Determination of 11 Low-Molecular-Weight Carbonyl Compounds in Marine Algae by High-Performance Liquid Chromatography

Vilma Mota da Silva[1], Márcia Cristina da Cunha Veloso[2], Eliane Teixeira Sousa[1], Gislaine Vieira Santos[3], Miguel C. Accioly[3], Pedro Afonso de P. Pereira[1], and **Jailson B. de Andrade[1,*]**

[1]Instituto de Química, Universidade Federal da Bahia—UFBA, 40170-290; [2]Centro Federal de Educação Tecnológica da Bahia – CEFET, 40625-650; and [3]Instituto de Biologia, Universidade Federal da Bahia—UFBA, 40170-290, Salvador, Bahia, Brazil

Abstract

A new analytical method is reported for the determination of 11 volatile carbonyl compounds isolated at room temperature from the headspace of marine algae. This method is based on the conversion of the carbonyl compounds to their 2,4-dinitrophenylhydrazone derivatives followed by high-performance liquid chromatography analysis. Using this method, 11 carbonyl compounds are detected and identified from the dynamic headspace sampling of 10 species of marine algae. Eight compounds are quantitated and the three remaining are only identified. Under optimized conditions, all carbonyl compounds are separated in 32 min. The detection and quantitation limits of the high-performance liquid chromatography method are, respectively, in the range of 0.26–0.85 ng/g of algae (formaldehyde) to 13.77–45.90 ng/g of algae (*E*)-2-hexenal. The calibration curves are linear in the concentration range of 2.0–1000 µg/L of solution, corresponding to 0.34–170.00 ng/g of algae. Acetaldehyde and propanal are the most abundant carbonyl compounds identified, with concentrations as high as 980 and 790 ng/g, respectively. The present work, as far as we know, is the first analytical methodology that has been developed to determine low-molecular-weight carbonyl compounds in marine algae. Because many species of marine algae are used as human food, the reported method should be useful to investigators studying the nutritional value as well as oxidative spoilage of fresh and preserved marine algae that is destined for human consumption.

Introduction

Marine macroalgae, which have served for millennia as an important component of the Asian human diet, possess a very complex and diverse mixture of lipids.The concentration of polyunsaturated fatty acids (PUFA) in some species is relatively high, and they are, therefore, of practical interest for food and drugs (1). Besides the traditional use as nourishment, algae have been utilized widely in the world as fertilizer and for the production of the phycocolloids (2).

Some low-molecular-mass carbonyl compounds are important contributors to the development of flavor and unpleasant flavors (off-flavors) in various lipid-containing foods (3). In addition, because these compounds are powerful electrophiles, they react readily with biomolecules. These reactions are deleterious because they result in reduced nutritional value of the food as well the formation of potential mutagens and carcinogens (4–6).

In the last two decades, several aldehydes, especially formaldehyde and acetaldehyde, have received a great deal of attention because of accumulating evidence that they can act as mutagens and carcinogens (7,8). Formaldehyde is classified as "a probable human carcinogen" (9), and acetaldehyde can induce nasal carcinomas in experimental animals (10). In the same way, malonaldehyde has often been used as a marker of oxidative damage in foods (11).

Although formaldehyde and other aldehydes are natural components of a variety of foodstuffs, there have been no systematic investigations of their levels in foods to provide a basis for estimating human population exposure (12–14). Available data suggest that the highest concentrations of formaldehyde in foods (i.e., 60 mg/kg) occur in some fruits and marine fishes (15).

Recently reported analytical methods for the determination of carbonyl compounds (CC) have commonly been based on the derivatization reaction between CC and 2,4-dinitrophenylhydrazine (DNPHi) (16–19). The hydrazones thus formed are then separated by high-performance liquid chromatography (HPLC) and detected by UV–vis spectrophotometry (3). Other recent methods are based on a variety of derivatization methods: (i) reactions with cysteamine to form thiazolidines, (ii) reaction with ethyl 3-oxobutanoate and ammonia to form the 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate derivative (21), and (iii) the Hantzsch reaction, which involves the cyclization reaction of ammonium, CC, and 2,4-pentanedione to form 3,5-diacetyl-1,4-dihydrolutidine (22).

