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Short Communication

Analytical Characterization of Pure and Blended Watermelon (*Citrullus lanatus*) oil: Impact of Blending on Oxidative Stability

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Abstract

Analytical characterization of pure, blended watermelon (*Citrulluslanatus*) oil and impact of blending on oxidative stability was investigated. Watermelon oil was added with mango (*Mangiferaindica* L.) kernel oil at four different concentrations 5, 10, 15 and 20% (B_1, B_2, B_3 and B_4) and referenced with a control (100% watermelon oil). All the blends were stored in transparent PET bottles at ambient temperature (25-28^{o}C) for 3 months; storage stability was assessed at the interval of 1 month. Free fatty acid, unsaponifiable matter, saponification value, refractive index and iodine value of watermelon seed oil and mango kernel oil was 1.38%, 0.34%; 0.71%, 1.68%; 198, 193; 1.468, 1.457; 107.51, 54.62, respectively. The α tocopherol content of watermelon oil, mango kernel oil, B_1, B_2, B_3 and B_4 was 127.49, 205.44, 135.24, 144.52, 156.81 and 169.34 mg/kg. δ tocopherol in watermelon oil, mango kernel oil, B_1, B_2, B_3 and B_4 was 55.26, 34.81, 53.64, 51.27, 50.14 and 48.23 mg/kg. Concentration of linoleic acid decreased from 50.78% to 30.17% when 40% mango kernel oil was added to watermelon oil. Oleic acid increased from 22.89% in watermelon oil to 25.19%, 28.84% and 30.64% in B_1, B_2, B_3 and B_4. The increase in peroxide value of watermelon oil, B_1, B_2, B_3 and B_4 was 10.07, 9.56, 7.62, 5.17 and 2.87 (meqO_2/kg) in a time dependent manner. Induction period of pure watermelon oil was less than mango kernel oil and blends. These results suggest that chemical characteristics and oxidative stability of pure watermelon oil can be improved by blending with mango kernel oil.

***Keywords**:* Watermelon oil; Mango kernel oil; Oxidative stability

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Introduction

The situation of food insecurity is getting worst in developing countries; the addition of about 2000 million people in Asian and African continents by the year 2050 will further spoil the current situation of food insecurity [1]. The potential of non-traditional sources of edible oil must be exploited to fulfill the needs of ever increasing population, unfortunately, out of 500,000 oil producing plants; only 12 are commercially utilized to feed a massive human population [2]. In spite of huge potential of oil production from indigenous sources, country has become the leading importer of edible oil in the world;the situation in many other developing countries is almost similar. With 6.8% of the area of vegetable oil production, around the world, watermelon seed oil accounts for 6.8% area. Watermelon (*Citrulluslanatus)* have a place in the family *Cucurbitaceae*, the production of watermelon on the globe was about 125 million tons in the year 2009, that can yield 12.29 million tonns of watermelon seed oil [3], the juicy or pulpy part is

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commonly used by the humans; seeds are regarded as a potential source of good quality edible oil and protein,oil content of seed varies from 25-50% golden yellow / pale yellow colour, oil contains about 65% polyunsaturated fatty acids, mango produces about 400,000 tons waste all over the world, which can yield approximately 121, 600 M. tons good quality edible oil [4]. Presence of considerably higher concentration of linoleic acidhas a great set back on storage stability of water melon seed oil. Vegetable oil can be stabilized by synthetic antioxidants; however, their role as a carcinogen has led to a considerable decline in their popularity and usage in developed countries. Mango (*Mangiferaindica* L.) kernel produces about 12-13% good quality edible oil with oleic acid as the major fatty acid which accounts for about 45%,followed by stearic acid, which requires no further processing[5, 6]. The use of mango kernel oil in large number of food preparations has been reported [7]. Oxidation of fats and oils decreases their quality, organoleptic characteristics and nutritional quality.This study aimed to determine the effect mango oil on oxidative stability of watermelon oil on the basis of some chemical characteristics.

