

## Original Paper

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## Chemical composition and fatty acid content of white food sorghums grown in different environments

Paola Pontieri<sup>1,2\*</sup>, Raffaele Di Fiore<sup>3</sup>, Jacopo Troisi<sup>3</sup>, Scott R Bean<sup>4</sup>, Earl Roemer<sup>5</sup>, Josephine Okot<sup>6</sup>, Pietro Alifano<sup>7</sup>, Domenico Pignone<sup>8</sup>, Luigi Del Giudice<sup>1,2</sup>, Domenica Rita Massardo<sup>1,2</sup>

<sup>1</sup>Istituto di Genetica e Biofisica "Adriano Buzzati-Traverso" (IGB-ABT), CNR, Naples, 80131, Italy

<sup>2</sup>Istituto di Genetica Vegetale, CNR, Portici (NA), 80055, Italy

<sup>3</sup>Laboratorio Chimico Merceologico Az.Spec. CCIAA, Napoli, 80143, Italy

<sup>4</sup>USDA-ARS, GMPRC, Manhattan, KS 66502, USA

<sup>5</sup>NU LIFE MARKET, 3005 N Sycamore Healy, Kansas 67850, USA

<sup>6</sup>Victoria Seeds Ltd, Kampala, Uganda

<sup>7</sup>Dipartimento di Scienze e Tecnologie Biologiche e Ambientali, Università del Salento, Lecce, 73100, Italy

<sup>8</sup>Istituto di Genetica Vegetale, CNR, Bari, 70126, Italy

\*Corresponding author: E-mail: pontieri@igb.cnr.it

### Abstract

The chemical composition and fatty acid content of both white sorghum hybrids and pure lines grown in various areas of the world were studied. Various attributes were investigated including moisture, protein, carbohydrate, dietary fiber, fat contents, and fatty acid composition. Slight variations in both protein and in fiber contents were observed among cultivars. Linoleic, oleic and palmitic were the most abundant fatty acids in all samples with little difference in their percentage content among the cultivars. Enzyme-linked immunosorbent assays (ELISA) demonstrated, for all sorghum flours analyzed, the absence of toxic protein sequences for celiac patients. The present results demonstrate that food-grade sorghum varieties are potentially able to be grown in Mediterranean countries in addition to regions where sorghum has been traditionally produced, i.e. either in arid tropical and sub-tropical regions of Africa and Asia or in dry regions of America.

**Keywords:** sorghum hybrid, sorghum pure line, chemical composition, fatty acids, enzyme-linked immunosorbent assay, gliadins

**Abbreviations:** GC: Gas Chromatography, kcal: kilocalorie, FID: Flame Ionization Detector, He: Helium, KPa: Kilopascal, RT: Retention Time, St.Dev.: Standard Deviation, ELISA: Enzyme-Linked Immunosorbent Assay

### Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], a tropical plant belonging to the family of *Poaceae*, is the fifth most important cereal crop in the world after wheat, rice, corn and barley (Awika and Rooney, 2004). Sorghum out-performs other cereals under various environmental stresses, requires little input during growth and is thus generally more economical to produce (ICRISAT/FAO, 1996). It is a vital crop for millions of people in parts of Africa and Asia (Anglani, 1998; Dicko et al, 2006; ICRISAT/FAO, 1996). With increasing world population and decreasing water supplies, it represents an important crop for future human use. Worldwide, more than 35% of sorghum is grown directly for human food. The rest is used primarily for animal feed, alcohol production and industrial products (FAO, 1995; Awika and Rooney, 2004). The United States is the largest producer and exporter of sorghum, accounting for 20% of world production, and almost 80% of world sorghum exports in 2001-2003 (USDA-FAS, 2003). World sorghum production was 57 million metric tons during this period (Awi-

ka and Rooney, 2004). Several improved sorghum varieties adapted to semi-arid and tropic environments are released every year by sorghum breeders. Selection of varieties meeting specific local food and industrial requirements from this great biodiversity is of high importance for food security. In developing countries in general, and particularly in West Africa, demand for sorghum is increasing. This is due to not only the growing population, but also to the countries policy to enhance its processing and industrial utilization (Akintayo and Sedgo, 2001; Dicko et al, 2006). More than 7000 sorghum varieties have been identified (Kangama and Rumei, 2005); therefore there is a need for further characterization of them at the molecular level with respect to food quality. The acquisition of good quality grain is fundamental to produce acceptable food products from sorghum.

