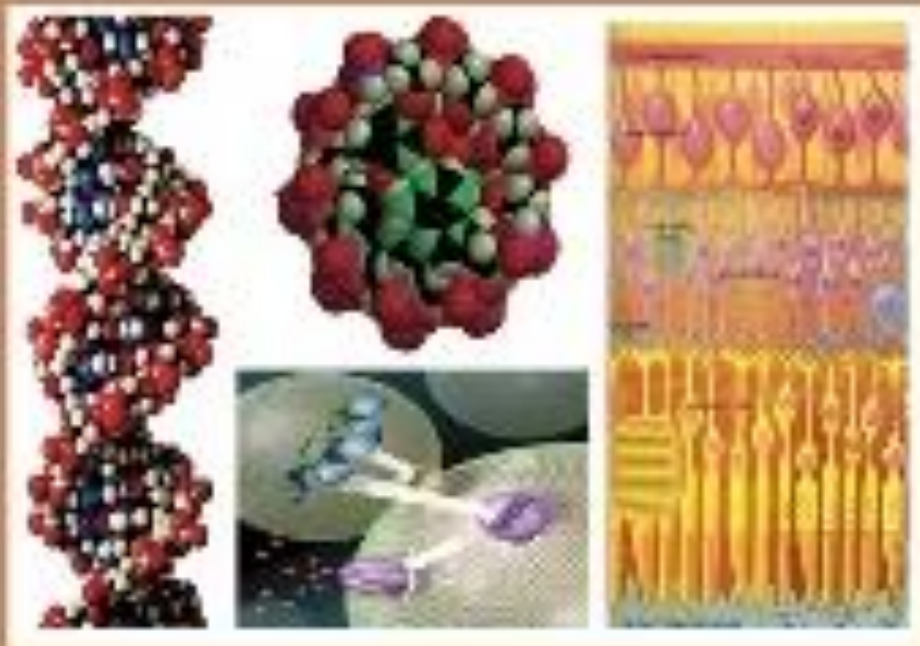




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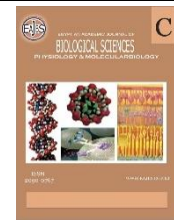
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Physicochemical Properties and Fatty Acids Composition of Moringa Seed Oil Compared with Olive Oil

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ABSTRACT

The study aimed to examine the physicochemical properties of moringa seeds and assess the oil extracted from the seeds. The seeds were collected from Eldamazine in the Blue Nile state and Alsamrab north of Khartoum. The oil extracted using hexane, the color of oil examined using a Lovibond tintometer, showed red and yellow colors (0.02, 3.9) for moringa and (2.3, 31.9) for olive oil respectively. Viscosity, density, and refractive index are (23.81, 0.90, 1.472 cp) for moringa oil and (73.5, 0.91, 1.551 cp) for olive oil, respectively. Chemical characteristics of moringa and olive are free fatty acid, peroxide value, iodine value, and saponification value (0.19%, 0.49 mg O₂/kg/oil 63.5 mg 1/kg/oil, 81.55 mg/kg/oil, 172 mg KOH/kg oil). GLC was used to detect the fatty acid composition of moringa oil which are Palmitoleic acid (0.1860%), Palmitic acid (3.248%), Linoleic acid (0.0690%), Oleic acid (71.57%), Linolenic acid (6.725%), Stearic acid (7.127%), Arachidic acid (0.9820%), Gadoleic acid (3.842%), Behnic acid (5.973 %) and Arachidonic acid (0.2320 %). Moringa oil contained a high content of tocopherol (257 mg liter).

INTRODUCTION

Moringa oleifera is a fairly large tree native to north India. It goes by a variety of names, such as drumstick tree, horseradish tree, or ben oil tree. Currently, the distribution of Moringa oleifera is cosmopolitan, mainly found in the tropical and subtropical regions (Ayerza., 2019). The leaves, seeds, bark, roots, sap, and flowers of Moringa oleifera are widely used in traditional medicine, also their leaves and immature seed pods are used as a food product in human nutrition. The leaf extracts exhibit high antioxidant activity, and various safety studies in animals involving aqueous leaf extracts indicate a high degree of safety. No adverse effects were reported in association with human studies (Arnarson A., 2018). Moringa provides a rich and rare combination of nutrients, amino acids, antioxidants, and anti-aging, and anti-inflammatory properties used for nutrition and healing processes. Moringa oil has been used in the industry for the manufacture of food and bio-lubricants and has the potential for the production of biodiesel (Liu *et al.*, 2018).

