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CHEMICAL COMPOSITION OF SELECTED FOOD-GRADE SORGHUM VARIETIES GROWN UNDER TYPICAL MEDITERRANEAN CONDITIONS

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ABSTRACT - Sorghum is a staple food grain in many semi-arid and tropical areas of the world, notably in Sub-Saharan Africa due to its good agronomic properties in harsh environments. At present, sorghum is widely found in the dry areas of Asia (India and China), the Americas and Australia. Due to its properties as a wheat-free food, interest is increasing in cultivating sorghum in Mediterranean countries. However, little is known about how the environment of Mediterranean countries would influence the chemical composition of sorghum. Thus, research has been conducted to compare the composition of selected food-grade white sorghum hybrids grown in Foggia (southern Italy) to hybrids grown in one of the primary sorghum growing regions of the US; Kansas. The sorghum grown in Italy were found to have a higher protein content than the sample grown in Kansas, though overall grain quality was comparable between the two regions. Immunosorbent assays (ELISA) showed for all sorghum flour samples analyzed, the absence of proteins that are toxic for celiac patients.

KEY WORDS: Sorghum hybrid; Sorghum pure line; Chemical composition; Energy value; Amino acids.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important crops grown in the semi-arid tropics. Sorghum contributes substantial amounts of energy and protein to the diets of poorer classes in many developing countries. It is consumed mostly in northern China, India, Africa and southern Russia, where about 85% of the crops are consumed directly as human food (DENDY, 1995; DICKO *et al.*, 2006). 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 2001-2002 (AWIKA and ROONEY, 2004).

Sorghum is higher in protein, ash and fiber compared to other cereals used for human consumption (AHMED *et al.*, 1996). However, the relative nutritive value of sorghum protein is low when compared to casein or other well-balanced protein sources (AHMED *et al.*, 1996). Based on the amino acid scores sorghum has the lowest lysine scores but total essential amino acids content is relatively high in comparison to other cereals. For example, the total content of essential amino acids, including cysteine and tyrosine have been reported at 38.9 (g/16 g nitrogen) for sorghum and 33.5 (g/16 g nitrogen) for wheat (CHUNG and POMERANZ, 1985). While such comparisons are typically based on limited number of samples grown across widely different environments, this demonstrates that sorghum is nutritionally similar to other cereals except for lysine levels as mentioned previously.

Sorghum can be used in a variety of foods. White "food-grade" sorghums can be milled into flour similar in appearance to wheat and processed into products such as expanded snacks, cookies and ethnic foods, and is gaining popularity in areas like Japan (ROONEY, 2001; AWIKA and ROONEY, 2004). Sorghum is often recommended as a safe food for celiac patients, those people with an that have a negative autoimmune response to wheat gluten and similar proteins in rye and barely.

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Sorghum has long been assumed to be a safe cereal due to its distant relationship to the Triticeae tribe of cereals which includes wheat, rye and barley (KASARDA, 2001; TAYLOR *et al.*, 2006). Sorghum is a member of Panicoideae sub-family which also includes maize (SHEWRY, 2002). Recently, *in vitro* and *in vivo* tests of the toxicity of sorghum proteins and peptides showed no toxicity (CIACCI *et al.*, 2007). Sorghum, therefore, provides a good basis for gluten-free breads and other baked products like biscuits, snacks and pasta. Recently, we have begun developing the cultivation of white, tan-plant, "food-grade" sorghum lines in the south of Italy (DEL GIUDICE *et al.*, 2008). Thus, the aim of the present study was to compare the chemical composition of a sorghum hybrids grown in typical US sorghum growing regions (Kansas) to sorghum hybrids grown in Foggia (south of Italy).