In many developing countries sorghum has traditionally been used in food products and various food items. For instance, in India, sorghum-based product types include Roti (unleavened flat bread), Sangati (stiff porridge), Annam (rice-like),

Kudumulu (steamed), Dosa (pancake), Ambali (thin porridge), Boorelu (deep fried), Pelapindi (popped whole grain and flour), Karappoosa (deep fried) and Thapala chakkalu (shallow fried). A nearly exhaustive list of recipes and culinary preparations based on sorghum are reported on the web site <http://www.fao.org/docrep/T0818e/T0818E0g.htm>

In addition, sorghum is often recommended as a safe food for celiac patients, which do not tolerate protein sequences contained in both the gliadins (Kagnoff et al, 1982) and glutenins (Van de Wal et al, 1999) of wheat gluten. In fact, sorghum is only distantly related to the triticeae tribe cereals wheat, rye and barley (Kasarda, 2001), being a member of the *Panicoideae* sub-family which also includes maize (Shewry, 2002). Therefore, the future promise of sorghum in the developed world is for wheat substitution for people with celiac disease or allergies to gluten (Fenster, 2003; Ciacci et al, 2007). Sorghum provides a good basis for gluten-free breads and other baked products like cakes and cookies (biscuits) and in snacks and pasta (Taylor et al, 2006).

The present study was conducted to compare the chemical composition and fatty acid content of sorghum varieties grown in different countries. Knowledge of the lipid composition of food-grade sorghums is important to predict the storage stability of food-grade sorghum flours as well as for nutritional reasons. Our effort is to provide scientific information useful to spread the cultivation of food-grade white sorghum varieties into Mediterranean countries.

## Materials and Methods

### Plant cultivars

Names, genotypes and sources of plant cultivars are listed in Table 1.

### Flour sample preparation

Grain samples were milled into flour using a two-roll mill (Chopin mod. Moulin CD1). After being milled to flour, the samples were sieved with a planetary sieve (Buhler), through a 120  $\mu\text{m}^2$  sieve opening.

### Humidity

After sieving, the humidity rate of all flours was determined immediately after (within 30 min), in order to prevent significant variations of the humidity rate in the samples. Humidity was determined according

to the AOAC (1995, 925.09) method. A well-known sample mass was weighed in a capsule previously desiccated at 100°C vacuum-packed (25 mm Hg) in an oven (ISCO mod. NSV9035) and chilled at room temperature in a silica gel dryer. Then, the humidity rate was removed from the sample (about 2 g), keeping it in the same temperature and pressure conditions for about five hours, until a constant weight was achieved. The humidity rate was estimated on weight loss.

### Crude protein content

Crude protein content was measured using the Kjeldahl method (AOAC, 1995, 920.87). Briefly, 2 g of flour samples were subjected to digestion at 450°C (PBI International mod. Mineral SIX) with 30 ml of 96%  $\text{H}_2\text{SO}_4$  in presence of 7 g of  $\text{K}_2\text{SO}_4$  and 0.7 g of  $\text{CuSO}_4$ . Digested were alkalized with 45% NaOH and then subjected to steam distillation by using a distiller (Buchi mod. B-324). The condensed distillate was gathered in an Erlenmeyer flask containing 25 ml of 0.25N  $\text{H}_2\text{SO}_4$ . The sulphuric acid not neutralized by the ammonia present in the distillate was titrated with 0.25N NaOH in presence of an indicator methylene blue/methyl red mix. The ammonia rate, estimated on the difference in sulphuric acid equivalents between those present before and after the ammonia distillate gathering, was converted into protein using 6.25 as conversion factor.