Although these alternative methods are reported to be more

* Author to whom correspondence should be addressed: email jailsong@ufba.br.

sensitive than derivatization with 2,4-dinitrophenylhydrazine under certain conditions, they are typically not applicable to more than one or a few carbonyl compounds.

Although DNPHi derivatization is the most widely used method for determining carbonyl compounds, it has not been commonly applied to the study of the volatile carbonyls formed from algae. This is probably attributable to analytical limitations associated with the direct reaction of biological material with the strongly acidic derivatization medium (20). To our knowledge, there are no reported HPLC methods for the determination of low-molecular-weight carbonyl compounds (LMWCC) in algae.

In sum, this paper describes a new analytical method for the determination of LMWCC in marine algae using headspace sampling followed by hydrazine derivatization and HPLC analysis. A primary advantage of this method is that sample decomposition is avoided by employing mild derivatization reaction conditions and short reaction time (30 min). Using this method, the identification and quantification of 11 volatile low-molecular-mass carbonyl compounds from a variety of species of marine algae harvested from the Atlantic coast of Bahia, Brazil is reported.

Experimental

Reagents and standards

HPLC-grade acetonitrile, 2,4-dinitrophenylhydrazine, propanal, pentanal, butanal, (E)-2-pentenal, (E)-2-hexenal, and hexanal were obtained from Aldrich Chemical Co. (Milwaukee, WI). HPLC-grade ethanol was obtained from Merck (Darmstadt, Germany). Purified water was obtained by distillation and filtration through an E-pure Alltech system (Alltech Associates, Deerfield, IL). Unless otherwise noted, all of the mentioned reagents were of analytical grade.

Preparation of 2,4-DNPHi solution

The 2,4-DNPHi solution (pH = 1.85) was prepared at 0.05% (w/v) in acetonitrile–H_2O–H_3PO_4 (20:79:1, v/v/v) and purified by liquid–liquid partitioning with CCl_4. This solution was stored in the dark at 4°C. The purity of the solution was verified by HPLC–UV analysis. A more detailed description of reagent preparation can be found elsewhere (23).

A second, non-acidic 0.05% (w/v) 2,4-DNPHi solution (pH = 6) was prepared for the study of the effect of pH on the derivatization reaction. This solution was prepared by dissolving the correct mass of 2,4-DNPHi in a mixture of acetonitrile/water (20:80, v/v). After adjusting the pH to the desired value (in the range 1.24–5.98) with HCl, this reagent was combined with aqueous solutions of propanal or hexanal and then allowed to react for 1 h at room temperature with magnetic stirring.

2,4-Dinitrophenylhydrazone standards

2,4-Dinitrophenylhydrazone (2,4-DNPHo) derivatives [formaldehyde, acetaldehyde, propanal, propanone, butanal, butanone, 2-butenal, benzaldehyde, pentanal, (E)-2-pentenal, (E)-2-hexenal, and hexanal] were synthesized from the reaction of 2,4-DNPHi with corresponding carbonyl compounds, according to the methods described elsewhere (24), and purified by recrystallization from ethanol. The purities of 2,4-DNPHo were verified by the comparison of their melting points with the literature (24).

Preparation of carbonyl–DNPHo calibration solutions

A stock solution (20 µg/mL) of the hydrazone derivative mixture was prepared by combining preweighed portions of each hydrazone and then dissolving the mixture in acetonitrile. Standard solutions were prepared by diluting the stock solution with acetonitrile to yield concentrations in the range of 2.0 to 1000 µg/mL. These solutions were all stored in the dark at 4°C. The standards were injected into the HPLC system (triplicate injections), and the average of peak height obtained was plotted against the concentration.

