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Seed Oil Extraction of *Cucurbita maxima* Duchesne Growing in Madagascar: Impact of Storage and Use of a Cineole–Rich Essential Oil as a Green Solvent

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Abstract: The performance of a cineole-rich essential oil as an extraction solvent for vegetable oil was studied. This essential oil was obtained from *Melaleuca quinquenervia* (Cav.) St Blake Myrtaceae, commonly called niaouli, an invasive plant on the eastern coast of Madagascar. The whole essential oil was used without preliminary isolation of terpenes. Experimentation was carried out with *Cucurbita maxima* Duchesne Cucurbitaceae seeds. The Soxhlet method was used for the oil extraction, and solvent recovery was performed by hydrodistillation. Niaouli essential oil allowed a better extraction yield of 39% compared to hexane, with 36%. The linoleic acid content of the oil was also higher: 37.19% against 7.25% for hexane. After 30 weeks of storage, the seed powder linoleic acid content increased to 44.99% for niaouli oil against 25.00% for hexane oil. With the screw press extraction method, the best yield of 13.00% was obtained with roasting pretreatment while heating at 60°C. However, the best oleic acid content of 45.36% was obtained at 50°C. It was demonstrated that the seed powder underwent biochemical changes during storage which were similar to intracellular catabolic reactions of fatty acids. The high expenses induced by the deterpenation process in terms of cost and energy limit the diffusion and the development of the use of terpenes as alternative solvents. The results of this research support the idea that it is possible to avoid this costly step by directly using the entire essential oil since this can provide a vegetable oil of good quality.

Keywords: Pumpkin seed oil; green solvent; *Melaleuca quinquenervia*; linoleic acid; skin care.

1. Introduction

The oil extracted from the seeds of *Cucurbita maxima* Duchesne Cucurbitaceae, commonly known as pumpkin, presents interesting bioactive properties including the regulation of blood cholesterol levels, the alleviation of diabetes, the improvement of urinary tract dysfunction, the prevention of cancer, prostatitis and high blood pressure [1]. This oil is rich in linoleic acid, a ω-6 polyunsaturated fatty acid. Although its positive impact on heart and blood circulation is controversial

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[2], linoleic acid is nonetheless considered as an essential fatty acid. It is particularly essential for skin health and hydration. It is among the components of ceramides, a constituent of the epidermal barrier [3]. Linoleic acid has a key role in reducing skin inflammation and also in the wound healing process. Both oral and topical uses are encouraged for optimal efficacy [4-6].

Due to health and environmental issues with petroleum-based solvents, many studies are being conducted on the possibility of using green solvents. Among them, terpenes from essential oils are identified to be good alternatives to hexane in the extraction of vegetable oils [7]. To the extent of our knowledge, research on the use of essential oils as direct solvents for extraction is still scarce, and this study is among the pioneers in exploring this possibility. If the essential oil is appropriately chosen, this would constitute a greener process that saves the extra energy and cost of deterpenation. 1,8-cineole, also called eucalyptol, has been identified as a potential eco-friendly substitute for conventional solvents [8]. In some countries, the acquisition of aromatic plants as raw materials in sufficient quantity for 1,8-cineole industrialized extraction is difficult. Furthermore, the process of its separation is expensive [9]. This justifies the use of the whole essential oil as a solvent instead of the isolated cineole.

In some countries like Madagascar, *Melaleuca quinquenervia* (Cav.) St Blake Myrtaceae, also called niaouli, is an invasive species. The plant with cineole chemotype is largely abundant on the eastern coast of Madagascar, and its industrial exploitation would help control its invasion [10]. The activity of its essential oil in cosmetics and dermatology is well stated [11, 12]. As vegetable and essential oils generally present good synergistic actions in skin care formulations, niaouli essential oil could potentially have a complementary effect with pumpkin seed oil [13, 14]. This research aims, on the one hand, at demonstrating the possibility of using niaouli essential oil in the extraction of pumpkin seed oil. The oil extracted was characterized to determine its potential uses as functional food, as an ingredient for cosmetic products, or for topical ointment in the treatment of cutaneous problems.

