Static Headspace GC-MS Analysis for Evaluation of Oxidative Stability in Rice Bran

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Abstract

A technique based on static headspace (SHS) analysis was applied to evaluate the major volatile compounds, which causes off-flavor in rice bran samples. This method can be carried out using small amount of sample and short total analysis time as compared with peroxide value method. Rice bran samples in Thailand were analyzed using peroxide value (PV) by standard methods and headspace analysis, followed by gas chromatography-mass spectrometry (GC-MS) to test oxidative stability. Correlation and stepwise multiple regression analysis of the data revealed a relation between PV and headspace concentration of the volatile lipid oxidation products hexanal (R^2=0.796) and 2-pentylfuran (R^2=0.764). It was shown that both methods are related to rancidity evaluation of rice bran samples and SHS-GC-MS technique can be used as alternative measurement of oxidative stability in rice bran.

Keywords: static headspace analysis, gas chromatography-mass spectrometer, peroxide value, rice bran, rancidity evaluation

Introduction

Oxidative stability is an important quality criterion for rice bran oil. The identification of the volatile compounds causing off-flavor is the key for quality improvement. Upon degradation of hydroperoxides, several molecules are formed such as aldehydes, alcohols, furans and aromatic compounds. Frankel et al. (1981, 1983), Frankel (1998), Lee et al. (2003), Min and Boff (2002) reported that heptanal, octanal, and nonanal, which are of characteristic fatty acids and oily flavors, were produced by oleate hydroperoxide decomposition. Linoleate and linolenate decomposition produced hexanal the major volatile compound of rice, which was described as grassy and fatty flavor. They also found that pentanal, pentanol, hexanal, pentylfuran, octanal and nonanal are the main volatile components in both head and broken rice.

Several analytical tools have been proposed to evaluate products of lipid oxidation of rice bran oil. The chemical methods such as peroxide value (PV), acid value (AV), thiobarbituric acid (TBA) and carbonyl value (COV) are simple methods commonly used to evaluate the oxidative deterioration in oil. Nevertheless, these methods require large amount of oil of at least 5 grams in experiment. They are not appropriate in the case of trial seed cultivar lines grown in greenhouse conditions which contain little amount of oil (<1 g) available for rancidity measurement.

A technique of gas extraction, called headspace sampling, which has been reviewed as a rapid and efficient technique for the analysis of volatile fractions in many food samples (Snow and Slack, 2002), was developed and applied in this study. Generally, there are a number of techniques for sampling headspace vapors and introducing them to analysis by a gas chromatograph which can be

divided into two categories; static and dynamic. If the gas phase flows through the sample and is later trapped on a sorbent, or somehow collected, the technique is termed dynamic headspace extraction. In static headspace (SHS) extraction, sample was sealed in a gastight vial with a septum and then withdrawn to GC by a gastight syringe. As a one-step extraction without the aid of any additional sorbents, static headspace sampling is the simplest way for headspace analysis. Nowadays most modern headspace-GC instruments employ static headspace sampling, where the gastight syringe is replaced by a heated transfer line that allows for rapid sample transfer. Hence, shorter analysis time is normally achieved when static headspace sampling is employed in headspace analysis. Moreover, static headspace sampling does not require cleaning between sample injections because only the volatile compounds are injected to the GC and the non-volatile part is maintained in the vial (Frankel, 2005). The analytical method employing static headspace or automated headspace sampling combined with GC has, thus, been applied extensively for volatile fraction analysis of various types of samples (Yonamine et al., 2003; Alvarado and Rose, 2004; Fliszar et al., 2004; Wasfi et al., 2004; Li et al., 2006). With regard to rice volatiles, the developed SHS-GC method was successfully applied for quantitative analysis of the impact of aromatic compound in fragrant rice (Sriseadka et al., 2006). Simple GC-MS was used to determine the degree of staleness in the flavor of stored brown rice (Suzuki et al., 1999). The volatile components in a group of aldehyde in milled rice can be detected and measured by GC-MS (Monsoor and Proctor, 2004). However, none has reported the development of SHS-GC-MS as a rapid and efficient analytical tool for the evaluation of oxidative stability in rice bran through the characterization and quantification of volatile lipid oxidation products.

