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Oil Extraction from Millet Seed –Chemical Evaluation of Extracted Oil

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ABSTRACT: The object of this study is to extract the oil from millets and evaluate the chemical properties of the extracted oil. Commercial millet oil was obtained by cold solvent extraction adopting soxhlet procedure. Quantitative and qualitative tests concerned with the non-saponifiable matter, fatty acid composition, phosphorus content representing total phospholipids, refractive index, peroxide, saponification, acid and iodine values were carried out on the extracted oil. The extracted non-saponifiable matter was fractionated into a number of chemical classes of compounds on TLC plates. The major two fractions; sterols and tocopherols were separated and extracted with ether. Gama tocopherol and β-sitosterol were the predominant tocopherol and sterol present in the millet oil. Prepared fatty acid methyl esters of the oil sample showed that linoleic (64.8 %), followed by oleic (24.2 %) and palmitic (6.1%) acids were the predominant fatty acids, in respective decreasing order. The oil contains considerable quantities of tocopherols which act both as vitamin E and antioxidant. The oil content of the seed depending on the types of extraction is low (4-7%) but due to its nature, being high in both linoleic acid and tocopherol, the purified oil might be employed as an ingredients in food formulation.

Keywords: *Chemical Evaluation, Fatty Acid Composition, Millet Oil, Non- Saponifiable Matter, Oil Extraction.*

Introduction

Millets belong to the small grains and are annual plants of hot and dry regions with high tolerance against dryness and salinity stress. The word millet originates from Latin word millesimum meaning one thousandth of root and the word mill means small and tiny, which is often used for referring to very small things. Millet is considered as the sixth crop in the world after wheat, rice, corn, barley and sorghum (Karyudi & Fletcher, 1999).

Millet loses its quality quickly after grinding due to some enzymatic activities on fatty components. Odor changes in millet flour stored at the temperature of 19˚C and relative humidity of 58% is seen during 4-5 days (Lai & Varriano, 1980).

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The seeds have generally low content of lipids approximately 6-7% on dry weight basis (Devittori *et al*., 2000).

Devittori *et al* investigated the extraction of millet bran oil by supercritical fluid and compared the resultant oil with the oil extracted by petroleum ether. Chromatography procedures and extraction methods indicated that linoleic (64%), oleic (23%) and palmitic (7%) acids were the predominant fatty acids present. It was also shown that the major tocopherol (70%) in the extracted oil was γ tocopherol (Devittori *et al*., 2000). The efficiency of the oil extraction and fatty acid composition by 8 different solvents were studied by Osagie and Kates (1984). Advance studies concerned with the extraction of millet oil and evaluation of the methods have not been carried out in detail. Due to the remarkable properties of millet oil namely high content

of tocopherols and linoleic acid, it is the aim of this study to evaluate the extraction methods and chemical characteristic of extracted oil.

Materials and Methods

Samples of commercial millet seeds were prepared, grinded and stored. The oil was extracted by cold solvent and soxhlet procedures. In the cold method, the seeds were initially grinded, dried, shaken in a mixer with hexane for 24 hours and finally the solvent removed by rotary evaporator. Soxhlet method was carried out according to AOCS standard No. BC-349(2000) by using n-hexane as solvent.

Free fatty acid was determined as oleic acid according to AOAC standard method No. 940/28 (1990) in triplicate order (Firestone, 1990).

Fatty acid composition of the oil was determined by preparation of methyl ester according to AOAC standard method No. 969/33 (Firestone, 1990). The analysis of the fatty acid methyl esters were carried out on gas chromatography apparatus equipped with 10% DEGS column and Flame Ionization Detector according to AOAC method No. Ce 1e-91 (Firestone, 1990).

The non-saponifiable matter of the oil was isolated by alcoholic potassium hydroxide saponification of the oil followed by the extraction of the non-saponifiable matter with ether according to the AOAC standard method No. 933/08 (Firestone, 1990). The nonsaponifiable matter was fractionated on a TLC plate into a number of chemical classes of compounds namely tocopherols and sterols.

Tocopherols were identified and quantified by HPLC apparatus according to AOCS standard method No. Ce 8-89 (Firestone, 1994).

Sterols were determined by GC equipped with HP5 capillary column and Flame Ionization Detector according to AOAC standard method 970/51 (Firestone, 1990).

The amount of phosphorus representing phospholipids content was determined according to IUPAC standard No. H.D. 16, 20 (Paquot, 1979).

Refractive index was determined according to AOCS standard No. Cc 1-25 (2000).

