

# Development of Rancidity in Wheat Germ Analyzed by Headspace Gas Chromatography and Sensory Analysis

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Wheat germ is susceptible to oxidation due to its high content of unsaturated oil. Volatile compounds in stored wheat germ were evaluated using dynamic headspace gas chromatography (HS-GC) and sensory analysis. Preliminary comparisons were also made between freshly prepared wheat germ and wheat germ subjected to microwave heating at 45 and 55 °C prior to storage at room temperature. The progress of oxidation was followed in untreated wheat germ for 4 weeks and in heat-treated wheat germ for 7 weeks by HS-GC and sensory evaluation. Significant ( $p < 0.05$ ) changes in rancid odor and flavor were observed in the untreated wheat germ after 3 weeks, whereas no corresponding difference was observed in the microwave-heated wheat germ after 7 weeks of storage. Identification of a total of 36 volatile compounds was performed according to their mass spectra and Kovats indices. The major volatiles were hexanal,  $\alpha$ -pinene, 1-hexanol, and 3-carene. In addition to analysis of a short period of storage, 30 volatile compounds were identified from the headspace of wheat germ stored for >1 year.

**Keywords:** *Wheat germ; oxidation; volatile compounds; headspace gas chromatography; sensory evaluation*

## INTRODUCTION

The germ is the most nutritious part of the wheat grain, representing ~3% of the whole grain (Haridas Rao et al., 1980). The oil content of wheat germ is 12%, consisting mainly of oleic, linoleic, and  $\alpha$ -linolenic acids (Mecham, 1978). Wheat germ has a versatile vitamin content, being especially rich in vitamin E. The germ contains ~28% protein and is a good source of essential amino acids when compared to other cereal products. Wheat germ is mainly used for fodder, although a minor part of the annual production of 16 million tons is used for human consumption (Appelt, 1986). Because of its unfavorable baking properties and susceptibility to oxidation, the germ is removed from the endosperm during milling.

High lipase and lipoxygenase activities as well as a high content of unsaturated oil are characteristic of wheat germ. Even slight oxidation may cause a destruction of essential fatty acids and vitamins (Appelt, 1986).

Oxidation may be prevented and shelf life prolonged by inactivating the enzymes by heat shock (Haridas Rao et al., 1980) or by removing the oil fraction from the wheat germ by extraction (Karwowska and Kostrzewa, 1988) or combined techniques (Appelt, 1986). Extrusion cooking (Frezdorff and Seiler, 1987; Ekstrand et al., 1993) and microwave heating (Kermasha et al., 1993) have been reported to be rapid and effective methods for inactivating lipase.

The aim of this work was to study oxidative changes occurring in wheat germ during the early stages of storage using dynamic headspace gas chromatography (HS-GC) and sensory analysis. Microwave heating for improving the oxidative stability of wheat germ was also examined.

## MATERIALS AND METHODS

**Wheat Germ Samples.** Wheat germ was obtained in two lots from the commercial wheat mill of Melia Ltd., Raisio, Finland. One lot was divided into paper bags, four 350 g replicates for each storage week. Samples were stored at room temperature in the dark for 4 weeks. The wheat germ was stored in paper bags to avoid condensation of water, which might have promoted microbial growth because the water content of the wheat germ was 11.9 %, somewhat higher than typical (Haridas et al., 1980). Another lot of wheat germ was heated using a microwave oven as explained below. Wheat germ was also obtained every week from the mill during the study for reference in sensory analysis.

**Microwave Heating.** Two identical samples of 400 g of freshly milled wheat germ were as duplicates repeatedly heated to a final temperature of 45 or 55 °C using an AEG Micromat 725–1200 W microwave oven (AEG) equipped with a temperature sensor. The microwave-heated samples (400 g) were stored for 7 weeks at room temperature.

**Gas Chromatographic (GC) and Mass Spectrometric (MS) Analysis.** The wheat germ samples were analyzed when fresh and twice a week during storage weeks 1–4 using a modified dynamic headspace system previously reported (Kallio, 1991). The microwave-heated germ was analyzed the next day after heating and after 3 and 7 weeks. A 6.0-g aliquot of wheat germ was weighed in a gastight 100-mL reagent bottle (Schott) connected with deactivated fused silica tubing (i.d. = 0.25 mm) via a six-port valve (4N6WT, Valco Instruments, Houston, TX) to a deactivated fused silica trap (1.5 m, 0.32 mm i.d.). Air was removed from the sample bottle by flushing for 5 min with helium. The bottle was allowed to stabilize for 5 min prior to collection of a headspace sample of 100 mL, which was focused over 3 min in the trap chilled in liquid nitrogen. After removal of the cold trap, volatiles were flushed into a DB-1701 column (30 m, i.d. = 0.25 mm,  $d_f = 0.25 \mu\text{m}$ ) and analyzed by a Varian 3700 gas chromatograph (Walnut Creek, CA) with a flame ionization detector connected to a Hewlett-Packard 3388A integrator. The column temperature program was as follows: from 28 °C (isothermal for 3 min) to 200 °C at 5 °C/min and an isothermal period at 200 °C for 10 min. The average linear flow rate of helium was 32 cm/s, and nitrogen was used as the makeup gas. Control experiments

