A Comparative Characterization of Different Non-Conventional Oilseeds found in Pakistan

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Summary: Hexane-extracted oil contents of rice bran of variety super kernel (*Oryza sativa*), muskmelon (*Cucumis melo*), watermelon (*Citrllus vulgaris*) and mango kernel (*Mangofera indica)* ranged from 10 to 45%. Other physical and chemical parameters of the extracted oils were as follow: Iodine value 117, 106, 83 and 50; refractive index (40°C) 1.466, 1.467, 1.468 and 1.461; density (40°C) 0.919, 0.926, 0.902, and 0.974; saponification value 183.0, 174, 205 and 189; unsaponifiable matter 5.64, 4.18, 0.68 and 1.00, respectively. The investigated oils were found to contain a high level of oleic acid ($C_{18:1}$ Omega-9) ranged 23-83% followed by linoleic acid ($C_{18:2}$ Omega-6) 1-59%. Linolenic acid ($C_{18:3}$ Omega-3) was only found in super kernel variety (2.4%). Many parameters of the investigated oils were quite comparable with those of different conventional oils.

Introduction

Vegetable oils have numerous commercial values. Oils and fats satisfy important nutritional requirements of human beings. Livestock and poultry also require oils and fats for better growth and production. Vegetable oils are important raw materials in vanaspati, soap, paints, varnishes and pharmaceutical industries. Certain industrial wastes, such as seeds of certain fruits and vegetables are rich and cheaper sources of fats and oils, provide important nutrients such as, protein, starch, minerals, vitamins and fats [1].

Due to the growing demand and scientific awareness about the nutritional and functional properties of oils [2], the quality assessment and composition analysis of oils from non-conventional oilseeds is a current focus of international research. Some reports are available on the characterization of non-conventional oilseeds from different geographical regions [3-5]. The use of cucurbit seeds as sources of oils and proteins have been reviewed by Jacks *et al.*, [6]. Most of its oils are composed of unsaturated fatty acids, thus giving them high nutritional values. Conjugated fatty acids in some cucurbit oils make them highly useful as drying oils, i.e. they combine readily with oxygen to form an elastic, waterproof film. Watermelon (*Citrullus vulgaris*) and grapes (*Vitis vinifera*) seeds oil was characterized and composition of different components was determined [7]. Yaniv *et al.*, [8] determined fatty acid profile of *Citrullus colocynthis.* In India, non-traditional oilseeds are gaining special importance. The mango kernel is a major by-product of the mango-processing industry. Extracted kernel fat is white, solid at room temperature like cocoa butter and tallow, and has been proposed as a substitute for cocoa butter in chocolate. Thus, mango kernel oil has good potential as an edible oil [9].

Fatty acid composition mainly determines the properties of the oil. Oleic oils are recently gaining importance because of their superior stability and nutritional benefits. Many of the chemical tests used to identify an oil or validate its purity rely on its fatty acid composition [3].

In view of cardinal role of dietary fats in human health and diseases [10-12] the chemical analysis and particularly the fatty acid composition of oil used for domestic consumption have become a research priority of lipid chemistry [13-16]. Saunders [17] has characterized rice bran for food potential.

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Prabharka and Hemavathy [18] have described the lipid content and fatty acid composition of three varieties of rice bran from India. In addition to its potential edible value, fatty acid monoesters of rice bran oil produced by esterification of fatty acids with monohydroxy compounds are widely used in industry due to their lubricating and softening properties [19].

The fatty acid composition of muskmelon (*Cucumis melo*) seeds was reported from different geographical regions such as Brazil, Canada, India, and Veitnam [5, 7, 20-21].