In this study an attempt has been taken to collect data regarding the rancidity quality of rice bran samples by determining the volatile components that could be used as measures of lipid oxidation for the experiment of plant breeding in which only small sample amounts are available. The relation was sought between analytical measurements of PV by standard method and the selected rancid volatile components formed during oxidation of rice oil by HS-GC-MS. We used correlation and stepwise multiple regression analysis to assess the relations between two methods for rancidity measurement.

Materials and Methods

Plant Materials

A set of 17 rice samples (15 Daw Dam and 2 Payaluemkang) were obtained from Pathum Thani Rice Research Center, Thailand (Table 1). Both varieties originated from different provinces in the North and Northeast of Thailand. The rice paddy was stored at room temperature 35^{o}C for 6 months. They were hulled and milled. Rice bran samples were stored at 4^{o}C for 7 days before use.

Chemicals

The solvents in this study were analytical-reagent grade. Acetic acid, chloroform and $Na_2S_2O_3$ were purchased from LAB-Scan (Stillorgan, Ireland). Potassium iodide and starch was purchased from Scharlau (Barcelona, Spain). Benzyl alcohol was purchased from Fisher (Loughborough, UK). Standard grade of hexanal, ethylbenzene, *p*-Xylene, heptanal, octanal, nonanal and 2,4-dimetylpyridine (2,4-DMP), used as internal standard for GC analysis, were purchased from Aldrich (Milwaukee, WI).

HS-GC-MS Instrument and Conditions

The headspace autosampler (Agilent Technologies, Welmington, DE) model G1888 was used to perform the experiment. The conditions were set as follows: oven temperature, 120^{o}C; vial equilibration time, 7 min with high-speed shaking; loop filling time, 0.05 min; pressurizing time, 1.5 min; and injection time, 0.4min. The sample loop and transfer line temperatures were set at 10 and 20^{o}C higher than the oven temperature, respectively.

Table 1 List of rice variety used in this study.

Accession	Cultivars' name	Species/subspecies	Region/province
3354	Daw Dam	*O.sativa/* Indica	Northeastern Thai/Si sa ket
5614	Daw Dam	*O.sativa/* Indica	Northeastern Thai/ Yasothon
5645	Daw Dam	*O.sativa/* Indica	Northeastern Thai/ Yasothon
5647	Daw Dam	*O.sativa/* Indica	Northeastern Thai/ Yasothon
5978	Daw Dam	*O.sativa/* Indica	Northern Thai/ Chiang Rai
6702	Daw Dam	*O.sativa/* Indica	Northern Thai/ Phrae
6710	Daw Dam	*O.sativa/* Indica	Northern Thai/ Phrae
7734	Daw Dam	*O.sativa/* Indica	Northern Thai/ Phayao
7751	Daw Dam	*O.sativa/* Indica	Northern Thai/ Phayao
12127	Daw Dam	*O.sativa/* Indica	Northeastern Thai/Nakhon Phanom
13852	Daw Dam	*O.sativa/* Indica	Northern Thai/ Lampang
19004	Daw Dam	*O.sativa/* Indica	Northern Thai/ Phrae
19086	Daw Dam	*O.sativa/* Indica	Northern Thai/ Phrae
19557	Daw Dam	*O.sativa/* Indica	Northern Thai/ Phrae
20904	Daw Dam	*O.sativa/* Indica	Northern Thai/ Uttaradit
7620	Payaluemkang	*O.sativa/* Indica	Northeastern Thai/Nakhon Phanom
14549	Payaluemkang	*O.sativa/* Indica	Northeastern Thai/Nakhon Phanom

The GC-MS analysis was performed with an Agilent Technologies (Wilmington, DE) model 6850 gas chromatograph equipped with an Agilent Technologies model HP 5973 mass-selective detector. The GC was carried out using a fused silica capillary column HP-5MS, biphenyl-dimethylpolysiloxane, with dimensions of 30m × 0.25 mm i.d. and 0.25 μm film thickness (Agilent Technologies). The injection port temperature was set at 230^{o}C. The column temperature program started at 45^{o}C, hold for 5 min and was increased at a rate of 3^{o}C min^{-1} to the final temperature of 100^{o}C. Purified helium, at a flow rate of 1.3 mL min^{-1}, was used as the GC carrier gas. The GC-MS transfer line temperature was 280^{o}C. The mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV, ion source temperature, 230^{o}C, quadrupole temperature, 150^{o}C, mass range *m/z* 29-350, scan mass rate, 6.35 scan s^{-1}, and electron multiplier voltage, 1432 V.

