Asian Journal of Chemistry Vol. 17, No. 1 (2005), 219–223

Natural Antioxidant and Anticarcinogenic Compounds from Two Varieties of Iranian Garlic

PARVIN RAMEZANI KHARAZI* and GHOLAMALI PEYVAST
Chemistry Department, Guilan University, Rasht, Iran

Conjugated linoleic acid (CLA) or its downstream metabolites are screened for its various antioxidant and antitumour activities in two varieties of Iranian garlic [*Allium sativum*, var. *sativum* (I) *and Allium ampeloprasum*, var. *holmens* (II)] that are cultivated in north of Iran. In this work, IR, GC and GC-mass have been used for the characterization of unsaturated fatty acid methyl esters (UFAMEs). The qualitative and quantitative analysis of garlic extract showed significant amount of 9,12-octadecadienoic acid methyl ester and 11,14-eicosadienoic acid methyl ester among other UFAMEs for (II).

Key Words: Antioxidant, Anticarcinogenic, Iranian garlic.

INTRODUCTION

Linoleic acid (18 : 2 n-6) is the most common polyunsaturated fatty acids, in plants and animal tissues. It was isolated in 1844 by Sacc[1]; after a long controversy, its exact structure[2] was clarified in 1939 and it was synthesized[3] only in 1950. Conjugated linoleic acid (CLA) is a mixture of 8 (and perhaps even more) different isomers of an 18-carbon fatty acid made by hydrogenation from linoleic acid (LA), the omega-6 (n-6) essential fatty acid (EFA)[4]. In nature, this is accomplished by bacteria in the stomach of beef, goats, sheep and other cud-chewing animals (which include deer, moose, caribou, elk, buffalo, yak, musk ox)[5, 6]. It cannot be synthesized by animals which must find it in plant foodstuff. Its determination is currently used as an assay of free radical activity, but recent studies have cast doubt on the specificity of the assay since bacteria are able to induce their formation without excluding any dietary origin[7]. The two double bonds in CLA are primarily in position 9 and 11, and 10 and 12 along the carbon chain. There can also be geometric changes: *cis* or *trans* configuration. Various antioxidant and antitumur activities have been attributed to CLA and its isomer[8]. Reported health benefits of dietary supplementation with CLA have been attributed variously to competitive inhibtion of Δ 6-desaturase and/or PPARY activation[9]. 11,14-Eicosadienoic acid is an uncommon naturally occurring PUFA

(poly unsaturated fatty acid). It inhibits the binding of [3H]-LTB4 to pig neutrophil membranes with a Ki of 3 μM[10]. Linoleic acid is the precursor of all the (n-6) series formed by desaturation and elongation. Two *trans* isomers of linoleic acid have been detected in seed oils. The 9c,12t-isomer was found in *Crepis rubra* and the 9t,12t-isomer was found in *Chilopsis linearis*.

To the best of our knowledge there has been no data available about the poly-unsaturated fatly acids in Iranian garlic. The aim of this study was, therefore, to compare the amount of these compounds in two kinds of garlic.

EXPERIMENTAL

The two *Allium* varieties were obtained directly from the growers in Astaneh (north of Iran). Solvent grade methanol and pentane, hexane, isopropanol and toluene were obtained from Merck Chemical Company without further purification. Anhydrous sodium sulfate was used as drying agent.

Gas chromatography/mass spectrometry: GC analysis performed on a 30 m capillary column of silicon-5, CB. The temperature programmed was 40°C (held for 2 min), increasing to 250°C. The carrier gas was helium with 10 psi pressure and the amount of sample injection was 1 μL.

GC-MS was obtained with a Fisons Instruments GC 8000/Trio 1000. Quantification was achieved using peak area calculation and compound identification was partly carried out using correlation between retention times[11-16].

Extraction and Separation of Garlic Components

Chopped garlic pieces (1000 g) were soaked in methanol (1000 mL) at 18°C. The methanol was replaced 3 times for 3 days by using a total of 3 L methanol[17]. The methanol extract was concentrated *in vacuo* to about 100 mL, diluted with water and extracted with ether (4 × 300 mL). The combined ether extract was dried by magnesium sulfate and concentrated *in vacuo* affording 3.5 g oily residue for variety **I** and 4.2 g for variety **II**. To the 50 mg of oily residue from var. *sativum* and *holmens* added 1 mL of toluene in a test tube[18], 0.5 M sodium methoxide in anhydrous methanol (2 mL) was added and the solution was maintained at 50°C for 10 min Glacial acetic acid (0.1 mL), followed by 5 mL of water, was then added. The required esters were extracted by hexane (2 × 5 mL), using a pasteur pipette to separate the layers. The hexane layer was dried over anhydrous sodium sulfate and filtered under reduced pressure on a rotary film evaporator. The sample was dissolved in hexane (containing 50 ppm BHT) for GC analysis. Compounds were characterized by direct comparison of their spectral data with standard data in literature (Table-1).

