Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 855–859
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Analytical Methods

# Fatty acid composition of seed oil of different *Sorghum bicolor* varieties

Sajid Mehmood [a], Ilkay Orhan [b], Zaheer Ahsan [c], Sinem Aslan [d], Muhammad Gulfraz [a,*]

[a] *Department of Biochemistry, University of Arid Agriculture Rawalpindi, Pakistan*
[b] *Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey*
[c] *Oil Seed Program, National Agriculture Research Center (NARC), Islamabad, Pakistan*
[d] *Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey*

Received 20 June 2007; received in revised form 11 December 2007; accepted 12 January 2008

**Abstract**

In order to find out new sources of premium quality edible oil in the country, seeds of ten varieties of *Sorghum bicolor* were initially analyzed for their total oil contents. The seed oil was later fractionated into eight fatty acids including two new saturated fatty acids. The oil contents were determined by Soxhlet method and compared with the results obtained by NMR analysis. The total oil contents in the seeds of sorghum ranged from 5.0 to 8.2 % (w/w), indicating non significant difference obtained by two different techniques. The results revealed that oleic acid (31.12–48.99%), Palmitoleic acid (0.43–0.56%), linoleic acids (27.59–50.73%), linolenic acid (1.71–3.89%), stearic acid (1.09–2.59%) and palmitic acid (11.73–20.18%) was present in the seed oil of different *sorghum* varieties when analyzed by GC–MS. It was observed that in most of the varieties polyunsaturated fatty acids (PUFA) were higher than monounsaturated fatty acids (MUFA). The two atypical SFAs, octanedioic (C8:0) and azelaic acid (C9:0) were found in some varieties. These results suggest that these *S. bicolor* varieties could be additional sources of edible oil due to presence of clinically important saturated and high concentration of unsaturated fatty acids. A large scale production of the seed oil after refining process can contribute towards alleviation of edible oil shortage in the country with increased use of premium quality oil.


*Keywords:* *Sorghum bicolor*; Fatty acid; Gas chromatography (GC); Nuclear magnetic resonance (NMR)

## 1. Introduction

One of the important facets of cereal crops is their diverse pool of fatty acids. The oilseeds containing peculiar fatty acids are industrially important because of their characteristic properties. The main constituent of all the oils is the fatty acids which may include saturated fatty acids (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) that contribute in human physiology in different ways. Vegetable oil not only provides high quality food, containing essential nutrients for the life, but also bestow bioactive compounds that have particular clinical significance. For examples, PUFAs are present either as component of membrane phospholipids in specific tissue or a precursor of hormone like prostaglandins (Patil & Gislerød, 2006). As the saturated fatty acids increase the risks of cardiovascular diseases, cancer and autoimmune disorders (Iso et al., 2002), oils being source of lipids, are of more nutritional value if they have more unsaturated to saturated fatty acid ratio (Aronson et al., 2001). Further more, the fatty acids with some pharmacological significance have caught the attention of both consumer and industries in the recent years.

*Sorghum bicolor* L. Monech is a drought resistant low input cereal crop grown throughout the world, can be an alternative source of oil having clinical advantages. Genus sorghum includes many species and subspecies, including grain sorghum, grass sorghum, sweet sorghum and broomcorn. It is used as food, animal feed, fibers as in wall board, fences, biodegradable packing material and for ethanol

* Corresponding author. Present address: School of Pharmacy, Reading University UK, Whiteknights, Reading RG6 6AP, United Kingdom. Tel.: +44 1183787017; fax: +44 1183786562.
*E-mail addresses:* M.Gulfraz@reading.ac.uk, gulfrazsatti@yahoo.com (M. Gulfraz).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.01.014

production (Rooney & Waniska, 2000). In most of the countries, sorghum is used primarily as animal feed, but in Africa and India, it is a staple food for million of people (Agrama & Tuinstra, 2003). Rooney and Waniska (2000) reported that crude fat contents of sorghum are higher than wheat and rice but lower than that of maize. Although the cereal crops with low oil contents may not confer much as domestic oil source but their importance is due to their fatty acid constituents having some atypical advantages. Today a variety of vegetable oil and fat products have been developed with knowledge of their physical and chemical properties obtained through application of scientific research and development (O'Brien, 1998).

