

# PHYSICOCHEMICAL CHARACTERIZATION OF *CUCURBITA PEPO* L. (CUCURBITACEAE) SEED OIL OBTAINED BY COLD ORGANIC EXTRACTION

## ABSTRACT

**Background:** African flora is full of many plant species used by populations for a long time as sources of food, timber, energy and remedies for various somatic or spiritual ailments. In tropical areas, there are many sources of vegetable oils available, but not exploited or not used optimally. Oilseeds are important sources of oils of food, industrial and pharmaceutical importance.

**Aim:** The aim was carry out to determine some of the physicochemical characteristics of *Cucurbita pepo* seeds oil in order to promote its valorization.

**Results:** After extraction of the oil by cyclohexane, we obtained a yield of 31.26 %. The study of physical parameters indicate that the oil is pure and belongs to the group of non-drying oils with values of  $0.915 \pm 0.01$  and  $1.468 \pm 0.01$  for density and refractive index respectively. This is confirmed by the results of chemical parameters study. Indeed the low values of the acid and peroxide index (respectively  $0.41 \pm 0.13$  mg KOH/g oil and  $8.92 \pm 1.08$  mEq O<sub>2</sub> / kg oil) confirm the stability of the oil, which allows it to better resist oxidation. The value of the iodine index ( $88.15 \pm 2.57$  g iodine /100 g oil) shows that the oil is of the oleic type. The high value of the saponification index ( $191.67 \pm 1.52$  mg KOH/g) oil proves that the oil has long carbon chains.

**Conclusion:** Based on these results, *Cucurbita pepo* seeds oil would be a good candidate in the food industry, phytomedicine and for the formulation of cosmetic or body care products.

**Key words:** Oil, *Cucurbita pepo*, physico-chemical values, organic extraction

## 1. INTRODUCTION

African flora is full of many plant species used by populations for a long time as sources of food, timber, energy and remedies for various somatic or spiritual ailments [1, 2]. In tropical areas, there are many sources of vegetable oils available, but not exploited or not used optimally [3].

*Cucurbita pepo*, commonly called pumpkin, is one of these sources. It is a herbaceous, creeping plant of the Cucurbitaceae family. It is native to the tropical regions of South America. It is a plant well known by populations thanks to its curative properties. Nutritionally, *C. pepo* is a vegetable of choice that helps meet current requirements for a balanced diet [4].

The Cucurbitaceae family includes a number of very different species and genera, all of which have significant economic importance. '*Cucurbita pepo*' is a species of plant in the Cucurbitaceae family, which includes several varieties of squash of very different shapes, colours and sizes, colours and sizes. For example, squash, melon, cucumber

and watermelon are all species that may be produced and marketed in Madagascar [5].

But, like all Cucurbitaceae, the seeds of *C. pepo* also contain biochemical compounds, in this case, fatty acids that can be used in food or for other purposes. In the food sector, lipids constitute, alongside carbohydrates, an important source of energy for the body. Seeds contain magnesium, which is involved in a number of vital physiological functions, including the production of ATP (adenosine triphosphate, the molecule that provides our body with energy). The synthesis of RNA (ribonucleic acid) and DNA (deoxyribonucleic acid), the pumping action of the heart, the proper formation of bones and teeth, the relaxation of blood vessels and good intestinal transit are also virtues of the *Cucurbita Pepo* seed [6]. Seeds of *C. pepo* is a source of zinc, which is important for immunity, cell growth and division, sleep, mood, the senses of taste and smell, eye and skin health, insulin regulation and male sexual function. Improves insulin regulation and prevents the complications of diabetes by reducing oxidative stress. Rich in good fats, antioxidants and fibre, may be beneficial for heart and liver health [6]. It is an important source of tryptophan, an amino acid (or protein building block) that our body converts into serotonin, which in turn is converted into melatonin, the 'sleep hormone' [6].

Also, vegetable oils are sought after for their quantities and qualities compared to animal fats which are rich in saturated fatty acids. These oils are used in the preparation of various dishes and in the manufacture of cosmetic products. Most often, they contain essential fatty acids and pigments essential for the harmonious functioning of the body and for maintaining the quality of these oils [7].

The objective of this study was to contribute to the study of *C. pepo* L seeds oil, which is very little exploited in Senegal, by determining the physicochemical characteristics following cold organic extraction.