Moringa is sometimes called "Mother's Best Friend" and Miracle Tree." Since 1998, the World Health Organization (WHO) has promoted Moringa as an alternative to imported food supplies to treat malnutrition (Johnson., 2005; Manzoor et al., 2007; Sreelatha and Padma., 2009). Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids, and fibers (Gafar and Itodo., 2011). Plant oil from seeds and leaves are in high demand for their medicinal value. The seed oil contains all the fatty acids found in olive oil, except for linolenic acid (Abdul Karim., 2011). The use of oil extracted from mature seeds by ancient Egyptians has been well documented. the oil was treasured by the Egyptians for skin protection against both infections and damage due to extremes of desert conditions. The benefits of the healthful attributes of the oil were later relayed to the ancient Greeks and Romans, who also used the oil for skin protection. The light and non-drying nature of the oil makes it a good massage oil, and also for aromatherapy applications. due to its tremendous cosmetic value, it was used extensively by the ancient Egyptians in body and hair care, as a moisturizer and conditioner. It was also used by ancient Egyptians, Greeks, and Romans in extracting floral fragrances used in perfumes, the oil has an excellent ability to retain fragrances extracted from flowers (Hasanah and Abdul Karim., 2011).

The Moringa seed oil is high in polyunsaturated fatty acid (80.4%) content (Ogbunugafor *et al.*, 2011), is a liquid at room temperature and is pale yellow in color. The oil extracted from Moringa seeds is rich in monounsaturated fatty acids, with greater than 70% oleic acid and 4.2% linoleic acid, presents high oxidative stability, and is similar to olive oil (Ayerza, 2019). The electronic nose analysis showed that the unrefined oil has a flavor similar to that of peanut oil, the melting point, estimated by differential scanning calorimetry, was found to be 19.0 °C (Abdulkarim et al., 2005). The chemical composition of the oil, with high

nutritional values associated with benefits to human health, by reducing the risk of cardiovascular diseases and lowering blood sugar levels, makes Moringa oil a nutraceutical food (Seifu and Teketay, 2020, Zhong *et al.*, 2018). Additionally, Moringa esters have the potential used as lubricants or as an ecological additive (Moreira *et al.*, 2020).

Mainly this work aimed to study the proximate composition of moringa seeds, physicochemical properties, and fatty acids profiles of moringa seed oil.

MATERIALS AND METHODS

The moringa seeds were obtained from the Aldamzine and Khartoum north areas. The seeds were cleaned by removing foreign particles and kept at room temperature in polyethylene bags for further analysis. Olive oil is brought from the local Market. The chemicals and reagents used are analytical grades and were purchased from the Lab. Line. Company, Khartoum Sudan.

1-Weight of A Hundred Seeds:

The weight of a hundred seeds was determined according to the Association of Official Analytical Chemists (AOAC., 1984) in which 100 seeds of moringa seeds were taken and weighed. The test was repeated three times and the values were recorded.

2. Mechanical Extraction of Moringa Oil (mechanical pressing):

The extraction of the oil from moringa seeds was done in the manner described by Balla (2001) with minor modifications. About one Kilogram of seeds was weighed after the removal of impurities, by using mortar, the size of the seeds was reduced to increase the surface area for oil extraction. The sample was transferred to a cloth bag, and then the oil was extracted from the seeds by using a cold press.

3. Physical Analysis of Moringa Oil:

3.1.Refractive Index:

The refractive index (RI) was determined by the Abbe 60 Refractometer as described by the AOAC method (1990). The double prism was opened using a screw head, and a few drops of oil were placed in a prism.