Therefore keeping in view the importance of biochemical analysis of sorghum, the present study was conducted to assess the fatty acid composition of oil obtained from different varieties of *Sorghum bicolor* by solvent extraction and subjected to GC and GC–MS.

## 2. Materials and methods

### 2.1. Plant materials

The seeds of *S. bicolor* used in this study were purchased from Millet station, Rawalpindi (Pakistan) and study was conducted during July–October, 2006. All chemicals used in this study were A grade and obtained from sigma.

The seed samples of *Sorghum* varieties were analyzed for their total oil contents, which was further fractionated into saturated and unsaturated fatty acids. The samples were prepared according to the methodology described by the Laboratory Analytical Procedure (Hames, 2004) and stored at −20 °C for further analysis.

### 2.2. Oil determination using Soxhlet and $^1H$ NMR

The oil contents were analyzed by AOAC method, 920.85 (AOAC., 1990) with Soxhlet apparatus. In the Soxhlet extraction procedure, 5 g of the crushed seeds (80 mash) was packed in a thimble and the oils were extracted with diethyl ether for 6 h. For $^1H$ NMR study, whole seeds were used after drying at 50 °C for more than 3 h subsequently cooled at room temperature in a desiccator. The NMR used in this study was Newport 4000 Analyzer from Oxford Analytical Instruments Ltd. UK, and study was conducted by following method described previously by Tomi, Bradesi, Bighelli, and Casanova (1995). The instrument was calibrated with one point calibration using one of the Sorghum variety 84-Y-00, (having less standard deviation as compared to other). The accuracy of NMR calibration was checked by the samples prepared by solvent extraction.

### 2.3. Analysis of fatty acids

The lipid extracts from the Sorghum varieties were mixed with boron trifluoride ($BF_3$)–methanol reagent (20%) and fatty acids were converted into the methyl ester derivatives (Morrison & Smith, 1964). The methyl esters of the fatty acids were dissolved in $CHCl_3$ and analyzed by GC and GC–MS.

### 2.4. Gas chromatography (GC) conditions

GC analysis was performed on an Agilent 6890N Network GC system, under the following conditions: column, HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 μm; oven temperature programme, the column held initially at 60 °C for 3 min after injection, then increased to 185 °C with 10 °C/min heating ramp for 1 min and increased to 200 °C with 5 °C/min heating ramp for 10 min. Then the final temperature was increased to 220 °C with 5 °C/min heating ramp for 20 min; injector temperature, 250 °C; detector (FID) temperature, 275 °C; carrier gas, He; inlet pressure, 40.65 psi; linear gas velocity, 39 cm/s; column flow rate , 2.7 ml/min; split ratio, 40:1; injected volume, 1 μL.

### 2.5. Gas chromatography–mass spectrometry (GC–MS) conditions

GC–MS analysis was performed on an Agilent 6890N Network GC system combined with Agilent 5973 Network Mass Selective Detector. The GC conditions were as follows: column: HP Innowax Capillary (60.0 m × 0.25 mm × 0.25 μm); oven temperature program: the column held initially at 60 °C for 3 min after injection, then increased to 185 °C with 10 °C/min heating ramp for 1 min and increased to 200 °C with 5 °C/min heating ramp for 10 min. Then the final temperature was increased to 220 °C with 5 °C/min heating ramp for 20 min; injector temperature: 250 °C; carrier gas: Helium; inlet pressure: 40.65 psi; linear gas velocity: 44 cm/s; column flow: 2.9 ml/min; split ratio: 40:1; injection volume: 1.0 μL. MS conditions were regulated as follows; ionization energy: 70 eV, ion source temperature: 280 °C; interface temperature: 250 °C; mass range: 35–450 atomic mass units.