## **2. MATERIALS AND METHODS**

### **2.1. Plant material**

The plant material used in this study consists of seeds of *C. pepo* L (Fig 1) collected from the Useful Plants Experimentation Garden of the Faculty of Medicine, Pharmacy and Odontology of the Cheikh Anta DIOP University of Dakar. The identification was made at the same laboratory.

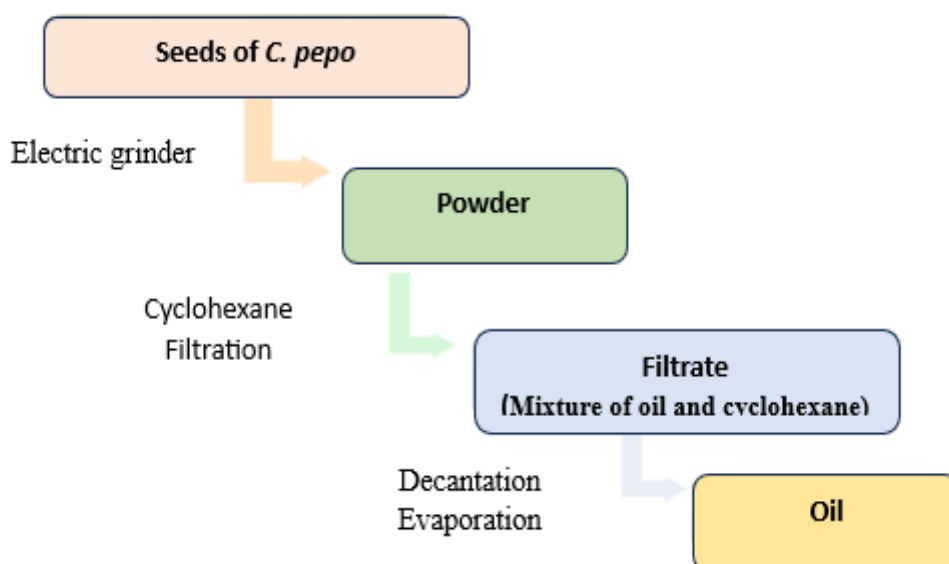


**Fig.1. Seeds of *Cucurbita pepo***

## **2.2. Methods**

### **2.2.1. Oil extraction**

The seeds of *C. pepo* L were pulverized using an electric grinder to give an oily powder. A double maceration of this powder, for 48 hours, in cyclohexane under agitation was carried out. The macerate thus collected after filtration was subsequently passed through a rotary evaporator at 45 ° C in order to separate the lipid extract from the organic solvent. The extraction process is illustrated in Fig. 2. The lipid phase is in turn decanted and filtered. A clear greenish-yellow oil is obtained. This oil will be the subject of physicochemical characterization. Fig. 2 illustrates the Protocol for extracting oil from *Cucurbita pepo* seeds.



**Fig. 2. Protocol for extracting oil from *Cucurbita pepo* seeds**

### **2.2.2. Organoleptic characteristics**

After extraction, the oil obtained was transferred to a clean, clear, transparent glass bottle. The bottle was then left for 24 hours at room temperature in the laboratory, protected from light. Subsequently, the color, odor, taste, and appearance of the oil were evaluated.

### 2.2.3. Physical indexes

#### 2.2.3.1. Density

The density was measured using three 25 ml volumetric flasks. The latter were first cleaned carefully before measuring their empty masses ( $m_0$ ) using a precision balance. Then, they were filled with distilled water (corresponding to a mass  $m_1$ ) and then with oil (giving a mass  $m_2$ ). The density was calculated using the formula below [8].

$$d = \frac{m_2 - m_0}{m_1 - m_0}$$

d : Density

$m_0$ : Mass (g) of the empty flask

$m_1$ : Mass (g) of the flask filled with water

$m_2$ : Mass (g) of the flask filled with pumpkin oil

#### 2.2.3.2. Refractive index

The refractive index of oils was measured using an ABBE refractometer of the OPL type. Thus, after cleaning the prism of the refractometer with distilled water, a few milliliters of oil are spread on it before covering it hermetically with the lid of the device. The sighting scope is then moved so that the dividing line of the light range and the dark range is located at the intersection of the reticle wires before reading [8].

