

# Chemical characterization of local “Beldi” almond oil for food and cosmetic purposes in northeastern of Morocco

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**Abstract.** The new trend of almond planting intensification aims for better profitability at the expense of biodiversity, which will lead to forsaking of local Beldiecotypes. Indeed, European late-flowering cultivars, especially *Ferragnes* and *Ferraduel*, were recently largely planted in northeastern Morocco. In parallel with our studies on these new almond crops, which are profitable in favorable areas, this research aims to conserve local almond biodiversity. Thus, it seeks added value for products from Beldialmonds, particularly for defective kernels (Doubles, malformed, split, and broken almonds). Therefore, these defective kernels could be used as raw materials for almond oil extraction to be used in cosmetology or even in food industry. In addition, this study is the first that has been conducted to determine the characterization of the Beldi ecotype has been carried out in this region. Low acidity and peroxide indexes values, which are respectively 0.44% and 2.67 meq O<sub>2</sub>/Kg, and a medium oxidative stability value (19 hours) were recorded for the studied almond oils obtained from seeds by mechanical pressing extraction. Total tocopherols content showed significant values 528.28 mg/Kg, with  $\alpha$ -tocopherol as dominant. The total phenolic content of the analyzed Beldi almond oil is 147.59 mg/kg. The analyzed fatty acid profiles show that their composition is mainly constituted by oleic acid (64.68%). Finally, it should be noted that despite its low production, the Beldiecotypes will continue to spread in the marginal agro-ecological zones due to their remarkable resistance to drought and their almond oils highly valued in cosmetic, especially for skin and hair care products.

## 1 Introduction

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Sweet almonds are edible nuts of *Prunus amygdalus*, (var. *dulcis*), belonging to the Rosaceae family [1], approximately 80% of the global almond cultivated area is concentrated in California and the Mediterranean region[2]. The majority of almond cultivars are self-incompatible and must be cross-pollinated, at least two independent genes expressed in the pistil or pollen control the self-incompatibility that explains the high polymorphism of almond cultivars[3,4], and the necessity to safeguard this biodiversity. The almond tree is a typically Mediterranean crop, with large economic importance. It is able to withstand the high temperatures during the summer and the intense winter cold. It can survive long periods of drought and be cultivated on very poor land and marginal areas [5].The Almonds have numerous nutritional advantages and potential health benefits, they are considered excellent sources of energy, fats, proteins, minerals, carbohydrates, unsaturated fatty acids, phenolic compounds and tocopherols[6–9]. In addition, the oil that is the main constituent of almond kernels, which is largely used in cosmetic especially for skin and hair care.

Currently, Morocco is the sixth-largest producer of almond nuts in the world, with an average production of 102.185 tons in 2019[2]. In marginal areas, in addition to the varieties of almonds commonly cultivated, the Beldi almond trees represent a set of local almond ecotypes reflecting the richness of biodiversity and showing high adaptation to diverse edaphoclimatic conditions of the different agro-ecological areas of Morocco. However, the early pre-flowering and a relative sensitivity to late frosts, which sometimes occur in February, could be limiting factors that could affect the flowering of these almond ecotypes and therefore affect their fruit production. [10].

The resulting variability from seedling leads to high levels of heterozygosity, and therefore to a strong heterogeneity of the fruits showing poor pomological quality (kernels of different shapes and sizes, high rate of doubles and defective kernels. However, based on the chemical analysis, Beldi almonds show excellent quality in terms of nutritional values and health benefits [11].

In Morocco, more than 50% of almond plantations are located in the regions of Taza-Taounate-Alhouceima and Sous-Massa-Daraa that represent 30% of national production. However, the Saïss region produces as much with only 6% of plantations; this is due to the more intensive nature of its plantations, the availability of water, use of high-yield varieties. Indeed, the production is highly variable due to drought and the incidence of frost, which affect flowering and subsequent fruit set. Therefore, in eastern Morocco, the latest almond planting program aimed at increasing farmer's profitability at the expense of biodiversity. This results in the introduction of new late-flowering European varieties, mainly *Ferragnes* and *Ferraduel*, which in the short term seem better adapted and more profitable but in the long term this could lead to the abandonment of the Beldi ecotypes and a loss of biodiversity.