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**Table 1. Fatty Acid Composition of Fresh Wheat Germ.**

fatty acid		mol % <sup>a</sup>
myristic acid	14:0	0.1 (8.1)
palmitic acid	16:0	17.7 (0.8)
palmitoleic acid	16:1( <i>n</i> -7)	0.2 (3.1)
stearic acid	18:0	0.4 (2.0)
oleic acid	18:1( <i>n</i> -9)	10.9 (0.7)
vaccenic acid	18:1( <i>n</i> -7)	1.2 (0.6)
linoleic acid	18:2( <i>n</i> -6)	58.2 (0.3)
$\alpha$ -linolenic acid	18:3( <i>n</i> -3)	9.3 (1.2)
arachidic acid	20:0	0.2 (2.1)
<i>cis</i> -5-eicosenoic acid	20:1( <i>n</i> -9)	1.3 (0.9)
<i>cis</i> -11,14-eicosadienoic acid	20:2( <i>n</i> -6)	0.1 (2.6)
brassicic acid	22:1( <i>n</i> -9)	0.2 (15.0)
lignoceric acid	24:0	0.2 (4.2)

<sup>a</sup> Values are means of six analyses with relative standard deviations in parentheses.

were carried out to certify that the headspace system was clean. Volatiles were identified by a Hewlett-Packard 5890 GC equipped with a headspace system connected to a Finnigan MAT TSQ-700 mass spectrometer (Finnigan Mat, San Jose, CA). Electron ionization mode was used at 70 eV. Identification was based on Kovats indices (co-injection of mixture of *n*-alkanes), the literature (Stenhagen et al., 1974), and the library of the mass spectrometry (NIST, National Technical Information Services, Springfield, VA).

**Oil Content and Fatty Acid Analyses.** Oil was extracted from homogenized wheat germ with a mixture of chloroform/methanol (2:1 v/v) using a modified Folch procedure (Folch et al., 1957; Ways and Hanahan, 1964). The fatty acid methyl esters were prepared by the alkaline-catalyzed sodium meth-

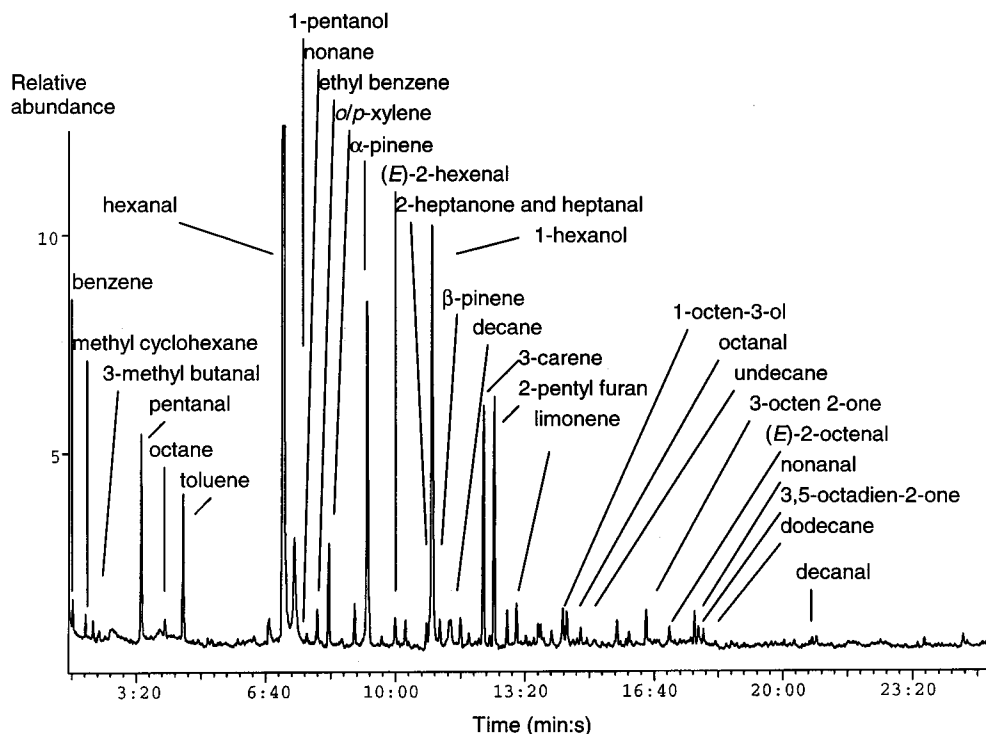
oxide transesterification method (Christie, 1982). The fatty acid methyl esters were analyzed by a Varian 3300 gas chromatograph (Limerick, Ireland) with an OV-351 fused silica capillary column (25 m  $\times$  0.32 mm i.d., film thickness = 0.2  $\mu$ m, Ohio Valley) using helium as carrier gas. The column temperature program was 120 °C for 2 min, raised to 230 °C at 3 °C/min, and held at 230 °C for 10 min. The injector temperature was 225 °C, and the temperature of the flame ionization detector was 240 °C. Fatty acid methyl ester samples were analyzed six times.