Identification of the components was assigned by comparison of their retention times and mass spectra with corresponding data from reference compounds and by comparison of their mass spectra with Wiley and Nist libraries.

### 2.6. Data analysis

Data analysis was performed using one way ANOVA variance and expressed in the form mean, standard deviation and % age values.

## 3. Results

The comparison of soxhlet and NMR analysis for total oil contents from seed of *S. bicolor* varieties (Fig. 1) and analysis of saturated and unsaturated fatty acids (Tables 1 and 2) by GC–MS (Fig. 2) are given in this section.

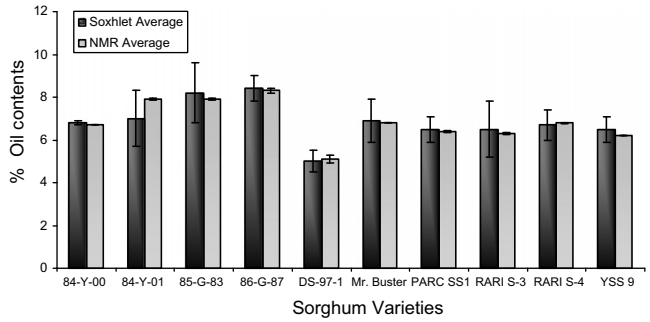


Fig. 1. Comparison (%) of total seed oil of *Sorghum bicolor* varieties analyzed by Soxhlet and NMR techniques. Data points represent the mean value from three experiments. The error bar represents the standard deviation.

According to the results obtained by Soxhlet method and their analysis by ANOVA, the highest percentage of oil among the ten varieties was 8.4% found in seed of variety 86-G-87. While the lowest was found in DS-97-1 seeds (5%). All other varieties were with less variation in their contents (Fig. 1).

NMR technique is used to determine the oil contents in oil seeds (having oil contents higher than 20%) on routine basis but not well reported in the seeds having low amount of oil. Therefore, NMR technique was optimized for this study and the results of both techniques were compared with each other (Fig. 1). No significant difference was found between the mean values of seed oil obtained from both techniques. Important distinguishing features between these methods are the time and the amount of solvent needed for an exhaustive extraction. Although Soxhlet extraction is the official method of determining oil content in oilseed crops (AOAC, 1990), but, the procedure is lengthy, laborious, and relatively hazardous. This technique fails when there is large number of seeds to be analyzed with in short period of time. Therefore, an alternate method is needed to analyze a large number of different seed samples in short time, such as NMR. As this method is non destructive, seed samples remain viable after analysis but because of its requirement for intensive calibration by solvent extraction methods it is classified as secondary analytical technique.

The principal fatty acid components in the Sorghum seed oils were palmitic (16:0), linoleic (18:2), and oleic (18:1) acids (Tables 1 and 2). Most of the varieties contain linoleic acid as a major unsaturated fatty acid when analyzed by GC–MS (Fig. 2) but three varietal seed, *i.e.* 86-G-87, RARI S-3 and RARI S-4, contain oleic acid as the major fatty acid constituent. Only one variety, DS-97-1,

Table 1
Percentage* (%) of saturated fatty acids analyzed by GC–MS and their retention times (Rt)

