Amaranth Oilseed Composition and Cosmetic Applications

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Abstract: Amaranth (*Amaranthus cruentus*) is a possible alternative to high-nutritional-value crops. Amaranth seeds are considered to be one of the few sources of phytosqualene (up to 8%). The use of squalene and its hydrogenated form squalane in skincare formulations has been steadily increasing, and the demand for these compounds is expected to rise continuously. The aim of this study was to investigate the amaranth oilseed as a potential ingredient for cosmetic applications. First, an experimental design and optimization were carried out in order to obtain amaranth oil rich in squalane instead of squalene through catalytic hydrogenation. Under the optimal conditions, the resulting oil was fully hydrogenated, with higher stability, and more suitable for cosmetic uses. Furthermore, the effect of the addition of amaranth oil and squalane on the rheological and sensory characteristics of moisturizing cream formulations was assessed. As expected, higher contents of oil and polyunsaturated fatty acids were obtained by supercritical CO_2 extraction, and were used for the next step of the experiment. Optimization of the experimental conditions resulted in fully hydrogenated amaranth oil, with higher stability and rich in squalane. Better quality of moisturizing cream formulations was achieved when W/O formulations were enriched with 2% oil, or by adding 1% oil and 1% squalane. The formulation rich in squalane showed a better overall quality compared to other formulations.

Keywords: Amaranth oil; squalene; squalane; catalytic hydrogenation; moisturizing cream

Citation: Sayed-Ahmad, B.; Urrutigoïty, M.; Hijazi, A.; Saad, Z.; Cerny, M.; Evon, P.; Talou, T.; Merah, O. Amaranth Oilseed Composition and Cosmetic Applications. *Separations* **2022**, *9*, 181. https://doi.org/10.3390/separations9070181

Academic Editors: Angela Sorbo, Claudia Zoani and Daniele Passeri

Received: 25 June 2022
Accepted: 17 July 2022
Published: 19 July 2022

1. Introduction

The Amaranthaceae family includes more than 60 species characterized by their ability to produce a large amount of biomass in a short period. Few of them can produce edible seeds, and *Amaranthus cruentus* or amaranth is one of these species [1,2]. In fact, it is a multipurpose plant providing seeds, leaves of high nutritional quality, and stems containing a bark and pith—both possibly usable in the materials industry [3,4]. It can also be grown as an ornamental plant, as a garden plant, or as a dye [5–7]. Currently, amaranth is cultivated in many countries of America, Southeast Asia, and Africa. The National Foundation of the United States has identified it as one of the six most promising crop plants [2,8]. Plantations have also been located in many European countries since the early 1990s. Amaranth, along with quinoa, buckwheat, and millet, is part of the family of pseudo-cereals. They are dicotyledonous (unlike plants recognized as true cereals, which are monocotyledonous plants), and the seeds of these plants are used for human nutrition, e.g., in cereals [7,9,10]. In fact, consumers and farmers, as well as the cosmetics industry, are increasingly interested in this unconventional plant due to the fact that its seeds are

a major source of minerals and vegetable oil rich in essential fatty acids, vitamins, and unsaponifiable substances—especially squalene [11].

The development of liquid–liquid emulsions is a common practice in the food and pharmaceutical industries. A system consisting of oil droplets dispersed in an aqueous phase is called oil-in-water (or O/W) emulsion, whereas one with water droplets dispersed in an oily phase is called a water-in-oil (or W/O) emulsion [12]. When O/W cream is applied to the skin's surface, most of the water evaporates, which makes it easily washable, while in the case of W/O cream, a certain amount of water is retained in the oil phase on the skin, making it stickier [13]. In recent years, scientists have sought to use active ingredients with specific cosmetic effects. Vegetable oils are considered to be a cheap natural source with excellent cosmetic and skincare properties. Oils such as soybean oil, corn oil, safflower oil, and linseed oil are widely used for cosmetic purposes in the form of W/O emulsions in pharmaceutical and cosmetic formulations [14]. In this context, the addition of amaranth oil in W/O emulsions seems to be a potential option due to its richness in a number of biologically active compounds. This oil is very compatible with the acidity of the skin, and is rich in precious substances, such as squalene, phytosterols, and tocopherols, as well as valuable fatty acids such as oleic and linoleic acids, all of which have soothing and protective effects on the skin.

Squalene is a triterpene whose summation formula is $C_{30}H_{50}$. It represents an intermediate in the phytosterol/cholesterol biosynthesis pathway in plants, animals, and humans (Figure 1). It displays some benefits for the skin's physiology as an emollient and antioxidant [15,16]. Squalene and its hydrogenated product, squalane, are used in pharmaceutical and cosmetic formulations for treatment of skin disorders due to their moisturizing activity and protection against external agents (e.g., air, light, UV rays, and environmental pollution) [17]. They are traditionally used in China in anti-fatigue and anti-aging products. They have also been used for antiviral emulsions [11]. Formulations prepared with either squalene or squalane show the same efficiency. However, squalane is preferred due to its higher stability, due to the absence of double bonds [18]. It can be easily emulsified in all formulation types without the need for any preservatives. It can also maintain its properties—including viscosity—unaffected in a range from −30 to 250 °C [19]. These exceptional properties and its high resistance to oxidation make it the best emollient in the cosmetics industry, included moisturizing creams, makeup, lipstick, and personal haircare products [20].

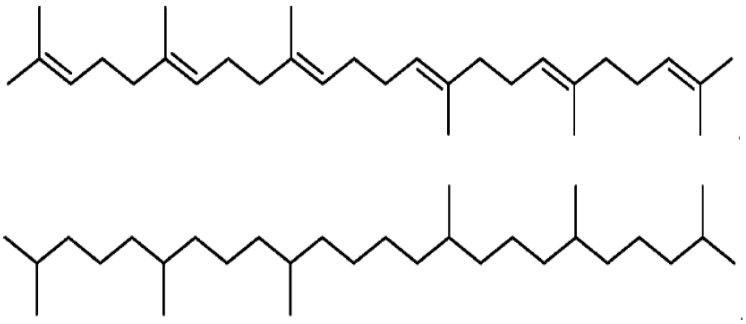

Figure 1. Chemical structure of squalene (**top**) and squalane (**bottom**).

