vacuum and subsequently freeze-dried. The freeze-dried material was dissolved in 25 mL of 10% methanol in water solution, after which 5 mL was loaded on a 500 mg preconditioned C18 cartridge (Supelco, Bellefonte, PA, USA) and eluted with 5 mL of methanol. The methanol from the eluent was removed under vacuum and the concentrate was filtered over a 0.20 μm nylon syringe filter (Millex, Billerica, MA, USA) and analyzed by liquid chromatography−mass spectrometry (LC-MS).

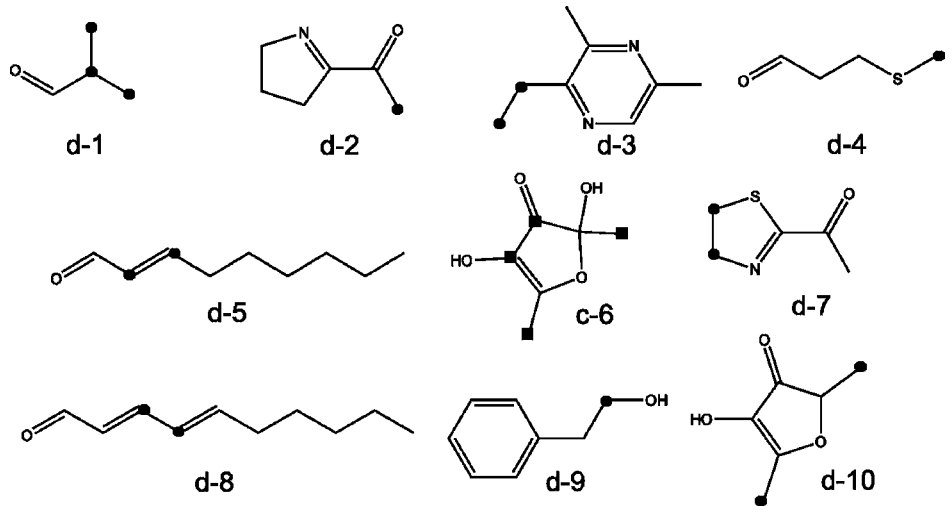

Figure 1. Structures of labeled aroma compounds (●/d, deuterium; ■/c, carbon-13): 2-methylpropanal (2MP, d-1); 2-acetyl-1-pyrroline (2AP, d-2); 2-ethyl-3,5-dimethylpyrazine (EDMP, d-3); methional (d-4); (*E*)-2-nonenal (d-5); 2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone (DHDMF, c-6); 2-acetyl-2-thiazoline (2A2T, d-7); (*E*,*E*)-2,4-decadienal (d-8); 2-phenylethanol (2PE, d-9); 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF, d-10).

Table 2. Odorants with FD Factor ≥16 and Difference ≥2 between Refined (RWF) and Whole Wheat (WWF) Bread Crust

compound[b]	aroma descriptor[c]	LRI		FD factor[a]		FD ratio (RWF/WWF bread crust)
		Wax	DB-5	RWF bread crust	WWF bread crust	
2-methylpropanal	malty	<800	594	128	512	0.25
2-acetyl-1-pyrroline	corn chip	1335	920	128	32	4
2-ethyl-3,5-dimethylpyrazine	earthy	1457	1080	32	64	0.5
methional	potato	1461	907	128	64	2
(*E*)-2-nonenal	cucumber	1534	1159	32	64	0.5
2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone	caramel	1540	979	64	16	4
2-acetyl-2-thiazoline	corn chip	1772	1102	128	32	4
(*E*,*E*)-2,4-decadienal	fatty	1815	1317	32	128	0.25
2-phenylethanol	flowery	1916	1112	128	64	2
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel	2043	1063	128	64	2

[a]Flavor dilution (FD) factors based on the average from two panelists. [b]Compound positively identified (LRI, MS, and authentic). [c]Odor described at the GC sniffing port during GC-O.