Iodine value was calculated by the AOCS standard method cd 1c-85 (Firestone, 1994).

Induction period was measured by using Metrohm Rancimat model 743 apparatus according to AOCS method No. cd 12-57 (2007).

The saponification value was determined by fatty acid composition according to AOCS method No. cd 3A-94.

Peroxide value of the oil was obtained according to AOCS standard method No. cd 8-53 (Firestone, 1994).

Results and Discussion

By adopting soxhlet method, it was revealed that millet seed contains approximately 7% oil. Oil percent depends on cultivar, location, soil fertility, seed grinding method, freshness of seeds, oil extraction method and type of solvent. Hot soxhlet method had higher efficiency than the cold method for oil yield. In cold method, oil percent was about 4.7% but the advantage of the cold method was that millet seeds, which are sensitive to heat, experienced less damage. Oil content in millet seed is lower than some other oil seeds such as sunflower (33-35 %), cottonseed (18-20%), canola (40-45%) and soybean (18-20%) (Hui, 1996).

The fatty acid composition of oil is presented in Figure 1. Linoleic acid is the predominant fatty acid followed by oleic acid. As indicated on average the oil is composed of 8.1% saturated, 25.2% mono-unsaturated and 65.5% poly-unsaturated fatty acids. The amount of linoleic acid in millet oil is higher in comparison with most other types of vegetable oils (Hui, 1996), which this might be regarded as a great

advantage when the oil is employed for consumption as salad oil or in food formulation.

The iodine value of millet oil is 135 which is similar to soyabean oil (135), higher than sunflower (129), cotton seed (111) and canola (110) oils. Therefore, in term of unsaturation it is almost similar to soybean oil understanding that the oil contains little amount of linolenic acid but higher concentration of linoleic acid as compared to soybean oil (Ghavami *et al*., 2008). The acid value does not identify the oil but indicates the quality of the oil or seed and is the measurement of the degree of hydrolytic rancidity and formation of free fatty acids, therefore the amount is a function of purity and freshness. According to the tests performed, free fatty acid content of millet oil is approximately 1.44% which indicates the storage condition and practices carried out prior to solvent extraction (Devittori *et al*., 2000; Osagie & Kates, 1984).

Peroxides are the initial product of oxidation of fatty substances and in general, the higher the unsaturation of the oils, the more the oil or fatty substance is susceptible to oxidation. When the amount of peroxide reaches a definite limit, various changes take place and aldehydes, ketones, acids and alcohols are produced, which contribute to unpleasant odors and tastes in the fatty substances. Peroxide indicates the progress of oxidation and its production in early phases is low and the speed it is formed may vary depending on the type of oil, storage conditions, temperature and some other factors (Parvaneh, 1998).

Refractive index expresses the intensity of the refraction of a beam of light while passing through the oil is shown in Table 1 along with some other chemical characteristics of the extracted oil (Ghavami *et al*., 2008).

The nonsaponifiable matter of an oil or fat which might be considered as an important fraction consists of numbers of classes of compounds which might be regarded as a key to verify and identify the oil and the fact that most potent compounds responsible to stabilize the oil are present in this fraction.