### 2.2.4. Chemical indices

#### 2.2.4.1. Acid value

In a small beaker, 1 g of the oil is dissolved in 25 ml of ether-ethanol solution. After complete dissolution, the mixture was titrated with 0.1N ethanolic KOH solution in the presence of 1 ml of 1% phenolphthalein, until after stirring, the pink color persists for at least 30 seconds. The tests were done in triplicate ( $n = 3$ ) and the acid number (AI) was expressed in mg of KOH/g of oil as mean  $\pm$  standard deviation. The degree of acidity (Da), expressed as a percentage of oleic acid, is deduced from the acid number [9].

$$Ia = \frac{V \times N}{m} \times 56,1$$

$$Da = \left( \frac{Ia}{56,1} \times 282,04 \right) \times 10$$

Ia: Acid number

V: Volume of KOH (0.1mol/l) in ml

N: Normality of the KOH solution (0.1mol/l)

m: Weight of the test sample in g

56.1: Molar mass of KOH

Da: Degree of acidity

282.4: Molar mass of oleic acid

#### 2.2.4.2. Peroxide value

In a 250 ml flask, is introduced between (0.5 to 2 g) of fat dissolved in 10 ml of chloroform. After adding 15 ml of glacial acetic acid and 1 ml of a saturated KI solution, the stoppered flask is shaken for 1 min then incubated in the dark and at room temperature for 5 min. Then, 75 ml of distilled water is added. Finally, the iodine released by 0.01N sodium thiosulfate is titrated in the presence of starch paste (2 to 3 drops) as an indicator. Three tests were carried out for each oil. A blank was also prepared under the same conditions [9]. The peroxide index (Ip) was expressed in milliequivalents of active oxygen per kilogram of sample as mean ± standard deviation according to the following formula.

$$I_p = \frac{(V_1 - V_0)}{m} \times N \times 1000$$

Ip: Peroxide value

V<sub>0</sub>: Volume of sodium thiosulfate solution for blank test in ml.

V<sub>1</sub>: Volume of sodium thiosulfate used in ml.

N : Normality of sodium thiosulfate solution 0.01N.

M : Mass of sample in grams.

1000 : Conversion factor from mg to g

#### 2.2.4.2. Iodine value

A test sample of approximately 0.13 g of oil is dissolved in 12 ml of chloroform. After adding 12 ml of WIJS reagent, the mixture is incubated in the dark for 1 hour. Then, 7 ml of a saturated KI solution and 50 ml of distilled water are added. The released iodine is titrated with 0.1N sodium thiosulfate until pale yellow discoloration. A few drops of starch paste are added, the color turns blue and then the titration is continued until complete discoloration. A blank test is carried out in parallel by replacing the sample with 2 ml of chloroform [10]. The iodine value (Ii) is expressed in g of iodine / 100 g of oil as the mean ± standard deviation according to the following formula.

$$I_i = \left( \frac{V_0 - V}{m} \right) \times N \times 1,27$$

Ii : Iodine value

V<sub>0</sub> : Volume in (ml) of sodium thiosulfate required to titrate the blank test.

V : Volume in (ml) of sodium thiosulfate required to titrate the sample.

M : Test portion (g) of the sample.

N : Normality of the sodium thiosulfate solution at (0.1N).

#### 2.2.4.2. Saponification index.

In a ground-neck Erlenmeyer flask, a test sample of oil (1-2g) and 25 ml of 0.5N potash solution were added. The mixture was brought to the boil under reflux for 1 hour. Then the excess potash in the solution was titrated with 0.5N hydrochloric acid, in the presence of 4 to 5 drops of 1% phenolphthalein solution as a colored indicator, until the red or pink color disappeared. A blank test (without oil) was also treated in the same way as previously [11]. The saponification index (Is) is expressed in mg of KOH/g of oil as a mean  $\pm$  standard deviation according to the following formula :

$$Is = \left( \frac{V_0 - V}{m} \right) \times N \times 56,1$$

Is : Saponification index

V<sub>0</sub> : Volume in ml of HCl used for the blank test

V : Volume in ml of HCl used for the sample to be analyzed

M : test portion in grams

N : normality of the HCl solution

56.1 : molar mass of KOH

### 3. RESULTS

#### 3.1. Extraction yield

From the test sample of 366.9 g of *C. pepo* seeds, 114.33 g of oil were obtained, giving a yield (oil content) of 31.26%.

