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# Extraction of amaranth seed oil using subcritical butane and sunsequent protein extraction from the cake generated in the process

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**SUMMARY:** The purpose of this research was to determine the technical feasibility of extracting amaranth seed oil with butane in a subcritical state and to take advantage of the cake generated. To this end, a type of non-germinated grain was characterized, oil was extracted from a germinated grain and the characterized one, the oil obtained was characterized, and the protein was extracted from the defatted cake of the non-germinated one. It was found that the non-germinated grain was made up of 13.33% protein, 7.24% fat, and 9.02% moisture, the optimum yield of this grain was 91%, for the germinated grain, a maximum value of 6.63% for oil mass. By comparing the characteristics of both oils, higher quality was found in the non-germinated oil, and the maximum protein extraction productivity was 5.15%. Thus, it has been concluded that this extraction method is technically feasible.

***KEYWORDS:*** *Amaranth oil; Amaranth protein; Oil extraction; Oil quality; Subcritical butane.*

**RESUMEN:** ***Extracción de aceite de semilla de amaranto utilizando butano subcrítico y uso de la torta generada para la extracción de proteínas.*** Esta investigación tuvo como propósito determinar la viabilidad técnica de la extracción de aceite de semilla de amaranto con butano en estado subcrítico y aprovechar la torta generada. Para lo cual, se caracterizó un tipo de grano no germinado, se extrajo aceite de un grano germinado y del caracterizado, se caracterizó el aceite obtenido, y se extrajo la proteína de la torta desengrasada del no germinado. Se encontró que el grano no germinado estaba conformado por 13,33% de proteína, 7,24% de grasa, y 9,02% de humedad, el rendimiento óptimo del grano caracterizado fue de 91%, para el grano germinado se obtuvo un valor máximo de 6,63% en masa de aceite, comparando las características de ambos aceites se encontró mayor calidad en el aceite no germinado, la productividad de extracción de proteína máxima fue de 5,15%. Concluyendo así que técnicamente es factible este método de extracción.

***PALABRAS CLAVE:*** *Aceite de amaranto; Butano subcrítico; Calidad de aceite; Extracción de aceite; Proteína de amaranto.*

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## 1. INTRODUCTION

The *Amaranthus L.* (Amaranthaceae) genus comprises between 70 and 80 species throughout the world. Most of them are located in the Americas, although only 3 of them are cultivated for commercial purposes: *A. caudatus*, *A. cruentus*, and *A. hypochondriacus* (Luis *et al.*, 2018; Riggins and Mumm, 2021). These plants are annual herbaceous or short-lived perennials that produce tiny flowers which grow at the end of their branches or above the mother center of the plant. These flowers can persist even after the death of the plant, and this is the place where the seeds are generated (Riggins and Mumm, 2021).

The amaranth seed has remarkable nutritional qualities. Its protein content is higher than other conventional cereals, ranging from 13 to 19%. Its contents in essential amino acids such as lysine, valine, methionine, phenylalanine, and threonine stand out. In addition, it has a low proportion of gluten, which makes it suitable for consumption by celiacs, not to mention its high digestibility, ranging from 80 to 92% (Arias-Giraldo and López-Mejía, 2021; Castel, 2010; Hernandez and Herrerías, 1998; Luis *et al.*, 2018). It also has a high content of dietary fiber, ranging from 2 to 9% (Arias-Giraldo and López-Mejía, 2021; Hernandez and Herrerías, 1998; Luis *et al.*, 2018).

The total or partial isolation of amaranth protein could have different types of uses. The most distinguished among them is its use as a nutritional supplement. Amaranth protein could be introduced as a component in different concentrated protein flours used by athletes. It also could be mixed with soy flours to create vegetable meet, which would be particularly attractive to the vegetarian population (Venskutonis and Kraujalis, 2013).

The oil content in the seed can vary considerably, depending on the species and variety of the grain, and ranges from 6 to 10.9% (Krist, 2020; Nasirpour-Tabrizi *et al.*, 2020). This oil has a high potential as a marketable product. In its fatty acid profile, the contents in stearic, palmitic, oleic, and linoleic acids stand out (Nasirpour-Tabrizi *et al.*, 2020). Its unsaponifiable matter is made up of phospholipids, tocopherols, tocotrienols, and phytosterols, but, above all, squalene, which represents an average value of 4.2% of the total oil and can rise to 8% for the *caudatus specie*. Therefore, amaranth oil could represent an alternative source for the obtention of squalene to the traditional one, which is shark liver. (Nasirpour-Tabrizi *et al.*, 2020). Thanks to its composition, amaranth oil could have multiple applications. In the pharmaceutical industry for example, it could be used to produce dietary supplements which would help to reduce blood cholesterol and blood pressure, and consequently the risk of heart and circulatory diseases. Thanks to its antioxidant, rejuvenating, and anti-inflammatory effect, it also could be used in the cosmetic industry for the production of creams, especially made for the elderly and those with sensitive or dry skin (Krist, 2020).