Preparation of DNPHi-impregnated cartridges (SEP-PAK C18)

The SEP-PAK C18 cartridges (360 mg) (Waters, Milford, MA) were washed with 2 mL of acetonitrile and impregnated with

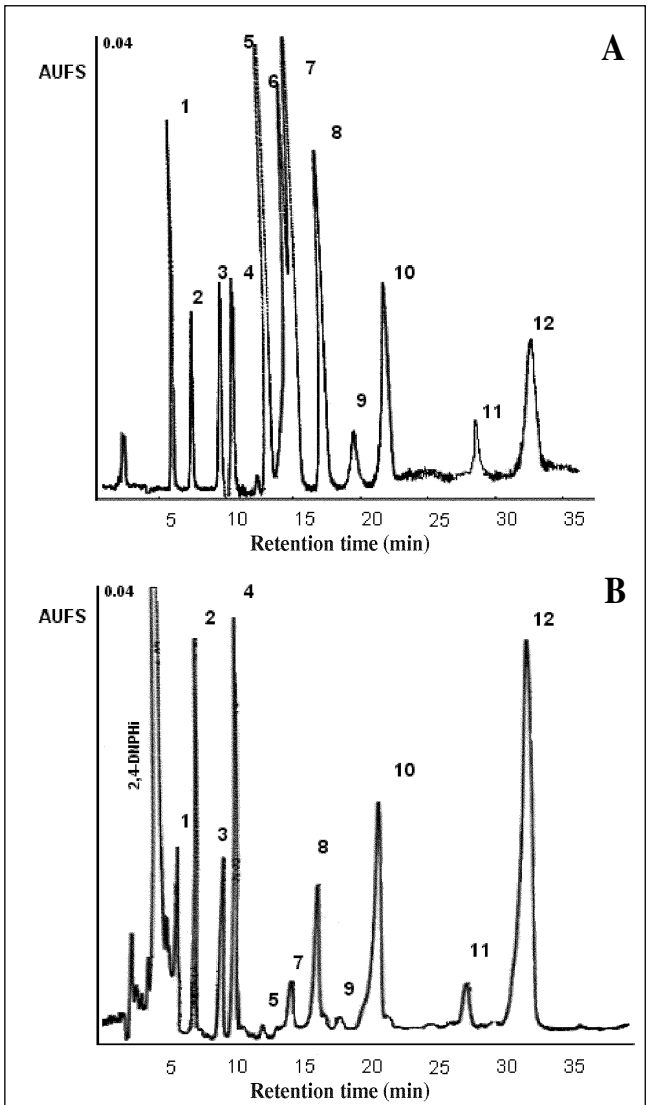

Figure 1. Typical chromatograms obtained for 2,4-DNPHo: DNPHo (A) and *Lobophora variegata* (B). Standards: formaldehyde, 1; acetaldehyde, 2; propanone, 3; propanal, 4; 2-butenal, 5; butanone, 6; butanal, 7; benzaldehyde, 8; (E)-2-pentenal, 9; pentanal, 10; (E)-2-hexenal, 11; and hexanal, 12.

4 mL of 2,4-DNPHi solution (0.05%). Excess liquid was blown out of cartridges with N_2 (carbonyl compound-free) for 10 min at 5 mL/min. The cartridges were wrapped in aluminum foil and then stored in a dessicator at 4°C (25).

Sample preparation

Marine algae were collected in the intertidal zone at low tide on the Atlantic Coast of Bahia, Brazil. The samples were immediately stored in a box with ice and transported to the laboratory. The algae were thoroughly cleaned manually to remove epiphytes, small invertebrates, and sand particles and gently washed with purified water. Excess water was allowed to drain from the samples and then further water was removed by placing each approximately 50-g sample on a 50-cm circle of qualitative cellulose filter paper for 30 s at room temperature. Each sample was individually packed in a polyethylene bag and stored at –15°C until analysis. A small sample of each algae species was used by the Institute of Biology–UFBA for taxonomical identification. The algae species studied were: Caulerpa cupressoide, Caulerpa racemosa, Digenia simplex, Gelidiela acerosa, Gracilariopsis lemaneiformis, Halimeda discoidea, Hipneia musciformis, Lobophora variegata, Spatoglossum schvoedevii, and Ulva lactuca.