MATERIALS AND METHODS

Plant cultivation

The plant cultivars and source employed in this study are indicated in Table1. Field trials were conducted in southern Italy at the Experimental Institute for Cereal Research of Foggia (41° 28' N, 15° 32' E and 75 m a.s.l.) on a clay-loam soil (Typic Chromoxerert) during 2008. On the basis of their potential to produce better quality cookies than other hybrids, the sorghum hybrid F1000 (designated as F1000/F in the remainder of the manuscript) and two sorghum cultivars PL-3 and PL-4, respectively, were chosen for the present study (Table 1). Statistical designs used to analyze the field data included a complete randomized design replicated three times with plots of 45 m^2 size. Individual plots were rows wide. Row length was 1.5 m. The test plots were planted and harvested with equipment designed for small-plot work. The sowing date was on May 8, 2008, and the plots were harvested on October 4. Nitrogen (120 Kg N ha^{-1}) was split applied, at the rate of 1/3 before sowing (incorporated by disk harrowing) as ammonium phosphate, and 2/3 N top-dressed applied at the tillering stage as ammonium nitrate. Weeds within the growing season were controlled by means of specific herbicides.

The sorghum hybrid F-1000 was also grown in western Kansas (designated as F1000/K in the remainder of the manuscript) in 2008.

Floor sample preparation and analysis

Approximately 500 g of grain samples were turned into flour with a two-roll mill (Chopin mod. Moulin CD1). Subsequently, the flours so obtained have been sieved with a planetary sieve (Buhler), through a 120 μm^2 sieve opening. Dry matter content was evaluated by drying the samples for 1h in a thermostatically controlled oven, at uniform temperature of 130°C. Subsequently, the sample was cooled in a desiccator, in presence of $CaCl_2$, and weighed. Nitrogen concentration was obtained by the Kjeldahl method (AOAC, 1920) and total protein content was estimated using a conversion factor of 6.25. Sorghum samples (1 g each) were analyzed using a Mineral Six Digester and an Auto Disteam semi-automatic distilling unit (International PBI, Milan, Italy).

Lipid content in the sorghum grain samples was determined by grinding samples (about 3 g each) with liquid nitrogen in a mortar and lyophilized using a FTS-System Flex-Dry™ instrument. The samples were extracted using a Soxhlet apparatus with chloroform ($CHCl_3$) for 4h, then were dried using a rotary evaporator to obtain the crude extracts which were weighed to obtain the amount of extracted fat. Samples were analyzed in triplicate. For ash measurement, sorghum samples (ca 3 g each) were weighed into shallow, relatively broad ashing dish that has been ignited at ~550°C, cooled in a desiccator, and weighed soon after reaching room temperature (AOAC, 1923). Starch was determined by amyloglucosidase/ -amylase method prescribed by Megazyme total starch analysis kit. K-TSTA (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co.Wicklow, Ireland) (AOAC, 1996). Total carbohydrates were determined as described by ARIENZO *et al.* (2003).

For total amino acid determinations, aliquots (ca 50 mg) of sorghum flour were hydrolized with 1.0 ml of 6M Hydrochloridric acid (HCl) containing 0.02% phenol and nor-Leu as internal standard at 110°C for 20h (ARIENZO *et al.*, 2003). Following hydrolysis, HCl was removed under vacuum and samples resuspended in 1.0 ml of 0.2 M lithium citrate buffer (pH 2.2). Aliquots (µl) were then analysed using a Biochrom 20 amino acid analyser (Biochrom, Cambridge, UK) equipped with polyvinyl sulphonate ionic exchange column (Biochrom, Cambridge, UK) and a post-column ninhydrin derivatization system. The detection was performed both at 570 nm and 440 nm for Proline detection. Protocols employed were suggested by the manufacturer. All hydrolyses and analyses were performed in duplicate. Total energy value of whole sorghum samples was measured by bomb calorimeter instrument as described by MERRIL and WATT (1973).

The RIDASCREEN® standard test kit [RIDASCREEN® Gliadin (Art. No R7001), R-Biopharm AG] sandwich ELISA based method was used to screen for the presence of protein sequences toxic to people with celiac disease in the sorghum samples in sorghum grain flour samples according VALDÉS *et al.* (2003) and the manufacturer's instructions.