The prism was closed firmly by tightening the screw head and the instrument was then left to stand for a few minutes before reading to equilibrate the sample's temperature with that of the instrument ($32 \pm 2^{\circ}\text{C}$ $32 \pm 2^{\circ}\text{C}$). The prisms were cleaned between readings by wiping off the oil with a soft cloth, then with petroleum ether, and left to dry, and the test was repeated three times.

3.2. Density:

The oil density was determined according to the AOAC (1990) methods, using a psycho-meter. An empty Stopped psycho-meter was weighed, and the psycho-meter was filled with water and kept at a constant temperature of 25°C in a water bath for 30 minutes. The weight of water at 25°C was determined by subtracting the weight of an empty psycho-meter from its weight filled with water. At the end of time, the Stopped psycho-meter was adjusted to the proper level, dried with a cloth, and weighted. In the same manner, the weight of the oil at 25°C was determined. The density was calculated as follows:

The density at $^{\circ}\text{C} = \text{W}/\text{W1}$

Where:

W = weight of oil at 25°C

W1 = weight of water at 25°C

3.3. Viscosity:

The viscosity of the oil samples was detected by using an Ostwald-U-tube viscometer according to the Cocks and Van Rede (1966). The viscometer was suspended in a constant temperature bath ($32 \pm 32 \pm 2^{\circ}\text{C}$), using the pressure on the respective arm of the tube, the oil was moved into the other arm so that the meniscus was 1cm above the mark at the top of the upper reservoir. The liquid was then allowed to flow freely through the tube and the time required for the meniscus to pass from the mark above the upper reservoir to that at the bottom of the upper reservoir was recorded.

Calculation:

The viscosity of the oil = $(T - T_0)/T_0$

Where:

T: flow- time of the oil

T₀: flow- time of the distilled water

3.4. Colour:

The color intensity of oil was recorded by using a Lovibond Tintometer as units of red, yellow, and blue, in the manner described by Balla (2001). The oil sample was filtered through a filter paper immediately before testing. An appropriate 5.25 inches was filled with oil and placed in the tintometer in a specific place. The instrument was switched on and looked through the eyepiece. Then slides were adjusted until a color match was obtained. The values obtained by matching were recorded as red, yellow, and blue color.

4. Chemical Analysis of Moringa Oil:

4.1 Peroxide Value:

The peroxide value (PV) of the oil samples was determined according to the AOAC method (2000). 5gm ($\pm 5gm$ $\pm 5gm$) of the samples were weighed into a 250ml stoppered conical flask, and 30 ml of acetic acid and chloroform solvent mixtures were added and swirled to dissolve. A 0.5 ml saturated potassium iodide solution was added with a Mohr pipette and stood for 1 minute in the dark with occasional shaking, and then about 30 ml of water was added. Slowly the liberated iodine was titrated with 0.1 N sodium thiosulphate solutions, with vigorous shaking until the yellow color almost disappeared. About 0.5 ml starch solution as an indicator was added and titration continued with vigorous shaking to release all Iodine gas from the CHCL layer until the blue color disappeared. If less than 0.5 ml of 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ was used 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$ was repeated. Blank determination (must be less than 0.1 ml 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$) was conducted. Peroxide value expressed as mille equivalent of peroxide oxygen per kg sample (Meq per Kg oil).

Calculation:

$$\text{Peroxide Value} = \frac{\text{Titre} \times N \times 1000}{\text{Weight of the sample used}}$$

Where:

Titer = ml of sodium Thiosulphate used (blank corrected)

N = Normality of sodium thiosulphate solution

4.2 Free Fatty Acid:

Free fatty acids were determined according to the AOAC method (2000). About 5 to 10 g of cooled oil sample was weighed in a 250 ml conical flask, 50 ml to 100 ml of freshly neutralized hot ethyl alcohol was added and about 1 ml of phenolphthalein indicator solution, the mixture was warmed

$$\text{Free fatty acids as oleic acid percent by weight} = \frac{28.2 \times V \times N}{W}$$

Calculation:

Where:

V = Volume in ml of standard sodium hydroxide used.

N = Normality of the sodium hydroxide solution.

W = weight in g of the sample.