### Total lipid content

Raw fat determination was carried out according to the AOAC (1995, 920.85) standards. Ten g of sample were weighed in a Soxhlet extraction thimble. Three g of anhydrous  $\text{Na}_2\text{SO}_4$  were added, and absorbent cotton was used as a seal. Fats were extracted with hexane by using an automatic extractor (PBI International mod. Soxhtraction). The hexane was removed with vacuum-packed distillation and then in a stove at 105 °C for 1 h. The extracted fat weight was compared to the initial 10 g of sample.

### Fiber content

Fiber content was determined according to the AOAC (1995, 962.09) method. In particular, fiber was considered to be the loss, after incineration, of a certain rate of the sample digested in an acidic environment by 0.25N  $\text{H}_2\text{SO}_4$ , followed by an alkaline digestion by 0.25N NaOH. Digestion was carried out with an automatic digester (Velp Scientific mod. FIWE3).

Table 1- List of sorghum cultivars.

Cultivar	Genotype	Field trials	Source
F1000/K	Fontanelle 1000 hybrid	Kansas (USA)	E. Roemer
F1000/I	Fontanelle 1000 hybrid	Foggia (South of Italy)	E. Roemer
C3 = 05MN5115	91BE7414 pure line	Foggia (South of Italy)	M. Tuinstra
Macia	Macia pure line	Foggia (South of Italy)	M. Tuinstra
Epu = Epuripur	2K x 17/B/1 hybrid	Nasarri (Uganda)	J. Okot

**Table 2** - Sorghum samples nutritional values.

	F1000/I	F1000/K	C3	Macia	EPU	Average	St.Dev%	St.Dev
Humidity (%)	13.72	12.44	11.35	14.13	11.12	12.55	10.8	1.36
Proteins (%)	7.44	7.88	9.66	8.37	9.08	8.49	10.6	0.90
Carbohydrates (%)	75.82	75.09	74.54	74.45	76.55	75.29	1.2	0.89
Ashes(%)	0.99	1.60	1.71	1.06	1.16	1.30	25.1	0.33
Fats (%)	2.03	2.99	2.74	1.99	2.09	2.37	19.6	0.47
Fiber (%)	1.08	0.97	0.77	1.39	0.40	0.92	39.9	0.37
Energy Value (kcal/100g)	353	361	363	352	362	358	1.4	5

#### Ash content

Ash content was determined according to the AOAC (1995, 900.02) method on ashes. About 10 g flour samples were weighed in a capsule previously calibrated at 550°C for 4 h and chilled in a silica gel dryer. Subsequently, the samples were burned on a little flame and then incubated overnight in muffle furnace (Heraeus mod. K1251F). Then, ash was chilled in a silica gel dryer and weighed soon after reaching room temperature. The ash rate was determined by the ratio between the remnant mass and the original sample mass.

#### Carbohydrate content

Carbohydrate content was determined by subtraction and was considered to be the amount of material left in after accounting for moisture, ash, protein and fat content.

#### Energy value

Energy value was theoretically derived from the content in fats (9 kcal/g), carbohydrates (4 kcal/g), proteins (4 kcal/g) and fibers (2 kcal/g) according to EU guidelines (1990).

#### Gas chromatography of fatty acids

Esterification of fatty acids from bulk populations was carried out by melting solid sorghum fat in a oven at 50°C in order to determinate its composition. A drop of fat was transferred into a 1.5 ml-vial with a glass Pasteur pipette heated to 50°C. One ml of hexane was heated to 50°C, added to the drop of fat and then the mixture was slowly chilled to 25°C. Next, 100µl of 2N KOH methanolic solution was added. The vial was vortexed for 5 min, and then left under static conditions for 5 min, in order to enable a complete stratification of the hexanic portion, which contains the methyl ester of the fatty acids. Chromatographic separation was carried out according to Ngeh-Ngwainbi et al (1997), with a GC Carlo Erba (mod. 5300 Mega), equipped with a Restek 2336 column. GC conditions were as follows. Carrier gas: He. Pressure: 75 kPa; injector temperature: 220°C; FID temperature: 250°C. Oven programme: 170°C for 8 min, 2°C/min to 185°C for 10 min, 1°C/min to 190°C for 12 min, 10°C/min to 240°C for 5 min.