**Sensory Analyses.** The detection of rancid odor and flavor in the stored samples was determined by the paired-comparison test (Amerine et al., 1965). Because fresh, unstored germ has a fairly strong odor already, which diminishes during the first days of storage, a sample stored for 1 week was used as a standard for nonrancid germ. This was compared with the samples stored for 2, 3, and 4 weeks. In addition, the microwave-heated germ was compared with the nonrancid sample after 7 weeks of storage. The paired comparison tests were carried out with 15 panelists (5 male, 10 female) to evaluate samples stored for 2, 3, and 4 weeks and with 20 panelists (10 male, 10 female) to evaluate microwave-heated samples stored for 7 weeks. The assessors were selected from the staff and students of the department, some of the assessors having former experience in sensory evaluations. The evaluations were conducted in individual assessment booths under fluorescent illumination. All samples were coded with three-digit numbers and served in random order. One session was conducted at each storage time. During each session, three replicate sets of sample pairs were presented, resulting in a total of 45 (3  $\times$  15) assessments. The microwave-heated samples were analyzed in two replicate sets of sample pairs,

**Table 2. Volatile Compounds of Fresh and Oxidized Wheat Germ Analyzed with a DB-1701 Capillary Column**

peak	compound	$I_K^b$	relative proportion (%) from identified compounds <sup>a</sup>									
			fresh		1 week		2 weeks		3 weeks		4 weeks	
			$x^c$	$s_x$	$x^d$	$s_x$	$x^e$	$s_x$	$x^e$	$s_x$	$x^e$	$s_x$
1	ethyl acetate	692	0.0		3.2	6.1	0.0		0.2	0.3	0.6	0.4
2	dichloromethane	698	1.7	1.5	4.3	1.7	0.5	0.5	1.2	1.7	6.4	6.2
3	heptane	700	0.5	0.3	0.6	0.5	0.7	0.1	0.6	0.1	0.8	0.6
4	benzene	712	0.7	0.3	0.5	0.1	0.9	0.2	0.7	0.1	1.3	0.9
5	methylcyclohexane	732	0.3	0.3	0.4	0.1	0.5	0.5	0.5	0.1	1.0	0.8
6	3-methylbutanal	742	1.0	0.5	0.3	0.3	tr		0.2	0.3	0.7	1.0
7	pentanal	784	0.0		tr		0.0		0.8	0.2	1.0	1.2
8	octane	800	0.5	0.0	0.6	0.1	0.0		0.6	0.4	0.1	0.2
9	toluene	817	46.1	8.6	4.8	1.5	4.8	1.7	3.4	0.3	4.7	2.6
10	3-methyl-1-butanol	863	8.7	0.7	8.1	4.2	7.1	4.4	3.8	3.2	6.2	6.2
11	hexanal	887	2.8	1.4	6.1	2.1	4.6	0.2	12.2	7.8	6.6	2.1
12	1-pentanol	891	1.9	0.4	2.3	1.6	5.7	1.2	6.7	3.1	5.3	4.2
13	nonane	900	4.6	7.2	0.4	0.5	tr		tr		tr	
14	ethylbenzene	910	1.9	0.1	1.5	0.4	1.1	0.1	0.9	0.2	1.3	0.7
15	<i>o/p</i> -xylene	919	7.5	1.2	5.2	1.3	3.6	0.2	2.7	0.6	3.9	1.8
16	$\alpha$ -pinene	948	3.7	0.8	16.6	2.4	14.9	4.2	14.0	2.4	13.6	3.5