TABLE 1 - *List of sorghum cultivars.*

Cultivar	Genotype	Field trials	Origin
F1000/K	Fontanelle 1000	Kansas (USA)	E. Roemer
F1000/F	Fontanelle 1000	Foggia (South of Italy)	E. Roemer
PL-3 = 05MN5115	91BE7414	Foggia (South of Italy)	M. Tuinstra
PL-4 = Macia	Macia	Foggia (South of Italy)	M. Tuinstra

TABLE 2 - *Chemical composition of whole sorghums.*

Component	Cultivar			
	F1000/K	F1000/F	PL-3	PL-4
Whole Kernel (%)	100	100	100	100
Dry Matter (%)	92.8	87.8	89.7	88.7
Protein (Nx6.25) (%)	10.4	13.1	10.8	10.8
Fat (%)	3.9	3.8	3.5	3.4
Ash (%)	1.5	1.5	1.9	1.3
Starch (%)	47.2	46.7	28.3	45.1
Total Carbohydrate (%)	80.9	69.3	73.5	73.2

RESULTS AND DISCUSSION

Chemical composition and energy value in sorghum cultivars

The main goal of the present study was to compare the chemical composition, energy value and the amino acids content of the hybrid F1000 grown in Kansas (USA) (F1000/K) and in Foggia (south of Italy) (F1000/F). Both sorghum pure lines, PL-3 and PL-4, were utilized as controls in the Mediterranean environment.

As shown in Table 2, the percentages of components in all sorghum cultivars analyzed were comparable among them. An exception is the protein content of F1000/F, which was about 1.25-fold higher than that of F1000/K. On the contrary, the total carbohydrate content of F1000/K was about 1.16-fold higher than that of F1000/F cultivar from Foggia (south of Italy). In addition, differences were also found in ash and starch contents, among PL-3 cultivar and the other three sorghum cultivars, F1000/K, F1000/F and PL-4. In fact, PL-3 exhibited a higher percentage of ash and a lower percentage of starch when compared to PL-4, F1000/K and F1000/F. These differences could be due to genotypic constitutions of the samples, though more research with multiple environments would need to be conducted to determine this (SERNA-SALDIVAR and ROONEY, 1995).

The calculated energy value of the F1000/K cultivar was slightly higher than that of F1000/F (Table 3). The higher energy value of F1000/K was consistent with the percentage of the three macromolecular components, i.e. proteins, fats and total carbohydrates, which together form the final energy value of whole sorghum (BRODY, 1998).

The relative content of protein amino acids obtained after hydrolysis demonstrated the same amino acid composition in all analyzed sorghum samples (Table 4).

TABLE 3 - *Energy value of whole sorghums.*

Cultivar	Energy value	
	Kcal/100g	KJ/100g
F1000/K	384.7	1630.1
F1000/F	364.2	1543.1
PL-3	368.7	1562.6
PL-4	366.6	1553.8

ELISA assays for revealing the absence of gluten in sorghum cultivars

Table 5 shows the results of immunochemical measurement of gliadin concentration in sorghum flour sample produced by milling of the sorghum samples utilized in this study. The results indicated that the "gluten" level in all sorghum cultivars analyzed was less than 5mg/Kg (ppm), showing little to no cross-reactivity to the anti-bodies in the ELISA test kit. That value is well below the 20 ppm threshold that has been proposed to be safe for celiac patients (VALDÉS *et al.*, 2003). This confirms the previous data of CIACCI *et al.* (2007) that sorghum is a safe food for people with celiac disease.

Sorghum is ranked fifth among cereals grown worldwide. It is a major food grain in Africa and parts of India and China (ANGLANI, 1998). More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal food, alcohol production and industrial products (AWIKA and ROONEY, 2004). In the USA and Japan, sorghum interest in utilization of sorghum as human