Method

Dynamic headspace sampling (purge and trap)

A 10-g aliquot of algae sample along with 2 mL of an aqueous solution of (E)-2-hexenal (100 µg/mL) (used as an internal standard to determine the efficiency of the extraction) were introduced into a 500-mL glass flask and purged with N_2 (0.369 L/min) for 30 min at room temperature. The effluent was passed through a pair of 2,4-DNPHi-impregenated SEP-PAK C18 cartridges that were connected in series (see previous section for cartridge-preparation procedure). Following the N_2 purge, the treated cartridges were eluted with 2 mL of acetonitrile. Under these conditions, all of the carbonyl compounds studied were trapped on the first cartridge. The acetonitrile eluent was subsequently subjected to HPLC–UV analysis to detect and quantitate 2,4-DNPHo derivatives.

Compounds separation

The derivatives were separated with a Zorbax ODS 5 µm (4.6 mm × 25 cm) column (Rockland Tecnologies Inc., Chadds Ford, PA) using an acetonitrile–water mixture (60:40, v/v) as a mobile phase at flow rate of 1.5 mL/min [Varian LC model 2510 equipped (Palo Alto, CA) with a Rheodyne (Cotati, CA) injector with a 10-µL sample loop]. Absorbance detection was employed using a Varian model 2550 UV–vis detector (flow cell path length 8 mm, 12-µL total volume) operated at 365 nm (absorbance units full scale = 0.04).

Table I. DL and QL of the HPLC Method

Carbonyl compounds	DL*	QL*
Formaldehyde	0.26	0.85
Acetaldehyde	0.37	1.24
Propanal	0.53	1.75
Propanone	0.54	1.80
Butanal	0.85	2.82
Butanone	0.88	2.94
Pentanal	6.00	19.72
Hexanal	7.82	26.01
(*E*)-2-Hexenal	13.77	45.90

* in ng/g algae.

Extraction efficiencies

The accuracy of the dynamic headspace sampling, used in the recovery of carbonyl compounds from algae, was determined by the addition of an aliquot (2 mL) of a solution containing pentanal, hexanal, and (E)-2-hexenal (200 µg/L in water) to different fresh species of algae (10 g) (without carbonyl compounds). The extractions were performed in triplicate, under the same experimental conditions as previously described (3).

Results and Discussion

Sample preparation techniques, used to preconcentrate or separate analytes from the matrix (or both), are of critical importance to the success of trace analysis of biological and food materials. Sample preparation conditions should be as mild as possible to avoid oxidation, thermal decomposition, or other analyte degradation reactions. For example, Josephson and Lindsay (26) reported that during the distillation of volatiles from the tissue of fishes and oysters, one of the volatile analytes, (E,Z)-2,6-nonadienal, is converted to (Z)-4-heptenal via a water-mediated retro-aldol condensation.

For the specific case of ketone and aldehyde derivatization with 2,4-DNPHi, the pH-dependence of the derivatization reaction causes problems when the acidic solution of 2,4-DNPHi reacts directly with neutral samples. For example, it was observed in a previous study (3) that the direct reaction of fish samples (pH 6.0–7.0) with an acidic solution of 2,4-DNPHi (pH 1.5–2.0) allowed quantitation of only the most eletrophilic aldehydes, formaldehyde, and acetaldehyde. In fact, as the reaction progresses and pH increases, there is even decreased yield of the 2,4-DNPHos derived from formaldehyde and acetaldehyde. Similar results were observed in the current study for the pH dependence of propanal and hexanal derivatization.

To avoid these problems, the method reported here first volatilizes the aldehydes and ketones in a room temperature stream of N_2 gas and then reacts the airborne analytes with an acidic solution of 2,4-DNPHi, directly deposited on a C18 cartridge. As a result, there is no direct contact between the algae samples and the acidic derivatization reagent. This method has allowed successful quantitation of all the compounds studied in algae samples.