LC-MS analysis was conducted using a Waters Acquity UPLC system interfaced with a Quattro Premier XE mass spectrometer (Waters, Milford, MA). A sample (2 μL) was separated on an Acquity UPLC BEH C18 1.7 μm column (2.1 mm × 50 mm) (Waters) at 25 °C. The mobile phase was maintained at a flow rate of 388 μL/min using a binary solvent system of 0.1% formic acid in water (A) and methanol (B). The elution gradient started at 10% B (0−1 min), linearly increased to 50% B (1−8 min), was held at 100% B (8−9 min), decreased to 10% B (9−10 min), and was held at 10% B (10−20 min). The MS operation parameters were as follows: positive electrospray ionization (ESI+); source temperature, 110 °C; desolvation temperature, 350 °C; capillary voltage, 3.0 kV. The mass analyzer was set for multiple reaction monitoring (MRM) mode. The ion transitions for FA along with the cone voltages and collision energies were as follows: 195.29 → 177 for unlabeled FA; 201.31 → 183.02 for labeled FA; cone, 20 V; collision, 10 V. Samples were analyzed in duplicate.

Refined (RWF) Bread Made with Flour Spiked with FA. Breads formulated with RWF spiked with FA were made as described previously with the exception that 68 mg FA/kg flour was blended with the flour prior to the addition of other ingredients. The baking and extraction protocol was as described above.

Quantification of Aroma Compounds. The selected compounds (Table 1) were quantified by stable isotope surrogate standards consisting of deuterium or carbon-13 labeled analogues (Figure 1). Those isotopically labeled compounds needed for this study that were not provided as previously mentioned were synthesized as previously reported: [$^2H_7$]-d-1,[27] [$^2H_5$]-d-3 and [$^2H_4$]-d-7,[28] [$^2H_2$]-d-5,[29] [$^{13}C_4$]-c-6,[21] and [$^2H_2$]-d-9.[30]

Bread samples were prepared as previously described with the exception that the labeled standard compounds were added to the initial extraction solvent. The amounts of labeled standards added to extraction solvent for RWF bread were as follows (reference corresponding compound names in Table 1): d-1 (2450 μg); d-2 (6.5 μg); d-3 (2 μg); d-4 (60 μg); d-5 (6 μg); c-6 (330 μg); d-7 (25 μg); d-8 (5 μg); d-9 (1200 μg); d-10 (7000 μg). Those for the WWF bread were as follows: d-1 (2830 μg); d-2 (1.5 μg); d-3 (4 μg); d-4 (220 μg); d-5 (10 μg); c-6 (220 μg); d-7 (25 μg); d-8 (20 μg); d-9 (600 μg); d-10 (5750 μg). Those for the RWF bread spiked with FA were as follows: d-1 (2830 μg); d-2 (1.5 μg); d-3 (4 μg); d-4 (220 μg); d-5 (10 μg); c-6 (220 μg); d-7 (25 μg); d-8 (20 μg); d-9 (600 μg); d-10 (5750 μg).

Analysis was performed on a Hewlett-Packard GC (Agilent Technologies, model 5890plus) equipped with a DB-Wax column (30 m × 0.25 mm i.d. × 0.5 μm film thickness; J&W Scientific) that was coupled with a mass detector (Agilent, model 5972) using chemical ionization (CI) with isobutane as the reagent gas, under selective ion monitoring (SIM) mode for maximum sensitivity. The ions monitored are listed in Table 1. The GC conditions were as follows: constant flow rate, 1 mL/min (helium); 1 μL of the sample was injected under splitless mode; inlet temperature, 200 °C; oven program, 40 °C held for 2 min, followed by an increase of 15 °C/min to 115 °C, and held for 10 min. The temperature was then increased at 5 °C/min to 165 °C and then increased at 25 °C/min to 230 °C and held for 5 min. Quantification was based on five-point calibration curves (r^2 > 0.97 for all compounds).

Sensory Evaluation. Descriptive sensory analysis was conducted on three bread samples (RWF bread crust, WWF bread crust, and WWF bread crust with added flavor compounds). The panel consisted of six females and four males, ages 22 to 46, and was trained on the "fresh refined bread crust" aroma attributes over 12 × 1 h sessions. All samples and standards were presented, under red lights, in amber bottles (60 mL), fitted with conical polyethylene lined lids, which were filled with 10 g samples for analysis. The intensity of the fresh refined bread crust aroma was based on the intramodality *n*-butanol scale in water.[31] Three standard references of 4, 6, and 8 on a 12 cm line scale were used and were determined to be an appropriate scale for the bread crust samples. Six test sessions were completed to familiarize the panelists with the butanol scale; each panelist could identify unknown reference samples within a unit of 1.