RESULTS AND DISCUSSION

By comparison of GC-mass spectra with standard data, a large amount of unsalurated fatty acid methyl esters (UFAMEs) have been detected in hexane fraction of (II): *cis*-9,*trans*-12-octadecadienoic acid methyl ester (26.4%), 7,10,13-hexa-decatrienoic acid methyl ester (8.7%), *cis*-9,*cis*-12-octa-decadienoic acid (22.5%), dodecanoic acid (7%) and 11,14,17-eicosatrienoic acid methyl ester (2.124%). This finding is surprising, because the sulfur compounds that are responsible for garlic characteristic were found only in very low concentration for II (Table-2). The main unsaturated fatty acids are *cis*-9,*trans*-12-octadecadienoic acid methyl ester and 7,10,13-hexa-decatrienoic acid methyl ester and *cis*-9, *cis*-12-octadecadienoic acid. 9,12-Octadecadienoic acid is a geometric isomer of linoleic acid that is reported to have anticarcinogenic properties and antiatherogenic and hypocholesterolemic effects. These results indicate that this type of Iranian garlic by high percentage of CLA may have different medicinal usefulness and also it can be used for production of alkyd resins to increase the drying time of normal vegetable oils.

TABLE-1
UNSATURATED FATTY ACID METHYL ESTERS (USFAMEs) IN *AMPELOPRASUM* VAR. *HOLMENS*.

Compounds	Name of compounds	GC RT min.	m.w.	%	Mass m/e intensity
$C_{11}H_{22}O_2$	Decanoic acid	48.890	186	6.375	186(M+, 1%), 101(10%), 87(90%), 74(100%), 55(65%), 43(80%), 41(70%), 29(35%)
$C_{19}H_{34}O_2$	*cis*-9, *trans*-12, octadecadienoic acid, methyl ester	52.803	294	26.400	294(M+, 1%), 262(2%), 150(5%), 109(40%), 95(70%), 81(100%), 67(85%), 55(80%), 41(70%), 29(60%)
$C_{17}H_{28}O_2$	7,10,13-hexadeca-trienoic acid, methyl ester	52.908	264	8.700	263(1%), 149(20%), 135(25%), 121(30%), 108(60%), 105(30%), 95(80%), 93(85%), 81(60%), 79(100%), 67(80%), 55(70%), 41(55%), 29(30%)
$C_{18}H_{32}O_2$	*cis*-9,*cis*-12-octa-decadienoic acid	54.530	280	22.500	280(M+, 1%), 150(2%), 109(20%), 96(30%), 95(50%), 82(40%), 81(75%), 67(100%), 55(60%), 41(70%), 29(30%)
$C_{12}H_{24}O_2$	Dodecanoic acid	49.710	200	7.106	200(M+, 20%), 120(30%), 85(30%), 73(70%), 60(65%), 55(60%, 43(100%), 41(85%), 29(50%)

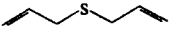
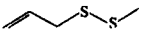
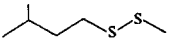
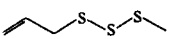
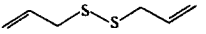
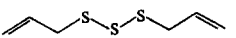
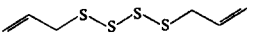
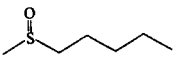
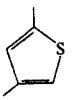
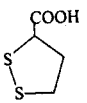
RESULTS AND DISCUSSION

By comparison of GC-mass spectra with standard data, a large amount of unsalurated fatty acid methyl esters (UFAMEs) have been detected in hexane fraction of (II): *cis*-9,*trans*-12-octadecadienoic acid methyl ester (26.4%), 7,10,13-hexa-decatrienoic acid methyl ester (8.7%), *cis*-9,*cis*-12-octa-decadienoic acid (22.5%), dodecanoic acid (7%) and 11,14,17-eicosatrienoic acid methyl ester (2.124%). This finding is surprising, because the sulfur compounds that are responsible for garlic characteristic were found only in very low concentration for **II** (Table-2). The main unsaturated fatty acids are *cis*-9,*trans*-12-octadecadienoic acid methyl ester and 7,10,13-hexa-decatrienoic acid methyl ester and *cis*-9, *cis*-12-octadecadienoic acid. 9,12-Octadecadienoic acid is a geometric isomer of linoleic acid that is reported to have anticarcinogenic properties and antiatherogenic and hypocholesterolemic effects. These results indicate that this type of Iranian garlic by high percentage of CLA may have different medicinal usefulness and also it can be used for production of alkyd resins to increase the drying time of normal vegetable oils.

TABLE-1
UNSATURATED FATTY ACID METHYL ESTERS (USFAMEs) IN *AMPELOPRASUM* VAR. *HOLMENS*.