The detection limit (DL) observed using this method varied between 0.26 ng/g of algae (for formaldehyde) and 13.77 ng/g [for (E)-2-hexenal] (Table I). Likewise, the quantitation limit (QL) was observed to vary between 0.85 ng/g of algae (for formaldehyde) and 45.90 ng/g [for (E)-2-hexenal], (Table I). The DL and QL were calculated according to the International Union of Pure and Applied Chemistry recommendation (27), using the expression:

$$(DL) = K\sigma/\mathbf{b} \qquad \text{Eq. 1}$$

where K is 3, σ is the standard deviation of the blank signal, and (**b**) is the slope of the calibration line (sensitivity). For QL, K is 10.

The analytical calibration curves (A = aC + b) were linear for the carbonyl compounds in the concentration range of 2.0 to 1000 µg/L (n = 6 points) as is shown in Table II. In all cases, the correlation coefficient for a linear curve fit was higher than 0.99, and the relative standard deviation of the mean peak height was below 3%.

The percent recovery of volatile carbonyl compounds from algae by this method, based on the standard addition of pentanal, hexanal, and (E)-2-hexenal was, as previously presented in the Experimental section, in the range of 92–95%. It is worthwhile to mention that this recovery efficiency is not definitive because the standards are not uniformly distributed within the algae's tissue in the same way as the endogenous carbonyl compounds.

Table II. Analytical Curves of Mixtures of Aldehydes Prepared, Concentrations Ranging between 2.0 and 1000 µg/L*,†

Carbonyl compounds	a	b	R^2
Formaldehyde	0.1962	0.9163	0.9982
Acetaldehyde	0.0896	0.5792	0.9991
Propanal	0.2414	1.1261	0.9984
Propanone	0.1100	0.7112	0.9980
Butanal	0.0204	–1.6973	0.9960
Butanone	0.0537	0.2451	0.9951
Pentanal	0.0081	–0.1622	0.9981
Hexanal	0.0062	–0.1865	0.9986
(*E*)-2-Hexenal	0.0196	–1.0839	0.9993

* *n* = 6 points.
† For each aldehyde, A = aC + b, equations showed the following linear correlation. Here, A = peak height, a = slope, C = concentration (µg/L), b = intercept, and R^2 = determination coefficient.

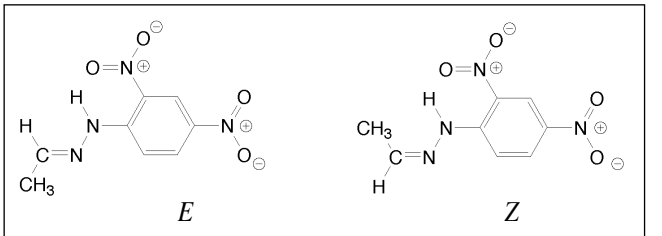

Figure 2. *E*- and *Z*-isomers of acetaldehyde-2,4-dinitrophenylhydrazone.

Under the optimized conditions, the HPLC analysis was completed in 32 min (Figure 1A). The chromatogram presented in Figure 1B illustrates the profile obtained for Lobophora variegata. Peaks were assigned on the basis of retention times, compared with authentic standards. With the exception of formaldehyde and symmetrical ketones such as acetone, the 2,4-DNPHo derivatives of aldehydes and ketones consist of a mixture of Z- and E-isomers (Figure 2). These isomers are typically difficult to separate by HPLC and were not separated under the conditions used in this work.

Algae samples were either analyzed fresh on the day of collection (up to 2 h after treatment) or following 30 or 90 days of storage at –15°C. Table III shows concentrations of the carbonyl compounds, determined 90 days after the storage. The most frequently detected analytes belonged to the homologous series of saturated carbonyl compounds ranging from C_1–C_6, with acetaldehyde and propanal being detected in all species analyzed, in concentrations as high as 980 and 790 ng/g, respectively. Formaldehyde was identified in the species Digenia simplex, Gelidiela acerosa, Lobophora variegata, and Ulva lactuca, and its highest concentration determined was 670 ng/g (Table III). For the algae species studied, the levels of acetone, butanal, pentanal, and hexanal were found to vary between zero and the following maximum concentrations: acetone (550 ng/g), butanal (404 ng/g), pentanal (200 ng/g), and hexanal (240 ng/g). Lobophora variegata was the only species to yield all of the C_1–C_6 carbonyl compounds studied, as well as the only species to yield (E)-2-hexenal.