Beldi almond kernels, showing defects in shape, size, and kernel development, unfit for human consumption, are used as raw material for almond oil extraction. This is a valuation operation for creating added value to farm products made from Beldi almonds and so for sustainable agriculture. This is a real opportunity for the small farmers from these marginal zones which constitutes a financial resource at the service of rural development and therefore to promote the cultivation of biodiversity preservation of the Beldi almond. Thus, our study concerns the chemical analysis of the oil extracted from Beldi almonds as a high benefit product for food and cosmetic uses. In Morocco, some studies have been conducted to determine the characterization of oil content and quality indices for almond cultivars [12–14]. However, no specific study is done on the Beldi ecotype except for preliminary work by Nadia et al and Kodad et al [11,15]

## 2. Material and methods

### 2.1. Almonds' harvesting and oil extraction

Harvesting of Beldialmonds was carried in crop year 2018 out on the southern side of the Tafoghalmountains in northeastern of Morocco known as ancestral area for traditional cultivation of Beldialmond trees. This area shows great biodiversity within the same field, where plantations were done by seed germination. Thus, Almonds nuts of Beldiecotype were harvested in August at the last phase of maturity in the rural zone of Sidi Bouhria (34°44'13.6'' N, 002°20'15.0'' W). Six kilograms of shelled almonds were harvested from three almond orchards, which are mainly rain-fed but receive additional irrigation during periods of drought. Almond oil is extracted using a mechanical oil screw press, at the speed of 70 RPM, oil's fine particles were removed by centrifugation at 3000 RPM for 15min. Finally, the obtained almond oil samples were stored for further analysis in the dark at a temperature of 4°C.

### 2.2. Analytical methods

The chemical quality parameters of Beldi almond oil were analyzed, its oxidation stability was tested, and its fatty acid and triacylglycerol profiles were determined. Each parameter is analyzed in triplicate

#### 2.2.1 Chemical parameters and oxidative stability of Beldialmond oil

Almond oil acidity (% oleic acid) and peroxide value (meq O<sub>2</sub>/Kg), The official European methods [16] used to determine the oil acidity (% oleic acid) and peroxide index (meq O<sub>2</sub>/Kg) of olive oil were maintained for the evaluation of the studied almond oil (AO) quality. 3g of almond oil heated at 100°C with an airflow of 15L/h, was used to determine the oxidative stability index (OSI) using a Metrohm Rancimat 743 apparatus [17]. OSI value is expressed as the oxidation induction time (hours).

#### 2.2.2 Phenol analysis

The Folin-Ciocalteu method described by Ollivier et al, (2004) was used for analyzing the total phenolic contents of almond oil, where the absorbance was made at 750 nm by using caffeic acid as a standard [18].

#### 2.2.3 Tocopherols analysis

HPLC-FLD equipped with an automatic injector was used for the tocopherols profile analysis, according to the AOCS method Ce 8-89 AOCS, [16] where  $\alpha$ ,  $\beta$ , and  $\gamma$  tocopherols which are the main tocopherol isomers were separated by using an Uptisphere 120A° NH<sub>2</sub> column (150 mm \*3 mm, 3  $\mu$ m, Interchim, Montluçon, France), with an injection volume of 10  $\mu$ L and a maintained temperature at 30 °C. Hexane/2-propanol (99:1, v/v) constitute the mobile phase which was eluted in isocratic conditions at a flow rate of 1mL/min. The tocopherols were identified and quantified by external standardization (Mixture of tocopherols:  $\alpha$ -tocopherol,  $\beta$ -tocopherols,  $\gamma$ -tocopherols,  $\delta$ -tocopherols) obtained from Sigma-Aldrich (Steinheim, Germany).