Compounds	Name of compounds	GC RT min.	m.w.	%	Mass m/e intensity
$C_{11}H_{22}O_2$	Decanoic acid	48.890	186	6.375	186(M+, 1%), 101(10%), 87(90%), 74(100%), 55(65%), 43(80%), 41(70%), 29(35%)
$C_{19}H_{34}O_2$	*cis*-9, *trans*-12, octadecadienoic acid, methyl ester	52.803	294	26.400	294(M+, 1%), 262(2%), 150(5%), 109(40%), 95(70%), 81(100%), 67(85%), 55(80%), 41(70%), 29(60%)
$C_{17}H_{28}O_2$	7,10,13-hexadeca-trienoic acid, methyl ester	52.908	264	8.700	263(1%), 149(20%), 135(25%), 121(30%), 108(60%), 105(30%), 95(80%), 93(85%), 81(60%), 79(100%), 67(80%), 55(70%), 41(55%), 29(30%)
$C_{18}H_{32}O_2$	*cis*-9,*cis*-12-octa-decadienoic acid	54.530	280	22.500	280(M+, 1%), 150(2%), 109(20%), 96(30%), 95(50%), 82(40%), 81(75%), 67(100%), 55(60%), 41(70%), 29(30%)
$C_{12}H_{24}O_2$	Dodecanoic acid	49.710	200	7.106	200(M+, 20%), 120(30%), 85(30%), 73(70%), 60(65%), 55(60%, 43(100%), 41(85%), 29(50%)

TABLE-2
COMPARISON OF ORGANOSULFUR COMPOUNDS AND UFAMEs IN *ALLIUM SATIVUM* VAR. *SATIVUM* AND *ALLIUM AMPELOPRASUM* VAR. *HOLMENSE*

Compounds	Name of compounds	% for (I)	% for (II)
	Dimethyl disulfide	0.980	–
	Diallyl sulfide	1.272	–
	Allyl methyl disulfide	5.900	–
	Isopentyl methyl disulfide	18.900	–
	Allyl methyl trisulfide	3.254	0.082
	Diallyl disulfide	2.250	0.483
	Diallyl trisulfide	5.500	6.313
	Diallyl tetrasulfide	2.160	5.000
	Butylmethyl sulfoxide	2.500	–
	2,3-Dimethyl thiophen	1.800	–
	2,4-Dimethyl thiophen	–	1.000
	1,2,3-Thiadiazole-5-methyl	4.800	0.252
	Cyclopentanethiol	–	1.500
	1,2-Dithiolan-3 carboxylic acid	1.400	–
$C_{11}H_{22}O_2$	Decanoic acid methyl ester	–	6.375
$C_{19}H_{34}O_2$	*cis*-9,*trans*-12-Octadecadienoic acid methyl ester	–	26.400
$C_{17}H_{28}O_2$	7,10,13-Hexadecatrienoic acid methyl ester	–	8.700
$C_{18}H_{32}O_2$	*cis*-9,*cis*-12-Octadecadienoic acid	–	22.500
$C_{12}H_{24}O_2$	Dodecanoic acid	–	7.100
$C_{21}H_{36}O_2$	11,14,17-Eicosatrienoic acid methyl ester	–	2.124

REFERENCES

1. F. Sacc, *Ann*, **51**, 213 (1844).
2. T.P. Hilditch, *J. Soc. Chem. Ind.*, **58**, 233 (1939).
3. R.A. Raphael, *Nature*, **165**, 235 (1950); *J. Chem. Soc.*, 2100 (1950).
4. S.R. Demaree, C.D. Gibert, H.J. Mersmann and SB. Smith, *J. Nutr.*, **132**, 3272 (2002).
5. Y.L. Ha, N.K. Grimm and M.W. Pariza, *J. Agr. Food Chem.*, **37**, 75 (1989).
6. S.F. Chin, W. Liu and J.M. Storkson, *J. Food Comp. Anal.*, **5**, 185 (1992).
7. M. Briton, *Clin Sci.*, **883**, 97 (1992).
8. T.D. Shultz, B.P. Chew and W.R. Seaman, *Cancer Lett.*, **63**, 125 (1992).
9. K.L. Houseknecht, J.P.V. Heuvel and S.Y. Moyacamarena, *Biochem. Biophys. Res. Commun.*, **244**, 678 (1998).
10. K.A. Yagaloff, L. Franco and B. Simko, **52**, 293 (1995).
11. P.H.A. Zollo, M.J.D. Youngo, B.F. Fekam, C. Menut, G. Lamaty and J.M. Bessiere, *Riv. Ital. Eppos*, **7**, 613 (1996).
12. S. Burton and P.T. Kaye, *Planta Med.*, **58**, 295 (1992).
13. Aldrich, Flavors and Fragrances, International Edn., Sigma-Aldrich, Milwaukee, USA.
14. S. Arctander, Perfume and Flavor Chemicals, Published by the Author, Montclair, USA.
15. M. Sasser, Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids, Microbial ID, Inc. ,Newark, DE, pp. 1–7 (1990).
16. C. Wilhelm, *Food Quality*, **7**, 50 (2000).
17. E. Bloke and S. Ahmad, *J. Am. Chem. Soc.*, **106**, 8296 (1984).
18. W.W. Christie, A Practical Guide to the Analysis of Conjugated Linoleic Acid (CLA), MRS Lipid Analysis Uni., Scottish Crop Research Institute, Invergowrie, Dundee (DD25DA), Scotland.

(*Received*: 9 March 2004; *Accepted*: 10 August 2004) AJC-3515