Materials and Methods

Experimental plan

Watermelon seeds (sugar baby variety) were obtained from Ayub Agricultural Research Institute, Faisalabad, ground (100 mesh size), and oil was extracted with *n*-hexane on a soxhlet distillation unit. Mango (Chaunsa) was procured from local market, conformed by a botanist; flesh was removed to obtain the stones, which were dried in a hot air oven at 40^{o}C for 3 hours (Memmert, Germany). Stones were broken to get the kernels, which were ground (100 mesh size), followed by oil extraction on a soxhelt unit using *n*-hexane, excess hexane was evaporated on a rotary evaporator (Buchi, Japan), oils were transferred to amber bottles and stored at -60°C in a freezer at -65oC (Sanyo) for till further usage in the current investigation. Water melon oil was added with mango kernel oil at four different concentrations; 5, 10, 15 and 20% (B_1, B_2, B_3 and B_4) and referenced with a control. All these blends were stored at ambient temperature for 3 months; storage stability was assessed at the interval of 1 month.

Analysis

α and δ in watermelon oil were determined according to the method prescribed by [8]. Proximate composition of watermelon seeds and mango kernel oil was determined by the standard methods [9]. Free fatty acids, peroxide value, saponification value, refractive index and iodine value were determined according to the standard methods [10]. Conjugated dienes and trienes were determined as per methods of [11]. Induction period was determined on Rancimat 679 [12]. Fatty acid methyl esters were prepared by methanolic HCl (14%) [13]. The experiment was conducted in a completely randomized design, the data was analysed by analysis of variance technique, the significant difference ($P<0.05$) among the blends was determined by Duncan Multiple Range Test using SAS 9.1 statistical software [14].

Results and Discussion

Proximate composition of watermelon seed revealed oil, protein, ash content, moisture and fibre content were 36.41%, 23.78%, 1.92%, 3.78% and 6.25%. Whereas, mango kernelcontained moisture content 45.9%, crude protein 5.89, oil 13.45%, ash content 3.22%. The results regarding chemical composition of watermelon seed in some Pakistani varieties is also reported by [15]. The results regarding the proximate composition of mango kernel obtained in this study are almost similar to the earlier findings [16].

Chemical composition of blends of watermelon oil and their blends

The results of chemical composition of watermelon oil, mango kernel oil and their blend are presented in (Table 1). Free fatty acids of blends of watermelon oil andmango kernel oil decreased with progressive addition of mango kernel oil ($P<0.05$). Free fatty acids of watermelon oil were 1.38%, which decreased to 0.82% when 40% mango kernel oilwas added in watermelon oil.

Table 1. **Chemical characteristics of watermelon oil, mango kernel oil and their blends.**

Parameter	WSO	MKO	B_1	B_2	B_3	B_4
FFA%	1.38±0.05a	0.34±0.02a	1.25±0.02b	1.12±0.03c	0.98±0.06d	0.82±0.05d
USM%	0.71±0.03f	1.68±0.04e	0.81±0.04d	0.95±0.02c	1.12±0.06b	1.25±0.03a
SV	198±5.62a	193±4.36a	197±3.54a	195±2.71a	194±1.62a	195±2.36a
RI	1.468±0.01a	1.457±0.02a	1.459±0.02a	1455±0.01a	1.453±0.04a	1.451±0.03a
IV	107.51±1.63a	54.62±0.53f	95.47.26±2.14b	91.22±1.12c	85.73±1.45d	81.27±0.95e
Red	2.8±0.1a	2.4±0.4c	2.8±0.2b	2.7±0.1b	2.7±0.3b	2.7±0.2b
Yellow	30±0.5a	22±0.2c	26±0.1b	26±0.2b	26±0.1b	24±0.1b

Means represented by a different letter in a row are not similar ($P<0.05$)
WSO: watermelon oil
MKO: Mango oil
B_1: 90% watermelon oil + 10% mango oil
B_2: 80% watermelon oil + 20% mango oil
B_3: 70% watermelon oil + 30% mango oil
B_4: 60% watermelon oil + 40% mango oil FFA: Free Fatty Acids
USM: Unsaponifiable Matter SV: Saponification Value
RI: Refractive Index (40^{o}C)

Table 2. **Fatty acid composition of watermelon oil, mango kernel oil and their blends.**