#### ELISA assay

The RIDASCREEN® standard test kit [RIDASCREEN® Gliadin (Art. No R7001) R-Biopharm AG]

sandwich ELISA based method was used to identify the presence of protein sequences reactive to gliadins in sorghum flour samples according to Valdés et al (2003) and manufacturer's instructions.

## Results

#### Sorghum field trials in different countries

Sorghum field trials were carried out during the 2009 growing season as detailed in Table 1. In particular, the F1000/K hybrid cultivar was grown in Kansas (USA), the Epu hybrid cultivar was cultivated in Kampala (Uganda, Africa), and the F1000/I hybrid and both C3 and Macia pure lines were grown in Foggia (south of Italy).

#### Chemical composition

The chemical composition of the different sorghum cultivars is shown in Table 2. For each measurement, both the average and standard deviations are shown (both in absolute terms and in percentage) Analysis of the data shows that the samples mostly differ in their less represented parameters. In particular, the samples exhibited the highest standard deviations in fiber (39.9%), ash (25.1%), and fat (19.6%). Nevertheless, composition profiles were nearly similar with slight variation either in protein percentage of the C3 cultivar, which was higher in comparison with that of the other cultivars, or in fiber percentage of the Epu cultivar, which was lower in comparison with that of the other cultivars.

#### Energy value

The calculated energy value was 358 kcal/100 g on average, with slight variation among sorghum cultivars. In fact the energy value of both F1000/I and Macia cultivars was slightly lower than those of the other cultivars (Table 2).

#### Fatty acid composition of total lipids

Fatty acid composition of total lipids of the sorghum cultivars is shown in Table 3. Linoleic was the predominant fatty acid in all extracts, followed by oleic acid and palmitic acid, which is consistent with previously published data (Osagie, 1987; Serna-Saldivar and Rooney, 1995). The results were confirmed by gas chromatograms of fatty acid methyl esters of the total lipids from F1000/K, Epu and C3 cultivars (Table 3 and Figures 1-2). It should be noted that the major differences can be found on the ground of the least