The uncontrolled growth in world population coupled with industrialization has widened the gap between demand and production of vegetable oils. This has resulted in ever-increasing imports, requiring the expenditure of valuable foreign exchange. Now, when sustainable socio-/agro forestry is gaining recognition as an appropriate mean to improve national economics, the search for alternative source of additional fats and vegetable oils has to play a crucial role. In view of growing demand and scientific awareness about the nutritional and functional properties of oils, the quality assessment and composition of oils from non-conventional oilseeds is of much concern. Until now, a full characterization of oil from these botanical materials indigenous to Pakistan has not been reported. This has promoted us to design an analytical protocol and investigate these oils. The ultimate objective of the present study was to look into detail analytical characterization and exploitation of these oils for edible as well as for other commercial purposes. In this context, as a part of previous studies [3] for characterization of some non-conventional oil seed crops, we have selected rice bran (*Oryza sativa*), muskmelon (*Cucumis melo*), watermelon (*Citrllus vulgaris*) and mango kernel (*Mangofera indica)* from different agroclimatic regions of Pakistan. The present study was made to assess their commercial potentials.

Results and Discussion

The hexane-extracted oil content of rice bran, muskmelon, watermelon and mango kernel was found 14.03, 45.0, 35.11 and 10.01%, respectively (Table-1). The oil content of rice bran was comparable to that reported from Korea and Bangladesh [4, 22]. The oil content (16.41%) of rice bran was found to exceed than cottonseed (10-12%) oil indigenous to Pakistan [23] and comparable to cottonseed grown in United States, Brazil, China and some other Asian and European countries [24]. Among cucurbitaceae family muskmelon seeds contained higher oil (45.0%) content than investigated watermelon. The oil content of muskmelon was higher [5] than Brazil muskmelon kernel.

Such variation in oil content with in the countries is attributed to possible changes in environmental and geological conditions of the region [25].

Free fatty acid (FFA) or acid value of a fat is a measure of extent to which hydrolysis has liberated the fatty acid from their ester linkage with parent triglyceride molecule. The FFA content among the investigated varieties was ranged from 0.38 to 56.03% (Table-1). Rice bran was found with highest FFA (56.03%), while mango kernel constituted significantly lower FFA value i.e., 0.38%. Acidity may develop during seed deterioration from lipase catalyzed hydrolysis of fat to FFA. Most of oils, high in Peroxide value (PV), were also found with low FFA or vice versa, yet there was lack of strong correlation between the data.

Saponification value (SV) is the index of mean molecular weight of the triglycerides comprising the fat. The range of saponification value and unsaponifiable matter among investigated varieties was within 174.89 to 205.47 and 0.68 to 5.64, respectively (Table-1). Mango kernel (189) and watermelon (205.47) hold significantly higher saponification values. This rise in SV may be attributed to the replacement of very long chain (C_{22}) fatty acids by a shorter chain C_{18} fatty acids [20]. The saponification values of investigated oils are in close agreement with those of shea nut oil [24]. Watermelon oil, which had the highest level of unsaponifiable matter among the investigated oils and fat.

Iodine value (IV) is a measure of the degree of unsaturation or number of double bonds present in an oil or fat. The fats were deteriorated due to oxidation and hence their iodine value was reduced. IV of all investigated varieties ranged from 50.09 to 117 (Table-1). Super kernel was found to show a slightly higher IV than that reported in the literature [24]. Thus, super kernel oil is much more exposed to oxidation but rice bran oil is highly stable due to presence of reported tocopherols [26]. IV of rice bran

Table-1: Physico- chemical characteristics of oils and fat

Sample	RBS	MM	WM	MK
Oil content (%)	14.03 ± 0.20	45.0 ± 0.96	35.11 ± 0.79	10.01 ± 0.36
Free fatty acid (%)	56.03 ± 0.83	1.92 ± 0.10	5.62 ± 0.25	0.38 ± 0.01
Density (mg/mL 40°C)	0.919± 0.04	0.926 ± 0.04	0.902 ± 0.03	0.974 ± 0.05
Saponification Value (SV) (mg of KOH/g of oil)	183 ± 3.33	174.89 ± 2.79	205.47 ± 4.38	189 ± 3.68
Unsaponifiable matter (%)	5.64 ± 0.20	4.18 ± 0.21	0.68 ± 0.03	1.11 ± 0.04
Iodine value (IV) (g of 1/100g of oil)	117 ± 1.22	106 ± 3.56	83.67 ± 2.43	50.09 ± 1.95
Refractive Index (40°C)	1.466 ± 0.04	1.467 ± 0.04	1.468 ± 0.03	1.461 ± 0.04
Peroxide Value (PV)	1.1 ± 0.05	5.1 ± 0.16	6.32 ± 0.27	2.1 ± 0.04