Fatty Acid	WSO	MKO	B_1	B_2	B_3	B_4
C16:0	15.47±0.41a	7.55±0.25f	13.78±0.67b	12.92±0.78c	11.69±0.19d	10.73±0.42e
C18:0	12.61±0.56f	36.88±0.09a	14.94±0.51e	16.27±0.33d	19.26±0.15c	21.136±0.41b
C18:1	20.53±0.88f	45.17±1.95a	22.89±1.64e	25.19±0.76d	28.84±1.27c	30.64±2.49b
C18:2	50.78±1.19a	5.31±0.17f	46.25±1.12b	41.35±1.34c	35.51±0.91d	30.17±0.64e
C18:3	0.14±0.02f	1.61±0.13a	0.23±0.01e	0.31±0.03d	0.42±.006c	0.51±0.02b
Oxidizibility	51.47±0.46a	8.78±0.45f	47.28±1.95b	42.73±2.64c	37.18±1.26d	32.21±2.42e

Means represented by a different letter in a row are not similar ($P<0.05$)
Refer Table-1 for the detail of treatments

Iodine value of all the blends decreased as a function of addition of mango kernel oil, the iodine value of B_1, B_2, B_3 and B_4 was 95.47, 91.22, 85.73, 81.27, 92.73 and 96.27, respectively. Iodine value of mango kernel oil was 55 [17]. Colour and saponification value of the blends were not different from the parent oils, however, refractive index and unsaponifiable were statistically different from the substrate oils. The higher extent of unsaponifiable matter in mango kernel oil was connected to hydrocarbons, sterols, triterphenols, carotenoid and tocopherols [18].

Fatty acid composition

Watermelon oil was characterized with appreciable amount of unsaturated fatty acids, linoleic acid accounted for 50.78% followed by oleic acid 20.53%, whereas, mango kernel oil was accounted for 45.17% oleic acid (Table 2). Concentration of linoleic acid decreased from 50.78% to 30.17% when 40% mango kernel oil was added to watermelon oil. Oleic acid increased from 22.89% in watermelon oil to 25.19%, 28.84% and 30.64% in B_1, B_2, B_3 and B_4. The contribution of fatty acids in process of auto-oxidation of is 24%, whereas the contribution of tocopherols in the inhibition of lipid peroxidation phenomenon was lesser than those contributed by the fatty acid profile [19]. Mango kernel oil is superior to other oils in terms of better oxidative stability which can be explained by the higher number of monounsaturated fatty acids and phenolic compounds, which can act as inhibitors of free radical mechanism [7]. Characterization of mango kernel oil revealed that oleic and stearic acid was the major fatty acids [20].Fatty acid composition of watermelon oil reveals its great perspective as a source of edible oil [4].

Peroxide value

The results of peroxide value of watermelon oil and mango kernel oil blends are presented in (Table 3). Blending of mango kernel oil in watermelon oil had a great effect on peroxide value of all the blends, oxidative stability of blends increased with progressive increments of mango kernel oil. The increase in peroxide value of watermelon oil, B_1, B_2, B_3 and B_4 was 10.07, 9.56, 7.62, 5.17 and 2.87 ($meqO_2/kg$) in a time dependent manner. The increase in generation of primary oxidation products during ambient storage period was dependent upon fatty acid profile and phenolic compounds present in mango kernel oil. The great contribution of fatty acid profile in auto-oxidation process in well established, however, the role of tocopherols as antioxidants is less important than fatty acid composition of fats and oils. [21] reported that the peroxide value in mango kernel oil can be used in industries without processing because of lower peroxide value. Peroxide value reported in this study was considerably less than reported during the storage stability of canola oil [22]. Addition of mango kernel oil in tallow significantly inhibited the auto-oxidation [23]. The peroxide value of blend of butter oil and Moringa oleifera oil was less than pure butter oil [24]. The generation of oxidation products must be controlled, 100 ($meqO_2/kg$) in oxidized fats and oils can have a neurotoxic effects [25] Described that total phenolic content of mango kernel oil was 9.87 mg/g. The superb oxidative stability of mango kernel oil describes its huge potential as a commercial source of edible oil [26]. [27] Stabilization of tallow was achieved by blending with mango kernel oil, addition of mango kernel oil at 3% concentration considerably inhibited the oxidative breakdown in tallow.