#### 2.2.4 Fatty acids analysis

Fatty acid profile of almond oils was identified by a standard composed of 37 methyl esters of fatty acids. Before analysis, fatty acids were converted into fatty acid methyl esters; after

adding 8 mL of hexane, the mixture was subjected to gas-chromatograph analysis using an HP 6890 series gas-chromatography system equipped with an FID detector and a capillary column (Supelco Omega wax: 30 m × 0.25 mm × 0.25 μm). The carrier gas was nitrogen, supplied at a flow rate of 1.7 ml min<sup>-1</sup>. The initial temperature of the injector was 50°C, it was gradually increased to 150°C at a rate of 30°C min<sup>-1</sup> and then at a rate of 4°C min<sup>-1</sup> up to 250 °C. The injection volume was 1 μl in the split-less mode. This method of analysis was described in by Mansouri & al[19].

### 2.2.5 Triacylglycerols analysis.

Analysis of molecular species of Triacylglycerols (TAGs) was carried out using 2 ml of Beldialmond oil dissolved in acetone (9%) and filtered through 0.45μm membranes. TAGs profile was determined by high-performance liquid chromatography (HPLC) coupled with a refractive index detector. The column used is type C18 reversed-phase column (ODS C18: 250× 5mm, 5μm), and the mobile phase consisted of acetone/acetonitrile (60% - 40%, v/v). Elution was conducted in isocratic conditions out at 1ml/min. TAGs peaks were identified by comparison with standards triglyceride (Sigma chemical products).

## 3. Results and discussion

### 3.1. Quality indexes, phenol and tocopherol of Beldialmond oil

Lipolysis and lipid oxidation, which causes undesired flavors and smells, are the main reasons for virgin almond oils spoilage, hence the importance of measuring the acidity, peroxide index, and oxidative stability index (OSI) values [20]. Those parameters are essential factors to determine the shelf-life and acceptability of almond oil via the determination of enzymatic or chemical hydrolysis of acylglycerols, as well as the secondary oxidation products such as ketones, aldehydes [21]. In this regard, the analysis results of physico-chemical characteristics shows a low acidity (0.43%), a low peroxide value (2.67 meq O<sub>2</sub>/kg) and a medium OSI value (19 hours) expressed as the oxidation induction time (Table 1)

Like all vegetable oils, the presence of natural antioxidants (phenols and tocopherols) in almond oil improves its stability, and therefore its storability. Thus, phenolic compounds present in almond oil are important secondary metabolites with great antioxidant capacity. In fact, Beldialmond oil's richness of phenols delays the oil oxidation speed by different actions and it is closely associated with the nutritional quality and health benefits. These natural antioxidants play a prominent role in the prevention of various diseases [22]. The total phenolic content of the analyzed Beldialmond oil is 147.59 mg/kg, (Table 1). This observed value is higher than that recorded for oils extracted from almond varieties (*Ferragnes*, *Ferraduel*, and *Marcona*) commonly cultivated in Morocco, which varied from 18.53 mg/kg to 237 mg/kg [23,24]. This variability is certainly attributed to the genotype and the agro-climatic conditions but also to the oil extraction methods.

In addition to their richness of phenols, almond oil is a good dietary source of tocopherols, which constitute a large class of fat-soluble compounds with vitamin E activities. In fact, vitamin E is a mixture of different forms of tocopherols and tocotrienols, with α-tocopherol being the most effective form of vitamin E [25]. Due to its high content of antioxidants, almond oil may help reduce the risk of cardiovascular disease and plays an important role in preventing diseases associated with aging and protecting cell membranes from oxidative damages [26,27]. Therefore, almond oil is a natural cosmetic product that helps soothe the skin from irritation, protects from UV damage, and restores the skin's moisture barrier [28]