Some foodstuffs still undergo biochemical changes that could be beneficial if stored under appropriate conditions. To cite an example, the maturation of wheat flour leads to an improvement in its physical and rheological properties [15]. The gluten extensibility decreases, and its elasticity increases. This results in better dough handling and better product quality [16]. The second focus of this study is to evaluate the effect of pumpkin seed powder aging on the fatty acid profile of the oil.

2. Materials and Methods

2.1. Plant Material and Essential Oil

The ripe undamaged fruit of *Cucurbita maxima* was purchased in a local market of Antananarivo, the capital of Madagascar, in January 2021. Seeds were manually separated from the pulp, washed with water, and oven-dried at 40 °C for 24 hours. *Melaleuca quinquenervia* essential oil was purchased from a local institute of natural products. Their technical data described a type-1 niaouli essential oil, with a relative percentage of 55.9 for the cineole compound.

2.2. Aging of Pumpkin Seed Powder

After grinding, the seed powder was stored in a cool and dry ambient place. It was packed in a container that was simply covered with a piece of cloth to induce an aerobic condition. Referring to a study showing that pumpkin fruit is degraded after 8 months of storage at ambient temperature [17], we have chosen to investigate the oil quality within 30 weeks of storage of the seed powder. Three samples were taken for extraction within this period: the first just after grinding, the second after 20 weeks, and the last at the end of 30 weeks.

2.3. Soxhlet Extraction Using Hexane

Hexane, laboratory-grade, was purchased from a local chemical importer. Pumpkin seed powder was put in a 250 ml flask with a ratio of 30 g for 150 ml of solvent. The extraction time was 8 hours. After cooling, the solvent was evaporated under a vacuum at 40°C. The yield was expressed as the mass percentage of oil per powdered seed.

2.4. Soxhlet Extraction Using Niaouli Essential Oil

To make a comparison with the hexane extraction, the same amounts of substrate and solvent were used. The ratio was 30g of seed powder per 150 ml of niaouli essential oil (niaouli EO). Since the essential oil takes longer to siphon off, the distillation took 12 hours. Following the extraction procedure previously described by other authors [18], solvent recovery was carried out by hydrodistillation. The mass percent oil yield was compared with conventional solvent extraction.

2.5. Screw Press Extraction

Yield optimization was studied for extraction using a screw press. Two pretreatment parameters were taken into account: roasting and slight heating. Three temperatures were chosen: ambient temperature, 50°C, and 60°C. 200 g of seed powder was extracted for each assay. The preheating process was performed using a water bath prior to pressing. Each extraction was carried out in triplicate, and the results are presented as the mean values.

2.6. Oil Quality Characterization

Specific gravity was determined using the pycnometer method. Refractive index assessment was performed using Abbe's refractometer. Gas Chromatography analysis of the oil fatty acid methyl esters was performed with a GC-14A SHIMADZU apparatus equipped with a Flame Ionization Detector. A SOLGEL-WAX column of 30m*0.53mm*1µm was used. The carrier gas was nitrogen, and the flow rate was 3 ml/min. 1µl of the sample was injected, and the oven temperature was set at 190°C. The detector temperature was 280°C.

The computer controlling the chromatograph was equipped with an internal database allowing the identification of the various compounds. To confirm the authentication of the results, data on samples previously tested within the same apparatus were taken into account. The results were also compared to the literature data.

3. Results and Discussion

3.1. Effect of Solvent Extraction on Oil Quality Prior to Aging

Extractions using Niaouli EO and hexane as solvent yielded 40.00 % and 36.00% of dark yellow viscous oils with characteristic odors of pumpkin seeds, respectively.

Table 1. Fatty acid compositions of freshly ground seed oils

Parameter / Fatty acid	Hexane extracted oil	Niaouli EO extracted oil
Extraction yield (%)	36.00	40.00
Specific gravity (25°C)	0.969	0.989
Refractive index (40°C)	1.475	1.504
Myristic acid (%)	2.34	0.42
Palmitic acid (%)	33.10	20.26
Palmitoleic acid (%)	-	-
Stearic acid (%)	15.90	9.88
Oleic acid (%)	36.32	30.55
Linoleic acid (%)	7.25	37.19
Linolenic acid (%)	1.98	-
Arachidic acid (%)	0.94	-
Gadoleic acid (%)	2.17	0.57

A very slight smell of essential oil was scented for the oil extracted with niaouli EO, but that odor was weakly perceived. Table 1 shows the fatty acid compositions of the two oils. Linoleic acid has a better affinity with niaouli EO. A significant difference is observed between its relative percentage with niaouli EO extraction (37.19%) and hexane extraction (7.25%). Arachidic acid is absent within the niaouli EO extracted oil, and gadoleic acid exists only in a small quantity.

