Physicochemical analysis and oil extraction yield of moringa (Moringa oleifera) seed
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ABSTRACT
Moringa oleifera (Moringaceae) is fast growing, drought resistant tropical tree mostly found in Asian countries. Moringa is known as miracle plant due to its numerous health benefits and biological properties. Seeds of M. oleifera contain up to 35-40% of nondrying and pale yellow oil that is being utilized for illuminations because it burns without producing smoke. The current research was aimed to investigate extracted oil percentage and physicochemical characteristics of moringa seed oil. Proximate analysis of moringa seed powder was carried out and resulted that it has moisture 6.64±0.343%, crude protein 30.60±1.105 %, crude fat 24.06±1.520, crude fiber 7.07±0.367, ash 3.61±0.046 and nitrogen free extract 28.00±1.41% Mineral profiling of moringa seed powder was done and calcium was found in higher amount of 356.67 ±15.53 mg/g than all other minerals. Concentration of magnesium was more prominent followed by calcium which was 285.71± 3.51 mg/g. Moringa oil was extracted by solvent and cold press extraction technique and oil percentage of solvent extracted was 24.06 % as compared to mechanical extracted which was 22.47%. Moringa oil extracted by the solvent extraction showed that it contained 0.026±0.005 % moisture, 184.473±0.567 mgKOH/g saponification value which is greater than the value obtained by the solvent extraction.

Keywords: Physicochemical, Cold press extraction, Solvent extraction, Proximate

1. INTRODUCTION
Moringa oleifera is one of important vegetable crops belonging to family moringaceae and order Brassica. Leaves, roots, flowers, pods containing seeds of moringa can be used as food and feed (Leone et al., 2015). Moringa is said to be originated from famous Indian cities like Oudh and Agra, South region of Himalayan mountain belt and Indian northwest region. Moringa tree has been introduced in almost 60 countries around the globe including Mexico, Argentina, Algeria, and Brazil (Boukandoul et al., 2017). Seeds of moringa are triangular shaped to round packed naturally within light wooded thick shell with papery wings. The moringa flowers are white to creamy white with seeds in green colour, cylindrical and about 60-70 cm long pods (Bhargave et al., 2015). It can grow in all soil types except heavy clay, sandy, stiff and it has less capability to tolerate frequently flooding and hard water (Stadtlande and Becker, 2017). Moringa oleifera is categorized due to its drumstick shaped elongated pods and among its parts, leaves are especially used in food and feed because it contains sufficient amount of essential amino acids, anti-oxidative components, β-carotene and nonorganic compounds (Olayemiv et al., 2016; Leone et al., 2016). Moringa leaves contains sufficient amount of Vitamin C, E and A. Copper, potassium, manganese, calcium, iron, phenolic compounds and proteins are found in leaves of moringa plant in significant amount (Hekmat et al., 2015). In Pakistan, moringa has been reported to be grown in province Sindh and other irrigated...
areas like Khyber Pakhtunkhwa. *Moringa oleifera* is considered as a non-wood forest plant in Pakistan. Inflorescences of moringa are movable panicles formed in drooping 10-25 cm lengthy. Moringa flowers are bisexual zygomorphic, cream white to white in color, up to 12mm long having 5 white petals, 5 green sepals and 5 stamens with and without anthers and staminoid, respectively (Heuzé, 2016). In Pakistan, annual production of edible oil is around 3 million tons and annual export is around 6.6 million tons to fulfill increasing needs of society. As edible oils have become necessity more than luxury, its consumption has increased from 2.6 to 3.0 million tons. Pakistan imports around 1.8 to 8.0 million tons of edible oil while its production status is around 0.7 million tons. Moringa is called as “miracle tree” because of its healing property for injury and also give protection from some chronic diseases (Bhargave et al., 2015). Stearic, behenic and palmitic acid are found in appropriate amount as 7.6%, 6.2%, and 7.8%, respectively in moringa oil. Fatty acids composition of moringa oil shows high level of oleic acid contents that proves its resemblance with olive oil (Rehman et al., 2014). Moringa oil is mainly composed of mono-unsaturated fatty acids including oleic acids, eicosanoid acids and palmitoleic. Saturated fatty acids are found in less quantity while reduced amounts of behenic acids, arachidic and palmitic acids and just traces of polyunsaturated fatty acids are found in moringa oil (Salaheldeen et al., 2014). *M. oleifera* contains oleic acid ranging from 66.5-81.7%, while *M. peregrina* has 71.1-77 % and in cultivar of *M. stenopetala*, oleic acid is found at range of 63-76%. Moringa species contains behenic acids in which *M. oleifera* contains 2.9 to 8.13%, *M. peregrina* contains 2.7-7.8% and 5.6-6.1% behenic acid is found in *M. stenopetala* (Melaku et al., 2017). Oil extraction is generally obtained by the method of extraction using hexane. Cold press method has least recovery of oil as compared to solvent extraction, While acidity and viscosity of oil obtained by cold press is high than solvent extraction (Ogunsina et al., 2014). It is too supportive in reducing cholesterol levels and increases wound healing process (Bollinger, 2016). Moringa seed oil is being used in traditional Ayurvedic medicines for thousands of years to cure many diseases including hypertension and arthritis (Aviara et al., 2015). Potential antitumor promoters have been found in various bioactive compounds obtained from moringa seeds. However, in various cancerous cell lines Moringa oil can be affected by cytotoxic observation by recent study (Elsayed et al., 2015). *M. oleifera*, *M. stenopetala* and *M. peregrina* are being moringa species and have been proved to interesting alternatives because of high percentage of oil, oxidative stability and ease in production (Fernandes et al., 2015).