17	( <i>E</i> )-2-hexenal	967	0.0		0.2	0.3	0.0		0.2	0.2	0.0	
18	2-heptanone	979	1.3	1.8	0.4	0.6	0.6	0.6	0.4	0.5	0.0	
19	heptanal	982	0.0		1.7	3.5	0.0		0.0		0.0	
20	1-hexanol	985	7.2	2.5	21.2	7.6	24.6	2.6	29.6	5.8	22.1	8.0
21	$\beta$ -pinene	995	0.0		0.9	0.1	0.7	0.7	0.9	0.1	0.9	0.5
22	decane	1000	1.0	0.5	1.3	0.4	1.5	0.1	1.0	0.3	1.0	0.7
23	methylethylbenzene	1002	0.0		1.2	0.1	1.4	1.2	1.2	0.1	0.8	0.7
24	methylethylbenzene	1008	0.6	0.2	1.0	0.4	1.5	0.0	1.0	0.2	1.5	0.8
25	trimethylbenzene	1014	0.0		0.3	0.3	0.0		0.1	0.1	0.1	0.3
26	3-carene	1024	0.8	0.3	8.8	1.3	10.0	1.8	8.4	0.8	9.5	1.6
27	2-pentyl furan	1030	0.9	0.3	2.2	0.2	4.6	1.4	3.4	0.5	3.8	0.6
28	methylethylbenzene	1039	0.4	0.2	1.2	0.2	1.7	0.1	1.1	0.2	1.7	0.7
29	limonene	1045	2.5	0.8	3.3	0.3	5.1	0.2	2.9	1.0	3.4	0.8
30	methylethylbenzene	1071	1.5	0.3	0.0		0.2	0.3	0.0		0.0	
31	1-octen-3-ol	1081	0.0		0.4	0.5	0.4	0.6	0.1	0.2	0.1	0.2
32	octanal	1083	0.6	0.6	0.9	0.7	2.3	0.8	0.9	0.3	1.6	0.6
33	undecane	1100	0.0		0.0		0.0		0.3	0.6	0.0	
34	nonanal	1169	1.0	0.5	0.0		0.9	0.7	0.1	0.2	tr	
35	decanal	1296	0.0		0.0		0.0		0.0		0.8	0.5

<sup>a</sup> Averages ( $\bar{x}$ ) and standard deviations ( $s_x$ ) of analysis. <sup>b</sup> Kovats index on a DB-1701 column. <sup>c</sup> Three replicates. <sup>d</sup> Four replicates. <sup>e</sup> Five replicates.



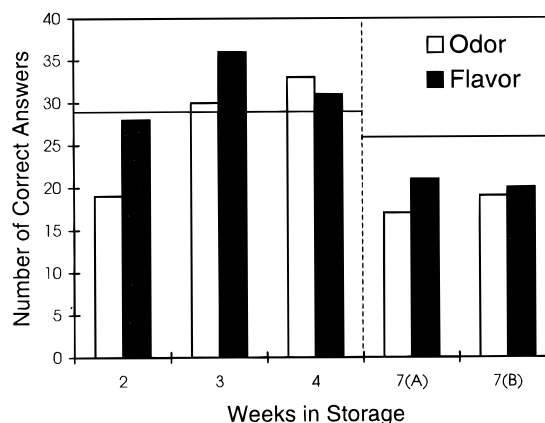
**Figure 1.** Identified volatile compounds of wheat germ stored for over 1 year at ambient temperature. Identification of compounds was based on mass spectra.

resulting in a total of 40 ( $2 \times 20$ ) assessments. The samples were prepared 2 h before evaluation by weighing 8 g of germ into 40-mL brown glass bottles, which were tightly capped. The assessors were asked to sniff the samples and, within each pair, designate the more rancid sample. After odor evaluation, the assessors waited in the booths for 2 min while, in the preparation area, the same samples were poured into glass cups labeled with another set of three-digit numbers and the presentation order was changed. The assessors were then asked to taste the samples, and within each pair designate again which of the samples had the more rancid flavor. At the beginning of each evaluation session, the assessors were presented reference samples of rancid and nonrancid germ. The reference samples of rancid germ had been stored for 4 months at 35 °C in paper bags. The germ stored in a paper bag for 1 week at room temperature was used as a nonrancid germ sample. Significant differences of the samples were determined according to the method of Roessler et al. (1978).