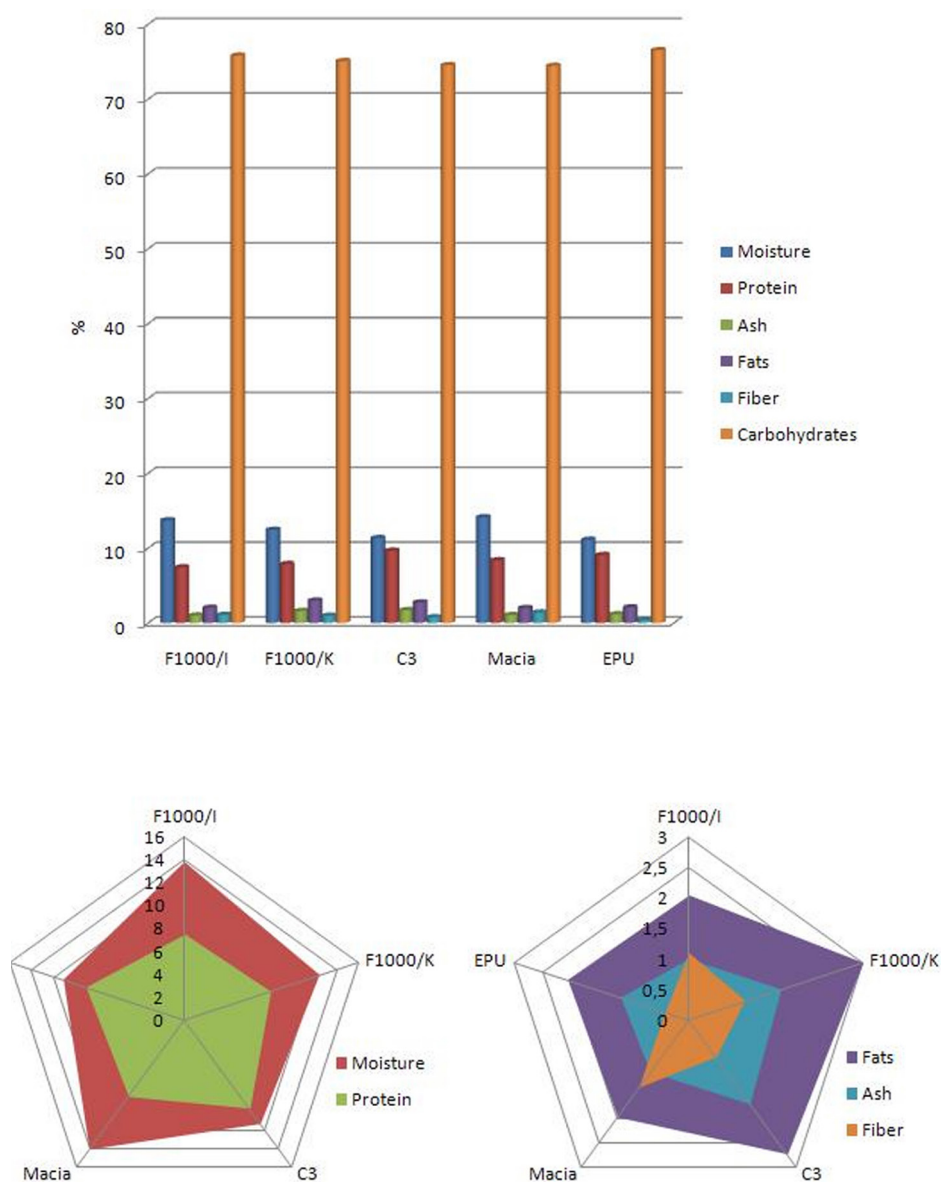


Figure 1- Bar graph (A) and radar representation (B) of chemical composition of sorghum cultivars

represented fatty acids, that is behenic acid (St.Dev. 91.4%) and both arachic and eicosenoic acids (St. Dev. 47%).

#### ***Immunochemical evidence for the absence of gluten in sorghum cultivars***

The results of immunochemical measurement of gliadin concentration in the sorghum flour cultivars F1000/I, F1000/K, C3, Macia and Epu demonstrated that gluten levels in all sorghum cultivars were less than 5 ppm (i.e. none detected) Those values are well below the 20 ppm threshold that has been proposed to be safe for celiac patients (Valdés et al, 2003).

#### **Discussion**

In the present study, to provide scientific information useful to cultivate food-grade sorghums in the Mediterranean area, we compared both the chemical composition and the fatty acid content of sorghum cultivars grown during the 2009 season in Kansas (USA), Kampala (Uganda, Africa) or Foggia (south of Italy). As shown in Table 2 and in Figure 1, the composition profiles of all sorghum cultivars were nearly the same among them, with a slight differences in either protein percentage of the C3 cultivar, which was higher in comparison with that of the other cultivars,