To obtain vegetable oils, multiple techniques have been used. By mechanical means, cold pressing produces high-quality oil and reduces costs associated with electricity; however, the extraction yield is generally low. Hot pressing allows an increase in the extraction yield, but the quality of the final product deteriorates. Furthermore, the process requires more time and costs associated with electricity consumption are a drawback (Bhargavi *et al.*, 2018; Hernández and Mieres-Pitre, 2005; Le Clef and Kemper, 2015). Regarding extractions with chemical solvents, the most widely used is the Soxhlet method. It generally uses hexane, petroleum ether, or acetone as solvents, and it usually generates high extraction yields; however, due to the toxicity of its solvents, the oil requires a subsequent refining process, in which the quality decreases (Bhargavi *et al.*, 2018). On the other hand, a novel vegetable oil extraction process has been studied over the last 2 decades, the supercritical oil extraction method. This technique uses a fluid in a supercritical state as a solvent, which is usually carbon dioxide. Thanks to the viscosity and solubility of supercritical fluids, this method generates high extraction yields and quality products. Unfortunately, the supercritical extraction technique has elevated costs associated with energy consumption, and the required machinery (Bhargavi *et al.*, 2018).

An interesting and new alternative for vegetable oil extraction is the sub-critical extraction. This technique does not require extremely high pressures, expensive machinery or large amounts of energy. The extraction yields obtained using fluids in a sub-critical state are usually high, as is the quality of the products (Han *et al.*, 2016; Sun *et al.*, 2018; Xu *et al.*, 2015).

This study aims to discover the technical feasibility of extracting amaranth oil using butane in a subcritical state, and to extract protein from the cake generated in the process.

## 2. MATERIALS AND METHODS

### 2.1. Materials

In this work, amaranth grains of the *caudatus* specie, Oscar Blanco variety were used. They were obtained from the supplier ASOVITA. These grains were grown in Bolivia, in the department of Chuquisaca, Zudáñez province, more specifically in the Sopachuy region. Its harvest season was between the months of March and April, 2022. The grains were stored for 6 months, at an average temperature of 23 °C, and an average relative humidity of 46%. For the assays, part of the grain was germinated and dried to a humidity content of 12%, which was done to study the effect of germination on the oil quality and protein content of the grains. For grinding, a YB-2500 A brand Yunbang (China) cereal mill was used, where the germinated grain was crushed for 3 minutes at 2,500 rpm, thus obtaining a "fine" granulometry flour. On the other hand, two flours of different granulometry were obtained for the ungerminated grain, the first grinding for a time of 1.5 minutes at 2,500 rpm to obtain a coarse granulometry, and the second with a grinding time of 3 minutes at 2500 rpm to obtain a fine granulometry. All reagents used were purchased from Merck Co. (Darmstadt, Germany) or Winkler Ltda. (Santiago, Chile).

### 2.2. Characterization of the non-germinated amaranth grain

The characterization of the non-germinated amaranth grain was made based on the standards stipulated by the Bolivian Institute for Standardization and Quality (IBNORCA). To determine the moisture content, NB 312026 was used, NB 312027 for fat, NB 312029 for protein, NB 312030 for ash, and NB 312031 for carbohydrates.

### 2.3. Extraction of amaranth oil from germinated and non-germinated grain

To carry out the extraction, a 5 LB bidirectional extractor was used in a Closed-Circuit brand Extractor Solutions (USA), which consists of a tank with butane content, a column for the extraction of the raw material with a diameter of 6 and a length of 12, an oil collection column of 12 in diameter and 18 in length, a dehydration column, a compressor, a condenser, and a chiller (see, Figure 1). The previously crushed raw material was introduced into the extraction column, and a paper filter was installed

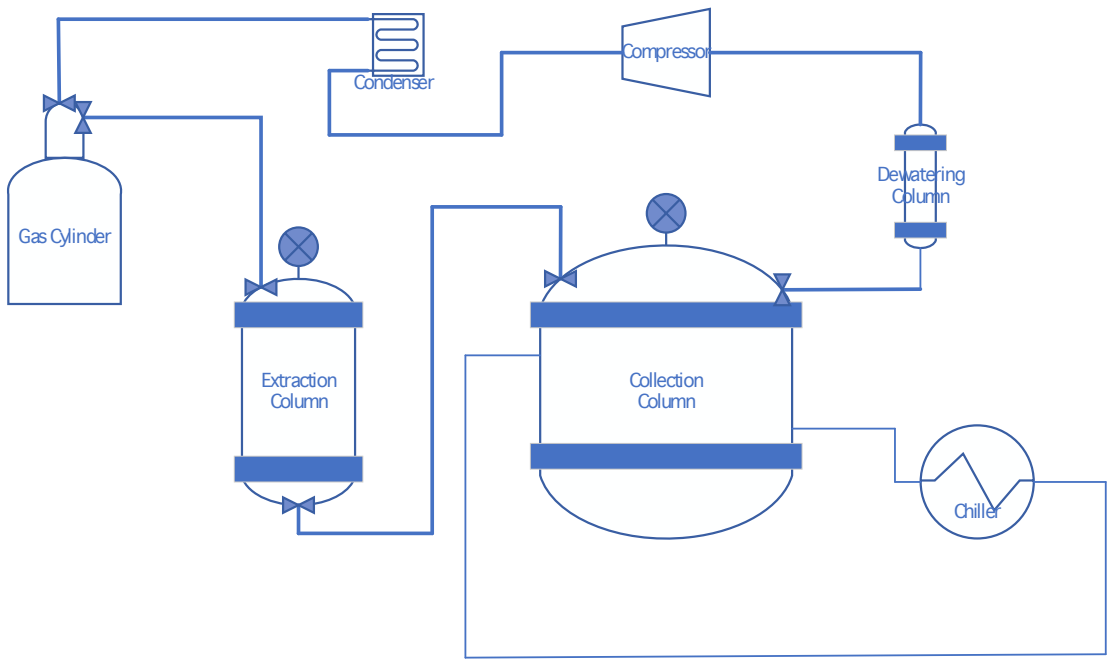


**Figure 1.** Diagram of the subcritical phase extractor used in the experimentation.

on a stainless-steel grid at the bottom to retain the raw material inside the extraction column. After installing the flour, the system was subjected to a pressure of 90 PSI with Nitrogen gas, to verify eventual leaks, then the extractor was evacuated. Later butane was allowed to flow through the upper part of the extraction tank, where the amaranth flour was contained, and it was left to soak for 30 min. Then, the solvent with the extract was allowed to flow to the collection column, where the solvent was evaporated and recovered into the butane tank by employing the extractor compressor and a cooling system.