The peak identification of rice bran headspace volatiles was mainly based on EI mass spectral data comparison with database libraries, NIST 98 and Wiley 275 Mass Spectral Library, supported by data of the retention indices (RI), which were calculated from the average retention times of the volatiles. Some sample spectra were compared with those obtained from the standard compounds. Determination of the amount of each identified headspace volatile was performed by semi-quantitation in terms of the relative amounts derived from the ratios of the peak areas of the volatiles and 2,4-DMP added into the rice bran samples at constant concentration as standard compound. The software used to process peak areas was ChemStation (Agilent Technologies, USA). Each sample of rice bran was analyzed in triplicate. The repeatability was estimated by means of relative standard deviation (RSD).

Sample Preparation for HS-GC-MS

One gram of each rice bran was placed in 10-mL glass headspace vials and 2.0 μL of 0.5 mg mL^{-1} 2,4-DMP in benzyl alcohol was added as internal standard. The headspace vials were then sealed instantly with PTEE/silicone septum and aluminum crimp cap and shaken well at a room temperature for 15 min before analysis.

Measurement of the Peroxide Value

The peroxide value (PV) in total lipid has been used to analyze flavor quality and stability of rice bran oil. The standard method of IUPAC 2.501 was used to determine PV. Twenty-five mL of acetic acid-chloroform (3:2 v/v) mixture was added in 5 g

of oil, followed by the addition of 1 ml of saturated KI solution and swirled for 1 min. The mixture was then kept in dark for 5 min and added with 75 mL of deionized water and shaken. After that the content was titrated with 0.01 N $Na_2S_2O_3$ solution in the 0.5 mL starch indicator solution (5%) until the blue color has just disappeared (end point) and record the volume of $Na_2S_2O_3$ used. Blank was also run to comprise all the solutions but without the oil sample. PV was measured in triplicate where average was calculated. The peroxide value (PV) was evaluated by measuring iodine released from potassium iodide titrated with sodium thiosulphate solution. The PV was expressed as milliequivalents of hydroperoxide per kg of oil.

Statistical Analysis

An analysis of variance (ANOVA) was conducted on the combined data set across peroxide value and eleven oxidation volatile compounds. Correlation analysis was performed to explore associations among the variables. These statistical analyses were carried out using CropStat 7.2 software (IRRI, 2007). To set up a model relating in PV to the volatile oxidation products, an initial selection of explanatory variables was made using stepwise multiple regression analysis (Statgraphics plus version 3.0). P value of less than 0.05 was considered significant.

Results and Discussion

Peroxide Value and Volatile Components from Oxidation of Fatty Acid in Rice Bran

The PV was analyzed in 17 samples of rice oil and the result is shown in Table 3. This data was used for relation study with HS-GC-MS method. The results obtained by HS-GC-MS showed the separation of at least 39 volatile components present in all rice bran samples, but only 11 volatile compounds were tentatively identified through reasonable RI and mass spectral data. The identification of some minor components was especially difficult due to their poor quality mass spectra and the fluctuation of their contents. An example of volatile components profile of the Daw Dam and Payaluemkang rice bran obtained by the HS-GC-MS method is shown in Figure 1.

Eleven volatiles were identified in headspace of the oxidized rice bran and are listed in Table 2. These rice bran volatiles were classified as aldehydes (hexanal, heptanal, *trans*-2-heptenal, octanal, *trans*-2-octanal, and *n*-nonanal), octanal (1-octanol), aromatic hydrocarbons (ethylbenzene, *p*-xylene and 1,3-dimethylbenzene) and heterocyclic compounds (2-pentylfuran). Among the compounds identified, hexanal and nonanal were found to be the major abundant compound in headspace of the rice bran. Other volatile components detected