## RESULTS AND DISCUSSION

Table 1 shows the fatty acid composition of wheat germ oil. The major fatty acids were linoleic, palmitic, oleic, and  $\alpha$ -linolenic acids, which constituted 96% of the fatty acids. Mecham (1978) published similar results, although showing slightly lower concentrations of linoleic and linolenic acids. The oil content of wheat germ was 11.1%, which is in accordance with the values published in previous papers (Haridas Rao et al., 1980; Mecham, 1978; Appelt, 1986).

The fresh wheat germ was found to have a distinctly intense and sharp odor compared to the stored samples in the preliminary sensory analysis. This odor was recognized to vanish after the first week. The preliminary paired comparison tests showed that 80% of panelists perceived 1-week-old wheat germ to be different from fresh wheat germ. One-week-old wheat germ was selected as a reference because comparison of fresh wheat germ to stored samples seemed to describe changes in flavor due to evaporation of low molecular



**Figure 2.** Pooled results of sensory analysis of stored wheat germ. Minimum number of correct answers (29/45) has been marked with a line on the left side of the figure for significant ( $p < 0.05$ ) rancid odor and flavor in wheat germ samples stored for up to 4 weeks. A correspondingly minimum number of correct answers (26/40) is marked on the right side of the figure. Microwave-heated samples are as follows: 7(A), 45 °C; 7(B), 55 °C.

weight compounds more than the level of oxidation. Gas chromatographic analysis showed that the proportion of nonane, and especially of toluene, decreased rapidly during the first week. Toluene has been reported to be one of the most abundant components in the dry purge headspace analysis of oat flavor volatiles (Heydanek and McGorin, 1986). Toluene has been also found in volatiles of unprocessed rice (Bullard and Holguin, 1977). The concentrations of  $\alpha$ -pinene, 1-hexanol, and 3-carene also increased clearly during the first week, while those of *o/p*-xylene and 3-methyl-1-butanol decreased gradually during storage.

Peaks overlapped in the beginning of the chromatograms (up to 2.5 min), and they were excluded, although volatile hydrocarbons such as pentane have been shown