The extracted oil was left to rest for two days in a closed, dark environment, and then purified using a rotary evaporator at 35 °C and a partial vacuum of -40 kPa for 2 h. Finally, the oil was stored in amber containers at room temperature.

For ungerminated amaranth, a factorial experimental design was made considering as variables the ratio of mass of solvent/mass of raw material and the granulometry. On the other hand, for germinated amaranth, 2 extraction tests were performed with fine granulometry and ratios of 1/1 and 2/1 solvent/flour. The same methodology was used for all treatments.

### 2.4. Measurement of the ratio and granulometry

To measure the ratio, 3.0 kg was established as the standard amount of ungerminated raw material and 2.4 kg of germinated raw material to be introduced into the extractor. These quantities were selected to fill the column where the flour had to be disposed. Then, the ratio was defined by the following equation:

$$Ratio = \frac{Solvent\ mass}{Flour\ mass}$$

To determine the solvent mass needed to satisfy the ratios required, the values of the ratio and the flour mass were simply substituted in the equation previously presented in this section, and then the solvent mass was calculated. A balanced was used to ensure that the solvent mass introduced into the extractor was the calculated one. Initially, the weight of the butane container was measured and then a mathematical subtraction of this value was done, according to the following equation:

$$Final\ mass\ of\ the\ container = Initial\ container\ mass - Solvent\ mass$$

Then, the solvent was allowed to flow and the mass of the container was controlled until the obtention of the final mass calculated.

To determine the granulometry of the flours, the NB 39012 standard was used, which is based on the sifting of the flour.

### 2.5. Performance and Productivity Indicators

To characterize the results of the tests, two indicators were established to express the effectiveness of the process. On the one hand, the extraction yield relates the amount of oil extracted to the oil content of the grain, as given in the following equation:

$$Ext.Yield = \frac{Oil\ extracted}{Grain\ oil\ content} * 100\%$$

This indicator was used exclusively for the extraction of oil from ungerminated amaranth grain. The oil content in the flour is obtained from the analysis carried out on a sample according to the standard procedure, in this case, a Soxhlet extraction.

On the other hand, the extraction productivity was defined as the amount of oil extracted per unit mass of flour, expressed as a percentage, which would be given by the following equation:

$$Productivity = \frac{Oil\ extracted}{Amaranth\ flour\ used} * 100\%$$

This indicator was used exclusively for the extraction of germinated amaranth grain oil.

### 2.6. Experimental design

A factorial experimental design was carried out for the extraction of amaranth oil from the non-germinated grain. The factors considered were the granulometry and the ratio of mass of solvent/mass of raw material. The response variable was the extraction yield. For the first factor, 2 levels were considered a coarse and a fine granulometry. For the second, 3 levels were considered, ratios of 8/15, 16/15, and 24/15 solvent/raw material. This is shown in

Table 1. Each test was done in duplicate, and the total number of tests was 12. The variables which remained constant were the pressure, with a value of 70 PSI and the soak time of the solvent with the raw material, which was 30 min.

### 2.7. Statistical analysis

The data was analyzed using the Minitab 2019 software, and a two-factor analysis of variance (ANOVA 2) was applied to find out if, statistically, the factors considered in the experimental design had an impact on the response variable, with a significance level of 95%. For the analysis of the normality of the residual values, an Anderson-Darling test was applied, and to determine the homogeneity of variances of the residuals, a Bartlett test was applied.

### 2.8. Characterization and profile of fatty acids in the oils

To characterize the germinated and non-germinated amaranth oil, 100-ml samples of both oils were taken. The characterization was carried out by the Centro de Investigaciones Químicas SRL in the town of Quillacollo (Cochabamba, Bolivia).

The physicochemical parameters determined were: humidity, based on the Bolivian standard NB 34010; density, based on NB 34021; iodine value, based on NB 34006; refractive index at 25 °C, based on NB 34003; peroxide index, based on NB 34008; and acid value, expressed as oleic acid. The fatty acid profile for both oils was determined using the GC-FID Method FAME AOAC 969.33.

Finally, an extraction of non-germinated amaranth oil was made by cold pressing, which was carried out by the company COMEDICA Bolivia. Subsequently, this oil was characterized according to the same parameters as those of the oils obtained with subcritical butane in order to make a comparison between the values obtained from both techniques and thus know how good the quality of the oil is as produced by the technique of extraction investigated. The cold pressing technique was chosen for comparison since it generally produces high-quality oils.