1.1. Objectives
By keeping in view the importance of moringa oil the current study was planned to attain following objectives:
- To extract the moringa seed oil and evaluate its yield
- To assess the physicochemical characteristics of moringa oil

2. MATERIALS AND METHODS

2.1. Procurement of material
All the chemicals and reagents used in the analysis were made available in the Department of Food Science and Technology as well as central laboratory of MNS-University of Agriculture, Multan. The seeds of moringa were obtained from obtained from Hafeez ghee and general Mills, Multan and also collected from the local plantation in adjacent areas of university. These seeds were cleaned, washed and sun dried for further usage.

2.2. Preparation of moringa kernel powder
The procured *Moringa oleifera* seeds were sundried and grinded to produce seed powder and kept at room temperature until further use
2.3. Proximate analysis of moringa seed powder

2.3.1. Moisture content

The weighed amount of 2g sample was taken in the pre washed china dish and placed in hot air oven at 105±2°C for 18-24 hours followed by method 930.15 (AOAC, 2005). The moisture % of the moringa powder was determined using the formula as:

\[
\text{Moisture %} = \frac{\text{Weight of sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of sample}} \times 100
\]

2.3.2. Crude protein

Concentration of crude protein content was obtained by using Kjeldahl method following the method 992.23 recommend by (AOAC, 2005). 2g sample was digested in Kjeldahl tube containing Conc. H_2SO_4 and digestion mixture. The digestion sample was carried out for distillation by using 40% NaOH and 4% boric acid solution and then was titrated with 0.01 N H_2SO_4 using methyl red as indicator till end point reached. The percentage of nitrogen (N) was determined using the formula:

\[
\text{N (\%)} = \frac{\text{Volume of 0.1 N H2SO4 used}}{\text{Volume of the diluted sample (ml)}} \times 0.0014 \times \frac{\text{Volume of the diluted sample used for the distillation}}{\text{Weight of sample}} \times 100
\]

\[
\% \text{ age of crude protein} = \text{N \%} \times 6.25
\]

2.3.3. Crude fat

Fat determination of moringa seed powder was assessed by using the Soxhlet assembly subsequent using n-hexane as solvent by method 948.22 (AOAC, 2005). Percentage of crude fat was measured by using following equation:

\[
\text{Crude fat (\%)} = \frac{\text{Weight of fat (g)}}{\text{Weight of the sample (g)}} \times 100
\]

2.3.4. Crude fiber

For determination of crude fiber contents sample of the moringa seed powder was digested in 1.25% H_2SO_4 for about 30 min and then after washing out digestion was done with 1.25% NaOH for 30 min. The sample was then transferred in the muffle furnace after oven drying by method 991.43 (AOAC, 2005).

\[
\text{Crude fiber (\%)} = \frac{\text{Weight of the dried residue (g)} - \text{Weight of ash (g)}}{\text{Weight of sample}} \times 10
\]

2.3.5. Ash content

Percentage of ash was obtained by the burning the sample at 600 °C using Muffle furnace according to the approved method 942.05 (AOAC, 2005). The % age of ash was found using the formula:

\[
\text{Crude ash (\%)} = \frac{\text{Weight of sample after ashing}}{\text{Weight of the sample (g)}} \times 100
\]

2.3.6. Nitrogen free extract (NFE)

NFE was determined by using this formula:

\[
\text{NFE} = 100 - (\text{moisture \%} + \text{crude protein \%} + \text{crude fat \%} + \text{crude fiber \%} + \text{ash \%})
\]

2.3.7. Mineral profiling of moringa seed

The mineral composition like Mg, Ca, Fe, Mn and Cu of moringa seed will be estimated by using flame photometer and atomic absorption spectrophotometer according by protocols followed by AOAC (2005) method.