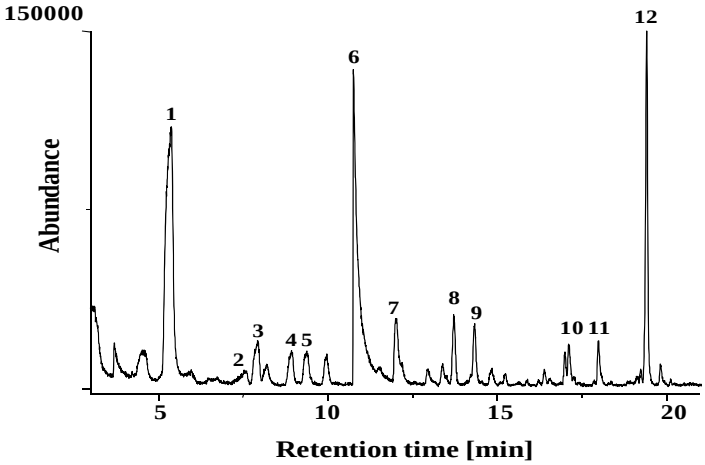

Figure 1 GC chromatogram of headspace volatiles of Daw Dam and Payaluemkang rice bran. Peak designation as follows: (1) hexanal, (2) ethylbenzene, (3) *p*-xylene, (4) 1,3-dimethylbenzene, (5) heptanal, (6) 2,4-DMP, (7) *trans*-2-heptenal, (8) 2-pentylfuran, (9) octanal, (10) *trans*-2-octenal, (11) 1-octanol, and (12) nonanal.

Table 2 The identified volatile components of Daw Dam and Payaluemkang rice bran.

Peak no.[1/]	Compound[2/]	RI[3/]	Match[4/] (%)
1	Hexanal[1,2,3]	803	94
2	Ethylbenzene[1,3]		90
3	*p*-xylene[1,3]		95
4	1,3-dimethylbenzene[1]		91
5	Heptanal[1,2,3]	902	90
6	2,4-dimetylpyridine[1,3]		95
7	*trans*-2-heptenal[1,2]	949	96
8	2-pentylfuran[1,2]	989	91
9	Octanal[1,2,3]	998	90
10	*trans*-2-octenal[1]		90
11	1-octanol[1,2]	1066	92
12	Nonanal[1,2,3]	1005	96

[1/] Number correspond to those labeled on the total ion chromatogram obtained by HS-GC-MS.
[2/] Identification: 1, mass spectrum (tentative); 2, Retention indices; and 3, standard compound.
[3/] Retention indices using a nonpolar dimethylpolysiloxane column.
[4/] Percentage of mass spectral matching quality against Wiley 275 or NIST 05 Mass Spectral Library.

in relatively low concentrations were not fully characterized for volatile oxidation products (Figure 1). The number of volatile compounds detected by HS methods depends on the particular technique and operating conditions (e.g. the sampling temperature and time) used for HS extraction. HS methods are suitable for detecting vastly volatile compounds but are not particularly suitable for detecting components with relatively low instability.

Several analytical reports have covered volatile components in raw brown, milled and cooked rice (Yasumatsu and Moritaka, 1964; Tsugita et al., 1980, 1983; Buttery et al., 1988; Suzuki et al., 1999; Lam and Proctor, 2003b). There have been no studies of the oxidative volatile components directly emitted from rice bran that contain most of rice oil. Relative contents of the identified volatile components in headspace of the 17 rice bran sample analysed by HS-GC-MS are shown in Table 3.

Relationship between PV and HS Volatile Compounds

To determine volatile compounds that could be used as measures of lipid oxidation, correlations were required between PV and the relative contents of many volatile compounds formed during oxidation of oils in the rice bran samples. The correlation matrix obtained from PV and oxidation volatile compounds is shown in Table 4. Analysis using combined data revealed that PV had significant positive correlations with hexanal and 2-pentylfuran. In addition, stepwise regression analysis of the data also revealed a statistically significant relationship between PV and the contents of hexanal and 2-pentylfuran which showed particularly good relation (R^2=0.796 and R^2=0.764, respectively) with the PV (Figure 2).

Hexanal is characterized by a fatty, oily, or grassy odor; and 2-pentylfuran is characterized by a beany or grassy odor (Krishnamurthy et al., 1967; Taylor and Mottram 1990). Both hexanal and

Table 3 Peroxide value and relative contents of the identified oxidative volatile components of 17 accessions of rice bran**.**