Among the carbonyl compounds identified in the present study, benzaldehyde, 2-butenal, and (E)-2-pentenal were not quantitated but were identified by matching their retention times with those of authentic standards. The 2-butenal was found only in the Lobophora variegata, and (E)-2-pentenal was found in the Lobophora variegata and Gracilariopsis lemaneiformis samples.

We could not ascertain the origin of formaldehyde, acetalde-

Table III. Highest Concentrations* (ng/g) of Carbonyl Compounds found in the Headspace of 10 Algae Species after 90 Days of Storage at –15°C

Species	*n*	Formaldehyde	Acetaldehyde	Propanal	Propanone	Butanal	Pentanal	Hexanal
Caulerpa cupressoide	3	nd	260	760	150	nd	nd	9
Caulerpa racemosa	2	nd	60	100	90	nd	10	20
Digenia simplex	2	40	420	220	nd	nd	30	130
Gelidiela acerosa	3	380	650	560	70	90	20	nd
Gracilariopsis lemaneiformis	1	nd	350	40	nd	40	30	10
Halimeda discoidea	2	nd	70	10	nd	nd	nd	20
Hipneia musciformis	2	nd	50	130	430	nd	nd	nd
Lobophora variegata	4	670	450	360	340	404	200	150
Spatoglossum schvoedevii	1	nd	280	100	80	40	130	240
Ulva lactuca	3	20	980	790	550	120	13	60

* The values are expressed as wet weight, *n* = number of different samples analyzed, and nd = not detected.

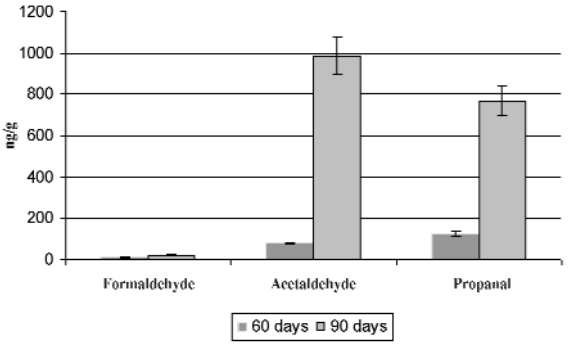

Figure 3. Amounts (ng/g) of formaldehyde, acetaldehyde, and propanal found in one sample of *Ulva lactuca* analyzed fresh and after 60 and 90 days of storage at –15°C.

hyde, butanal, and pentanal in the samples. Propanal probably originates from the breakdown of w-3 PUFA, such as linolenic acid, through sequential reactions catalyzed by lipoxygenase and fatty acid hydroperoxide lyase (28). Hexanal presumably arises from the analogous breakdown of (w-6)-PUFAs, primarily arachidonic and linoleic acids. Hexanal is described as having a "green and cut grass-like" odor (29) and is an important odor-active component in food because of its low-odor threshold (4.5 ppb in water). Hexanal levels have been used to gauge the extent of lipid oxidation and as a quality index for food products (30); however, there is no data on its oral toxicity. The (E)-2-hexenal probably forms from the isomerization of (Z)-3-hexenal (28). Benzaldehyde, which is described as possessing an almond-like odor, has been reported as an important flavor in crayfish tail meat (31) and contributes to the sweet odor of algae (32).

The level of volatile aldehydes and ketones found in algae samples increases dramatically during storage at –15°C. For example, Figure 3 shows the concentration of formaldehyde, acetaldehyde, and propanal found in Ulva lactuca following 60 and 90 days of storage (none of these analytes was detected in fresh samples of Ulva lactuca). While formaldehyde concentrations remained low throughout the 90-day storage period, the concentrations of acetaldehyde and propanal increased sharply during this period.

For a number of reasons, our results suggest that acetaldehyde and propanal may prove to be good indicators of food quality. First, this pair of aldehydes was found in relative high concentrations in all species studied. Second, their concentrations increased sharply with storage time. Finally, the presumed origin of propanal from the oxidative decomposition of w-3 fatty acids should allow quantitative assessment of the decomposition of this important class of nutrients.