RWF and WWF bread samples for sensory evaluation were prepared as previously described. Immediately after baking, the crust was separated from the bread, frozen in liquid nitrogen, and finely ground. The crust of each sample was then stored in glass jars with Teflon-lined lids at −80 °C under a blanket of argon and used within 24 h after baking. Two hours prior to analysis, the samples were removed from the freezer to come to room temperature. For the additional WWF bread sample with added flavor compounds, the five compounds in Table 4 were added in a 10 μL ethanol solution to 10 g of WWF bread crust 30 min prior to sensory evaluation and mixed gently, via rotation, until analysis.

Products were evaluated in duplicate over two sessions in one day. The samples were presented to the panelists in coded amber bottles in random order. The data were analyzed by a general linear model analysis of variance test with sample, panelist, and their interaction as variation factors. Statistical differences between samples were analyzed by Tukey's pairwise comparison (Statistix 9.0, Tallahassee, FL, USA).

RESULTS AND DISCUSSION

Differences in the aroma composition of bread crust formulated with refined wheat (RWF) versus whole wheat (WWF) flour were characterized by comparing the AEDA FD values for each sample. Compounds that had a FD value ≥16 with at least a 2-fold difference between the two samples were selected (shown in Table 2) and were subsequently quantified (Table 3). In the RWF bread crust, 2AP, HDMF, 2PE, 2A2T, and DHDMF, were reported to be higher in concentration, by 4.0-, 3.0-, 2.1-, 1.7-, and 1.5-fold, respectively; whereas EDMP, (*E*,*E*)-2,4-decadienal, and (*E*)-2-nonenal were present at lower concentrations by 6.1-, 2.1-, and 1.8-fold, respectively. No

Table 3. Quantification of Select Aroma Compounds in Refined (RWF), Whole Wheat (WWF), and RWF Spiked with Ferulic Acid (FA) Bread Crust Samples

	concentration (μg/kg crust)			
compound	RWF bread crust	WWF bread crust	RWF bread with added 68 mg FA/kg flour[a]	concentration ratio (RWF/WWF bread crust)
2-methylpropanal	4868	5517	nd	0.9
2-acetyl-1-pyrroline	10.4	2.6	0.94	4.0
2-ethyl-3,5-dimethylpyrazine	0.07	0.43	0.21	0.2
methional	109.3	124.6	nd	0.9
(*E*)-2-nonenal	1.2	2.2	1.1	0.5
2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone	640	436	213	1.5
2-acetyl-2-thiazoline	30.7	18.1	7.4	1.7
(*E*,*E*)-2,4-decadienal	0.7	1.5	0.7	0.5
2-phenylethanol	311	149	93	2.1
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	3417	1149	713	3.0

[a]nd, not determined.

significant differences between 2MP and methional were observed between RWF and WWF samples (which are within expected error of FD values).

Many of the compounds reported in Table 2 have been previously identified as important wheat bread odorants [e.g., 2AP, EDMP, methional, (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, 2PE, and HDMF].[8] Notably, 2AP, the compound with the largest concentration reduction in WWF bread crust (in comparison to RWF bread crust, Table 3), is considered to be one of the most important bread aroma compounds.[32] The perception of this compound is facilitated by an extremely low odor threshold (0.0073 μg/kg in starch),[33] which is known to degrade rapidly after baking, and is thought to also play a major role in stale bread flavor.[34]