Entry	Accession	Relative content[1/]											
		PV	Hexanal	Ethylbenzene	*p*-xylene	1,3-dimethylbenzene	Heptanal	*trans*-2-heptenal	2-pentylfuran	Octanal	*trans*-2-octenal	1-octanol	Nonanal
		(---------- meq kg^{-1} ----------											)
1	3354	1.750	1.150	0.070	0.140	0.100	0.105	0.170	0.125	0.116	0.070	0.075	0.520
2	5614	2.410	1.100	0.055	0.095	0.070	0.105	0.105	0.120	0.087	0.050	0.070	0.500
3	5645	1.810	0.547	0.075	0.195	0.110	0.150	0.120	0.075	0.084	0.055	0.075	0.480
4	5647	2.540	1.180	0.175	0.410	0.200	0.170	0.155	0.140	0.145	0.075	0.120	0.720
5	5978	1.340	0.385	0.145	0.345	0.165	0.085	0.095	0.060	0.085	0.045	0.065	0.400
6	6702	1.780	0.650	0.045	0.100	0.060	0.085	0.075	0.070	0.073	0.040	0.070	0.425
7	6710	1.420	0.380	0.155	0.425	0.215	0.075	0.060	0.065	0.061	0.030	0.055	0.350
8	7620	1.560	0.435	0.040	0.080	0.050	0.095	0.055	0.060	0.070	0.030	0.075	0.475
9	7734	1.610	0.275	0.095	0.240	0.120	0.070	0.055	0.050	0.060	0.030	0.060	0.345
10	7751	1.820	0.495	0.095	0.265	0.140	0.160	0.060	0.085	0.075	0.035	0.065	0.420
11	12127	1.780	0.615	0.095	0.285	0.150	0.076	0.035	0.080	0.060	0.020	0.060	0.480
12	13852	2.010	0.535	0.070	0.230	0.125	0.055	0.035	0.075	0.042	0.020	0.075	0.475
13	14549	1.080	0.289	0.080	0.230	0.120	0.079	0.030	0.055	0.042	0.020	0.040	0.275
14	19004	2.890	1.915	0.110	0.285	0.175	0.191	0.050	0.240	0.088	0.040	0.070	0.475
15	19086	2.870	1.805	0.045	0.105	0.095	0.098	0.045	0.170	0.079	0.030	0.070	0.415
16	19557	2.330	1.414	0.050	0.160	0.105	0.085	0.030	0.150	0.075	0.030	0.160	0.925
17	20904	2.020	0.543	0.095	0.255	0.130	0.120	0.035	0.095	0.075	0.030	0.130	0.640
	Mean	1.942	0.807	0.088	0.226	0.125	0.106	0.071	0.101	0.078	0.038	0.079	0.489
	5%LSD	0.053	0.167	0.022	0.042	0.022	0.021	0.019	0.014	0.014	0.007	0.014	0.047
	Prob	**	**	**	**	**	**	**	**	**	**	**	**
	CV	1.6	9.8	11.9	8.9	8.1	9.1	12.4	6.8	8.5	9	8.6	4.6

[1/] The ratio of peak area of each rice volatile and 2,4-dimethylpyridine added into the rice sample at constant concentration as standard compound; arbitrary unit** Significant at $p < 0.01$.

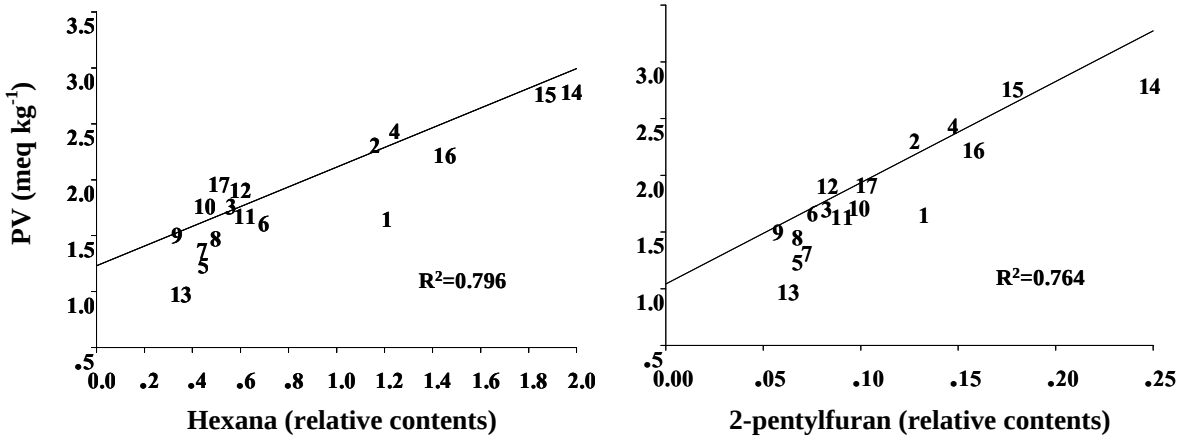

Figure 2 Regression analysis between PV (meq kg^{-1}) and and Hexanal and between PV (meq kg^{-1}) and 2-pentylfuran for 17 samples of rice.