#### 3.2. Organoleptic characteristics

The oil from *Cucurbita pepo* seeds was greenish yellow in color and liquid in consistency with a characteristic odor (Fig. 3).



Fig. 3. Oil of *Cucurbita pepo* by organic extraction

### 3.3. Density and refractive index

The density of the oil from the seeds of *Cucurbita pepo* was of the order of  $0.915 \pm 0.001$  as for the refractive index, we found a value of  $1.468 \pm 0.001$ .

### 3.4. Chemical characteristics

The determination of the different chemical indices gave the results mentioned in Table 1. *Cucurbita pepo* oil has a low acidity ( $0.41 \pm 0.13$  mg KOH/g of oil). Regarding the iodine and saponification indices, respective values of  $88.15 \pm 2.57$  g of iodine /100 g of oil and  $191.67 \pm 1.52$  mg of KOH/g of oil were obtained. For the peroxide index, it was  $8.92 \pm 1.08$  mEq O<sub>2</sub> / kg of oil. As for the ester index, its value was  $191.26 \pm 0.82$  mg of KOH/g of oil.

**Table 1. Chemical indices of *Cucurbita pepo* L. seed oil.**

Parameters	Results
Indice d'acide	$0.41 \pm 0.13$ mg KOH/g oil
Degrés d'acidité (en acide oléique)	$2.08 \pm 0.6$ % of oleic acid
Indice de peroxyde	$8.92 \pm 1.08$ mEq O <sub>2</sub> / kg oil
Indice d'iode	$88.15 \pm 2.57$ g iodine /100 g oil
Indice de saponification	$191.67 \pm 1.52$ mg KOH/g oil

## 4. DISCUSSION

Cyclohexane used as a nonpolar organic solvent for the extraction of oil from *Cucurbita pepo* seeds was chosen for its ability to solubilize fats. Cold extraction resulted in a yield (oil content) of 31.26%. This value is within the range of yields obtained by studies conducted by [12] on species of the genus *Cucurbita* (9.8 - 52.1%) and varieties of the species *Cucurbita pepo* (31.2 - 51.0%). However, this yield is quantitatively higher than the yields found from seeds collected in Madagascar in 2010 (24.32%) [13] and seeds collected in Algeria in 2018 (26.81%) [14]. In contrast, the oil content observed in this study is considerably lower than that reported by a study conducted on European varieties of *Cucurbita pepo* in 1996 (54.9%) [15] and Egyptian in 2001 (51%) [16]. These yield variations may be due to several factors including the degree of maturity of the seeds, the interaction with the environment (climate type, soil, etc.), as well as the time of harvest and the extraction method [17].

Density is one of the purity criteria that indicates the presence of foreign bodies and provides information on the group to which an oil belongs. The density of the *C. pepo* seed oil found was  $0.915 \pm 0,001$ . This value is within the range of densities (0.903 to 0.926) reported by studies conducted on *Cucurbita pepo* varieties [18]. In addition, this density is almost the same as those found in 2010 in Madagascar and in 2022 in Congo, respectively equal to  $0.9145 \pm 0.007$  and  $0.915$  [13, 19]. The density of *Cucurbita pepo* seed oil found in this study complies with the *Codex Alimentarius* standards (0.910 to 0.916), which suggests that the oil is pure [20].

The refractive index provides information on the purity and group of the oil. It is proportional to the molecular weight of fatty acids as well as their degree of unsaturation. In this study, the refractive index of *Cucurbita pepo* oil was  $1.468 \pm 0.001$ . This value is close to those found by the studies of Jafari et al., 2012 [21] in Iran ( $1.4683 \pm 0.000$ ) and [13] from seeds collected in Madagascar (1.468). The value of the refractive index found in this study makes it possible to classify *Cucurbita pepo* oil among non-drying oils with a refractive index that is between 1.468 and 1.470 [18].

The acid value of *C. pepo* oil that we obtained was  $0.41 \pm 0.13$  mg KOH/g of oil. According to the Codex standards, a refined vegetable oil must have an acid value less than or equal to 0.6 mg of KOH per g of fat. In principle, an oil is considered edible if its free acid content is less than 1% [22]. The low value of the acid value of *Cucurbita pepo* oil gives it the characteristics of a refined vegetable oil in addition to its good stability. However, there is a considerable difference between the acid value that we found and those reported by other studies. Indeed, [23] had found an acid value of  $0.78 \pm 0.02$  mg of KOH. The same is true for [24, 25] who had respective acid index values of  $0.84 \pm 0.00$  mg KOH / g of oil and  $0.94 \pm 0.05$  mg KOH / g of oil. In our study, the acidity was equal to  $2.08 \pm 0.6\%$  oleic acid. This acidity is lower than that recommended for an edible oil (3%) [26].