Previous studies have focused on developing new methods of isolation of squalene and hydrogenation processes for squalane production. The first industrial vegetable source of squalene is olive oil, but it cannot be directly extracted from oil, due to its low concentration (150–170 mg/100 g) [21]. It is preferably recovered from the condensed unsaponifiable matter of olive oil called "deodorization distillates (DD)". One kilogram of DD contains 100 to 300 g of squalene. Thus, further extraction processes are used in order to obtain higher levels of purity [22]. Recently, a potential process for microbial-derived sugarcane phytosqualane production has also been developed [23].

After the isolation and purification of squalene, different hydrogenation methods are implemented in order to obtain a completely saturated squalane (Table 1).

Table 1. Previous catalytic hydrogenation methods used for squalane production.

Squalene Origin	Catalyst Type	H2 Pressure (Bar)	Temperature (°C)	Time (Hour)	Ref.
Shark liver oil	0.05% nickel-kieselguhr	4	200	3–4	[24]
Olive oil	Ni-based	5 bar/30 bar	200	4 h/3 h	[23]
Sugarcane	5% Pd/C	150	160	16	[25]

Shark liver oil is considered to be the richest source of squalene (79.6% of liver oil of *Centrophorus squamosus*) [21]. However, for the preservation of marine life, researchers are looking for new natural sources of squalene—especially from plant oil. Squalene was detected in trace or low amounts in almost all plant oils, including palm oil, wheat-germ oil, and race bran oil [16,17]. An interesting concentration of squalene is found in olive oil (0.2–0.5%), but the greatest amount is identified in amaranth seed oil (6–8%) [11,21]. Several health benefits are attributed to amaranth and olive oils due to the presence of squalene [26,27]. However, all previous studies focused on the hydrogenation of squalene isolated from these different sources (Table 1), but none of them has addressed the overall hydrogenation of amaranth oil as source of phytosqualene, along with different fatty acids and sterols.

Therefore, this work was designed to first compare the fatty acid profiles and the contents of squalene and phytosterols in amaranth oils extracted through solvent and supercritical CO_2 extraction methods. Furthermore, we were interested in finding the optimal conditions to obtain hydrogenated amaranth oil rich in squalane. Alongside squalane, hydrogenated oil is rich in saturated fatty acids as well as phytosterols. Thus, the resulting oil is highly resistant to oxidation, and usable in a wide variety of cosmetic products. The effects of the addition of amaranth oil and squalane on the rheological and sensory properties of W/O moisturizing emulsions were also studied.

2. Materials and Methods

2.1. Vegetable Oil Extraction

A Soxhlet apparatus was used in order to obtain amaranth oil through solvent extraction. A sample of 25 g of ground amaranth seeds was used for oil extraction, using cyclohexane as a solvent, for 5 h. Then, the solvent was evaporated using a rotary evaporator at 40 °C for 3 h. Oil obtained by supercritical CO_2 extraction was provided by Sigma-Aldrich (Saint-Louis, MO, USA). Both oils were kept at 4 °C.

2.2. Fatty Acid Analysis

Vegetable oils were first methylated and converted into fatty acid methyl esters (FAMEs). One milliliter of MTBE (methyl tert-butyl ether) was added to 20 mg of vegetable oil. One hundred microliters was then transferred to an insert. Fifty microliters of a solution of trimethylsulfonium hydroxide (TMSH) in methanol (0.2 M) was added. The solution was moderately stirred. Fatty acid methyl esters were analyzed via GC/FID using a capillary column (Agilent, Les Ulis, France) (CP-Select CB for FAME fused silica WCOT, 50 m × 0.25 mm; film thickness was 0.25 µm). The previously optimized temperature program used was as follows: The initial temperature of the column was 185 °C, maintained for 40 min. Then, it rose at a rate of 15 °C min^{-1} up to a final temperature of 250 °C, and the latter was held for 10.68 min. The temperatures of both the injector and the detector were 250 °C. The carrier gas used was helium, at a flow rate of 1.2 mL min^{-1}, with a split ratio of 1:100.

2.3. Extraction and Identification of the Unsaponifiable Fraction

A sample of 100 mg of vegetable oil was saponified with 2 mL of KOH (1 M) in ethanol at 75 °C for 20 min, in the presence of cholestanol as an internal standard. After cooling at room temperature, 1 mL of distilled water and 6 mL of cyclohexane were added, followed by vortex agitation. The organic layer was then recovered. Forty microliters of BSTFA and 1% TMCS were added to 160 µL of organic layer, and the mixture was analyzed by gas chromatography. BSTFA (bis (trimethyl-silyl) trifluoro-acetamide) was used as a silylating reagent, and TMCS (trimethylchlorosilane) was added to BSTFA as a catalyst to increase the silyl donor's strength. Unsaponifiable samples were analyzed by GC using an FID PerkinElmer (Waltham, MA, USA) chromatograph equipped with a CP-SIL 8CB capillary column (30 m × 0.25 mm; film thickness, 0.25 µm). The carrier gas was helium with a pressure of 16 psi at the column head, and the injection was 1 µL on-column. Analyses were performed according to the temperature program presented in Figure 2.