Values are means ± SD of three samples of each variety analyzed individually in triplicate
Rice bran super kernel, RBS; Muskmelon, MM; Watermelon seed, WM; Mango kernel, MK

Table-2: Fatty acid composition (g/ 100g of fatty acids) of oils and fat studies and its comparison with literature

Fatty Acid	Rice bran	Reported (24)	Muskmelon	Not Reported	Watermelon	Reported (27)	Mango Kernel	Reported (24)
C_{12}	Trace	Trace	Trace	—	Trace	Trace	Trace	Trace
C_{14}	1.75 ± 0.02	0.5	Trace	—	2.03 ± 0.06	0.6	Trace	Trace
C_{16}	21.11 ± 0.22	16	8.96 ± 0.32	—	1.20 ± 0.04	33.28	10.25 ± 0.36	6.9
$C_{18:0}$	2.64 ± 0.04	1.5	6.15 ± 0.18	—	1.91 ± 0.07	14.90	42.20 ± 2.21	41.4
$C_{18:1}$	42.37± 0.45	42.5	23.4 ± 0.49	—	83.73 ± 3.57	15.32	35.39 ± 1.76	44
$C_{18:2}$	25.58 ± 0.62	35.5	59.09 ± 2.12	—	1.39 ± 0.04	31.17	6.91 ± 0.23	4.6
$C_{18:3}$	2.44 ± 0.04	1.0	Trace	—	Trace	2.07	Trace	0.3
$C_{20:0}$	1.58 ± 0.09	0.5	1.46 ± 0.04	—	1.50 ± 0.04	—	5.25 ± 0.21	2.5
$C_{20:1}$	2.53 ± 0.06	0.5	0.94 ± 0.03		8.24 ± 0.33	—	Trace	Trace

Values are means ± SD of three samples of each variety analyzed

oil was comparable with that of corn, cottonseed, mustard seed, rapeseed and sesame seed [24], while the IV of muskmelon oil was in good agreement with that reported by Maria et al [5] and is little bit lower than Brazil muskmelon [5].

PV was varied between 1.1 to 6.32 (Table-1) among oils and fat. High oxidative stability of the rice bran oil may be attributed due to presence of certain oryzanols [26], while muskmelon (5.1) and watermelon (6.32) oils were noted to be less stable and easily undergo deterioration when exposed to atmospheric oxygen because of high PV values.

These oils can be stabilized by adding an antioxidant or by matting suitable oil blends.

The value of density and Refractive index (RI) was ranged between 0.902-0.974 (at 40°C) and 1.461-1.468 (at 40°C), respectively (Table-1). Among the samples density of watermelon was lowest (0.902) with highest R.I value (1.468), while mango kernel was having highest density (0.974) with lowest R.I value (1.461).

The fatty acid composition of different non-conventional oils and fat together with literature values are given in (Table-2). In rice bran, musk-melon, watermelon and mango kernel oils the content of total saturates; make up 27.08, 16.57, 6.64 and 57.7%, respectively. Palmitic acid was the dominant fatty acid in rice bran and muskmelon while oleic acid dominated in watermelon oil. The investigated oils were found to contain a high level of monounsaturated fatty acids i.e., 44.9, 24.34, 91.97 and 35.39 %, respectively. Oleic acid was the predominant fatty acid in watermelon, which accounted for 83.73 %. The content of linoleic acid was 25.58, 59.09, 1.39, and 6.91%, respectively. Linolenic acid was only found rice ban oil i.e., 2.44%. These acids are medically very important and had been a research priority during last decades [3]. The concentration of major fatty acids, $C_{18:2}$, $C_{18:1}$, $C_{18:0}$, $C_{16:0}$ of rice bran was in close agreement with that reported for the rice bran oils indigenous to India [18]. The fatty acid composition of the investigated oils was quite similar in contents of $C_{18:1}$ with those of rice bran oil indigenous to Korea [4]. However, amount of $C_{18:2}$ varied to some extent.