4.3 Iodine Value:

The iodine value of the oils, which quantifies their unsaturation level, was determined according to the AOAC method (2000). Approximately, 0.2 gm of oil was accurately weighed and placed in a dry and clean flask specially offered for the test. A 25 ml of pyridine sulfated dibromide solution was added to the content of the flask. The flask was then stopped and the mixture was allowed to stand for 10 minutes in a dark place. The stopper and the side of the flask were rinsed with enough amount of distilled water, the contents of the flask were then shaken and titrated against 0.1N sodium thiosulphate solution by using the starch liquid as an indicator. A blank determination was carried out simultaneously.

Calculation:

$$\text{Iodine value} = \frac{12.69(B - S)N}{W}$$

Where, **B** = volume in ml of standard sodium thiosulphate solution required for the blank

S = volume in ml of standard sodium thiosulphate solution required for the sample

N = normality of the standard sodium thiosulphate solution

W = weight in g of the sample

4.4. Saponification Value:

The determination of saponification value (SV) was carried out according to the AOAC method (2000). 2 gm of oil sample were weighed accurately into the 200 ml conical flask, 25 ml of 0.5 N alcoholic KOH

for about 5 minutes, and titrated while hot against standard alkali solution shaking vigorously during the titration. The weight of the oil was taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration must not exceed 10 ml.

solutions was added, and the content of the flask was boiled under reflux for 1 hr. with frequent rotation. 1 ml of phenolphthalein indicator was added, while the solution was still hot, and the excess alkali was titrated with 0.5 N HCL. The ml of HCL required (a) were noted. The same process was repeated without oil and the numbers of ml of the acid required (blank) were also recorded simultaneously.

Calculation:

$$SV = \frac{(B - A) \times 28.05}{S}$$

Where:

A: ml of HCL for sample

B: ml of HCL for blank

S: weight of oil (gm)

4.5. Saponification and Etherification of Moringa Oil Method Before Injecting Into the GLC:

Eight drops of moringa oil were transferred to a 25 ml volumetric flask, 5 ml 0.5 M methanolic NaOH was added and the flask was to stand in a water bath at 65 °C with stopper open. The content was cooled under cold water and then 7-8 ml methanolic BF₃ reagent was added. The flask was left to stand in the water bath at 65 °C for three minutes and then cooled under cold water. 1 ml of heptanes was added and shaken well. After that saturated NaCl solution was added until the heptane layer came to the top. A small amount of anhydrous Na₂SO₄ was sprinkled through the heptane layer to remove the rest of the water. The heptane layer was transferred to avail for injection into the GLC device.

4.6. Determination of Fatty Acids Composition in Moringa Oil:

The fatty acid composition of the oil was determined by gas chromatography

apparatus (Py E-UNICAM model GCD) according to the acid-catalyzed method lipid technology 2.42-49 1900) as follows:

1 ml of oil was taken in 100 ml round-bottomed flask 100 ml, 6 ml of 0.5ml methanolic was well shaken, 6 ml 1% methanolic Hs 50 was well shaken, and the mixture was left overnight at 50 °C, then 2ml Hexane was added and shaken, enough saturated sodium chloride was added to bring the level to the neck of the flask, 1ml of the upper layer was taken into Stopped tube and some anhydrous sodium sulfate was added to remove the moisture, then sample now was ready for injection in GLC. Exact 0.5 oil was injected in GLC with a conductivity detector. The area of each peak was calculated by the triangulation method, and the ratio of the constituents was determined by measuring the area of all peaks and the percentage represented by each.

4.7. Determination of Vitamin (E)

Content:

1 ml of oil was depressed with 1 ml of ethanol, then the solution was extracted with 1 ml of chloroform. The extract was shaken for 5 min. before centrifugation. The extracted layer was evaporated to dry under nitrogen. The dried extract was dissolved in 100 ml of methanol. All reconstituted antioxidants are mixed before injecting into the HPLC system (Carpenter., 1979).

5. Statistical Analysis:

The data were statistically analyzed by using the student's t-test and analysis of Variance (ANOVA). The means separation was assessed by Duncan's Multiple Range Test (DMRT) (Peterson., 1985).