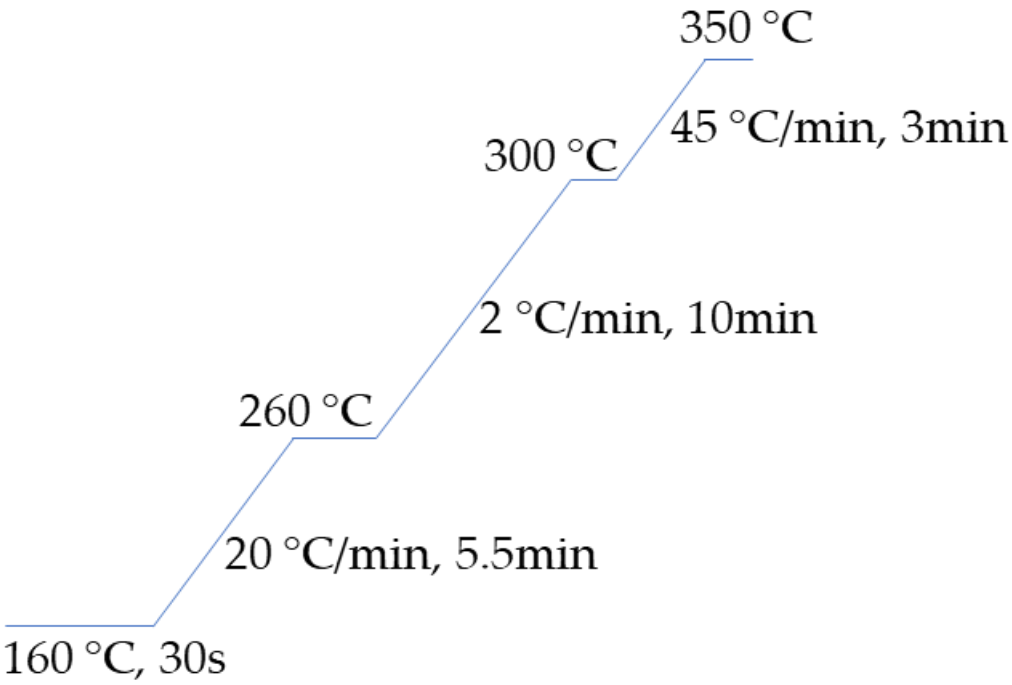

Figure 2. Temperature program used for the analyses of unsaponifiables in amaranth seeds.

2.4. Catalytic Hydrogenation of Amaranth oil Processes (Typical Procedure)

A mixture of 1 g of amaranth oil in 6 mL of cyclohexane and 100 mg to 250 mg of commercial 5 wt.% platinum on charcoal (Pt/C) was introduced to a 100 mL stainless steel autoclave (TOP-Industries) with magnetic stirring. Cyclohexane was added in order to decrease the viscosity of the oil. The reactor (Parr Instruments, St. Moline, IL, USA) was purged 3 times with hydrogen, and then heated in an oil bath to 170 °C, pressurized under 10 or 20 bars of H_2, and the reaction mixture was stirred at 300 rpm for the desired time. At the end of the reaction (after a period of 8 h), the oil bath was removed and the reactor was cooled to reach room temperature and then slowly depressurized. The reaction mixture was recovered and then filtered through a Buchner filter funnel. The solvent was evaporated using a rotary evaporator (Laboratoires Humeau, La-Chapelle-sur-Erde, France). The crude oil was analyzed by gas chromatography. After catalytic hydrogenation experiments, the GC-FID analysis of experiments 1–3 (Table 2) showed a non-squalene hydrogenation at 10 bars of H_2, even with addition of 250 mg of Pt/C (see experiment 3 in Table 2), as previously reported [23,28].

Table 2. Experimental run plan for the hydrogenation catalytic experiments *.

Experiments	5% Pt/C Mass (mg)	Pressure Conditions (Bars)
1	100	10
2	200	10
3	250	10
4	200	20
5	250	20

* Amaranth oil used in each assay = 1 g, oil bath temperature = 170 °C, stirring speed = 300 rpm, reaction time = 8 h.

Quantitative Determination of Platinum

Hydrogenated oil was first mineralized by adding nitric acid and hydrochloric acid (20:1, *v/v*). The assembly was then heated on a plate at 90 °C for at least 12 h. After complete digestion and removal of acid, the samples were adjusted to 5 mL with pure water, making them ready for measurement. Platinum quantitative analysis was then performed using inductively coupled plasma mass spectrometry (ICP-MS) apparatus.

2.5. Water-in-Oil Moisturizing Cream Formulations

The components of the formulations are presented in Table 3. A typical procedure was used to produce water-in-oil emulsions by heating the oily phase in a glass beaker up to 75 °C. The aqueous phase was mixed with the oily phase and homogenized with an Ultraturrax (F25 IKA®-Werke, Staufen, Germany) at 13,000 rpm for 15 min. The sample was then cooled at room temperature under moderate stirring (150 rpm), and finally stored at ambient temperature. The amaranth oil extracted by solvent and hydrogenated was used for the cosmetic formulation.

Table 3. Ingredients of formulations A–C based on oil and squalane from amaranth seeds.

Phase	Ingredients (INCI Name)	Content (wt.%)		
		Formulation A	Formulation B	Formulation C
Phase A (aqueous)	Aqua/water	57	57	57
	Cholorphenesin	0.2	0.2	0.2
	Carbomer	0.5	0.5	0.5
Phase B (oil)	Cetyl alcohol	2	2	2
	Stearic acid	2	2	2
	Paraffinum liquidum	25	23	23
	Amaranthus cruentus vegetable oil	0	2	1
	Squalane	0	0	1
	Decyl oleate	7	7	7
	Ceteraeth 12	3	3	3
Phase C	Propylene glycol	0.02	0.02	0.02
Phase D	Triethanolamine	0.9	0.9	0.9
	Aqua/water	1.68	1.68	0.68
	Phenoxyethanol	0.7	0.7	0.7

2.5.1. Measurement of Rheological Properties

The measurements of the rheological properties of the released formulations were performed using the cone-plate geometry on a modular rheometer (MCR 302, Anton Paar, Austria). The diameter of the cone was 25 mm. A 1 Hz frequency was used during

the oscillatory stress sweep tests. In doing so, the linear viscoelasticity range was used. The shear rates used varied from 1 to 100 s^{-1}. The generated flow curves expressed the viscosity as a function of the applied shear rate. A 25 °C temperature was used during the measurement.