Conjugated dienes and trienes (CD and CT)

The results of specific extinctions recorded at 232 and 268 nm are given in (Table 4 and 5). The concentration of CD and CT went on boosting throughout the storage period of three months, the increase in extent of CD and CT during the storage period was significantly different in watermelon oil, mango kernel oil and their blends. The progressive addition of mango kernel oil inhibited the generation of oxidation products, the coefficient of correlation was 0.94. The CD value of three months stored water melon seed oil, B_1, B_2, B_3 and B_4 was 7.91, 6.18, 3.07 and 2.43, respectively. Similarly, the inhibition of CT during the storage period was in the order of B_4> B3 > B_2> B_1 and watermelon oil. The great inhibition of oxidation products during the storage period could be correlated to considerable modification in fatty acid composition as function of blending of mango kernel oil. Generation of aldehydes of short and medium length are usually associated with rancid and objectionable flavours. The potential role of oxidation products in the development of nutrition related health disparities has been well understood due to their perceived role in carcinogenicity and heart diseases. According to the cited literature, potential inhibition of oxidation products in this study could also have a health benefits that can enhance the suitability of watermelon oil as a source of edible oil. HPLC characterization of mango kernel oil showed that the occurrence of tannin and vanillin, gaillic acid, cumarin, caffeic acid, mangiferrin, ferrolic acid and cinamic acid [28].

α and δ tocopherol content of watermelon oil

The results of α and δ tocopherol content of mango kernel oil and their blends is presented in (Table 6). The α tocopherol content of watermelon oil, mango kernel oil, B_1, B_2, B_3 and B_4 was 127.49, 205.44, 135.24, 144.52, 156.81 and 169.34 mg/kg. δ tocopherol inwatermelon oil, mango kernel oil, B_1, B_2, B_3 and B_4 was 55.26, 34.81, 53.64, 51.27, 50.14 and 48.23 mg/kg. Among tocopherols, δ tocopherol possesses the highest antioxidant activity; even then watermelon oil is susceptible to auto-oxidation. Two factors are primarily involved in auto-oxidation of fats and oils, fatty acid composition and presence of antioxidant substances, the role of fatty acid profile is even more important than tocopherols [19]. Existence of higher extents of tocopherols in mango kernel oil is already reported in literature [6].

Induction period

The results of oxidative stability indicated an overall increase in induction period of blends as function of mango kernel oilin a dose dependent manner (Fig. 1). Mango kernel oil registered a highest induction period, followed by B_4, B_3, B_2, B_1 and watermelon oil. Watermelon oil showed a distinctly lower induction period as compared to blends. The induction period of crude mango kernel oil in three varieties ranged from 58.8-85.2 hours [29]. Crude mango kernel oil can be used as a great source of antioxidants for the preservation of fats and oils [30]. Has reported the strong antioxidant activity of mango kernel oil. The mangiferrin and xanthon-C-glycoside, tocopherols have been isolated from mango kernel oil, the anti-oxidative characteristics of these phenolic compounds is scientifically proven [14]. The catechin mixture of mango kernel oil had a greater antioxidant capacity than BHT [27] Addition of mango kernel oil at 1% concentration, significantly inhibited the lipid peroxidation in sunflower oil, the inhibition was even greater than positive control (200-ppm BHT) [31].

***Table 3.* Peroxide value of watermelon oil, mango kernel oil and their blends.**

Treatments	0-Day	30-Days	60-Days	90-Days	Increase*
WSO	1.31±0.06n	3.45±0.11h	8.62±0.27c	11.38±0.44a	10.07
MKO	0.95±0.09p	1.36±013	2.15±0.08k	3.24±0.11i	2.29
B_1	1.24±0.10n	3.14±0.19i	6.95±0.22d	10.74±0.31b	9.56
B_2	1.25±0.08n	2.85±0.10j	4.83±0.13f	8.77±0.48c	7.62
B_3	1.10±0. 14o	2.19±0.15l	3.55±0.05h	6.27±0.35e	5.17
B_4	1.08±0.12o	1.84±0.13m	2.64±0.09j	3.95±0.37g	2.87