RESULTS AND DISCUSSION

The Moringa oil produced in the dry season was submitted to the refining processes. After refining, a significant reduction (<39%) was observed in the initial volume of the oil. This loss is observed, as the refining process removes impurities (Pereira et al., 2015). It is important that degumming removes the phosphatides and proteins, and the neutralization process removes free fatty acids. According to Tsaknis (1998), after the degumming process, a light-yellow oil is

obtained at room temperature,

By removing the free fatty acids, the acidity decreases after the neutralization process (Sanchez-Machado et al., 2015). The cultures of Moringa under different environmental conditions and the chemical composition of the oil extracted from the seeds are decisive factors for choosing the Moringa (Moreira et al., 2020).

1. Physical properties of Moringa oil:

1.1. Density:

The density of moringa oil was 0.9081 g/ml which is slightly higher than the value reported by Tsaknis (1999) (0.88) and lower than the value reported by Salah (2006) (0.94) and Tsaknis & L alas (2002) (0.90). The result obtained showed that the density value of moringa seeds was lower than the density of olive oil in this study.

1.2. Viscosity:

The value of viscosity of moringa oil (23.81 centipoises) is very low compared to the value obtained by Tsaknis et al., (1999) (57 centipoises) and L alas & Tsaknis (2002) (45.5 centipoises) and Salah (2006) (45.8 centipoises) respectively. The value obtained is very low compared with the viscosity of the olive oil in this study (73.5 centipoise).

1.3. The Colour:

The moringa oil in this study showed a deep yellow-red color which is an indication of the presence of carotenoids. The degree of color of the moringa oil was shown in **Table (1)** (0.02) for the red color and 3.9 for the yellow color. The value reported for red color is lower than the value reported by (Salah., 2006) who reported 1.5, and also lower than the red value of olive oil in this study (2.3). The yellow color was 3.9 which is lower than the value reported by (Anwer And Rashid., 2007) who reported (7.12). The value is very low compared with the value of the yellow color of the olive oil (31.9).

2. Refractive Index:

In this study, the value of the refractive index is shown in Table (1). The refractive index of moringa seeds oil was found to be (1.472 at 40 C°), which agreed with the value reported by (Tsaknis et al., 1999) who reported (1.454 at 40 C°) and also

agreed with the value reported by (Anwar and Rashid., 2007) who reported (1.4608 at 40 C°), the result obtained was agreed with the refractive index of the olive oil (1.551) in this study.

Table 1: Physical properties of moringa and olive oil.

Parameters	Value	
	Moringa oil	Olive oil
Refractive Index	1.472 ^b	1.551 ^a
Relative Density/ 20C°	0.9081 ^a	0.91 ^a
Viscosity/ (Cent Poise)	23.81 ^b	73.5 ^a
Colour	Mean values	
Red	0.02 ^b	2.3 ^a
Yellow	3.9 ^b	31.9 ^a
Blue	0.0	0.0

* Each test was repeated three times and the average was calculated.

3. Peroxide Value:

The peroxide value is the most used method to determine the degree of oxidation, the primary oxidation products of oil and fats are the hydroperoxides, and they can be quantitatively measured by determining the amount liberated by its reaction with potassium hydroperoxide iodide (Hui., 1999 c). In this study, the peroxide value of the moringa oil is shown in Table (2). The peroxide value of moringa seeds oil was (0.49 mg O₂/ Kg of oil), the value is agreed with the value reported by (Anwar and Rashid., 2007) who reported 1.83 mg O₂/ Kg of oil), and also lower than the value reported by (Tsaknis, 2002 0.59mg O₂/ K of oil). But this value is lower than the value reported by (Salah., 2006) who reported (9.0 mg O₂ / Kg of oil). In this study, the peroxide value of moringa

oil is lower than the peroxide value of the olive oil (0.07 mg O₂/ Kg of oil).

4. Free Fatty Acids:

The amount of NaOH needed to neutralize an oil sample is used to determine the level of free fatty acids present. The value is usually presented as a percent of Oleic acid (Hui., 1996 b). The percent of free fatty acids in moringa oil is shown in Table (2). The value of free fatty acids in moringa seed oil was (0.19%), a value lower than the value reported by (Khattab., 2011) who reported 0.282% and very low compared with the value reported by (Salah., 2006., 1.128%). In this study, the percent of free fatty acids in moringa oil is lower compared with the free fatty acids of olive oil.