2.5.2. Determination of Peroxide Value

A sample of four grams was weighed in a flask. The dissolution was carried out in 30 mL of a solution made of acetic acid and chloroform (3/2). Then, under continuous shaking, KI and distillated water were added at rates of 0.5 mL and 30 mL, respectively. This step was followed by a titration with 0.01 N sodium thiosulfate solution ($Na_2S_2O_3$) under vigorous shaking until reaching a constant yellow color. As described by Sapino et al. [29], 0.5 mL of starch solution was then added, and the titration continued with vigorous shaking to liberate whole I_2 from the chloroform layer, until the discharge was a blue color. The control test was performed under the same conditions (V_0 of 0.01 N $Na_2S_2O_3$ solution), and represented formulation A (used for V_0 determination).

$$\text{PV (meq/kg)} = 10\frac{(V1 - V0)}{P}$$

where P is the exact mass (g) of the weighted formulation, V_1 is the volume (mL) of the 0.01 N $Na_2S_2O_3$ solution used for the test, and V_0 is the volume (mL) of the 0.01 N $Na_2S_2O_3$ solution used for the control test.

2.5.3. Sensory Evaluation

Twenty female panelists (aged 23–30 years) completed a special questionnaire concerning four parameters. The general concept of the study was first explained to the assessors, comprising a detailed explanation of the test, and then of the sensory descriptors used. A category scale was used for rating each studied parameter, with predefined descriptive terms. A random three-digit code was attributed to each tested sample. Similar containers were used to present all of the samples.

All cream samples to be tested were given to the panelists, along with an analysis form. The latter contained all of the instructions needed for comparing the different formulations. For each sensory attribute under evaluation, the panelists had to choose the descriptive term that best described it. The back of the left hand was the place where the panelists had to apply each product. The product mass to apply was about 2 mg.

2.6. Statistical Analysis

Experiments were carried out in triplicate. Results were displayed as the mean ± standard deviation (SD). The significance of the results was tested by one-way ANOVA. The means were compared pairwise using Tukey's test at a 5% probability level.

3. Results and Discussion

3.1. Fatty Acid and Unsaponifiable Composition of Amaranth Oilseed

Amaranthus cruentus vegetable oils obtained by solvent and supercritical CO_2 extraction were analyzed for their fatty acid composition, and the results are shown in Table 4. A significant difference was generally observed between the different fatty acid contents obtained via these two extraction methods. Linoleic acid was the main fatty acid identified in both analyzed samples (44.2% and 48.6%, respectively). Similar levels of this fatty acid were obtained by Ogrodowska et al. [9] and Singhal and Kulkarni [30], with 47.0% and 46.7%, respectively. Much lower contents were determined by León-Camacho et al. [11] (38.2%), Gamel et al. [6] (40.0%), and Palombini et al. [31] (38.0%), while Becker et al. [32] and Lyon and Becker [33] found higher contents (51.4% and 62.1%, respectively). Nevertheless, these observed results should be considered with caution because of the different origins of the two amaranths used in this study.

Table 4. Comparison between the fatty acid and unsaponifiable composition of amaranth oils obtained by solvent and supercritical CO_2 extraction methods.

	Fatty Acid Profile (%)	
Component	Solvent Extraction	Supercritical Extraction
Myristic acid (C14:0)	0.3 ^{a} ± 0.0	0.2 ^{a} ± 0.0
Palmitic acid (C16:0)	19.2 ^{b} ± 0.1	20.2 ^{a} ± 0.1
Palmitoleic acid (C16:1n7)	0.1 ± 0.0	-
Stearic acid (C18:0)	4.2 ^{a} ± 0.1	4.0 ^{a} ± 0.1
Oleic acid (C18:1n9)	28.6^{a} ± 0.1	23.4 ^{b} ± 0.1
C18:1n7	1.2 ^{a} ± 0.0	1.2 ^{a} ± 0.0
Linoleic acid (C18:2n6)	44.2 ^{b} ± 0.2	48.6 ^{a} ± 0.2
Arachidic acid (C20:0)	1.0 ^{a} ± 0.0	0.8 ^{b} ± 0.0
Linolenic acid (C18:3n3)	0.6 ^{b} ± 0.0	1.1 ^{a} ± 0.0
Gondoic acid (C20:1n9)	0.2 ± 0.0	-
Behenic acid (C22:0)	0.4 ± 0.0	-
MUFA	30.0 ± 0.3	24.7 ± 0.2
PUFA	44.8 ± 0.4	49.8 ± 0.4
SFA	25.0 ± 0.1	25.4 ± 0.1
Unsaponifiable fraction (mg/100 g oil)		
Squalene	4909.1 ^{a} ± 434.0	5311.2 ^{a} ± 669.0
Campesterol	30.8 ^{a} ± 7.6	28.8 ^{a} ± 7.2
Stigmasterol	48.8 ^{a} ± 5.6	11.7 ^{b} ± 1.7
β-Sitosterol	786.2 ^{a} ± 56.1	766.3 ^{a} ± 7.6
Δ5-avenasterol	21.1 ± 5.0	-
Δ7-avenasterol	275.9 ^{a} ± 22.2	334.8 ^{a} ± 39.8
Gramisterol	50.8 ± 5.0	-
Citrostadienol	81.5 ^{a} ± 7.3	109.4 ^{a} ± 8.1
Cycloartenol	12.7 ± 0.5	-
Methylene cycloartanol	26.7 ^{a} ± 1.4	45.6 ^{a} ± 9.0
Total	6244.7	6607.7

^{a,b} Average values followed by different letters in the same line are significantly different at 0.05 probability. MUFA: Monounsaturated fatty acid. PUFA: Polyunsaturated fatty acid. SFA: Saturated fatty acid.