In general, wheat bread formulated with RWF generated more Maillard-type products with desirable corn chip, caramel, and floral notes (Tables 2 and 3). DHDMF, also known as acetylformoin(e), has been identified in honey[35] and aged sweet fortified wines.[36] Although DHDMF has not been previously identified in bread, it has been formed in proline–monosaccharide model systems[21,37] and is a precursor of HDMF[38] and pronyl-L-lysine, the latter an antioxidant in bread crust.[39] 2PE can be produced by the reduction of the Strecker aldehyde, phenylacetaldehyde, as well as from fermentation pathways. Wheat bread with WWF produced more compounds with cucumber and fatty notes [(*E*)-2-nonenal and (*E*,*E*)-2,4-decadienal, respectively], which are typically generated through lipid oxidation.[40] WWF bread crust also had a higher concentration of a select pyrazine, EDMP, with earthy notes. Between the decreased quantity of 2AP and the generation of lipid oxidation compounds during bread storage, WWF bread crust shows many similarities in aroma to stale bread flavor.[34] The increase in lipid oxidation products in WWF bread (Table 3) can be related to the additional lipid material (germ) and more particularly the hydroperoxide content of the oil.[9]

The observed differences between RWF and WWF bread samples, with respect to chemical composition, were in agreement with the descriptive sensory analysis. A trained sensory panel reported the intensity of fresh refined bread crust aroma was significantly lower in the WWF versus the RWF bread crust (α = 0.05; see Figure 2). To further study the importance of the Maillard-type aroma compounds in RWF bread crust aroma, an aroma recombination study was conducted. The compounds reported to be higher in concentration in RWF bread crust (2AP, DHDMF, 2A2T, 2PE, and HDMF) were added at equivalent concentrations (to the RWF bread crust) to the WWF sample (see Table 4).

Table 4. Aroma Recombination Model: Whole Wheat (WWF) Bread Crust with Equivalent Aroma Concentration of Five Selected Compounds in Comparison to Refined Wheat (RWF) Bread Crust

compound	quantity added in WWF bread crust (μg/kg)
2-acetyl-1-pyrroline	8
2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone	200
2-acetyl-2-thiazoline	12.5
2-phenylethanol	160
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2270

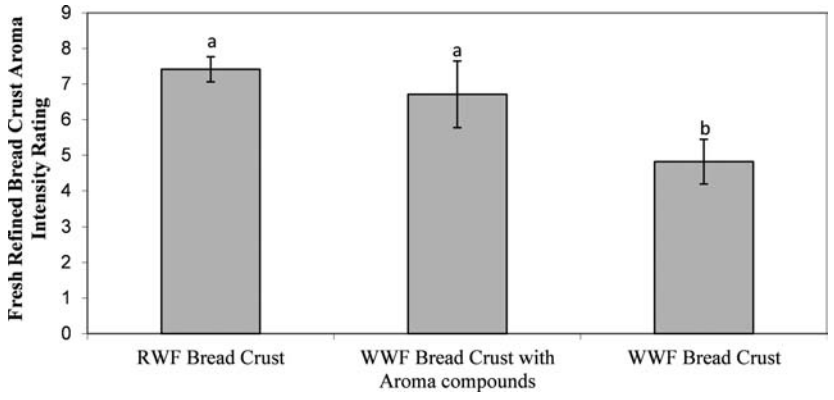

Figure 2. Mean intensity rating scores for fresh refined bread crust aroma in three bread crusts: (1) refined wheat (RWF), (2) whole wheat (WWF) with aroma compounds [2-acetyl-1-pyrroline (2AP), 2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone (DHDMF), 2-acetyl-2-thiazoline (2A2T), 2-phenylethanol (2PE), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF)], and (3) whole wheat (WWF). Samples with the same letter are not significantly different (α = 0.05).

Adding these compounds back to the WWF bread crust increased the fresh refined bread crust aroma and resulted in no statistical differences in the intensity of this aroma character when compared to the RWF sample (Figure 2). This indicated that the lower concentration of Maillard reaction compounds is a major factor in the observed difference in aroma between the two types of bread (Table 4). These results further relate back to model systems where ferulic acid (FA) was shown to inhibit the formation of aroma compounds upon heating.[11,12]

Although the amount of Maillard reaction products was corrected for by adding back aroma compounds to WWF crust, that sample still differed from RWF crust in terms of lipid oxidation concentration. These compounds, (*E*)-2-nonenal and (*E*,*E*)-2,4-decadienal, appear to have a lower effect on fresh refined bread crust aroma compared to compounds generated via the Maillard reaction. This indicates that the major mechanism in differences in aroma between the two types of bread is related to modulation of the Maillard reaction during processing.