### 2.9. Extraction of protein from defatted ungerminated amaranth flour

In the extraction of protein, the effect of the particle size on the extraction productivity was consid-

**Table 1.** Variables, levels, and results of the experimental design

| Variables and Levels | | | | | |
|---|---|---|---|---|---|
| **Independent variables** | **Units** | **Symbols** | **Level coding** | | |
| | | | **-1** | **0** | **1** |
| Granulometry | Micrometers | X1 | - | 291 | 393 |
| Ratio | Dimensionless | X2 | 8/15 | 16/15 | 24/15 |

| Experimental Results | | | | | |
|---|---|---|---|---|---|
| **Treatment** | **X1** | **X2** | **Replication Yield 1 (%)** | **Replication Yield 2 (%)** | **Mean value (%)** |
| 1 | 0 | -1 | 76.66 | 61.29 | 68.98 ± 7.69 |
| 2 | 0 | 0 | 92.46 | 89.54 | 91.00 ± 1.46 |
| 3 | 0 | 1 | 92.55 | 89.83 | 91.19 ± 1.36 |
| 4 | 1 | -1 | 62.06 | 72.34 | 67.20 ± 5.14 |
| 5 | 1 | 0 | 78.05 | 76.95 | 77.50 ± 0.55 |
| 6 | 1 | 1 | 80.59 | 79.35 | 79.97 ± 0.62 |

| Statistical Results of ANOVA 2 | | |
|---|---|---|
| **Fountain** | **F-value** | **p-value** |
| Ratio | 12.63 | 0.007 |
| Granulometry | 7.79 | 0.032 |
| Ratio*Granulometry | 1.29 | 0.343 |

*($P < 0.05$) It is accepted that the ratio and the granulometry have a significant effect on the extraction yield and that there is no interaction between the variables.

ered, for which 2 tests were carried out, one with the defatted cake of coarse particle size (9.8 % humidity and 1.49 % fat by weight) and another with the defatted cake of fine particle size (9.9 % moisture and 0.72 % fat by weight). These defatted cakes were obtained from previous oil extractions made with butane. The values considered for the basic and acid pH to which the suspension was brought were obtained from the investigations of Salcedo-Chávez *et al.* (2002), Das *et al.* (2021) and Cordero de los Santos *et al.* (2005), since they obtained optimal yields and a quality product under these conditions.

The extraction technique used was in an alkaline medium followed by a precipitation of the protein at the isoelectric point. A random sample of 200 g of defatted cake was suspended in water (1:9, m/m), and subsequently, the solution was basified up to pH 9 by adding 1 N NaOH solution, stirring the solution with a FLOC-6 model paddle stirrer, Raypa brand (Spain) at an angular velocity of 200 rpm, at 25 °C, and for 1 h. The pH was controlled throughout the process using a pH meter Edge brand Hanna (Romania). The solution was then centrifuged in a Pro–Hospital LL model centrifuge Centurion brand (England) at 2,100 g, for 30 min and the supernatant was transferred to another container to finally store it in a Samsung brand model RT53K65145L refrigerator (South Korea) at a temperature of 5 °C overnight. The following day, the protein concentrate was acidified to pH 5 using 1 N hydrochloric acid. The pH was controlled with a Hanna (Romania) Edge pH meter, shaking the solution with a Raypa FLOC-6 model paddle stirrer (Spain) at an angular velocity of 200 rpm for 1 h. Then the solution was centrifuged with an acceleration of 2100 g, for 30 min. At the end of the process the supernatant was discarded and the decantant was recovered as protein concentrate. The protein concentrate was neutralized with 1 N sodium hydroxide, and finally, it was lyophilized with a BK – FD10PT model lyophilizer, brand Biobase (China) for 3 days.

To verify the net protein content, the Kjeldahl analysis was performed for the 2 extractions, specified by standard NB 312029. The factor used to convert from nitrogen to protein was 6.25.

The productivity of the protein extraction from the ungerminated grain was defined as a percentage of the protein mass fraction recovered per unit of defatted cake used to perform the extraction.

$$\text{Protein extraction productivity} = \frac{Protein\ extracted\ (g)}{Defatted\ amaranth\ flour\ (g)} * 100\%$$

## 3. RESULTS

### 3.1. Physicochemical properties of ungerminated amaranth grain and particle size

The physicochemical properties of ungerminated amaranth are shown in Table 2. Krist (2020), in her book *Vegetable Fats and Oils*, makes a compilation of oil values in amaranth for the *caudatus variety*, which oscillate between 6 to 9 g of oil/100g of seed, values which agree with the result obtained of 7.24 g of oil/100 g of seed. Calderón-Vásquez (2017), who worked on the physicochemical characterization of a non-germinated amaranth grain with the same geographical origin as the grain used, obtained a value of 7.46 %, almost the same as that obtained in this study.

**Table 2.** Physicochemical properties of amaranth grain

| Properties | **Mean ± SEM (g/100 g of seed)** |
|---|---|
| Humidity | 9.02 ± 0.05 |
| Fat | 7.24 ± 0.12 |
| Protein | 13.33 ± 0.16 |
| Ash | 2.32 ± 0.01 |
| Carbohydrates | 68.09 |

*The tests for each parameter were carried out in duplicate as specified by the Bolivian standard. These data are expressed as mean ± SEM with the exception of carbohydrates, which result from the subtraction of the means of each of the components from 100 g of flour.

Regarding protein, the result obtained was 13.33 g of protein/100 g of seed, which is close to the lowest values given in the literature of 13 g/100 g of seed (Arias-Giraldo and López-Mejía, 2021; Castel, 2010; Hernandez and Herrerías, 1998; Luis *et al*., 2018). This may be due to the species and variety used. Calderón-Vásquez (2017), who used an amaranth grain of the same species and variety, reported a value of 14.30 g of protein/100 g of seed, a result close to that obtained in this work.