| *Sorghum* varieties | Octanedioic acid Rt, 17.33 min | Azelaic acid Rt, 18.71 min | Palmitic acid Rt, 19.77 min | Stearic acid Rt, 23.56 min | Total SFA |
|---|---|---|---|---|---|
| 84-Y-00 | – | – | 12.85 ± .01 | 1.27 ± 0.02 | 14.12 |
| 84-Y-01 | – | – | 11.73 ± 0.08 | 1.09 ± 0.05 | 12.82 |
| 85-G-83 | – | – | 12.82 ± 0.01 | 1.21 ± 0.001 | 14.03 |
| 86-G-87 | – | 0.23 ± 0.01 | 12.19 ± 0.65 | 1.85 ± 0.64 | 14.27 |
| DS-97-1 | – | 1.15 ± 0.30 | 20.18 ± 1.02 | – | 21.33 |
| Mr. Buster | – | 0.19 ± 0.01 | 14.21 ± 0.77 | 1.40 ± 0.006 | 15.80 |
| PARC SS1 | 0.09 ± 0.008 | 0.04 ± 0.001 | 11.83 ± 0.05 | 1.32 ± 0.07 | 13.28 |
| RARI S-3 | 0.30 ± 0.01 | 1.88 ± 0.14 | 18.46 ± 1.09 | 1.82 ± 0.21 | 22.46 |
| RARI S-4 | 0.37 ± 0.02 | 1.78 ± 0.12 | 17.33 ± 1.24 | 1.75 ± 0.14 | 21.23 |
| YSS 9 | – | 0.16 ± 0.06 | 13.44 ± 0.57 | 2.59 ± 1.77 | 16.19 |

SFA: saturated fatty acid.
* Values represent the average of three replicates ± standard deviation (SD).

Table 2
Percentage* (%) of unsaturated fatty acids analyzed by GC–MS and their retention times (Rt)

| *Sorghum* varieties | Oleic acid Rt. 24.13 min | Palmitoleic acid Rt. 20.16 Min | Linoleic acid Rt. 25.37 Min | Linolenic acid Rt. 27.24 min | Total MUFA | Total PUFA |
|---|---|---|---|---|---|---|
| 84-Y-00 | 36.19 ± 0.09 | 0.50 ± 0.002 | 47.38 ± 0.09 | 1.82 ± 0.02 | 36.69 | 49.2 |
| 84-Y-01 | 39.67 ± 0.61 | 0.44 ± 0.08 | 45.21 ± 0.58 | 1.88 ± 0.03 | 40.11 | 47.09 |
| 85-G-83 | 31.12 ± 0.006 | 0.51 ± 0.01 | 50.73 ± 0.24 | – | 31.63 | 50.73 |
| 86-G-87 | 45.73 ± 1.38 | 0.56 ± 0.02 | 37.50 ± 1.24 | 1.92 ± 0.10 | 46.29 | 39.42 |
| DS-97-1 | 39.84 ± 0.70 | – | 39.45 ± 1.02 | – | 39.84 | 39.45 |
| Mr. Buster | 34.64 ± 1.30 | 0.48 ± 0.03 | 43.96 ± 2.20 | 1.78 ± 0.08 | 35.12 | 45.74 |
| PARC SS1 | 35.34 ± 0.48 | 0.43 ± 0.003 | 46.79 ± 0.02 | 3.89 ± 0.01 | 35.77 | 50.68 |
| RARI S-3 | 48.99 ± 1.66 | 0.53 ± 0.16 | 27.59 ± 0.45 | – | 49.52 | 27.59 |
| RARI S-4 | 44.40 ± 3.25 | – | 29.67 ± 1.88 | – | 44.40 | 29.67 |
| YSS 9 | 39.33 ± 0.87 | 0.44 ± 0.02 | 42.75 ± 1.98 | 1.71 ± 0.07 | 39.77 | 44.46 |

MUFA: monounsaturated fatty acid.
PUFA: polyunsaturated fatty acid.
* Values represent the average of three replicates ± standard.