Linoleic acid is an essential fatty acid that plays a vital role in human nutrition, disease prevention, and the maintenance of healthy skin. High levels of oleic and palmitic acids were also obtained in the two studied oils: 28.6–23.5% and 19.2–20.3% by solvent and supercritical CO_2 extraction, respectively. These results are comparable to those reported in the literature. Indeed, Gamel et al. [6] found 19.4% and 32.9% contents of oleic and palmitic acids, respectively, in *A. cruentus* oilseed. Ogrodowska et al. [9] and Becker et al. [32] obtained 23.4%, 24.6% and 20.3%, 19.4% contents of oleic and palmitic acids, respectively. It has been reported that oils rich in oleic acid have beneficial effects on the treatment of cancer, as well as autoimmune and inflammatory diseases, in addition to their ability to facilitate the healing of wounds [34]. Palmitic acid is an important component of both the skin barrier and the acid layer of the skin and, along with ceramides and cholesterol, it protects the skin against penetrating substances from the outside. It is also stable against oxidation [35]. Low levels of stearic acid were obtained (around 4%), confirming the data reported by Berganza et al. [36]—who determined the stearic acid content to be between

3.0% and 3.8%—and Ogrodowska et al. [9], with a 4.2% value. Other acids were found in traces in both oils, such as myristic and linolenic acids. On the other hand, palmitoleic, gondoic, and behenic acids were only detected in the oil extracted by solvent. In the same way, behenic acid was found by Palombini et al. [31] in amaranth oil. Traces of gondoic and behenic acids were also present in the amaranth oil analyzed by Hlinková et al. [37].

The oil extracted by organic solvent contained a higher level of monounsaturated fatty acids (MUFAs) than that obtained by supercritical extraction: 30.0% vs. 24.7%, respectively. Consequently, opposite results were logically obtained for the polyunsaturated fatty acid (PUFA) contents: 44.8% and 49.8%, respectively (Table 4). These differences can be attributed to the extraction method, but also to the effect of the genotype. In addition, the year of culture—including the effect of the environment, as well as abiotic stressors such as precipitation, drought, and temperature—can significantly alter fatty acid levels in *A. cruentus* seeds [37].

The contents of squalene and phytosterols in *A. cruentus* seeds are given in Table 4. Squalene is a terpenoid that is ubiquitous in the unsaponifiable fraction in virtually all vegetable oils—although in the majority of them it is found only in minimal or trace quantities. Only amaranth seeds are a rich source of vegetable squalene. In our study, the highest content of squalene was found in the oil obtained by supercritical CO_2 extraction (5.3 g/100 g oil, or 5.3%). These results are similar to those described in the literature. For example, Czaplicki et al. [38] obtained a higher squalene content by supercritical extraction (7.0 g/100 g of oil) than that resulting from extraction by organic solvent (6.0 g/100 g of oil) from *A. cruentus* seeds. Gamel et al. [6] determined a 4.8% squalene content in the oil extracted through the Soxhlet method. Berganza et al. [36] obtained similar values—between 2.3% and 5.9%—for the oil extracted with *n*-hexane from different varieties of *A. cruentus* seeds. In addition, Ogrodowska et al. [9] and Singhal and Kulkarni [30] found 5662 and 4884 mg/100 g of squalene, respectively (i.e., 5.7% and 4.9%, respectively).

Sterols are probably the most important minor components, and constitute the majority of the unsaponifiable matter of most vegetable oils. As shown in Table 4, the dominant sterol in amaranth seeds was β-sitosterol, followed by Δ7-avenasterol. The contents of the two first dominant sterols were not significantly different in the two oils. In addition, minor sterols (Δ5-avenasterol, gramisterol, and cycloartenol) were not detected in the oil obtained by supercritical CO_2 extraction. Lower levels of campesterol, stigmasterol, citrostadienol, and others were found in both extracted amaranth oils. When comparing the results of our research on the sterol composition of amaranth oil with the different methods used so far, we found that the identification of sterols is debatable. Grajeta [39] found that spinasterol is predominant, ranging from 46% to 54% of the total sterol content. Sitosterol and α-spinasterol were the dominant sterols identified by Czaplicki et al. [38] in the seeds of *A. cruentus*. Ologunde et al. [8] showed that sitosterol, Δ5-avenasterol, and stigmasterol were the major constituents of amaranth oil. Marcone et al. [40] reported that they found only β-sitosterol, stigmasterol, and campesterol in different amaranth seed varieties. León-Camacho et al. [11] found that the main phytosterol component of amaranth oil was clerosterol.

3.2. Catalytic Hydrogenation of Amaranth Oil

Squalene constitutes the main component of the unsaponifiable fraction of amaranth oil. It was quantified directly by GC-FID, using cholestenol as an internal standard. The absence of reduction can be attributed to the presence of waxes in vegetable oil; thus, harsher conditions are required [23]. Therefore, the hydrogen pressure was increased to 20 bars, along with the use of 200 mg of Pt/C (see experiment 4 in Table 4). After reaction, the recovered oil was solid at ambient temperature, meaning that the fatty acids present were saturated. We should note that saturated oils are more stable and do not become rancid as quickly as unsaturated oils [39,41]. Further experiments were carried out in order to reduce the whole amount of current squalene. Thus, the quantity of catalyst used was increased to 250 mg (experiment 5).