The peroxide index is a measure for estimating the amount of peroxide present in a fat. It is linked to the storage conditions and extraction methods. It is a very useful criterion with satisfactory sensitivity for assessing the first stages of oxidative deterioration. The higher the peroxide index, the more the material is oxidized [27]. We found in this study a peroxide index of the oil equal to  $8.92 \pm 1.08$  mEq O<sub>2</sub> / kg of oil. This value is not far from that obtained by [25] in Greece ( $9.20 \pm 0.59$  mEq O<sub>2</sub> / kg of oil).

Nevertheless, it remains lower than 10 mEq O<sub>2</sub> / kg which characterizes most conventional edible oils such as soybean, corn and sunflower [20]. The peroxide index obtained leads us to believe that the oil studied does not deteriorate easily by oxidation. This can be explained by the oil's richness in natural antioxidant substances (tocopherols, polyphenols, carotenoids) [28]. This argues for the possible use of the oil in food and cosmetics.

In the analysis of vegetable oils, the iodine index is the most useful constant, because it is in relation to the values of this index that the important division of vegetable oils into drying, semi-drying and non-drying oils is based. Indeed, the iodine index informs us about the degree of establishment of the fatty acids contained in a given oil. It is directly related to the degree of oxidation of an oil. Thus, the more unsaturated an oil is, the higher its iodine index. This value can be used as a basis to assess the ease with which the oil becomes rancid, given that the more establishments it contains, the more sensitive it will be to oxygen [8]. In this study the iodine value found was  $88.15 \pm 2.57$  g iodine/100 g oil. This iodine value is lower than those obtained in 1997 in Greece ( $107 \pm 0.58$  g iodine/100 g), in 2011 in Iran ( $104.36 \pm 0.04$  g iodine/100 g) and in 2014 in Pakistan ( $97.9766 \pm 0.057413$  g iodine/100 g *Cucurbita pepo* oil) [23, 24, 25]. Despite its inferiority to the results obtained by other studies, this value allows us to classify this oil among the non-drying oils, whose iodine indices are between 0 and 110 g of iodine / 100 g of oil. This confirms the conclusion that we had previously drawn with the refractive index ( $n_D = 1.468 \pm 0.001$ ). The variation of the iodine index can be linked to several factors such as the variety of plant, the climate, the harvest period as well as the degree of maturity of the seed. Based on this iodine index value of *Cucurbita pepo* oil between 80 and 100, this oil would be of an oleic type with an average unsaturation number of 1. The conservation of this oil could be done without too much risk of auto-oxidation.

The saponification index of *Cucurbita pepo* oil was  $191.67 \pm 1.52$  mg KOH/g oil. It is in agreement with the values attributed to the Cucurbitaceae family whose saponification index should be between 174 and 197 (Nichols and Sanderson, 2003). In addition, the saponification index of our oil is close to that of [23] ( $190.69 \pm 1.40$  mg KOH / g), ( $194.606 \pm 0.57$  mg KOH / g) as well as that of [19] (192.59 to 195.27 mg KOH / g). However, the value observed in this study is lower than those reported by a author [25]. The latter had obtained  $201 \pm 3.72$  mg/KOH oil and 215.0 mg/KOH oil from seed oils collected in Greece and Saudi Arabia, respectively. On the other hand, a lower saponification index ( $175.37 \pm 0.72$  mg/KOH oil) was obtained from seed oil collected in Iran [21].

## 5. CONCLUSION

The objective of this study was to determine some physicochemical characteristics of *Cucurbita pepo* seed oil in order to promote its development. Cold organic extraction allowed to obtain a good extraction yield. Density and refractive index of *Cucurbita pepo* oil obtained after extraction shows that the oil is of good quality and belongs to the group of non-drying oils.

The chemical indices obtained was within the standard established by the *Codex Alimentarius* and support the use of *C. pepo* oil in food but also in cosmetology.

However, further studies should be undertaken to assess its chemical composition in fatty acids and its toxicity before considering its use.

## 6. DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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