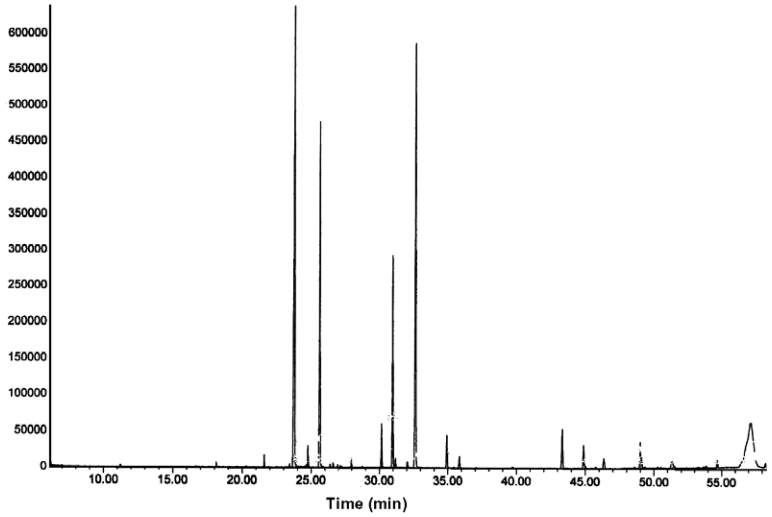


Fig. 2. Spectra of GC–MS for fatty acid analysis from different *Sorghum bicolor* varieties.

contains equal percentage of linoleic and oleic acids. Other unsaturated fatty acid includes linolenic acid (18:3), present in many varieties and palmitoleic acid (16:1) present in all varieties except DS-97-1 and RARI S-4. Two new saturated fatty acids not reported earlier in *S. bicolor* found in this study were, octanedioic acid (C8:0) and azelaic acid (C9:0) Total amount of unsaturated fatty acids in Sorghum seeds varies from 74.07% in RARI S-4 to 87.2% in 84-Y-01. In most of the varieties, PUFAs were higher than MUFAs except 86-G-87, RARI S-3, and RARI S-4 where the concentration of MUFA is higher than PUFA. It was observed that variety DS-97-1 showed different behavior as it contained an equal percentage of PUFA and MUFA, lacking in palmitoleic acid and containing higher concentration of palmitic acid (11.73–20.18%). The fatty acids composition of seed oil , linoleic acid (27.59–50.73 %,) , oleic acid (31.12–48.99%,) palmitoleic acid (0.43–0.56%) linolenic acid (1.71–3.89%) and stearic acid (1.09–2.59%) was similar to that of corn fat but was more unsaturated (Rooney & Waniska, 2000) These results suggested that the *S. bicolor* seed oils may serve as a potential dietary source of MUFA and PUFA.

## 4. Discussion

It has been recognized that a diet rich in MUFAs may be an alternative choice to low-fat diet, which may lower blood cholesterol levels (Hargrove, Etherton, Pearson, Harrison, & Kris-Etherton, 2001), modulate immune function decrease susceptibility of oxidation of LDL and improve the fluidity of HDL (Villa et al., 2002). The PUFAs enriched diet may also be important for the structure and function of many membrane proteins, including receptors, enzymes, and active transport molecules (Yaqoob, 2002).

Some of Sorghum varieties also contains two atypical saturated fatty acids, (Table 1), octanedioic acid (suberic acid, 8:0) and nonanedioic acid (azelaic acid, 9:0) those were not previously reported in *S. bicolor*. Octanedioic acid was present only in three varieties, PARC SS 1, RARI S-3, and RARI S-4, while azelaic acid was absent in three varieties, 84-Y-00, 84-Y-01, and 85-G-83. The important facet of azelaic acid is that it serves as antibacterial, reduces the growth of bacteria in the follicle (Tapiero, Ba, Couvreur, & Tew, 2002), keratolytic and comedolytic, returns to normal the disordered growth of the skin cells lining the follicle (Hung et al., 2000, a scavenger of free radicals, and reduces inflammation (Passi, Picardo, De Luca, Breathnach, & Nazzaro-Porro, 1991). In all Sorghum varieties investigated, palmitic acid was present in higher percentage among the saturated fatty acids. Less variation was also observed in stearic acid contents among all the varieties studied.