With all other ingredients being equal, the major differences in aroma between the two types of bread must be due to the inclusion of the additional parts of the wheat seed in WWF bread. The change in flavor related to lipid oxidation appears to be due to the inclusion of the germ, whereas the alteration of Maillard-type product generation in WWF bread may be related to the phenolic content in the bran portion of WWF. Hydroxycinnamic acid (HCA) phenolic compounds, native to wheat, have been reported to alter Maillard chemistry in model systems by forming new compounds.[11,12] One such compound formed in a glucose/glycine/FA model system was proposed as a 4-vinylguaiacol−glycolaldehyde adduct, a compound that was also directly produced after glycolaldehyde was substituted for glucose.[11] These findings were further substantiated after the decarboxylated form of FA, 4-vinylguaiacol, was used in place of FA and was also found to significantly reduce the generation of Maillard-type aroma compounds.[12] Proline/methylglyoxal systems used to mimic bread flavor formation have found similar reduction in aroma compound formation, specifically 2AP, with the addition of FA,[11] findings substantiated with time course experiments detailing the significant reduction of methylglyoxal over time.[14] Adduct formation with reactive intermediates, such as dicarbonyls, are not unique to HCAs and can occur with other phenolic compounds such as flavonoids,[15−19] but distinctive to HCAs is also the ability to form adducts with amino acid reaction products.[11,12] Although much work has focused on the effect of HCAs to alter aroma compounds generated during the Maillard reaction, to the best of our knowledge they have not been further extrapolated to food, such as wheat bread. The role of HCAs in WWF bread on Maillard-type flavor development was further investigated in the present study.

The concentrations of free, soluble conjugate, and insoluble conjugate (bound) FA content were determined in the WWF and RWF utilized (see Table 5). FA was found to be present predominately in the bound form, which is consistent with the literature (Table 5); however, enzymatic and fermentation treatments,[41] as well as high temperatures,[42] such as those processes that happen during bread production, have been shown to increase the bioaccessibility of FA. Because the free form of FA was considered to be the primary reactive species affecting Maillard chemistry[11,12] and the potential for bound FA to be converted into a free form during fermentation and baking, a method was developed to measure liberated FA. $^{13}C_6$-

Table 5. Concentration of Bound, Soluble Conjugate, and Free Ferulic Acid (FA) in Refined (RWF) and Whole Wheat (WWF) Flour

type of phenolic	type of flour	FA concentration[a] (mg/kg)
bound	RWF	nd
	WWF	250
soluble conjugate	RWF	nd
	WWF	2.3
free	RWF	nd
	WWF	1.3

[a]All concentrations are based on the same starting weight for each portion. nd, not detected.

Benzene ring labeled FA was synthesized, and a solution was applied to the top of the dough prior to baking to estimate the "free FA" content liberated in the bread crust. LC-MS analysis of the crust revealed approximately 68 mg FA/kg flour. This value is considered a best estimate, as the calculation is based on the stability of the internal standard (carbon-13 FA). Because it is expected that FA is liberated, in part, during the baking process, the internal standard could potentially undergo more extensive degradation because it was added prior to baking as a consequence of receiving a higher thermal dose. Nevertheless, this analysis provided a quantitative estimate of the free FA available during bread manufacture. On the basis of the initial free FA content in WWF (1.3 mg/kg, Table 5), approximately 68 mg free FA/kg flour was liberated during the manufacturing process (fermentation and baking), which accounted for 27% of the bound phenolic content.