Today, the physiological and behavioral effects of traces of metals on the human population are of growing concern. Contamination by these metals is an important health problem because of their toxicity even at low concentrations. Thorough purification was thus required to remove most of the leached Pt/C from the final product in order to reach the maximum allowable levels of toxic platinum in a cosmetic product. ICP-MS analysis was used to determine the platinum remaining in the hydrogenated oil after filtration. The obtained result was in the order of 4.5 ppm (i.e., <5 ppm). This is an acceptable concentration, and is consistent with the results already reported in the case of hydrogenation of squalene using palladium as a catalyst [42,43].

3.3. Rheological Properties Analysis

Comparative rheological studies were carried out for the three cosmetic systems of skin creams (formulations A, B, C) based on W/O emulsions. Formulation A represents the control solution; formulation B contained only amaranth oil (2%), while formulation C contained both amaranth oil (1%) and squalane (1%) (Table 2). The flow behavior is among the most important factors, as it makes it possible to determine the physical properties of the emulsions, e.g., absorption, propagation on the surface of the skin, ease of distribution of the packaging, etc. This is therefore an important aspect of the evaluation of the quality of cosmetics [13]. Figure 3 shows the flow properties of the W/O emulsions, with the generated flow curves reflecting the viscosity behavior of the emulsions with respect to the shear rate of formulations A, B, and C. Two distinct regions are shown in this figure: the low-shear-rate region, up to 27 s^{-1}, where the viscosity of the emulsions exhibits a strong non-Newtonian behavior, in which the apparent viscosity decreases considerably; and the second, high-shear-rate region, >27 s^{-1}, where the viscosity of the emulsions shows a Newtonian profile. For the high-shear-rate region, all curves approach one another, forming a single main curve, which means that the viscosity then becomes shear-rate-independent. This behavior is called rheofluidifying (pseudoelastic), with the viscosity decreasing in all formulations with the increase in the shear rate (Figure 3). This behavior is typical for emulsions of cosmetic cream types, as it facilitates their skin application, thus improving the sensory impression perceived by the consumer when they use the product.

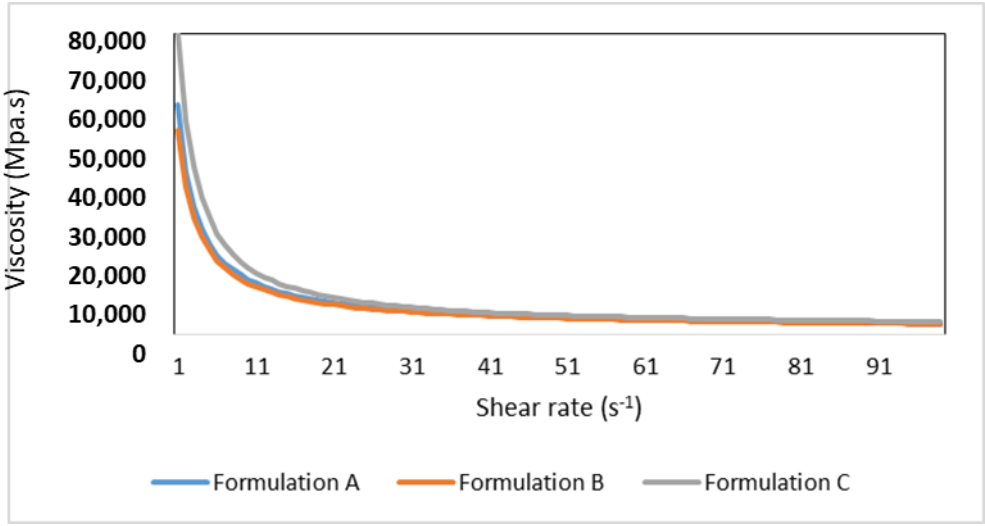

Figure 3. Viscosity of emulsions with respect to the shear rate of formulations A, B, and C.

Rheological oscillation tests are widely used to characterize the rigidity and integrity of multiphase viscoelastic systems, along with their internal structure. The typical parameters measured are the storage modulus G' (a measure of the deformation energy stored by the sample during the shearing process, representing the elastic behavior of the material) and

the loss modulus G″ (a measure of the deformation energy used by the sample during the shear process, representing the viscous behavior of the material) [44].

It has been established that the rheological properties of emulsions, along with their stability, are affected by the chemical composition of each phase. Oscillation sweep tests are usually used to determine the linear viscoelastic range (LVE). The results are shown in Figure 4. The limit of the LVE region indicates the maximum deformation tolerated by the sample before the internal structure is destroyed. Based on the curves of Figure 4, the deformation and shear stress limit values of the LVE range of the samples were estimated, and they are listed in Table 5. Formulations B and C showed shear stress and G′ values higher than those of formulation A. Such values were expected, as vegetable oils are generally used as thickening agents (i.e., binders) in cosmetic formulations [45].

Table 5. Rheological parameters determined from oscillation sweep tests, and peroxide indices for formulations A, B, and C.

	LVE Region		**Crossover Point**	**Peroxide Index (meq/kg Oil)**
	Deformation	**Shear Stress (Pa)**	**Storage Modulus (Pa)**	
Formulation A	0.003	5.4	218.7	Control
Formulation B	0.007	8.2	301.1	3.88 ^{a} ± 0.21
Formulation C	0.003	8.5	828.6	1.37 ^{b} ± 0.17

^{a,b} Average values followed by different letters in the same line are significantly different at 0.05 probability.

In fact, the rheological characteristics of W/O emulsions are strictly related to the formation of lipid crystals in the oily phase. These increasing values in the presence of amaranth oil and squalane (formulations B and C, respectively) could be attributed to the micellar interaction or elongated micellar transition to a spherical form. As a result, more nuclei can grow into larger crystals (nucleation phenomenon) [46]. Such a structure imparts higher mechanical stability to the formulation and, thus, enlarges the LVE domain.