Finally, moisture, ash, and carbohydrates, with values of 9.02 g, 2.32 g, and 68.09 g/100 g of seed, respectively, fit correctly into the values given in the literature of 9-12 g/100 g of seed for the first, 2-4.6 g/100 g of seed for the second, and 66-71 g/100 g of

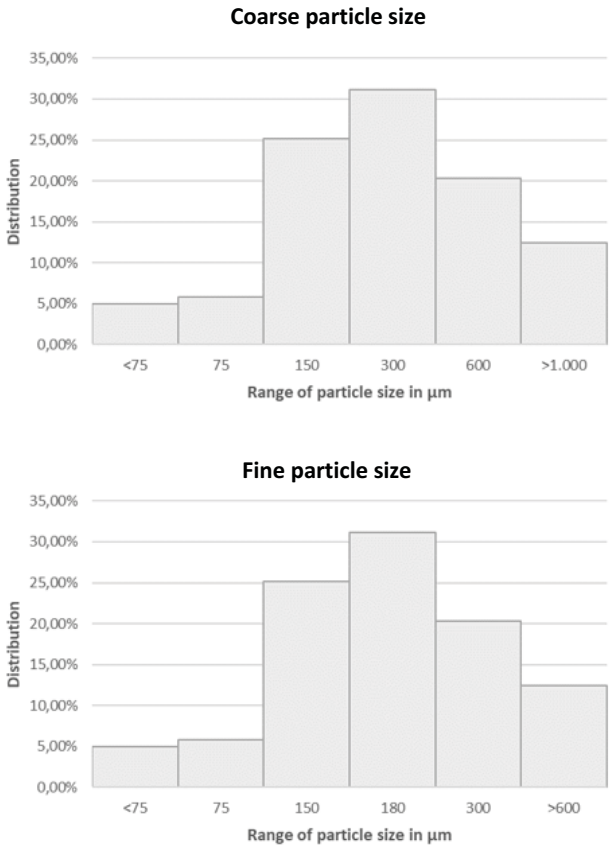


**Figure 2.** Frequency histogram showing the percentage by mass of coarse and fine flour retained on sieves of different sizes.

seed for the third (Arias-Giraldo and López-Mejía, 2021; Hernandez and Herrerías, 1998; Luis *et al.*, 2018).

Regarding the granulometry Figure 2, it was determined that the flour called coarse had an average particle size of 393 µm, on the other hand, the average particle size of the fine flour resulted in 291 µm.

### 3.2. Extraction yield

The extraction yields obtained for non-germinated amaranth are shown in Table 1. It can be seen that the highest yields correspond to the replicates of treatment 6, in which a ratio of solvent to flour of 24/15 was used, a fine granulometry of 291 µm; yield values of 92.55 and 89.83%, respectively, were obtained. This result was expected, since increasing the contact surface of the grain allows for a greater penetration of the solvent through the pores of the amaranth, and increasing the solvent ratio allows for a thorough extraction of the oil. Therefore, the smaller the granulometry of the flour used and the higher the ratio, the higher the extraction yield obtained will be. This can be seen more clearly in Figure 3.

Even though treatment 6 resulted in a higher extraction yield, treatment 5 was considered to be the

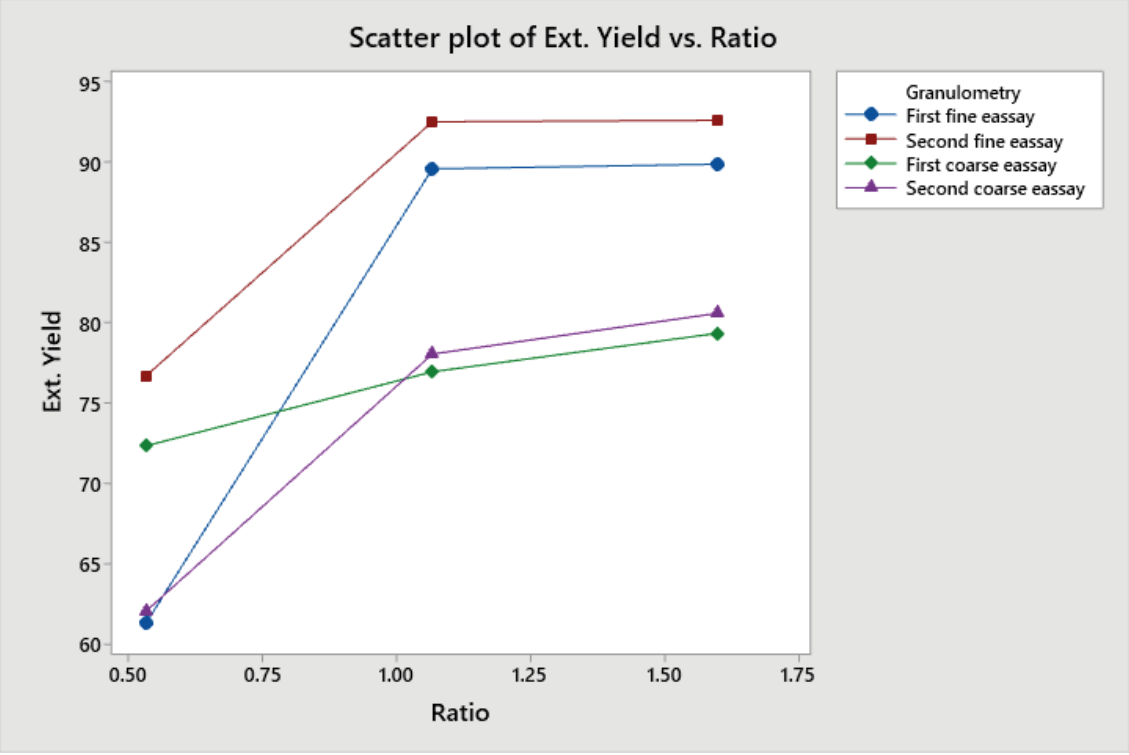


Figure 3. Scatter graph of the oil extraction yield as a function of the solvent ratio and granulometry.