The influence of the available free FA content in WWF bread on flavor development was subsequently evaluated by adding an equivalent concentration (68 mg FA/kg flour) to RWF prior to breadmaking. The concentrations of select flavor compounds were quantified and are reported in Table 3. Overall, the addition of FA to the RWF resulted in a bread aroma profile similar to that of the WWF bread sample. When FA was added to RWF bread, the concentrations of five Maillard-type compounds were reduced (2AP, DHDMF, 2A2T, 2PE, and HDMF), whereas the EDMP content increased. The addition of FA to the RWF bread, however, did not influence the generation of lipid oxidation products [(*E*)-2-nonenal and (*E*,*E*)-2,4-decadienal], even though FA is a well-known free radical scavenger capable of inhibiting lipid oxidation reactions.[43] This suggested the unique lipid composition of the germ or the existence of hydroperoxides or the aldehydes themselves in WWF was related to the increased concentration of these oxidation products in WWF bread crust. Wheat germ contains high amounts of the polyunsaturated fatty acid linoleic acid,[44] which is a known precursor of (*E*)-2-nonenal and (*E*,*E*)-2,4-decadienal.[45] Noel et al.[46] suggested that (*E*)-2-nonenal can form from two different reaction mechanisms: linoleic acid autoxidation and lipoxygenasic activity of the wheat seed.

Whereas there are other differences between breads made with the two types of flour, such as changes in amino acids and differences in levels of moisture, this was corrected for by adding only free FA to RWF bread. This supports the findings that FA can react with Maillard reaction products, thereby changing the aroma profile of bread. The most notable decrease is that of 2AP. This might indicate that FA plays a major role in quenching the precursors of the compound, supporting

previous model system data.[11,14] The facts that 2AP is considered to be the most important wheat bread aroma compound,[32] with its absence a major factor in stale flavor,[34] and FA is present in such large amounts in the WWF could be predominant reasons for the difference in aroma between RWF and WWF breads. Additionally, the amount of reduction of aroma compounds in the RWF bread with added FA model was generally lower than the WWF bread sample. This could be related to the fact that during normal bread baking, FA is gradually released with time, whereas in this model bread system, it was added all at one time.

In summary, these findings indicate the phenolic component of WWF, in part, influences the mechanisms of Maillard-type flavor generation in bread. This leads to a reduction in the development of desirable flavor notes (i.e., 2AP) in WWF compared to RWF formulated products. The observation that FA can be liberated during baking also provides further insight into whole grain food chemistry and related changes in product quality.

AUTHOR INFORMATION

Corresponding Author

*E-mail: dgp@umn.edu. Phone: 1 (612) 624-3201. Fax: 1 (612) 625-5272.

Funding

This work was supported by the USDA-NIFA program, Project 2009-35503-06066.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Smaro Kokkinidou for the synthesis of [$^2H_4$]-2-acetyl-2-thiazoline, Deshou Jiang for the synthesis of [$^{13}C_6$]-ferulic acid, Dr. Gary Reineccius for donating [$^2H_3$]-2-acetyl-1-pyrroline, [$^2H_3$]-methional, [$^2H_2$]-(*E*,*E*)-2,4-decadienal, and [$^2H_6$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and ConAgra for donating the flour.

ABBREVIATIONS USED

2A2T, 2-acetyl-2-thiazoline; 2AP, 2-acetyl-1-pyrroline; 2MP, 2-methylpropanal; 2PE, 2-phenylethanol; AEDA, aroma extract dilution analysis; BHT, butylated hydroxytoluene; DHDMF, 2,4-dihydroxy-2,5-dimethyl-3(2*H*)-furanone; EDMP, 2-ethyl-3,5-dimethylpyrazine; FA, ferulic acid; FD, flavor dilution; HCA, hydroxycinnamic acid; HDMF, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone; RWF, refined wheat flour; WWF, whole wheat flour.

REFERENCES

(1) USDA. *Dietary Guidelines for Americans*; U.S. Department of Agriculture: Washington, DC, 2010.

(2) Vitaglione, P.; Napolitano, A.; Fogliano, V. Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends Food Sci. Technol.* **2008**, *19*, 451−463.

(3) Bakke, A.; Vickers, Z. Consumer liking of refined and whole wheat breads. *J. Food Sci.* **2007**, *72*, S473−S480.

(4) Schieberle, P.; Grosch, W. Identification of the volatile flavour compounds of wheat bread crust − comparison with rye bread crust. *Z. Lebensm. Unters. Forsch.* **1985**, *180*, 474−478.

(5) Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 111−113.

(6) Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252−257.

(7) Schieberle, P.; Grosch, W. Potent odorants of rye bread crust-differences from the crumb and from wheat bread crust. *Z. Lebensm. Unters. Forsch.* **1994**, *198*, 292−296.