Conclusion

The new analytical method reported here has permitted the identification and quantitation of 11 volatile low-molecular-weight carbonyl compounds and offers two distinct advantages over previous methods: (i) low DLs and (ii) simple sample preparation. In principle, this method should be easily adapted for the analysis of almost any other lipid-containing foodstuff.

Acknowledgments

The authors thank Gizelia Vieira Stantos for collecting the initial algae samples and for help with algae species identification. The present work was supported by the National Research Council of Brazil (CNPq), Fundação de Apoio a Pesquisa do Estado da Bahia (FAPESB)-PRONEX/FAPESB/CNPq, and Financiadora de Estudos e Projetos (FINEP). The authors thank Prof. Dr. José Oscar N. Reis and Prof. Dr. Sergio L.C. Ferreira for useful discussions.

References

1. S. Popov, I. Elenkov, K. Stefanov, and S. Dimitrova-Konaklieva. Effect of salinity on lipid composition of C*ladophora Vagabunda*. *Phytochemistry* **42:** 39–44 (1996).
2. E.C. Oliveira. Marine algae: an under-exploited Brazilian resource – in Portuguese - *Panorama de Aqüicultura* **5/6:** 24–26 (1977).
3. J.B. de Andrade, M.C.C. Veloso, V.M. da Silva, and G.V. Santos. Determination of aldehydes in fish by high performance liquid chromatography (HPLC). *J. Chromatogr. Sci.* **39:** 173–76 (2001).
4. G.M. Siu and H.H. Draper. A survey of the malonaldehyde content of retail meats and fish. *J. Food Sci.* **43:** 1147–49 (1978).
5. A.H. Khalil and E.H. Mansour. Control of lipid oxidation in cooked and uncooked refrigerated carp fillets by antioxidant and packaging combination. *J. Agric. Food Chem.* **46:** 1158–62 (1998).
6. E. Decker and S. Zhou. Ability of amino acids, dipeptides, polyamines, and sulfhydryls to quench hexanal, a saturated aldehydic lipid oxidation product. *J. Agric. Food Chem.* **47:** 1932–36 (1999).
7. National Research Council, Committee on Aldehydes. *Formaldehyde and Other Aldehydes*. National Academy Press, Washington, D.C., 1981.
8. B.M. Goldschmidt. Role of aldehydes in carcinogenesis. *J. Environ. Sci. Health.* **2:** 231–49 (1984).
9. J.D. Thrasher, K.H. Kilburn, and S.J. Rothenberg. Embryo toxicity and teratogenicity of formaldehyde. *Archives of Environ. Health.* **56:** 300–12 (2001).
10. T. Shibamoto and T. Miyake. Quantitative analysis by gas chromatography of volatile carbonyl compounds in cigarette smoke. *J. Chromatogr. A* **693:** 376–81 (1995).
11. A.J. St. Angelo. Lipid oxidation in foods. *Crit. Ver. Food Sci. Nutr.* **36:** 175–224 (1996).
12. World Health Organization. "Formaldehyde". In *Environmental Health Criteria Series*, vol. 89. WHO, Geneva, Switzerland, 1989, p. 219.
13. E.A. de Oliveira and J.B. de Andrade. Simultaneous determination of formaldehyde and acetaldehyde and their respective hydroxyalkylsulphonic acids by high performance liquid chromatography (HPLC). (in Portuguese) *Quím. Nova.* **17:** 13–16 (1994).
14. J.B. de Andrade, M.B. Bispo, M.V. Rebouças, M.L.S.M. Carvalho, and H.L.C. Pinheiro. Spectofluorimetric determination of formaldehyde in liquid samples. *Am. Lab.* **28:** 56–58 (1996).
15. IPCS. *Formaldehyde*. World Health Organization, International Program on Chemical Safety (Concise International Chemical Assessment Document No. 40), WHO, Geneva, Switzerland, 2002, pp. 1–75.
16. S. Uchiyama, M. Ando, and S. Aoyagi. Isomerization of aldehyde-2,4-dinitrophenylhydrazone derivatives and validation of high-performance liquid chromatographic analysis. *J. Chromatogr. A* **996:** 95–102 (2003).
17. L.J.S. Liu, R.L. Dills, M. Paulsen, and D.A. Kalman. Evaluation of media and derivatization chemistry for six aldehydes in a passive sampler. *Environ. Sci. Technol.* **35:** 2301–2308 (2001).