**Table 3. Volatile Compounds of Microwave-Heated Wheat Germ Analyzed with a DB-1701 Capillary Column**

peak	compound	$I_K^c$	relative proportion (%) from identified compounds <sup>a</sup>											
			fresh <sup>b</sup>				3 weeks				7 weeks			
			45 °C		55 °C		45 °C		55 °C		45 °C		55 °C	
$x^d$	$s_x$	$x^d$	$s_x$	$x^e$	$s_x$	$x^e$	$s_x$	$x^d$	$s_x$	$x^e$	$s_x$			
1	ethyl acetate	692	1.0	0.7	0.3	0.5	0.4	0.7	0.1	0.2	0.0	0.0		
2	dichloromethane	698	1.3	1.0	0.0		5.7	4.6	0.1	0.3	1.9	2.6	2.9	2.6
3	heptane	700	1.3	0.0	0.9	0.1	0.4	0.4	0.7	0.2	0.0		3.1	4.1
4	benzene	712	1.2	0.1	0.7	0.2	0.2	0.2	0.5	0.2	tr		7.9	11.6
5	methylcyclohexane	732	1.2	0.3	0.6	0.2	0.3	0.3	0.3	0.3	tr		7.8	11.7
6	3-methylbutanal	742	0.9	0.6	0.7	0.3	0.1	0.2	tr		0.0		4.2	4.7
7	pentanal	784	0.7	0.2	0.4	0.6	0.6	0.6	tr		0.5	0.7	0.4	0.4
8	octane	800	2.7	0.1	1.6	0.3	0.8	0.7	0.8	0.2	0.8	1.1	1.0	0.6
9	toluene	817	6.7	0.3	4.5	2.0	3.6	0.8	3.1	0.3	8.0	3.0	4.1	1.8
10	3-methyl-1-butanol	863	6.8	4.8	10.1	9.0	0.0		3.5	1.5	0.0		1.1	1.1
11	hexanal	887	8.3	2.6	17.8	16.2	5.9	1.3	6.0	1.6	11.4	5.7	10.3	6.1
12	1-pentanol	891	1.6	0.3	5.4	1.2	4.2	2.4	6.0	2.6	5.2	1.7	0.5	0.9
13	nonane	900	0.7	0.0	tr		tr		tr		tr		0.5	0.3
14	ethylbenzene	910	1.7	0.1	1.3	0.2	1.4	0.4	1.3	0.1	2.4	0.3	1.7	0.9
15	<i>o/p</i> -xylene	919	5.3	0.0	3.6	0.7	4.6	0.7	5.1	0.3	7.8	0.3	5.7	2.8
16	$\alpha$ -pinene	948	21.4	3.0	14.6	2.3	18.3	2.2	21.8	2.8	19.9	2.3	14.2	7.0
17	( <i>E</i> )-2-hexenal	967	0.0		0.0		0.3	0.6	0.6	0.6	tr		0.8	0.4
18	2-heptanone	979	0.0		0.0		0.0		0.0		0.0		0.1	0.1
19	1-hexanol	985	16.9	8.8	24.0	4.7	23.1	5.3	17.5	0.8	22.5	3.8	13.3	6.0
20	$\beta$ -pinene	995	1.0	0.1	0.8	0.2	1.5	0.2	2.2	0.2	0.8	1.2	1.6	0.9
21	decane	1000	2.0	0.0	1.6	1.0	1.2	0.3	1.3	0.0	0.9	1.2	1.4	0.8
22	methylethylbenzene	1002	1.6	0.2	0.0		1.3	0.0	1.7	0.1	0.4	0.6	0.9	0.4
23	methylethylbenzene	1008	1.1	0.1	0.4	0.5	1.1	0.3	1.5	0.1	2.3	0.3	1.6	0.9
24	trimethylbenzene	1014	tr		0.0		tr		0.3	0.3	0.0		0.5	0.3
25	3-carene	1024	6.8	1.2	3.8	1.7	12.4	1.3	13.7	0.9	7.3	0.2	6.3	3.1
26	2-pentylfuran	1030	4.3	0.2	2.3	0.5	5.6	1.1	5.4	0.4	3.9	0.0	3.2	1.7
27	methylethylbenzene	1039	0.9	0.2	0.3	0.4	1.2	0.4	1.4	0.1	2.2	0.2	1.2	0.6
28	limonene	1045	1.8	0.1	1.5	0.7	3.8	1.1	3.1	0.2	1.8	0.2	1.8	0.9
29	<i>p</i> -cymene	1066	0.0		0.0		0.0		0.0		0.0		0.5	0.1
30	methylethylbenzene	1071	0.0		0.3	0.4	0.0		0.0		0.0		0.2	0.3
31	1-octen-3-ol	1081	0.0		1.2	0.8	0.1	0.2	tr		tr		0.2	0.1
32	octanal	1083	0.9	0.2	1.3	0.6	1.5	0.4	1.1	0.2	tr		0.7	0.3
33	undecane	1100	0.0		0.0		0.0		0.0		0.0		0.2	0.1
34	nonanal	1169	0.0		0.0		0.3	0.3	0.0		0.0		0.0	
35	decanal	1296	0.0		0.0		0.0		0.0		0.0		0.2	0.1

<sup>a</sup> Averages ( $\bar{x}$ ) and standard deviations ( $s_x$ ) of analysis. <sup>b</sup> Analyzed after heating. <sup>c</sup> Kovats index on a DB-1701 column. <sup>d</sup> Duplicates. <sup>e</sup> Three replicates.

to reflect oxidative degradation (Bigalli, 1977; Löliger, 1990). Thus, 35 compounds for untreated wheat germ and microwave-heated wheat germ were selected for investigation of oxidative changes. The compounds were the same except that heptanal was found in untreated samples, whereas *p*-cymene was identified in heated wheat germ. Compounds eluted later possessed lower intensity than compounds in the beginning of chromatogram, which might distort the proportion of some detected compounds.

Table 2 shows the identified volatile compounds of fresh and oxidized wheat germ headspace identified according to their mass spectra and Kovats indices ( $I_K$ ). Identifications matched with volatiles of oxidized oat (Heydanek and McGorin, 1981) as was expected due to a resemblance in fatty acid composition. However, the enzyme activities are different in these two products. The compounds were mainly aldehydes, alcohols, alkanes, and aromatic hydrocarbons. Aldehydes are known to cause a typically rancid odor (Selke and Frankel, 1987; Ullrich and Grosch, 1987; Robards et al., 1988). Hexanal has been reported by several authors to indicate oxidation of lipids (Fritsch and Gale, 1977; Frankel et al., 1989), and an increase in hexanal was also observed after 1 week of storage of wheat germ in this study. Aromatic hydrocarbons were also expected to be found in the headspace analysis of wheat products (Bullard and Holguin, 1977). Aromatic hydrocarbons