## 5. Conclusion

It is concluded that seed oil obtained from different varieties of Sorghum could be alternative source of edible oil due to presence of all saturated and unsaturated fatty acids required for human health. This is the first study on the fatty acid compositions of *S. bicolor* varieties and it is expected that further studies on the quality aspects of *Sorghum bicolor* varieties will highlight its future ranking as a high grade edible oil source.

## Acknowledgements

The authors thank to Higher Education Commission (HEC), Pakistan, for providing the funds under the project No. 286 to Dr. Muhammad Gulfraz. We are also grateful to Department of Pharmacognosy, Ghazi University Turkey for providing the facilities of GC and GC–MS studies.

## References

Agrama, H. A., & Tuinstra, M. R. (2003). Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. *African Journal of Biotechnology, 2*, 334–340.

AOAC. 1990. *Methods of the association of official analytical chemists*, Vol. II. 15th ed. Method No. 920.85. Method No. 920.85. Arlington, Virginia, USA; AOAC. p. 780.

Aronson, W. J., Glaspy, J. A., Reddy, S. T., Reese, D., Heber, D., & Bagga, J. (2001). Modulation of omega-3/omega-6 polyunsaturated ratios with dietary fish oils in men with prostate cancer. *Urology, 58*, 283–288.

Hames, B. (2004). *Preparation of samples for compositional analysis*. Biomass analysis technology team. Laboratory analytical procedure. National Renewable energy Laboratory (NREL). pp. 1–9.

Hargrove, R. L., Etherton, T. D., Pearson, T. A., Harrison, E. H., & Kris-Etherton, P. M. (2001). Low-fat and high-monounsaturated fat diets decrease human low-density lipoprotein oxidative susceptibility in vitro. *Journal Nutrition, 131*(6), 1758–1763.

Hung, P., Gu, J. Y., Kaku, S., Yunoki, S., Ohkura, K., Ikeda, I., et al. (2000). Dietary effects of eicosapentaenoic acid and docosahexaenoic acid esters on lipid metabolism and immune parameters in Sprague–Dawley rats. *Bioscience Biotechnology Biochemistry, 64*, 2588–2593.

Iso, H., Sato, S., Umemura, U., Kudo, M., Koike, K., Kitamura, A., et al. (2002). Linoleic acid, other fatty acids, and the risk of stroke. *Stroke, 33*, 2086–2093.

Morrison, W. R., & Smith, L. M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride—methanol. *Journal of Lipid Research, 5*, 600–608.

O'Brien, R. D. (1998). Fats and oil analysis. In *Fats and oils* (pp. 181–250). Lancaster: Technomic Publishing.

Patil, V., & Gislerød, H. R. (2006). HR The importance of omega-3 fatty acids in diet. *Current Science, 90*(7), 908–909.

Passi, S., Picardo, M., De Luca, C., Breathnach, A. S., & Nazzaro-Porro, H. (1991). Scavenging activity of azelaic acid on hydroxyl radicals "in vitro". *Free Radical Research Communication, 11*, 329–338.

Rooney, L. W., & Waniska, R. D. (2000). Sorghum food and industrial utilization. In C. W. Smith & R. W. Frederiksen (Eds.), *Sorghum, origin, history, technology and production* (pp. 689–729). New York: Wiley.

Tapiero, H., Ba, G. N., Couvreur, P., & Tew, K. D. (2002). Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomedical Pharmacotherapy, 56*, 215–222.

Villa, B., Calbresi, L., Chiesa, G., Rise, P., Galli, C., & Sirtori, C. (2002). Omega-3 fatty acid ethyl esters increase heart rate variability in patients with coronary disease. *Pharmacology Research, 45*, 475–478.

Tomi, F., Bradesi, P., Bighelli, A., & Casanova, J. (1995). Computer aided identification of individual components of essential s oils using carbon-13 NMR spectroscopy. *Journal of Magnetic Research and Analytical, 1*, 25–34.

Yaqoob, P. (2002). Monounsaturated fatty acids and immune function. *European Journal Clinical Nutrition, 56*, 9–13.