The present fatty acid composition of watermelon kernel oil in our study was quiet different than watermelon characterized in China [27], while investigated oil had high degree of unsaturation than watermelon reported and oleic acid was dominant in investigated oil while linoleic acid was dominant in other one [7]. It is a well-known fact that the fatty acid composition of seed oils depends on the climatic conditions and cultivars of the fruit [28]. Mango kernel fatty acid composition was quiet comparable with that reported in the literature [24].

In Pakistan, cottonseed is the main oilseed crop. Other oil sources include soybean, rapeseed, sunflower and canola seeds. Despite the fact Pakistan has an overwhelmingly an agrarian economy, it is unable to produce edible oils sufficient for domestic requirements and substantial amount of foreign exchange is being spent on the import of oils. Pakistan has vast fertile plains, agricultural lands, and a good irrigation system for almost all types of vegetation. Rice is the second most important food crop of Pakistan which ranks 13th among 112 rice producing countries [29]. Pakistan produced a significant amount of rice bran that could be used as a useful oil source that might be an acceptable substitute for some dietary high oleic oils as well as employed for developing nutritional balanced, high-stability blended formulations with other high linoleic oils.

In addition to this botanical wastes are being used for oil extraction and different for technical purposes.

We found that rice bran contained more oil than cottonseed grown in Pakistan. Rice bran oil could be used for edible as well as for industrial purposes. This oil has a unique fatty acid composition for fatty acid ratio of saturated/monounsaturated/ polyunsaturated for the healthy edible oils. Rice bran oil also perceived good stability but only problem associated with the oil is to reduce the free fatty acids by treating rice bran as early as possible after dehulling from rice during polishing.

Seeds of the cucurbitaceae family (water-melon and muskmelon) contained significant amounts of oil (35-45%), which is very important for technical uses. According to More *et al.*, [30] muskmelon oil content was highest in Pakistan than other ten countries collections. In addition to above uses of oils, watermelon oil and mango kernal fat could be used as stabilizers because they give stability to highly unsaturated oils (having good proportion of oleic acid) when blend with them in suitable proportions.

Experimental

Materials

Bran from variety super kernel (*O. sativa*), was collected from Gujranwala, Punjab muskmelon (*C. melo*), watermelon (*C. vulgaris*) and mango kernel (*M. indica)* were collected from Karachi, Sindh, Pakistan. Pure standards of fatty acid methyl esters from Sigma Chemical Co. (St. Louis, Mo, USA) were used for identification and quantification of fatty acids.

Extraction of oil

The air-dried bran, kernels of muskmelon, watermelon and mango after grinding to get appropriate mesh particles was used to feed Soxhelt extractor alternatively, fitted with a 2-L round bottom flask and a condenser. The extraction with hexane was executed for 5 h. The solvent was distilled off under vacuum in a rotary evaporator. The oils were used in all analysis.

Analysis of extracted oils

Determination of density, refractive index, iodine value, peroxide value, saponification value and unsaponifiable matter of the extracted oils was carried out according to various standard AOCS methods [31].

Fatty acid composition Fatty acid methyl esters were prepared according to standard IUPAC method 2.301 and analyzed on Perkin-Elmer gas chromatograph model 8700 fitted with a methyl lignoserate coated (film thickness = 0.22μm), polar capillary column Sp-2340 (60m×0.25mm), and a flame-ionizing detector. Oxygen free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. Other conditions were as follow: initial oven temperature 150°C; ramp rate (increasing temperature rate) 5°C/min; final temperature 220°C; injector temperature, 260°C; detector temperature, 270°C; and temperature hold, 5 min before the run and 6 min after the run. A sample volume of 1μL was injected, and total analysis time was 20 min. Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of the authentic standards of fatty acid methyl esters obtained from Sigma Chemical Co. All quantifications were done by a built in data handling program provided by the manufacturer (Perkin-Elmer) of gas chromatograph. The data was transferred on an Epson LX-800 printer attached to the instrument through an RS-232-C port. The fatty acid composition was reported as a relative percentage of the total peak area.

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