On the other hand, the LVE range of all of the formulations was included in a region where G′ is greater than G″ (Figure 4); the materials thus showed a gel-like (elastic) character [44]. Starting from the crossover point of the curves G′ and G″ (i.e., the deformation for which G′ = G″), the gel-like character of the system was transformed into a liquid (viscous) character, and this point was called "pour point". After this point, G″ then became greater than G′.

The results of the crossover points presented in Table 5 show high stress values. Indeed, such values are a sign of more durable elastic properties for a formulation that has dominant elastic properties [47]. The results also show that the higher the elasticity (higher G′ value), the higher the crossover point (i.e., the greater the elastic properties). Therefore, the most elastic cream (formulation C) had the most durable elastic properties under increasing stress. Moreover, the highest values for formulation C indicate increased consistency in this formulation. Similar results were obtained by Mydul Alam et al. [48] in the case of the addition of squalane to a gel emulsion.

3.4. Peroxide Index Analysis

Oxidative stability is an important parameter for the quality evaluation of cosmetic emulsions. Auto-oxidation is affected by atmospheric oxygen, and the oxidation process proceeds via free radical reactions involving unsaturated fatty acids. The primary products formed are hydroperoxides, which then decompose in a series of complex reactions to produce secondary products such as alcohols and carbonyl compounds, which are mainly responsible for offshore flavors (rancidity) and odors. The peroxide index indicates the amount of primary oxidation products [49]. The results of the peroxide values of formulations B and C are shown in Table 5. Formulation B shows a significantly higher peroxide value than formulation C. This higher peroxide value means less oxidative stability. The

increase in the peroxide value could be attributed to the increased presence of amaranth oil and, thus, to the higher contents of unsaturated fatty acids, which are sensitive to oxidative reactions [50]. On the other hand, 50% of the amaranth oil in formulation C was replaced by squalane, which is completely saturated, thus leading to a reduced peroxide value.

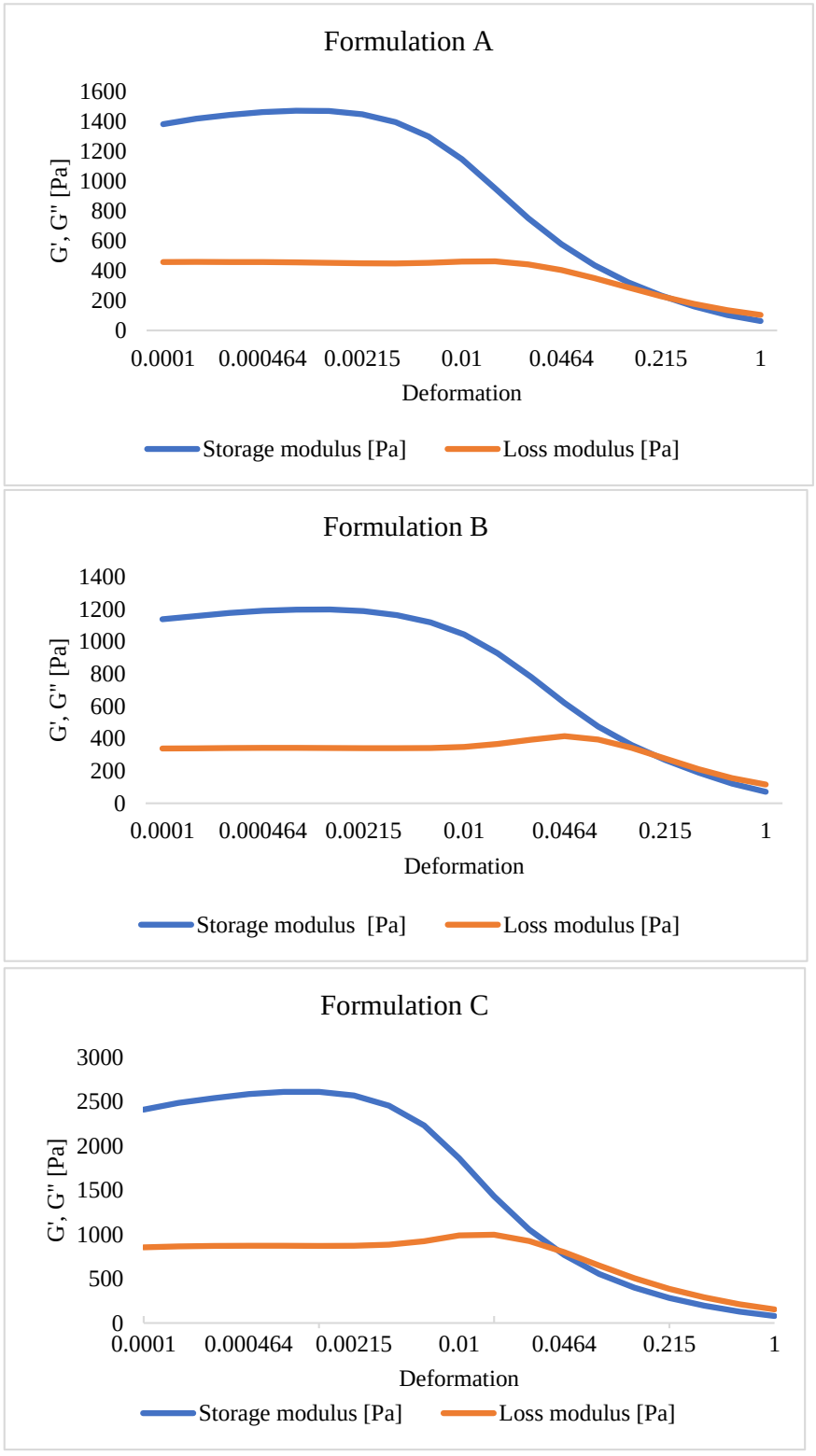

Figure 4. Oscillation sweep tests of formulations A, B, and C.