2.3.8. Oil extraction

The moringa seed was subjected to oil extraction using two different methods (solvent and mechanical extraction) according to their respective protocols using hexane as a solvent and cold press extraction machine, respectively. The recovered oil was assessed using the formula:

\[
\text{Moringa seed oil yield (\%)} = \frac{\text{Weight of oil (g)}}{\text{Weight of moringa seed (g)}} \times 100
\]

2.4. Physicochemical analysis of moringa seed oil

2.4.1. Determination of free fatty acid

The Moringa seed oil was assessed for free fatty acids composition by following the instructions as described in AOCS (2009). 5 g of moringa oil was added into 50 ml of solvent (Ethanol) in conical flask. Phenolphthalein indicator was added in 3-4 drops. The sample was then subjected to titration against 0.1 KOH and continuously shaking till pink color end point. Free fatty acid was determined using the formula:

\[
\text{FFA (\%)} = \frac{V \times N \times 28.2}{W} \times 100
\]

\[
V= \text{Volume of the titrant} \quad N= \text{Normality solution} \quad W= \text{weight of the sample (gm)}
\]
2.4.2. Determination of peroxide value

Peroxide value of Moringa seed oil was determined by following the approved method (AOCS, 2009). The oil sample 5g was added into conical flask with 30ml POV solution and also added 0.5ml potassium iodide solution. The conical flask was placed in dark area. After that 30ml distilled water was added in conical flask and was titrated with 0.01N solution of sodium thiosulphate till colorless end point was reached by using the starch solution as indicator. Peroxide value was calculated by the formula:

$$\text{Peroxide value} = \frac{(S - B) \times N}{W} \times 1000$$

S= titration sample  B= blank of titration reading  N= sodium thiosulphate normality

2.4.3. Determination of saponification value

Moringa seed oil was subjected to determination of saponification value according to protocol (AOCS 2009). The oil sample 2.0g was added in the conical flasks containing 0.5N ethanolic potassium hydroxide. The flask was run on the reflux condenser for 45 min with random shaking. After that the content was titrated with 0.5N H$_2$SO$_4$ in which phenolphthalein was used as indicator. A blank was undergone through the same treatment and value was calculated using the following formula:

$$\text{Saponification value} = \frac{(B - S) \times N \times 56.1}{W}$$

B= blank sample reading  S= sample of titration reading  N= HCl normality

2.4.4. Statistical analysis

The resulted data obtained from research was investigated statistically in triplicates by a software named Statistix 8.1 using two way ANOVA technique. (Montgomery, 2008).

3. Results and discussions

3.1. Proximate analysis of moringa seed powder

Proximate analysis of moringa seed powder was carried out and resulted that it has moisture 6.64±0.343%, crude protein 30.60±1.105 %, crude fat 24.06±1.520 %, crude fiber 7.07±0.367 %, ash 3.61±0.046 % and nitrogen free extract 28.00±1.41% which are shown in table 1. These values showed high oil contents (24.06±1.520 %), in the seed which was a good indicator for moringa seed oil and this property of moringa seed oil is suitable to its use as an alternative and proficient source of edible oil. The outcomes are comparable in accordance to (Waheed et al., 2010) as well as (Hussein et al.2011) with minute changes which is due to variations in variety and climatic factors. Results of moringa seed powder proximate analysis are given in table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Content (%)</th>
</tr>
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<tbody>
<tr>
<td>Moisture</td>
<td>6.64±0.34</td>
</tr>
<tr>
<td>Ash</td>
<td>3.61±0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>24.06±1.52</td>
</tr>
<tr>
<td>Fiber</td>
<td>7.07±0.36</td>
</tr>
<tr>
<td>Protein</td>
<td>30.62±0.87</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>28.00±1.41</td>
</tr>
</tbody>
</table>