2-pentylfuran are derived from the autoxidation of linoleic acid (Berlitz and Grosch, 1999). During storage of rice, hexanal is one of the major flavor contributors, arising from the decomposition of hydroperoxides of unsaturated fatty acid and the action of enzyme lipoxygenase-3 (Sekhar and Reddy, 2006). Aliphatic aldehydes establish a main portion of the volatiles generated by lipid oxidation. They are known to cause a typically rancid odor and are the most significant type of odorous compounds that are formed from the autoxidation and photoxidation of unsaturated fatty acids (Pugh, 2000).

Table 4 Correlation matrix of peroxide value and oxidative volatile compound[1/].

	PV	Hexanal	Ethyl benzene	*p*-xylene	1,3-dimethyl benzene	Heptanal	*trans*-2-heptenal	2-pentylfuran	Octanal	*trans*-2-octenal	1-octanol	Nonanal
PV	1											
Hexanal	0.892**	1										
Ethyl benzene	-0.104	-0.15	1									
p-Xylene	-0.151	-0.215	0.960**	1								
1,3-Dimethyl benzene	0.017	-0.006	0.927**	0.971**	1							
heptanal	0.512*	0.451	0.296	0.205	0.284	1						
trans-2 -Heptenal	0.056	0.132	0.271	. 538	. 539	0.373	1					
2-Pentylfuran	0.881**	0.964**	.-114	.-598	0.146	0.589	. 859	1				
octanal	0.448	0.486*	0.352	0.138	0.19	0.631	0.824	0.477	1			
trans-2-Octenal	0.246	0.304	0.297	. 747	. 988	0.548	0.962	0.285	0.925	1		
1-Octanol	0.438	0.349	.-184	.-186	. -19	0.203	. 314	0.352	0.390	0.208	1	
nonanal	0.491*	0.441	.-398	.-458	. -62	0.236	0.125	0.430	0.461	0.287	0.958	1

[1/] * Significant at $p < 0.05$; ** Significant at $p < 0.01$.

Thai rice, Daw Dam and Payaluemkang, was used as plant material in this experiment. Suzuki et al. (1999) reported that peroxidation products of unsaturated fatty acids are lower in Daw Dam bran fraction during storage than in rice varieties with Lox-3 in their seeds. These results suggested that the absence of Lox enzymes in rice grains alleviate oxidative deterioration. The appearance concentration of hexanal probably due to enzymatic activity because they are indicator of the presence of enzyme such as lipoxygenase.

Conclusions

In summary, the HS-GC-MS developed in this study has proven to be an accurate and sensitive analytical technique for the determination of lipid oxidation which requires only small sample size. This method also has advantages over other methods that the sample preparation step and the total analysis time are much shorter and it is fully automated and solvent-free. Thus, the technique can be used as an alternative approach for rice breeders to evaluate oxidative stability of rice bran and can be applied in breeding strategies for genetic improvement of rice bran flavor.

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References

Alvarado, J.S. and C. Rose. 2004. Static headspace analysis of volatile organic compounds in soil and vegetation samples for site characterization. Talanta. 62: 17–23.

Berlitz, H.D. and W. Grosch. 1999. Food Chemistry. 2nd ed. Springer-Verlag, Berlin.

Buttery, R.G., J.G. Turnbaugh and L.C. Ling. 1988. Contribution of volatile to rice aroma. J. Agric. Food Chem. 36: 1006–1009.

Frankel, E.N. 1998. Lipid Oxidation. The Oily Press, Dundee, Scotland.

Frankel, E.N. 2005. Lipid Oxidation, 2nd ed. The Oily Press, Bridgewater, England.

Frankel, E.N., W.E. Neff and E. Selke. 1981. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VII. Volatile thermal decomposition

products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate, and linolenate. Lipids 16: 279–285.