**Table 3** - Fatty acid content (%) of food-grade sorghum cultivar total lipids.

		F1000/I	F1000/K	C3	Macia	EPU	Average	St.Dev%
Palmitic	C16	13.24	11.88	13.04	12.99	14.18	13.07	6.3
Palmitoleic	C16-	0.59	0.42	0.54	0.50	0.71	0.55	18.9
Margaric	C17	0.03	0.05	0.09	0.06	0.10	0.07	41.1
Margaroleic	C17-	0.13	0.14	0.12	0.11	0.10	0.12	14.8
Stearic	C18	1.29	1.09	1.15	1.33	1.64	1.30	16.5
Oleic	C18-	36.41	33.26	33.96	42.49	43.81	37.98	12.9
Linoleic	C18--	46.12	49.37	48.65	39.63	35.51	43.86	13.8
Arachic	C20	0.12	0.13	0.15	0.18	0.33	0.18	47.4
Linolenic	C18---	1.61	2.14	1.98	1.99	1.35	1.81	17.9
Eicosenoic	C20-	0.33	0.14	0.21	0.38	0.52	0.31	47.1
Behenic	C22	0.09	0.04	0.10	0.11	0.37	0.14	91.4

or in fiber percentage of the Epu cultivar, which was lower in comparison with that of the other cultivars. It was reported that sorghum composition could vary significantly due to genetics and environment (Serna-Saldivar and Rooney, 1995).

From a nutritional point of view, food-grade sorghum flour turns out to be a very interesting product. In fact, its nutritional value is comparable to those belonging to the ordinary flours, obtained from the noble cereals (Anglani, 1998; Dicko et al, 2006; Taylor et al, 2006). The percentage of fats in all sorghum samples analyzed was comparable among them, with only slight variations. In fact, the total lipid content of both F1000/K and C3 cultivars was, in average, 1.4-fold higher than those of the other cultivars (Table 2 and Figure 1). The fatty acid composition for all sorghum cultivars was qualitatively and quantitatively similar with slight variation among them. Similarly to the nutritional values, it could be noticed that the main differences could be found on the ground of the least represented fatty acids (behenic St.Dev. 91.4 and both arachic and eicosenoic St.Dev. 47,25 on average) (Table 3 and Figure 2). Linoleic was the predominant fatty acid in all sorghum cultivars, followed by oleic acid and palmitic acid, confirming previous data on fatty acid composition of sorghums (Osagie, 1987). From these results it appears that, in all sorghum cultivars analyzed, the saturated fatty acid rate is low (14.8% on average) and essentially composed of palmitic acid.

The monounsaturated fatty acid rate is 38% on average, and essentially composed of oleic acid, while the polyunsaturated fat rate is about 44%, and essentially composed of linoleic acid. The considerable amount of essential fatty acids, in particular  $\Omega 3$  (linoleic) (1,8%) and  $\Omega 6$  (linoleic) (44%), is of great importance. Moreover, it must be pointed out that the Macia and EPU samples have an inverted ratio of C18-/C18--, compared to the other samples. This may be due to sorghum ripening degree, since a similar inverted ratio between oleic and linoleic acid can be found in other oleic and linoleic acid-rich vegetable species, including olive and its oil (Matos et

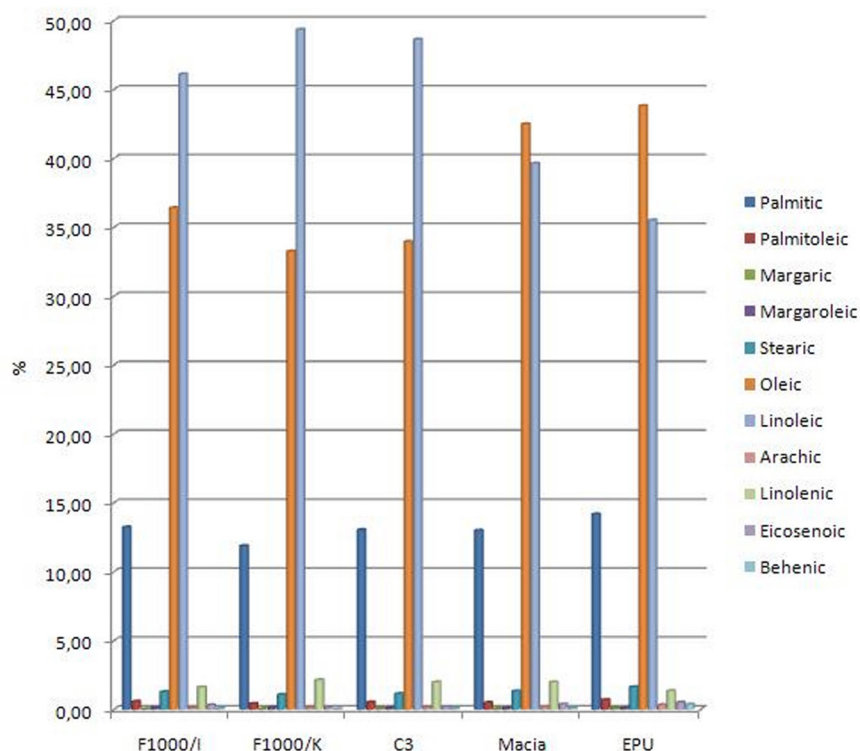
al, 2007).