Table 1. Moringa (Moringa oleifera) seed powder proximate analysis

3.2. Oil Extraction

Moringa (Moringa oleifera) seed oil was subjected to extraction by solvent (S.E) extraction and mechanical extraction (M.E). The total yield of oil obtained by solvent extraction and mechanical extraction techniques were 24.06±1.520 % and 22.47±0.058 %, respectively. Oil percentage of solvent extraction is high due to the use of an organic solvent that has better potential to extract oil as compared to mechanical extraction in which oil loss is occurred due to the wastage during extraction and sufficient remained in the equipment. Moringa oil percentage indicated by (Abdulkarim et al., 2005) and (Ogunsina et al., 2014) was 40 % and 36.7 %, respectively and this change in the oil percentage was due to changes in environment, variety and cultivar difference or soil condition. The results of oil extraction yield are given in table 2.
Physicochemical analysis of moringa seed oil obtained by solvent extraction showed that it contained 0.033±0.005% moisture, 183.72±5.073 mg KOH/g saponification value which is less than the value obtained by the mechanical extraction. Peroxide value of moringa oil obtained by solvent extraction was 1.11±0.25 Mg KOH/g. Melting point was observed 24.66±1.154 ºC and free fatty acids contents were 3.55±0.335%. Specific gravity and refractive index of moringa oil obtained by solvent extraction were 0.876±0.02 and 1.46±0.005%. Color is pale yellow for S.E moringa oil which is quite similar with the moringa oil extracted by M.E.

Moringa oil extracted by the solvent extraction showed that it contained 0.026±0.005% moisture, 184.47±0.567 mg KOH/g saponification value which is greater than the value obtained by the solvent extraction. Peroxide value of moringa oil obtained by mechanical extraction was 1.06±0.23 Mg KOH/g. Melting point was 25.66±0.577 ºC. Specific gravity of oil was 0.88±0.15 while the free fatty acids contents were 2.25±0.015 %. Color was pale brown for M.E moringa oil which is quite similar with the oil extracted by S.E.

Rancidity was not detected in both types of oils that are the best sign of its suitability to be used as a healthy alternative of conventional shortenings. The results of physicochemical analysis of oil are given table 3.

### Table 2. Oil extraction yield (%) of moringa seed

<table>
<thead>
<tr>
<th>Method</th>
<th>Solvent Extraction (S.E)</th>
<th>Mechanical Extraction (M.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24.06±1.520 a</td>
<td>22.47±0.058 b</td>
</tr>
</tbody>
</table>

Mean±SD

### 3.3. Physicochemical analysis of moringa Seed oil

Minerals are inorganic chemical components that are present in all the cells, fluids and tissues of human body and their existence is compulsory for the normal ongoing processes of life. Main requirements of these minerals in our body include monitoring the body fluids outside and inside of tissues and cells, building the teeth and strong bones and changing the food into the energy. Concentrations of various minerals including sodium, calcium, magnesium, zinc and iron found in the moringa seed powder are shown in the following figure 1. Calcium (Ca) was found in higher amount of 356.67 ±15.53 mg/g than all other minerals. Concentration of magnesium (Mg) was more prominent followed by calcium (Ca) which was 285.71 ± 3.51 mg/g. Calcium is necessarily required for the teeth and bones in combination with magnesium. Minimum amount of copper was present in seed which was 1.99 ±0.015 mg/g. Amount of Iron (Fe) was 10.99 ±0.49 mg/g while amount of manganese (Mn) was 5.96 ± 0.48 mg/g which are higher than amount of copper while cadmium was not detected in the Moringa seed powder. The obtained results of minerals present in moringa seed powder was similar as described by (Aja et al., 2013)
with the slight modification. This minute change in the mineral contents was due to changes in environment, variety and cultivar difference or the condition and chemistry of the soil in which moringa plant is grown.

Figure 1. Mineral profiling (mg/g) of moringa seed powder

4. CONCLUSION
Moringa oleifera acquires distinctive position in terms of its quality attributes, health benefits and cost effective production technology. The research findings of the present study revealed that moringa seeds have good nutritional profile and contain sufficient quantity of valuable oil percentage that has a good nutritional profile and great amount of unsaturated fatty acids which can be used to fulfil the valuable food processing needs in near future. It has been found to be an excellent alternative of daily used shortenings and fats in near future due to its dense nutritional profile.

5. ACKNOWLEDGEMENT
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6. REFERENCES
AOAC. 2005, Official Methods of Analysis (18th Edn.). Association of Official Analytical Chemists International, Maryland, USA.
AOCS. 2009. Official methods and recommended practices of the AOCS, 6th edn. American Oil Chemists’ Society, Champaign, IL.
Elsayed, E.A., M.A. Sharaf-Eldin and M.