might also originate from contamination from the natural environment (Heydanek and McGorin, 1986). Alcohols have been known to be produced during oxidation. In the headspace of soybean oil, the content of 1-pentanol followed oxidation rate, as did most of the minor constituents, but small changes were observed in its relative percentage (Snyder et al., 1988). However, 1-pentanol was found to be a possible indicator of oxidation level in wheat germ because its proportion increased gradually during storage. 1-Pentanol and 2-pentylfuran might be derived from the decomposition of secondary oxidation products of linoleate (Frankel, 1982). The proportion of 2-pentylfuran increased also during the first weeks of storage. Monoterpenes were also abundant among identified compounds in the headspace. A dramatic increase was observed in the proportions of  $\alpha$ -pinene and 3-carene after 1 week of storage. The relative amount of  $\alpha$ -pinene slightly decreased and the relative amount of 3-carene remained approximately the same after 1 week.

In addition to analysis of wheat germ stored for a short period, we tentatively identified compounds in wheat germ stored for >1 year (expiration date exceeded) at ambient temperature in a commercial package. Four additional volatile compounds were detected due to the long period of oxidation compared with those found in wheat germ stored for 4 weeks. A headspace chromatogram of wheat germ stored for >1 year is

shown with peak identification in Figure 1. The volatile compounds were mainly aldehydes, ketones, alcohols, and hydrocarbons. Hexanal,  $\alpha$ -pinene, 1-hexanol, 3-carene, and 2-pentylfuran were also major volatiles in this sample. Figure 1 shows a typical pattern of rancid wheat germ, although the intensity of the aroma compounds may have decreased due to drying and volatile losses through the carton package during storage. The reference samples of rancid germ for sensory analysis were also analyzed using gas chromatography. After storage of germ for 4 months at 35 °C in paper bags, hexanal was the most abundant volatile compound; concentrations of 2-pentylfuran and 1-hexanol were also high. The high concentration of 1-hexanol is probably due to enzymatic activity because C5 and C6 alcohols and aldehydes are indicators of the presence of enzymes such as lipoxygenase (Heydanek and McGorin, 1986).

Sensory analysis was applied in addition to HS-GC investigations to estimate the detection of odor and flavor during storage. Figure 2 shows pooled results of the sensory analysis of stored wheat germ. After 3 weeks storage at room temperature, the wheat germ was perceived to be significantly ( $p < 0.05$ ) more rancid than the germ sample stored for 1 week.

The effect of microwave heating on the stabilization of wheat germ was investigated. We twice heated the germ rapidly to the final temperatures of 45 and 55 °C using a microwave, whereas Kermasha et al. (1993) heated wheat germ for 20 min at 90 °C to prevent oxidation. In this study, the flavor and odor of the microwave-heated wheat germ were not evaluated to be significantly more rancid after 7 weeks of oxidation than 1-week-old germ in the paired comparison test (Figure 2). However, even rapid heating seemed to cause drying of the germ together with some other changes in both color and flavor.

The number of volatiles remained the same, and 35 compounds were identified in the headspace of the microwave-heated wheat germ (Table 3). Temperature seemed to affect the formation of 1-hexanol because the relative amount of 1-hexanol increased during storage of germ heated to 45 °C but decreased in the product heated to 55 °C. The water content of the samples may have had an effect on the proportions of the volatiles. Hexanal was also found in microwave-treated samples, but it overlapped with 1-pentanol and other compounds, which complicated quantification.

## CONCLUSIONS

This paper presented development of rancidity of wheat germ and during 4–7 weeks after milling followed by determination of selected volatiles using dynamic HS-GC and sensory analysis. Preliminary comparisons were also made between freshly prepared wheat germ and wheat germ subjected to microwave heating at 45 and 55 °C prior to storage at room temperature. Oxidation of wheat germ was detected by sensory evaluation after 3 weeks of storage at room temperature, and 1-week-old wheat germ can be differentiated from a fresh sample. Identification of 36 volatile compounds was performed. The levels of oxidation were observed in the relative amounts of hexanal, 1-hexanol, and 1-pentanol in the headspace of wheat germ.  $\alpha$ -Pinene and 2-pentylfuran were also possible indicators of storage time. No statistical difference of odor or flavor was observed in the microwave-heated wheat germ after 7 weeks of storage. Results suggested

that even mild microwave heating increases the oxidative stability of wheat germ.

## ABBREVIATIONS USED

HS-GC, headspace gas chromatography;  $I_K$ , Kovats index; MS, mass spectrometry.