(8) Grosch, W.; Schieberle, P. Flavor of cereal products − a review. *Cereal Chem.* **1997**, *74*, 91−97.

(9) Jensen, S.; Oestdal, H.; Clausen, M. R.; Andersen, M. L.; Skibsted, L. H. Oxidative stability of whole wheat bread during storage. *Food Sci. Technol.* **2011**, *44*, 637−642.

(10) Shahidi, F.; Naczk, M. Phenolic compounds in cereals and legumes. In *Food Phenolics: Sources, Chemistry, Effects, Applications*; Shahidi, F., Naczk, M., Eds.; Technomic Publishing: Lancaster, PA, 1995; pp 9−51.

(11) Moskowitz, M. R.; Peterson, D. G. Hydroxycinnamic acid − Maillard reactions in simple aqueous model systems. In *ACS Symposium Series 1042: Controlling Maillard Pathways to Generate Flavors*; Mottram, D. S., Taylor, A. J., Eds.; American Chemical Society: Washington, DC, 2010; pp 53−62.

(12) Jiang, D.; Chiaro, C.; Maddali, P.; Prabhu, K. S.; Peterson, D. G. Identification of hydroxycinnamic acid-Maillard reaction products in low-moisture baking model systems. *J. Agric. Food Chem.* **2009**, *57*, 9932−9943.

(13) Wang, Y. *Effects of naturally occurring phenolic compounds on the formation of Maillard aromas*. Ph.D. dissertation, Rutgers University, 2000.

(14) Peterson, D. G.; Noda, Y. Role of phenolic reactions on Maillard reaction flavors. In *Recent Highlights in Flavor Chemistry: Proceedings of the 8th Wartburg Symposium*; Hofmann, T., Meyerhof, W., Schieberle, P., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Eisenach, Germany, 2008; pp 97−103.

(15) Colahan-Sederstrom, P. M.; Peterson, D. G. Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via epicatechin addition. *J. Agric. Food Chem.* **2005**, *53*, 398−402.

(16) Schwambach, S. L.; Peterson, D. G. Reduction of stale flavor development in low-heat skim milk powder via epicatechin addition. *J. Agric. Food Chem.* **2006**, *54*, 502−508.

(17) Totlani, V. M.; Peterson, D. G. Reactivity of epicatechin in aqueous glycine and glucose Maillard reaction models: Quenching of C_2, C_3, and C_4 sugar fragments. *J. Agric. Food Chem.* **2005**, *53*, 4130−4135.

(18) Totlani, V. M.; Peterson, D. G. Influence of epicatechin reactions on the mechanisms of Maillard product formation in low moisture model systems. *J. Agric. Food Chem.* **2007**, *55*, 414−420.

(19) Noda, Y.; Peterson, D. G. Structure−reactivity relationships of flavan-3-ols on product generation in aqueous glucose/glycine model systems. *J. Agric. Food Chem.* **2007**, *55*, 3686−3691.

(20) Totlani, V. M.; Peterson, D. G. Epicatechin carbonyl-trapping reactions in aqueous Maillard systems: identification and structural elucidation. *J. Agric. Food Chem.* **2006**, *54*, 7311−7318.

(21) Hofmann, T. Acetylformoin − a chemical switch in the formation of colored Maillard reaction products from hexoses and primary and secondary amino acids. *J. Agric. Food Chem.* **1998**, *46*, 3918−3928.

(22) AACC. *Optimized Straight-Dough Bread-Making Method, 10-10B*; American Association of Cereal Chemists: St. Paul, MN, 1995.

(23) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation − a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237−241.

(24) Krygier, K.; Sosulski, F.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *J. Agric. Food Chem.* **1982**, *30*, 330−334.

(25) Sosulski, F.; Krygier, K.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* **1982**, *30*, 337−340.