optimal treatment because fewer resources (time, electrical energy, and inputs) were used to obtain similar extraction yields, which were 92.5 and 89.5%, almost the same as treatment 6 of 92.6 and 89.3%. This small difference in extraction yield is due to the ratios used, since the first treatments was completely saturated and insufficient to dissolve all the oil contained in the amaranth. As the ratio increases, there is greater solvent entry, for a constant mass of amaranth, therefore, the amount of solvent that enters ends up exceeding the amount necessary to dissolve the oil contained in the amaranth.

The effect of ratio and granulometry on the extraction yield can be statistically corroborated with a two-factor analysis of variance (ANOVA 2) as shown in Table 1, for which a significance level of 5% was considered ($P < 0.05$). The values obtained for P were 0.032 for the granulometry, 0.007 for the ratio, and 0.343 for the interaction between ratio and granulometry, thus concluding that the granulometry and the ratio affected the extraction yield and that there was no interaction between the two variables.

Regarding the productivity of the germinated amaranth grain shown in Table 3, absolute values quite like those obtained with non-germinated amaranth were found.

**Table 3.** Extraction productivity of germinated amaranth grain

| Treatment | Mass ratio (kg sol/kg MP) | Granulometry | Productivity |
|---|---|---|---|
| 1 | 1/1 | Fine | 6.55% |
| 2 | 2/1 | Fine | 6.63% |

*Each treatment was carried out once.

No investigations have been found on the extraction of amaranth oil using subcritical butane. However, there are studies where other types of vegetable oils were extracted with this solvent, such as that of Han *et al.* (2016), where he found that the yield in the extraction of soybean germ oil with subcritical butane was significantly equal to that obtained by carbon dioxide in the supercritical state or with hexane. Gu *et al.* (2017), studied the extraction of fenugreek seed oil using subcritical butane as a solvent and also found high extraction yields.

The extraction yields found in this research are similar to those found with other extraction techniques, such as that of Westerman *et al.* (2006), who studied the extraction yield of amaranth oil using carbon dioxide in a supercritical state.

### 3.3. Physicochemical properties of the oils obtained.

The physicochemical properties of the ungerminated and germinated amaranth oil obtained with subcritical butane and the ungerminated oil obtained by cold pressing can be observed in Table 4.

Making a quality comparison between the germinated and non-germinated grain amaranth oils obtained with subcritical butane, it is notable that the quality of the non-germinated with respect to germinated Amaranth grains is highly superior. This represented the great difference that exists between the peroxide indices of 3.78 for the first and 0.47 for the second, iodine 92.98 for the first and 106.93 for the second, and acidity 13.93 for the first and 0.52 for the second. This difference may be due to a degradation in the oil of the grain during the germination process and subsequent processes of drying and grinding.

Comparing the quality of non-germinated amaranth oil obtained with subcritical butane and that obtained by cold pressing, the following is notorious. Starting with the acid value of 0.52 for the first and 1.17 for the second and the peroxide value of 0.47 for the first and 3.04 for the second, a greater amount of free fatty acids and oxidation through cold pressing were evident. this may be due to the fact that, with cold pressing, there is a heating of the flour due to the friction and high pressures that may reach values above 80 °C. They are considerably higher than those during extraction with butane, which ranged between 10 to 15 °C. In addition, throughout the extraction process with butane, neither the flour nor the oil came into direct contact with ambient air. On the other hand, almost no water was extracted during extraction with butane, and the oil is quite dry in comparison with the oil extracted with cold pressing where significant amounts of water were extracted together with oil. Regarding the iodine value, a value of 106.93 was obtained for the first and 70.12 for the second, which indicates that a greater amount of unsaturation was obtained using butane as a solvent.

### 3.4. Fatty acid profile of the oils obtained.

The fatty acid profile obtained for the different oils is shown in Table 4. In the three of them,

**Table 4.** Physicochemical properties and Profile of fatty acids of the obtained oils.

| **Physicochemical properties of the obtained oils** | | | | |
|---|---|---|---|---|
| **Parameters** | **Unit** | **Ungerminated obtained with subcritical butane** | **Sprouted obtained with subcritical butane** | **Ungerminated obtained by cold pressing** |
| Humidity | g/100g | <0.100 | <0.100 | 0.14 |
| Density | g/ml | 0.915 | 0.919 | 0.917 |
| Iodine value | gI /100g | 106.93 | 92.98 | 70.12 |
| Refractive index 25°C | nD | 1,465 | 1,462 | 1,472 |
| Peroxide value | meq$O_2$/kg | 0.47 | 3.78 | 3.04 |
| value expressed as oleic acid | %Oleic acid | 0.52 | 13.93 | 1.17 |