Means represented by a different letter in a rows and columns are not similar (P<0.05)
*Increase: increase from initial value
Refer Table-1 for the detail of treatments

***Table 4.* Conjugated dienes of watermelon oil, mango kernel oil and their blends.**

Treatments	0-Day	30-Days	60-Days	90-Days	Increase*
WSO	0.27±0.02n	2.21±0.07h	4.79±0.09d	8.18±0.03a	7.91
MKO	0.18±0.04n	0.62±0.04	1.19±0.09l	1.84±0.16i	1.66
B_1	0.25±0.03n	1.92±0.09j	4.31±0.04e	7.79±0.22b	7.54
B_2	0.25±0.02n	1.72±0.015k	3.24±0.06f	6.43±0.15c	6.18
B_3	0.22±0.01n	1.14±0.11l	2.77±0.08g	3.29±0.18f	3.07
B_4	0.20±0.05n	0.95±0.06m	1.79±0.12k	2.63±0.09g	2.43

Means represented by a different letter in a rows and columns are not similar (P<0.05)
*Increase: increase from initial value
Refer Table-1 for the detail of treatments

***Table 5.* Conjugated trienes of watermelon oil, mango kernel oil and their blends.**

Treatments	0-Day	30-Days	60-Days	90-Days	Increase*
WSO	0.09±0.02lm	0.91±0.07h	2.17±0.014e	5.76±0.13a	5.67
MKO	0.04±0.01lm	0.15±0.03l	0.42±0.04j	0.79±0.12h	0.75
B_1	0.08±0.01lm	0.75±0.04i	2.21±0.09e	4.54±0.23b	4.46
B_2	0.06±0.01lm	0.67±0.11i	1.78±0.17f	3.64±0.29c	3.58
B_3	0.05±0.01lm	0.45±0.03j	1.15±0.06g	2.79±0.15d	2.74
B_4	0.05±0.01lm	0.24±0.02k	0.85±0.16h	1.44±0.10	1.39

Means represented by a different letter in a rows and columns are not similar (P<0.05)
*Increase: increase from initial value
Refer Table-1 for the detail of treatments

Table 6. Induction period and tocopherol content of watermelon oil, mango kernel oil and their blends.

Parameters	WSO	MKO	B_1	B_2	B_3	B_4
IP	4.52±0.16e	19.27±0.27a	6.77±0.31e	8.47±0.12d	9.12±0.45c	10.95±0.14b
α tocopherol	127.49±2.56f	205.44±6.73a	135.24±1.37e	144.52±2.66d	156.81±0.95c	169.34±2.27b
δ tocopherol	55.26±1.34a	34.81±0.16c	53.64±1.16b	51.27±0.76b	50.14±1.63b	48.23±1.44b

Means represented by a different letter in a row are not similar
IP: Induction Period
B_4

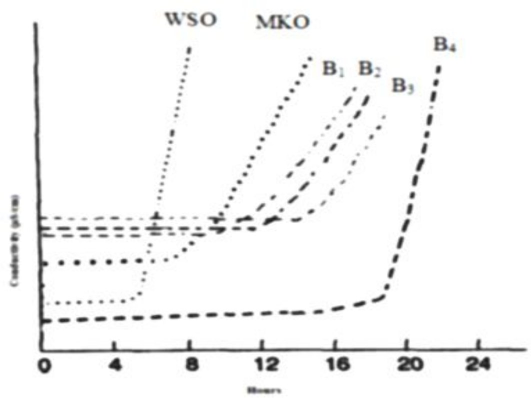

Figure 1. **Rancimat Plot of WSO. MKO and Their blends**

Conclusions

Supplementation of watermelon oil with mango kernel oil had a great effect on the modification of fatty acid composition from linoleic acid to oleic acid. Oxidation in long terms storage and accelerated oxidation conditions indicated that that the blends of watermelon oil and mango kernel oil had lower peroxide value with greater induction period and tocopherol content.

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