(26) Robbins, R. J.; Schmidt, W. F. Optimized synthesis of four isotopically labeled (^{13}C-enriched) phenolic acids via a malonic acid condensation. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 797−806.
(27) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366−2371.
(28) Cerny, C.; Grosch, W. Quantification of character-impact odour compounds of roasted beef. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 417−422.
(29) Lin, J.; Welti, D. H.; Vera, F. A.; Fay, L. B.; Blank, I. Synthesis of deuterated volatile lipid degradation products to be used as internal standards in isotope dilution assays. 1. Aldehydes. *J. Agric. Food Chem.* **1999**, *47*, 2813−2821.
(30) Gassenmeier, K.; Schieberle, P. Potent aromatic compounds in the crumb of wheat bread (French-type) − influence of pre-ferments and studies on the formation of key odorants during dough processing. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 241−248.
(31) ASTM. *Standard Practices for Referencing Suprathreshold Odor Intensity, E544-10*; ASTM: Philadelphia, PA, 2010.
(32) Adams, A.; De Kimpe, N. Chemistry of 2-acetyl-1-pyrroline, 6-acetyl-1,2,3,4-tetrahydropyridine, 2-acetyl-2-thiazoline, and 5-acetyl-2,3-dihydro-4*H*-thiazine: extraordinary Maillard flavor compounds. *Chem. Rev.* **2006**, *106*, 2299−2319.
(33) Rychlik, M.; Grosch, W. Identification and quantification of potent odorants formed by toasting of wheat bread. *Lebensm.-Wiss. u.-Technol.* **1996**, *29*, 515−525.
(34) Schieberle, P.; Grosch, W. Changes in the concentrations of potent crust odourants during storage of white bread. *Flavour Fragrance J.* **1992**, *7*, 213−218.
(35) D'Arcy, B. R.; Rintoul, G. B.; Rowland, C. Y.; Blackman, A. J. Composition of Australian honey extractives. 1. Norisoprenoids, monoterpenes, and other natural volatiles from Blue Gum (*Eucalyptus leucoxylon*) and Yellow Box (*Eucalyptus melliodora*) honeys. *J. Agric. Food Chem.* **1997**, *45*, 1834−1843.
(36) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.* **1999**, *47*, 2837−2846.
(37) Cutzach, I.; Chatonnet, P.; Henry, R.; Dubourdieu, D. Identifying new volatile compounds in toasted oak. *J. Agric. Food Chem.* **1999**, *47*, 1663−1667.
(38) Schieberle, P. Formation of Furaneol in heat-processed foods. In *ACS Symposium Series 490: Flavor Precursors*; Teranishi, R., Takeoka, G. R., Güntert, M., Eds.; American Chemical Society: Washington, DC, 1992; Vol. 3, pp 164−174.
(39) Lindenmeier, M.; Faist, V.; Hofmann, T. Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II enzyme modulating activity. *J. Agric. Food Chem.* **2002**, *50*, 6997−7006.
(40) Eriksson, C. Aroma compounds derived from oxidized lipids. Some biochemical and analytical aspects. *J. Agric. Food Chem.* **1975**, *23*, 126−128.
(41) Anson, N. M.; Selinheimo, E.; Havenaar, R.; Aura, A.-M.; Mattila, I.; Letinen, P.; Bast, A.; Poutanen, K.; Haenen, G. R. M. M. Bioprocessing of wheat bran improves in vitro bioaccessibility and colonic metabolism of phenolic compounds. *J. Agric. Food Chem.* **2009**, *57*, 6148−6155.
(42) Rose, D. J.; Inglett, G. E. Two-stage hydrothermal processing of wheat (*Triticum aestivum*) bran for the production of feruloylated arabinoxylooligosaccharides. *J. Agric. Food Chem.* **2010**, *58*, 6427−6432.
(43) Graf, E. Antioxidant potential of ferulic acid. *Free Radical Biol. Med.* **1992**, *13*, 435−448.
(44) Dunford, N. T.; Zhang, M. Pressurized solvent extraction of wheat germ oil. *Food Res. Int.* **2003**, *36*, 905−909.
(45) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277−282.
(46) Noël, S.; Liégeois, C.; Lermusieau, G.; Bodart, E.; Badot, C.; Collin, S. Release of deuterated nonenal during beer aging from labeled precursors synthesized in the boiling kettle. *J. Agric. Food Chem.* **1999**, *47*, 4323−4326.