Frankel, E.N., W.E. Neff and E. Selke. 1983. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VIII. Volatile thermal decomposition of hydroperoxy cyclic peroxides. Lipids 18: 264–270.

Fliszar, K., J.M. Wiggins, C.M. Pignoli, G.P. Martin and Z. Li. 2004. Analysis of organic volatile impurities in pharmaceutical excipients by static headspace capillary gas chromatography. J. Chromatogr. A. 1027: 83–91.

Krishnamurthy, R.G., T.H. Smouse, B.D. Mookherjee, B.R. Reddy and S.S. Chang. 1967. Identification of 2-pentylfuran in fats and oils and its relationship to the reversion flavor of soybean oil. J. Food Sci. 32: 372–374.

Lam, H.S. and A. Proctor. 2003. Milled rice volatile and odor changes during storage. J. Food Sci. 68: 2676–81.

Lee, J.Y., S. Min, E.O. Choe and D.B. Min. 2003. Formation of volatile compounds in soy flour by singlet oxygen oxidation during storage under light. J. Food Sci. 68: 1933–1937.

Li, Z., L.K. Jacobus, W.P. Wuelfing, M. Golden, G.P. Martin and R.A. Reed. 2006. Detection and quantification of low-molecular-weight aldehydes in Pharmaceutical excipients by headspace gas chromatography J. Chromatogr. 1104: 1–10.

Min, D.B. and J.M. Boff. 2002. Chemistry and reaction of singlet oxygen in foods. Comp. Rev. Food Sci. Food Safety 1: 58–72.

Monsoor, M.A. and A. Proctor. 2004. Volatile component analysis of commercially milled head and broken rice. J. Food Sci. 69: 632-636.

Pugh, S. and J.T. Guthrie. 2000. Development of taint and odor in cellulosic carton-board packaging systems. Cellulose 16: 247–262.

Sekhar, B.P.S. and G.M. Reddy. 2006. Studies on lipoxygenase from rice (*Oryza sativa* L.) J. Sci. Food Agric. 33: 1160–1163.

Snow, N.H. and G.C. Slack. 2002. Head-space analysis in modern gas chromatography. Trends Anal. Chem. 21: 608-617.

Sriseadka, T., S. Wongpornchai and P. Kitsawatpaiboon. 2006. Rapid method for quantitative analysis of the aroma Impact compound, 2-Acetyl-1-pyrroline, in fragrant rice using automated headspace gas chromatography. J. Agric. Food Chem. 54: 8183-8189.

Suzuki, Y., K. Ise, C. Li, I. Honda, Y. Iwai and U. Matsukura. 1999. Volatile Components in Stored Rice [Oryza sativa (L.)] of Varieties with and without Lipoxygenase-3 in Seeds. J. Agric. Food Chem. 47: 1119-1124.

Suzuki, Y., T. Yasui, U. Matsukura, J. Terao. 1996. Oxidative stability of bran Lipids from rice variety [*Oryza sativa* (L.)] lacking lipoxygenase-3 in seeds. J. Agric. Food Chem. 44: 3479-3483.

Taylor, A.J. and D.S. Mottram. 1990. Composition and odor of volatiles from autoxidized methyl arachidonate. J. Sci. Food Agric. 50: 407–17.

Tsugita, T., T. Kurata and H. Kato. 1980. Volatile components after cooking rice milled to different degrees. Agric. Biol. Chem. 44: 835–840.

Tsugita, T., T. Ohta and H. Kato. 1983. Cooking flavor and texture of rice stored under different conditions. Agric. Biol. Chem. 47: 543-549.

Wasfi, I.A., A.H. Al-Awadhi, Z.N. Al-Hatali, F.J. Al-Rayami and N.A. Al-Katheeri. 2004. Rapid and sensitive static headspace gaschromatography–mass spectrometry method for the analysis of ethanol and abused inhalants in blood. J. Chromatogr. 799: 331–336.

Yasumatsu, K. and S. Moritaka. 1964. Fatty acid compositions of rice lipid and their changes during storage. J. Biol. Chem. 28: 257–264.

Yonamine, M., N. Tawil, R.L. de. M. Moreau and O.A. Silva. 2003. Solid-phase micro-extraction–gas chromatography–mass spectrometry and headspace-gaschromatography of tetrahydrocannabinol, amphetamine, methamphetamine, cocaine and ethanol in saliva samples. J. Chromatogr. 789: 73–78.

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