Squalane is not very sensitive to peroxidation. At a high concentration (up to 1%), it appears to function in the skin as a neutralizer of singlet oxygen, protecting human skin surfaces from lipid peroxidation caused by exposure to UV light and other sources of oxidative damage [17]. Similar results have been reported in the literature, where the addition of squalane extracted from olive oil showed a better protective action than the same amount of mixed tocopherols in cosmetic formulations [51].

3.5. Sensory Profile Analysis

Sensory tests were performed in order to evaluate the products' overall acceptability by the consumer, and to compare various characteristics (i.e., spreadability, oiliness, freshness, and absorbency) of the three tested formulations (A, B, and C). Sensory evaluation is also useful for improving products and quality control. The results of the tests are shown in Figure 5. The analysis of the sensory profiles revealed that formulations containing vegetable oil and squalane (B and C) received high ratings for their spreadability and absorbency parameters at levels close to the maximum (6 points)—especially for formulation C, which contained 1% *Amaranthus cruentus* vegetable oil and 1% squalane. Low smoothness results were also obtained with formulations B and C. In fact, squalane is considered to be one of the best emollients, which are absorbed quickly and effectively into the skin, restoring its flexibility without leaving an oily residue [17].

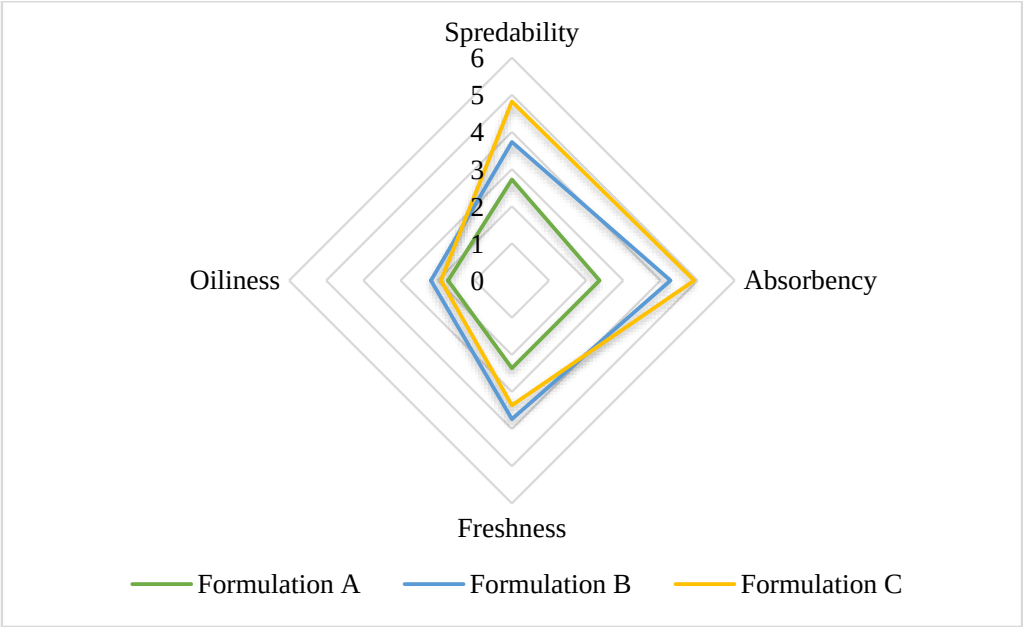

Figure 5. Sensory evaluation of formulations A–C following their application to the skin.

4. Conclusions

The obtained results underline the interest in amaranth oil and its components for various industries, including cosmetic uses. Indeed, as amaranth has the richest plant seeds in terms of squalene—known for its antiaging properties—it can be used for diverse cosmetic preparations. As expected, higher performances (i.e., yield and composition) were observed when amaranth seeds were extracted through supercritical CO_2 extraction, compared to solvent extraction. On the other hand, the optimal conditions to obtain a fully hydrogenated amaranth oil were revealed, and the resulting oil had higher stability as it was rich in squalane instead of squalene. Moreover, different W/O emulsions were prepared to investigate the added values of amaranth oil and squalane for the formulations' moisturizing effects. Better quality was achieved when W/O formulations were enriched with 2% amaranth oil, and with 1% oil and 1% squalane. However, the addition of squalane gave the best results in terms of rheological properties, peroxide index, and sensory at-

tributes. Further investigations are needed to compare the extraction methods using raw materials of the same origin and year of cultivation. Furthermore, it could be interesting to investigate the rheological effects of higher levels of oil and squalane than those tested in this study.

Author Contributions: Conceptualization, T.T., M.U., B.S.-A., A.H. and O.M.; methodology, B.S.-A., M.U., Z.S., M.C. and T.T.; software, B.S.-A., M.U., P.E. and O.M.; validation, T.T., M.U., O.M., A.H. and Z.S.; formal analysis, B.S.-A., M.C. and P.E.; investigation, B.S.-A., M.U., O.M. and P.E.; data curation, B.S.-A., M.C., M.U., P.E. and O.M.; writing—original draft preparation, B.S.-A., T.T., A.H., M.C. and O.M.; writing—review and editing, M.U., Z.S., P.E. and O.M.; supervision, M.U., T.T., A.H., Z.S. and O.M.; project administration, A.H., T.T. and O.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. The twenty female panelists gave their written informed consent before participating in the study. The study was approved by the Ethics Committee of Institut National Polytechnique de Toulouse, France (date of approval: 21 June 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: Bouchra Sayed-Ahmad obtained a dual doctorate thesis grant from the Lebanese University and the National Polytechnic Institute of Toulouse, and was supported both by the Lebanese University and the National Polytechnic Institute of Toulouse.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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