3.2. Effect of Seed Powder Aging on the Oil Quality and the Fatty Acid Composition

As shown in Table 2, the extraction yield did not change during the 30 weeks of seed powder storage.

Table 2. Evolution of oil quality extracted with hexane during seed powder storage

Parameter/Fatty acid	Freshly ground	After 20 weeks	After 30 weeks
Extraction yield (%)	36.01	36.93	36.19
Specific gravity (25°C)	0.969	0.978	0.911
Refractive index (40°C)	1.475	1.483	1.475
Myristic acid (%)	2.34	-	-
Palmitic acid (%)	33.10	26.23	23.86
Palmitoleic acid (%)	-	-	-
Stearic acid (%)	15.90	12.22	10.75
Oleic acid (%)	36.32	34.32	32.42
Linoleic acid (%)	7.25	19.87	25.00
Linolenic acid (%)	1.98	0.79	0.47
Arachidic acid (%)	0.94	0.69	0.55
Gadoleic acid (%)	2.17	1.26	0.91

A decrease in palmitic acid content is noticeable (33.10% down to 23.86%), concomitantly with an increase in linoleic acid content (7.25 % up to 25.00 %). Synthesis of fatty acid starts from the formation of palmitic acid from Acetyl-CoA [19]. Then follows the reaction of elongation to stearic acid. Desaturation of oleic acid and linoleic acid occurs after the elongation process. Thus the remarkable decrease in stearic acid content and the slight diminution of the percentage of oleic acid may also be partially explained by conversion to linoleic acid. These phenomena suppose the existence of biochemical reactions during seed powder storage.

3.3. Oil Extraction from Aged Seed Powder Using Different Extraction Methods

The decrease in palmitic and stearic fatty acid contents is confirmed when comparing the extraction results using niaouli EO before and after seed powder aging. The relative percentage of linoleic acid has increased. The small percentage of palmitoleic acid corroborates the existence of reactions of desaturation during storage. Contrary to the hexane-extracted oil, arachidic acid is absent, and gadoleic acid has a low relative percentage for niaouli EO extracted oil. The extraction yield remains around 39%.

Niaouli EO allows a better extraction yield than a screw press. Roasting accompanied by preheating allows for improving the yield of the mechanical extraction. However, linoleic acid content decreases if heating exceeds 50°C. Roasting of the seeds contributes to a better aroma of the oil [20], but it reduces its oxidation stability [21].

3.4. Discussion

Pumpkin seed oil quality undergoes a large variation according to process and crop season. A previous study has shown that proportions between saturated and unsaturated fatty acids were affected by ambient temperature during extraction [22]. Oil quality also differs according to pumpkin varieties [23]. For the Madagascan variety used in this study, the linoleic acid content is lower compared to the literature data. However, the aging of the seed powder allows an increase to 44.99%, which is suitable for the general fatty acid profile of pumpkin seed oils. This process can thus be suggested as a method to enhance the quality of the oil regarding fatty acid profile. We recommend roasting the seed powder prior to extraction. It has been demonstrated that this treatment does not affect the fatty acid profile while improving the oil stability [24].

This study is among the pioneers in investigating the impact of seed powder storage on oil quality. Previous research has reported the oil stability after extraction. The suggested shelf life was one year [25]. In this current work, we recommend not to store the seed powder for more than 30 weeks.