| **Profile of fatty acids of the obtained oils** | | | | |
|---|---|---|---|---|
| **No.** | **Parameters** | **Result (%)** | | |
| | | **Sprouted oil obtained with butane** | **Non-sprouted oil obtained with butane** | **Cold pressing** |
| 1 | **Saturated fatty acids** | 27.05 | 27.8 | 23.55 |
| | Myristic acid C14:0 | - | - | 0.15 |
| | Palmitic acid C16:0 | 17.32 | 22.51 | 19.28 |
| | Heptadecanoic acid C17:0 | - | - | 0.08 |
| | Stearic acid C18:0 | 7.87 | 4.47 | 3.54 |
| | Arachidic acid C20:0 | 1.68 | 0.82 | - |
| | Heneicosanoic acid C21:0 | - | - | 0.01 |
| | Behenic acid C22:0 | - | - | 0.18 |
| | Lignoceric acid C24:0 | - | - | 0.31 |
| 2 | **Monounsaturated fatty acids** | 29.30 | 30.69 | 29.11 |
| | Myristoleic acid C14:1 | | | 0.07 |
| | Palmitoleic acid C16:1 | 1.02 | - | 0.22 |
| | Heptadecenoic acid C17:1 | 0.41 | - | 0.59 |
| | Methyl *trans*-9 octadecanoic acid C18:1n9 | 1.83 | - | - |
| | Oleic acid C18:1n9c | 26.04 | 30.69 | 28,23 |
| 3 | **Polyunsaturated fatty acids** | 23.48 | 41.26 | 47.33 |
| | Linolelaidic acid C18:2n6t | | | 0.07 |
| | Linoleic acid C18:2n6c | 21.48 | 40.26 | 46.99 |
| | Linolenic acid C18:3n3c | 2.00 | 1.00 | 0.27 |

stearic, palmitic, oleic, and linoleic acids stand out, with values of 7.87, 17.32, 26.04, and 21.48%, respectively, for the extraction of germinated grain with butane; with values of 4.47, 22.51, 30.69, and 40.26%, respectively, for the extraction with butane of non-germinated grain; and values of 3.54, 19.28, 28.23 46.99%, respectively, for extraction by cold pressing. It can be seen that that there is a slightly larger fraction of unsaturated fatty acids in the saponifiable matter of the oil obtained by cold pressing than in the oil obtained with butane. This is in contrast with the iodine value, which indicates that there is a greater quantity of unsaturation obtained using butane than with cold pressing. This indicates that, proportionally, the unsaturated fatty acids represent a higher proportion of the saponifiable matter in the oil obtained by cold pressing, but in absolute terms, there is a greater amount of unsaturation obtained with butane, which would indicate that the saponifiable fraction of the oil obtained with butane is greater than that obtained by cold pressing.

### 3.5. Extraction of protein from ungerminated amaranth cake

Table 5 shows the results obtained for the protein extraction of ungerminated amaranth cake. The fine grain, which had a smaller particle size and a smaller amount of fat, had a productivity of 5.51%, which was higher than the value obtained for the coarse grain, which was 3.57%. This result was expected, since the reduced particle size increased the contact surface, and the penetration of the basic water solution into the pores of the flour was more effective.

Table 5. Results of protein extraction from defatted cake

| Grain type | Defatted cake dough on dry basis | Extracted Protein concentrate | Net protein extracted | Productivity |
|---|---|---|---|---|
| Fine | 180.26 | 11.5 | 9.29 | 5.15% |
| Coarse | 180.42 | 8.1 | 6.45 | 3.57% |

*Each treatment was carried out once.

It is important to mention that the product obtained after lyophilization was not pure protein, instead, it was a protein concentrate. Table 5 shows the concentrates extracted from the defatted cake, with a value of 11.50 g for the fine grain, which represented 6.38% of the cake used with a net protein content of 9.29 g, and a purity of 80.78%. For the coarse grain, the protein concentrate extracted was 8.1 g, which represented 4.49% of the cake used. It had a net protein content of 6.45 g, with 79.63% purity. A mass balance was released to know the protein content in the fine and coarse grains. For the first one, it was determined to be proximately 28.1 g and the net protein extracted was 9.29 g, so it represented 33.1% of the total. On the other hand, the protein in the coarse grain was determined to be proximately 27.9 g and the net protein extracted was 6.45 g, representing 23% of the total.

Further research is necessary to determine whether the protein concentrate extracted would be considered safe for human consumption. Nevertheless, it is not because of the sub-critical butane extraction method, given that the process of lyophilization eliminates all small butane residues, but because of the process of protein isolation.

The results obtained are similar to those of Cordero de los Santos *et al.* (2005), who obtained somewhat higher amaranth protein extraction values, but with a lower pH during the precipitation stage. As shown by Salcedo-Chávez *et al*. (2002) and Cordero de los Santos *et al*. (2005), protein extraction productivity can be further increased by reducing the pH to a value of 4.

## 4. CONCLUSION

It can be concluded that the extraction of amaranth oil using subcritical butane is technically feasible. The optimal values to generate the highest extraction yield were around 1/1 (mass of solvent/mass of amaranth) for the ratio and a particle diameter of less than 300 μm, considering a saturation pressure of 70 psi and a soak time of 30 min to obtain germinated and non-germinated grain oil. However, since there is no interaction between the factors considered, and the relationship between the granulometry and the extraction yield are inversely proportional, and that the relationship between the ratio and the extraction yield are directly proportional, higher values can be found appropriate to optimize the number of resources used.

The physicochemical parameters and the fatty acid profile of the oil obtained with subcritical butane were similar to those obtained by cold pressing, which denotes good quality in the oil. However, further studies are necessary to determine the components of the unsaponifiable fraction of the oil and the proportion they represent.

Finally, it is possible to take advantage of the defatted cake obtained by butane in a subcritical state to obtain amaranth vegetable protein with good productivity. Nevertheless, further research is necessary to determinate whether it is safe for human consumption.