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## LITERATURE CITED

- Amerine, M. A.; Pangborn, R. M.; Roessler, E. B. *Principles of Sensory Evaluation of Food*; Academic Press: Orlando, FL, 1965.
- Appelt, G. Nutritional value and stability of extruded wheat germ products. *Getreide Mehl Brot* **1986**, *40*, 176–180.
- Bigalli, G. Determination of pentane formed during autooxidation of oils contained in solid samples. *J. Am. Oil Chem. Soc.* **1977**, *54*, 229–232.
- Bullard, R. W.; Holguin, G. Volatile components of unprocessed rice (*Oryza sativa* L.). *J. Agric. Food Chem.* **1977**, *25*, 99–103.
- Christie, W. W. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *J. Lipid Res.* **1982**, *23*, 1072–1075.
- Ekstrand, B.; Gangby, I.; Åkesson, G.; Stöllman, U.; Lingnert, H.; Dahl, S. Lipase activity and development of rancidity in oats and oat products related to heat treatment during processing. *J. Cereal Sci.* **1993**, *17*, 247–254.
- Folch, J.; Lees, M.; Stanley, G. H. S. A simple lipid method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.
- Frankel, E. N. Volatile lipid oxidation products. *Prog. Lipid Res.* **1982**, *22*, 1–33.
- Frankel, E. N.; Hu, M.-L.; Tappel, A. L. Rapid headspace gas chromatography of hexanal as a measure of lipid peroxidation in biological samples. *Lipids* **1989**, *24*, 976–981.
- Frezdorff, B.; Seiler, K. The effects of twin-screw extrusion cooking on cereal enzymes. *J. Cereal Sci.* **1987**, *5*, 73–82.
- Fritsch, C. W.; Gale, J. A. Hexanal as a measure of rancidity in low fat foods. *J. Am. Oil Chem. Soc.* **1977**, *54*, 225–228.
- Haridas Rao, P.; Kumar, G. V.; Rang Rao, G. C. P.; Shurpalekar, S. R. Studies on stabilization of wheat germ. *Lebensm. Wiss. Technol.* **1980**, *13*, 302–307.
- Heydanek Jr., M. G.; McGorin, R. J. Gas chromatography mass spectroscopy investigations on the flavor chemistry of oat groats. *J. Agric. Food Chem.* **1981**, *29*, 950–954.
- Heydanek Jr., M. G.; McGorin, R. J. Oat flavor chemistry principles and prospects. In *Oats: Chemistry and Technology*; Webster, F. W., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1986.
- Kallio, H. Method of sensitive analysis of wine volatiles based on selective capillary column trapping. *J. Chromatogr. Sci.* **1991**, *29*, 438–443.
- Karwowska, K.; Kostrzewa, E. A new technology for the production of valuable vitamin extracts from wheat and rye germs. *Nahrung* **1988**, *32*, 491–495.
- Kermasha, S.; Bisakowski, B.; Ramaswamy, H.; Van De Voort, F. Comparison of microwave, conventional and combination heat treatments on wheat germ lipase activity. *Int. J. Food Sci. Technol.* **1993**, *28*, 617–623.
- Löliger, J. Headspace gas analysis of volatile hydrocarbons as a tool for the determination of the state of oxidation of foods stored in sealed containers. *J. Sci. Food Agric.* **1990**, *52*, 119–128.
- Mecham, K. D. Lipids. In *Wheat*; Pomerantz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1978; pp 393–451.

- Robards, K.; Kerr, A. F.; Patsalides, E.; Korth, J. Headspace gas analysis as a measure of rancidity in corn chips. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1621–1626.
- Roessler, E. B.; Pangborn, R. M.; Sidel, J. L.; Stone, H. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. *J. Food Sci.* **1978**, *43*, 940–943, 947.
- Selke, K.; Frankel, E. N. Dynamic headspace capillary gas chromatographic analysis of soybean oil volatiles. *J. Am. Oil Chem. Soc.* **1987**, *64*, 749–753.
- Snyder, J. M.; Frankel, E. N.; Selke, E.; Warner, K. Comparison of gas chromatographic methods for volatile lipid oxidation compounds in soybean oil. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1617–1620.
- Stenhagen, E.; Abrahamsson, S. A.; McLafferty, F. W. *Registry of Mass Spectra Data*; Wiley: New York, 1974.
- Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation on linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.
- Ways, P.; Hanahan, D. J. Characterization and quantification of red cell lipids in normal man. *J. Lipid Res.* **1964**, *5*, 318–328.

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