5. Iodine Value:

A higher Iodine value is an indicator of a greater number of double bonds that are more unsaturated (Hui., 1996 b). The traditional classification of fats and oils by iodine number into nondrying “>90”. Semi-drying (90-130) drying oil “> 130” is being used less and is being grouped into their content of fatty acid as follows: Oleic and Linoleic group, Palmitic group (Swern., 1979). The result given by the moringa oil shown in Table (2), showed a lower iodine value (63.5 mg of I/Kg of oil), this value is slightly agreed with the value reported by (69.45), (Anwar and Rashid., 2007), (65.58 mg of I/Kg of oil) and (Lalas & Tsaknis., 2002), But (Salah., 2006) reported higher iodine value to the moringa oil (88 mg of I/Kg of oil). In this study, the moringa oil reported a lower iodine value than the value of the olive oil (81.5 mg O₂/ Kg of oil).

Table 2: Chemical properties of moringa and olive oil.

Parameters	Value	
	Moringa oil	Olive oil
Peroxide value (mg O ₂ /k oil)	0.49 ^b	0.7 ^a
Free fatty acids (%)	0.19 ^b	0.95 ^a
Saponification number mg KoH/100g oil	187 ^a	178 ^b
Iodine number mg of I/ 100 oil	63.5 ^b	81.5 ^a
Total tocopherol content mg/ L	257	Not detect

* Each test was repeated three times and the average was calculated.

6. Saponification Number:

The saponification value of the moringa seed oil was (187 mg of KOH/kg of oil) which agreed with the value reported by (Anwar and Rashid., 2007) who reported 186.67 mg of KOH/kg of oil but was higher than the value reported by (Lalas and Tsaknis., 2002) who reported (182mg of KOH/kg of oil). In this study, the saponification number of moringa oil is lower than the saponification number of olive oil 172 mg O₂/ Kg of oil.

7. Tocopherol:

In this study, moringa oil reported a higher value of tocopherol (257 mg/L) this value is lower than the value reported by (Hasana & Abdulkarim., 2011) who reported 3.91mg/L for total tocopherol. Moringa seed oil is rich in several antioxidant compounds with therapeutic properties, such as tocopherols (fat-soluble vitamins) stand out. Moringa seed contains α -, γ - and δ -tocopherols, with α -tocopherol (vitamin E) being the most abundant. α -Tocopherol is associated with the prevention of cardiovascular diseases, additionally preventing lipid oxidation in the body, such as that of polyunsaturated fatty acids and cellular components (membranes and organelles) (Wiltshire *et al.*, 2022). Thus, Moringa seed oil is a green source of liposoluble vitamins and a potential source of bioproducts in industries (Gharsallah *et al.*, 2021).

8. Fatty Acid Profile of Moringa Oil:

The extracted oil from moringa seeds resembles olive oil in its fatty acid composition. The results in Table (3), showed that moringa oil contains a high percentage of oleic acid (71.57%) as a dominant fatty acid in moringa oil also contains (7.127%) stearic acid, (6.725%) linolenic, (5.973%) of Behenic acid, (3.842%) of Gadoleic acid and (3.248%) of palmitic acid. small contents of fatty acids are also present in moringa seed oil such as Arachidic acid (0.982%), Arachidonic acid (0.232%), Palmitoleic acid (0.1860%), and Linoleic acid (0.0690%).

Table 3: Fatty Acid Composition of Moringa Oil.

No.	Name	Area%
1.	Palmitoleic	0.1860
2.	Palmitic	3.248
3.	Linoleic	0.0690
4.	Oleic	71.57
5.	Linolenic	6.725
6.	Stearic	7.127
7.	Arachidic	0.9820
8.	Gadoleic	3.842
9.	Behenic	5.973
10.	Arachidonic	0.2320

Declarations:

Ethical Approval: It is not applicable.

Conflict of interests: All the authors read and approved the manuscript and declare that there is no conflict of interest

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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