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## DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

## REFERENCES


Arias-Giraldo S, López-Mejía M. 2021. Uses, nutritional properties and sensory evaluation for amaranth, quinoa and grape and coffee by-products. *Ing. Compet.* **24** (1), 1–16. https://doi.org/10.25100/iyc.24i1.11000

Bhargavi G, Nageswara Rao P, Renganathan S. 2018. Review on the Extraction Methods of Crude oil from all Generation Biofuels in last few Decades. *IOP Conf. Ser. Mater. Sci. Eng.* **330** (1), 1–19. https://doi.org/10.1088/1757-899X/330/1/012024

Calderón-Vásquez S. 2017. *Estudio Físico- Químico del Grano de Amaranto (Amaranthus caudatus), Variedad Óscar Blanco para su Aprovechamiento con Fines Industriales.* Universidad Mayor de San Andres, La Paz. http://repositorio.umsa.bo/xmlui/handle/123456789/12117

Castel MV. 2010. *Estudio de las propiedades funcionales, tecnológicas y fisiológicas de las proteínas de amaranto.* Universidad Nacional del Litoral, Santa Fe de la Vera Cruz. http://hdl.handle.net/11185/212

Cordero de los Santos MY, Osuna-Castro JA, Borodanenko A, Paredes-López O. 2005. Physicochemical and functional characterisation of amaranth (amaranthus hypochondriacus) protein isolates obtained by isoelectric precipitation and micellisation. *Food Sci. Technol. Int.* **11** (4), 269–280. https://doi.org/10.1177/1082013205056491

Das D, Mir NA, Chandla NK, Singh S. 2021. Combined effect of pH treatment and the extraction pH on the physicochemical, functional and rheological characteristics of amaranth (Amaranthus hypochondriacus) seed protein isolates. *Food Chem.* **353** (3), 1–12. https://doi.org/10.1016/j.foodchem.2021.129466

Gu LB, Liu XN, Liu HM, Pang HL, Qin GY. 2017. Extraction of fenugreek (Trigonella foenum-graceum L.) seed oil using subcritical butane: Characterization and process optimization. *Molec*. **22** (2), 1–14. https://doi.org/10.3390/molecules22020228

Han JH, Wu QF, Xu B, Zhou SL, Ding F. 2016. Quality characteristics of soybean germ oil obtained by innovative subcritical butane experimental equipment. *Qual. Assur. Saf. Crop. Foods*. **8** (3), 369–377. https://doi.org/10.3920/QAS2015.0625

Hernández C, Mieres Pitre A. 2005. Rendimiento de la extracción por prensado en frío y refinación física del aceite de la almendra del fruto de la Palma Corozo (Acrocomia aculeata). *Rev. Ing. Uc.* **12**, 68–75. http://www.ciiq.org/varios/peru_2005/Trabajos/IV/7/4.7.02.pdf

Hernandez R, Herrerías G. 1998. Amaranto: Historia Y Promesa. *Horiz. del tiempo*. **1**, 1–18.

Krist S. 2020. *Vegetable Fats and Oils*. Springer Nature Switzerland. https://doi.org/10.1016/s0037-6337(12)70310-8

Le Clef E, Kemper T. 2015. *Sunflower: Chemistry, Production, Processing, and Utilization*. AOCS Press. https://doi.org/10.1016/B978-1-893997-94-3.50014-3

Luis GM, Hernández-Hernández RB, Caballero VP, Torres-López G, Adrián V, Martínez E, Pacheco LR. 2018. Current and potential uses of Amaranth (Amaranthus spp.). *J. Negat. No Posit. Results*. **3** (6), 423–436. https://doi.org/10.19230/jonnpr.2410

Nasirpour-Tabrizi P, Azadmard-Damirchi S, Hesari J, Piravi-Vanak Z. 2020. *Nutritional Value of Amaranth*. Intech Open. https://doi.org/10.5772/intechopen.91381

Riggins CW, Mumm RH. 2021. Amaranths. *Curr. Biol.* **31** (13), 834–835. https://doi.org/10.1016/j.cub.2021.05.058

Salcedo-Chávez B, Osuna-Castro JA, Guevara-Lara F, Domínguez-Domínguez J, Paredes-López O. 2002. Optimization of the Isoelectric Precipitation Method To Obtain Protein Isolates from Amaranth ( Amaranthus cruentus ) Seeds. *J. Agric. Food Chem.* **50**, 6515–6520. https://doi.org/https://doi.org/10.1021/jf020522t

Sun J, Shi J, Mu Y, Zhou S, Chen Z, Xu B. 2018. Subcritical butane extraction of oil and minor bioactive components from soybean germ: Determination of migration patterns and a kinetic model. *J. Food Process Eng.* **41** (5), 1–10. https://doi.org/10.1111/jfpe.12697

Venskutonis PR, Kraujalis P. 2013. Nutritional Components of Amaranth Seeds and Vegetables: A Review on Composition, Properties, and Uses. *Compr. Rev. Food Sci. Food Saf.* **12** (4), 381–412. https://doi.org/10.1111/1541-4337.12021

Westerman D, Santos RCD, Bosley JA, Rogers JS, & Al-Duri B. 2006. Extraction of Amaranth seed oil by supercritical carbon dioxide. *J. Supercrit. Fluids*. **37** (1), 38–52. https://doi.org/10.1016/j.supflu.2005.06.012

Xu L, Ye L, Qiu R, Qin G, Huang X. 2015. Extraction process and quality research of macadamia nut oil by subcritical butane. *J. Chinese Cereal. Oils Assoc.* **30** (6), 79–83.