Table 3. Quality of oil extracted within different conditions after seed powder aging

Parameter / Fatty acid	Niaouli EO	NR 25	NR 50	NR 60	R 25	R 50	R 60
Yield (%)	39.29	8.71	10.42	12.44	10.35	12.18	12.92
Myristic acid (%)	0.14	0.69	0.74	0.44	0.62	1.45	1.73
Palmitic acid (%)	16.99	16.94	16.89	16.69	17.30	15.67	17062
Palmitoleic acid (%)	0.16	0.15	0.11	0.17	0.14	0.14	0.15
Stearic acid (%)	8.44	8.36	7.86	7.93	8.32	8.02	7.87
Oleic acid (%)	27.95	28.86	28.20	28.96	29.89	28.73	30.74
Linoleic acid (%)	44.99	44.73	45.18	44.81	43.10	45.36	41.06
Linolenic acid (%)	0.26	0.19	0.15	0.15	0.16	0.12	0.17
Arachidic acid (%)	-	-	0.41	0.53	-	-	0.44
Gadoleic acid (%)	0.55	-	0.12	0.15	0.47	0.51	0.21

NR 25: Not roasted, no heating**; NR 50:** Not roasted, preheated at 50°C**; NR 60:** Not roasted, preheated at 60°C**; R 25:** Roasted, no heating**; R 25:** Roasted, preheated at 50°C**; R 25:** Roasted, preheated at 60°C**.**

Niaouli EO shows a good extraction performance for pumpkin seed oil. Before the aging process of seed powder as well as after aging, the extraction yield is higher if compared to hexane. The oil is richer in linoleic acid with niaouli EO extraction. For a cosmetic product design, this oil can enter skin care formulations. Niaouli EO has recently been studied for nano-emulsification for acne treatment [26]. For oral use, long-term toxicity should be checked because of the trace of residual niaouli essential oil.

Regarding the cost-to-benefit ratio, eco-labeled products have a greater market value with a higher price compared to conventional ones [27]. For skincare products, there is a strong market trend for eco-designed formulated cosmetics [28]. In the present study, the essential oil was extracted from an invasive plant. This brings the double advantage of acting favorably for the ecology by limiting its proliferation while gaining in the cost of raw materials. Different plant species provide cineole-rich essential oils, such as plants in the genera Eucalyptus as well as certain varieties of rosemary [31] and lavender [32]. This wide availability could help lower the cost of essential oil. Additionally, recent research findings suggest using biotechnology like tissue culture, hairy roots culture, and microorganism culture to help achieve better production yield [33]. Concerning essential oil extraction, the process is very cheap for the particular case of this research, as the niaouli plant is proliferous, and the energy for the distillation is provided by its own branches and its trunk. The only expense related to its distillation is then the labor cost; however, this contributes to the promotion of local jobs. To gain price competitiveness in the essential oil production process, we recommend the use of cost-effective technologies like microwave extraction [34] or hybrid solar distillation [35].

4. Conclusion

Niaouli EO is a good alternative solvent for the purpose of extraction of linoleic acid-rich vegetable oil: 37.19% before aging and 44.99% after aging of the seed powder. It yields 39 % oil against 36% for hexane. Screw press yields around 12%. The extraction method could be chosen depending on the desired application. The existence of biochemical reactions during seed powder storage was demonstrated, which was in favor of a higher concentration of linoleic acid. The palmitic acid content has decreased as it is the precursor of C18 saturated and unsaturated fatty acid in normal metabolic processes inside the biological cell environment. This new understanding should help to improve the qualities of pumpkin oil to have a stable fatty acid composition despite seasonal variations or varietal changes.

The contribution of this study has been to confirm the possibility of using essential oil as a solvent without any prior deterpenation. This latter is an expensive process in terms of cost and energy. This method of extraction of pumpkin oil using cineole-rich essential oil may be applied to other oilseeds. The insights gained from this study can be useful in targeting a greener extraction process. The essential oil has been well chosen because it is extracted from an invasive species. Thus, the ecological advantage is not only to limit the use of petroleum-based solvents. It is also to help control the proliferation of invasive species. The leaves of this plant can be harvested to provide essential oil, whereas the wood can be used as renewable energy for its distillation.

A perspective for this work would be the study of the evolution of unsaponifiable matters during seed powder storage. This would concern the phytosterols and the tocopherols, which also have good biological activities on the skin. Experimentation using other extraction techniques like ultrasonication should also be conducted using this essential oil as a solvent.

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