Unsaturated fatty acids are of great importance to diet, because they are significant components of the biological membranes, and play the role as fluidity modulators in them. Moreover, unsaturated fatty acids are not cholesterogenic (at variance with saturated fatty acids), and lower the risk of thrombosis, due to their anti-aggregating activity on blood lipoprotein particles. These two features make them strongly recommended to lower the risk of atherosclerosis (Dicko et al, 2006; Taylor et al, 2006).

Modern screening studies show that celiac disease is much more prevalent than previously thought. The cornerstone treatment for celiac disease is the total lifelong avoidance of gluten ingestion. This means that wheat, rye, and barley have to be avoided, including durum wheat, spelt wheat, kamut, einkorn, and triticale (Kasarda, 2001; Kasarda and D'Ovidio, 1999). Recently, there has been increased interest in sorghum as a gluten-free cereal to substitute the gluten-rich cereals in the diet of people suffering from celiac disease (Fenster, 2003). The development of white, tan-plant, so-called food-grade, sorghum lines has enabled white, bland-tasting flour to be produced from sorghum grain. This flour is useful in food products because it does not impart unusual colors or strong flavors, and it may be desired over maize flour for these reasons (Waniska and Rooney, 2002). The development of new food types along with their identity preservation in the marketing system provides significant amounts of superb-quality sorghums for processing. This could lead to increased use of sorghum in many products over the processing.

The present study was planned to evaluate the impact of environment on growth of tan-plant, food-grade sorghum varieties with the aim to promote sorghum cultivation and flour production and use as valuable food for humans in Western countries, which traditionally use sorghum mostly for animal feed. The results of the present work demonstrate that neither the sorghum varieties nor the environment seems to have significant influence on its composition profile.

A



B

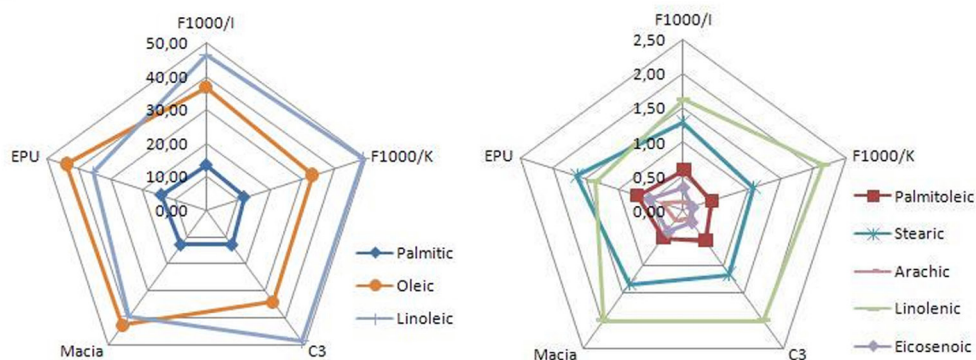


Figure 2 - Bar graph (A) and radar representation (B) of fatty acids contents of sorghum cultivars.

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