TABLE 4 - *Amino acid compositions in percent of whole sorghums.*

Amino acid[a]	Cultivar			
	F1000/K	F1000/F	PL-3	PL-4
Asx	8.84±0,10	8.57±0.62	8.53±0.65	9.15±0.39
Thr	3.66±0.14	3.26±0.23	3.20±0.00	3.26±0.11
Ser	6.71±0.14	5.74±0.41	6.23±0.49	5.68±0.13
Glx	12.86±0.00	15.39±1.11	14.19±1.17	13.79±0.06
Gly	11.92±0.09	9.59±0.69	9.40±0.15	10.28±0.06
Ala	17.01±0.56	20.00±1.44	19.27±0.10	18.70±0.17
Val	3.46±0.06	3.79±0.27	3.52±0.25	3.67±0.07
Cys	1.24±0.20	1.28±0.09	1.19±0.10	1.38±0.30
Met	1.59±0.11	1.90±0.14	1.74±0.05	2.02±0.31
Ile	1.50±0.07	3.14±0.23	1.54±0.32	2.05±1.03
Leu	12.03±0.35	14.60±1.05	13.82±0.09	12.73±0.15
Tyr	3.56±0.40	4.10±0.29	4.43±0.59	3.59±0.32
Phe	6.14±0.31	6.58±0.47	6.44±0.02	6.31±0.20
Lys	3.39±0.10	2.46±0.18	2.12±0.05	2.78±0.10
His	2.59±0.12	2.48±0.18	2.19±0.06	2.51±0.24
Arg	3.48±0.01	2.49±0.18	2.19±0.20	2.09±1.54

a Tryptophan was detected in acid hydrolysate.

TABLE 5 - *Measurement of gliadin (as ppm) in sorghum flours using ELISA assay.*

Cultivar	Content[1]
F1000/K	<5
F1000/F	<5
PL-3	<5
PL-4	<5

1 mean values from 3 measurements.

food is increasing due to its use in snacks and cookies (Rooney and Waniska, 2004). The future promise of sorghum in the developed world is for use as a wheat substitute for people allergic to gluten (Fenster, 2003; Ciacci *et al.*, 2007).

To demonstrate the feasibility of sorghum cultivation in Mediterranean countries, we have conduced field trials of both selected white sorghum hybrid and pure lines during the growing season 2008. Chemical composition and amino acid composition were assessed and compared among whole sorghum grains grown either in Kansas (USA) or in south of Italy (Foggia). The composition of the components in all sorghum samples analyzed were comparable However, a difference was found between the protein content of F1000/F cultivar grown in Foggia (south of Italy) and that of F1000/K grown in Kansas (USA). In fact, the protein content of F1000/F was about 1.25-fold then that of F1000/K. It is well known that grain composition can vary significantly due to genetics and environment. For example, high-nitrogen fertilizer levels increase grain protein content and decrease the amount of total carbohydrates (Serna-Saldivar and Rooney, 1995). Since the cultivars F1000/K and F1000/F have identical genotype, the influence of the environment is the principal reason in the differences in chemical composition found for both F1000/K and F1000/F cultivars analyzed. Environmental influence is also evidenced by the difference in the energy values between F1000/K, with higher energy value, and F1000/F, with lower energy value, since the final energy value of whole sorghum is formed by adding up the percentage of the three macromolecular components, i.e. proteins, fats and total carbohydrates (Table 2) (Brody, 1998).

The protein amino acids composition in all sorghum cultivars analyzed was the same with low lysine content among other essential amino acids. It is well known that sorghum is low in lysine (Serna-Saldivar and Rooney, 1995).

Celiac disease continues to be a major health problem in many countries; the highest reported prevalence is in northern Europe and in countries of European ancestry (Kasarda, 2001). As a result of

this, various efforts are being made to improve the quality of foods available to persons with celiac disease. 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 present study was aimed to demonstrate that food-grade, tan-plant, white sorghum cultivation for flour production and use as valuable food for humans in Mediterranean countries, which traditionally use sorghum only for animal feed. Results here reported show that chemical composition of sorghum cultivars grown in south of Italy is comparable with that of the same sorghum cultivar grown in Kansas (USA). These data encourage spreading cultivation of food-grade white sorghum varieties in Mediterranean countries, and raising the chance to design some new foods based on sorghum flour, especially for people suffering from gluten-intolerance disease. Novel sorghum foods could also be introduced into Mediterranean countries to take advantage of the numerous phytochemical compounds present in sorghum (AWIKA and ROONEY, 2004) for both celiac and non-celiac consumers.

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