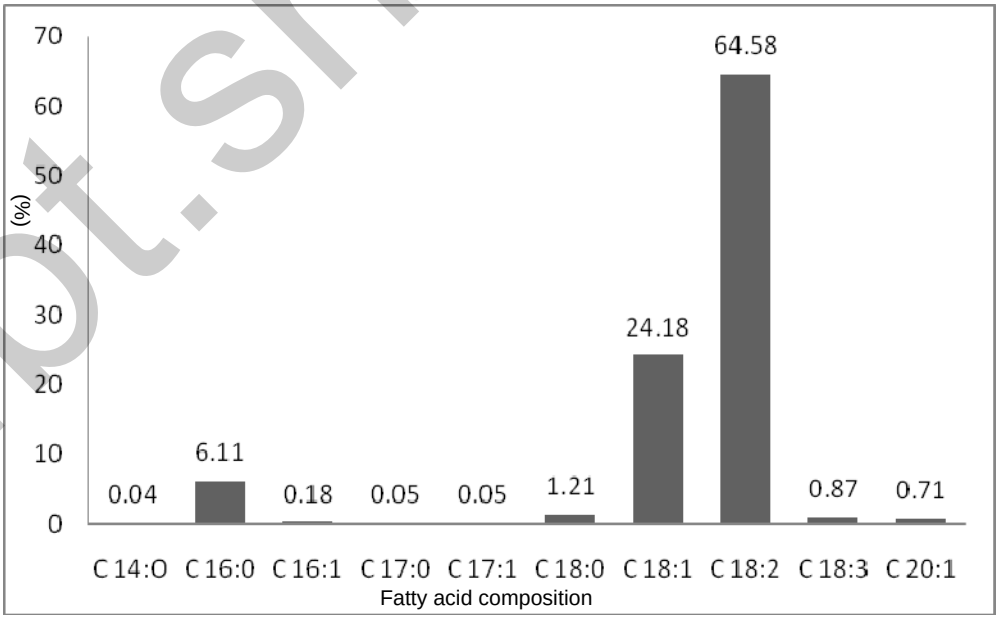

Fig. 1. Fatty acid composition of millet oil

Table 1. Chemical properties of millet oil

Characteristic	Crude millet oil
Acid value	1.44
Iodine value	135
Peroxide value (meq/Kg)	1.97
Refractive index	1.4658
Phospholipids (% of the total lipid)	1.4
Induction period at 110 ˚C (h)	4.34
Saponification index(mg KOH/g)	184
Unsaponifiable matter (%)	3.52
Tocopherol (ppm)	
α	86.01
β	115.40
γ	236.57
δ	38.48
Sterol (%)	
β- sitosterol	55.20
Campesterol	20.25
Citostanol	17.10
Stigmasterol	6.07
Δ^7 stigmasterol	0.50
Δ^5 avenasterol	0.10

Table 2. Unsaponifiable matters of millet oil

Zones	RF Value (×100)	Fluorescence Under UV	Percent of Nonsaponifiable Matter
First zone: Point of start up to sterol	0.00-5.55	Light Green	1
Second zone: sterols, 4 methyl sterols, triterpane alcohols	5.55-30.55	Golden yellow	40
Third zone: Tocopherols:α,β,γ,δ	30.55-55.55	Violet	23
Forth zone: Tocopherol dimmers	55.55-84.60	Violet	9
Fifth zone: Hydrocarbons: Squalene	84.60-100	Yellow	27

Fractionation of the nonsaponifiable matter on TLC plate separates the sterols, 4 methyl sterols, triterpane alcohols, tocopherols, tocopherol artifacts and

dimmers and hydrocarbons after development in hexane and ether (4:1) and spraying with a mixture of acetic anhydride, ethanol and sulfuric acid as shown in Table 2.

The analysis of the sterols, the major fraction of the nonsaponifiable matter as shown in Table 1 indicates that β-sitosterol was the predominant sterol followed by campesterol, citostanol and stigmasterol in respective decreasing order. Traces of Δ^7 stigmasterol and Δ^5 avenasterol were also present.

The tocopherol fraction, consisting of α, β, γ and δ tocopherols acting as antioxidants and having vitamin E activity were analyzed by the application of this fraction into HPLC apparatus. The results indicated that γ tocopherol was the predominant tocopherol followed by β, α and δ tocopherols (Table 1). It has been proved that the antioxidant activity of tocopherols depends on the kind of tocopherol as well as the concentration. γ and δ tocopherols at the concentrations of 0.01 to 0.04 were shown to exhibit the best activities (Hudson & Ghavami, 1984).

Considering the fatty acid composition of millet oil and the amount of unsaturation present, it might be concluded that the presence of tocopherols might protect the unused oil to some extent during storage and increases its shelf life. However, due to high content of linoleic acid, the oil is quite susceptible to oxidation and therefore it might not be recommended for cooking or frying practices.

Examination of the extracted oil indicated that the phospholipids content presented 1.4% of the total extractable lipids and the fatty acids esterified to the phospholipids had the same composition as those sterified to triglycerides.

The high quantities of phospholipids might create problems; therefore, this fraction should be removed prior to refining.

The induction period of the oil representing its resistance to oxidation at 110°C is shown in Table 1. The value for this oil is approximately similar to those for soyabean and sunflower oils, the two most popular oils in the market. The induction periods might be affected by the degree of unsaturation and the amount of antioxidant present in the oils.

The saponification value of the oil representing the mean value of the molecular weight of the fatty acids in the triglycerids is 184 (mg KOH /g oil) which is similar to other vegetable oils except rapeseed oil which has lower saponification value due to the presence of erucic acid.

Conclusion

Generally millet seed might be considered a remarkable product due to the protein, fiber and oil contents. The seed after milling might be employed in many applications because of the high content of fiber and high quality oil. Although it contains approximately seven percent oil, the oil due to the fatty acid composition namely high linoleic acid and high amount of tocopherols present might be used in many food and pharmaceutical formulations and products.

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