18. S. Wen, Y.L. Feng, Y.X. Yu, X.H. Bi, X.M. Wang, G.Y. Sheng, J.M. Fu, and P.A. Peng. Development of a compound-specific isotope analysis method for atmospheric formaldehyde and acetaldehyde. *Environ. Sci. Technol.* **39:** 6202–6207 (2005).
19. S. Uchiyama, E. Matsushima, S. Uchiyama, M. Ando, and S. Aoyagi. Simultaneous determination of C1-C-4 carboxylic acids and aldehydes using 2,4-dinitrophenylhydrazine-impregnated silica gel and high-performance liquid chromatography. *Anal. Chem.* **76:** 5849–54 (2004).
20. A. Yasuhara and T. Shibamato. Quantitative analysis of volatile aldehydes formed from various kinds of fish flesh during heat treatment. *J. Agric. Food Chem.* **43:** 94–97 (1995).
21. G. Burini and R. Coli. Determination of formaldehyde in spirits by high-performance liquid chromatography with diode-array detection after derivatization. *Anal. Chim. Acta* **511:** 155–58 (2004).
22. H.Y. Yuan, S.E. Wang, and D. Xiao. Spectrophotometric method with silica-gel beads for determination of trace formaldehyde in air. *Spectros. Letters* **38:** 121–30 (2005).
23. J.B. de Andrade, J.O.N. Reis, M.V. Rebouças, H.L.C. Pinheiro, and M.V. Andrade. Determination of formaldehyde and acetaldehyde in drinking water and alcoholic beverages by high performance liquid chromatography (HPLC). *Quím. Anal.* **15:** 144–47 (1996).
24. R.L. Shriner, R.C. Fuson, and D.Y. Curtin. *The Systematic Identification of Organic Compounds.* John Wiley & Sons, New York. NY, 1964.
25. J.B. de Andrade and A.H. Miguel. The use of reverse phase microcolumns in the sampling of aldehydes (as 2,4-dinitrophenylhydrazone) emitted from alcohol fueled motor vehicles. *Quím. Nova* **8:** 356–57 (1985).
26. D.B. Josephson and R.C. Lindsay. Retro-aldol degradation of unsaturated aldehydes: Role in the formation of c4-heptenal from t2,c6-nonadienal in fish, oyster and other flavors. *J. Am. Oil Chem. Soc.* **64:** 132–38 (1987).
27. International Union of Pure and Applied Chemistry. Nomenclature, symbols, units and their usage in spectrochemical analysis—II. Data interpretation, analytical chemistry division. *Spectrochim. Acta B* **33:** 241–45 (1978).
28. E.N. Frankel. Formation of headspace volatiles by thermal decomposition of oxidized fish oil vs. oxidized vegetable oils. *J. AOCS* **70:** 767–72 (1993).
29. C. Prost, T. Serot, and M. Demaimay. Identification of the most potent odorants in wild and farmed cooked turbot (*Scophtalamus maximus L.). J. Agric. Food Chem.* **46:** 3214–19 (1998).
30. T. Lee and H.A. Zhang. A novel silica gel adsorption/near-infrared spectroscopic method for the determination of hexanal as an example of volatile compounds. *J. Agric. Food Chem.* **45:** 3083–87 (1997).
31. W. Vejaphan, T.C.Y. Hsieh, and S.S. Willians. Volatile flavor components from boiled crayfish (*Procabarus clarkii*) tail meat. *J. Food Sci.* **53:** 1666–70 (1988).
32. H. Sugisawa, K. Nakamura, and H. Tamura. The aroma profile of the volatile in marine green algae (*Ulva pertusa). Food Rev. Int.* **6:** 573–89 (1990).

Manuscript received March 8, 2005;
revision received October 21, 2005.