Beldialmond oil was analyzed for their quantitative and qualitative tocopherol contents by HPLC-FLD. Total tocopherols content and proportion of each homolog ( $\alpha$ -,  $\beta$ -, and  $\gamma$  tocopherols) are summarized in Table 1. Beldialmond oil has a high content of tocopherols estimated at 528.28 mg/kg, with  $\alpha$  tocopherol as the major homolog (516.33 mg/kg) followed by  $\gamma$ -tocopherol (9 mg/kg) and  $\beta$ -tocopherol (2.95 mg/kg). This oil is very rich in tocopherols (528.28 mg/kg) compared to that from *Ferragnes* and *Ferraduel* cultivars where tocopherol contents range between (385-433 mg/kg)[13,29]. This high content of tocopherols a (particularly  $\alpha$ -homologue, the most effective form of vitamin E), make this oil a product of nutritional interest and beneficial for human health. Actually, this oil is considered an excellent source of natural antioxidants for food and cosmetic purposes. Therefore, these benefits will encourage the extension of Beldialmond cultivation for oil production. Besides this will help farmers' improving incomes, contribute to rural development, and led to protect the biodiversity of almond trees in marginal areas.

Table 1: Quality indexes, phenols and tocopherol contents in analyzed Beldialmond oil

Physicochemical parameters	values
Free acidity (% C18:1)	0.43± 0.1
peroxide value (meqO <sub>2</sub> /kg)	2.67± 0.05
Total phenols (mg/Kg)	147.59± 14.50
$\alpha$ -tocopherol (mg/Kg)	516.33± 14.01
$\beta$ -tocopherol (mg/Kg)	2.95± 0.25
$\gamma$ -tocopherol (mg/Kg)	9.00± 1.50
total tocopherol (mg/Kg)	528.28± 15.26
oxidative stability index (hours)	19.08± 0.1

The oxidative stability of almond oil should be understood as the oil's sensitivity to lipid oxidation, which leads to unwanted flavors and odors. It could be affected by the storage conditions of the oil, namely light, temperature, and exposure to oxygen, but also by its content of natural antioxidants. It is, therefore, necessary to measure the OSI of Beldialmond oil, which is a key parameter that refers to the susceptibility of the oil to lipid oxidation and which provides information about oil quality and its aptitude for storage [33]. The result of the analyzed Beldialmond oil showed an OSI value of 19.08 h, which's comparable to those of literature that range from 10.2 to 24.2 h[23,34]. The richness in antioxidants of Beldialmond oil would explain its resistance to oxidation and therefore its good stability during storage

by comparing these results with other type of vegetable oil such as olive oil, the latter presents high phenol values with 316.59 mg / kg and SOI with 44.55 h for the picholine ecotype. For tocopherol olive oil has less tocopherol concentration with 254.13 mg/Kg [30]. regarding of oil characteristics of non-industrial hemp (*Cannabis sativa* L.), the total tocopherol content was between 376.46 and 796.06 mg kg<sup>-1</sup> of oil, with a dominance of  $\gamma$ -tocopherol [31], which is not the case for almond oil with a dominance of  $\alpha$ - tocopherol. for argan oil, the total phenolic content was lower than 10 mg/Kg. the total tocopherol content range between (383-485 mg/Kg), the oils from unroasted seeds presented same stability of almond oil with value ranged between 16h and 32h [32]

### 3.2 Fatty acids and triacylglycerols profiles

The fatty acids are classified into three groups, including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). They play multiple roles in human's health and other organisms [35]. The content of saturated fatty acids in Beldialmonds oil is very low (10.03%) while the proportion of unsaturated FA (UFA= MUFA+ PUFA) is very important (89.89%). The most important unsaturated fatty acids are oleic acid and linoleic acid, the contents of which are estimated at 64.68% and 24.37% respectively. Table 2 presents the Beldialmond oil fatty acids profile, the mean values of SFA, MUFA, PUFA, and the ratios UFA/SFA and O/L. Among the eleven fatty acids identified, four of them are major and which are respectively in order of quantitative importance C18:1 (64.68%), C18:2 (24.37%), and C16:0 (7.22%), C18:0 (2.62%). The GC-FID analysis allows the detection and identification of seven other fatty acids in the form of traces, as stated by several studies that refer to the benefits of unsaturated FA intake on risk factors for cardiovascular disease, the consumption of Beldialmond oil will provide nutritional and health benefits. In addition, its O/L ratio (2.65) reflects its richness in oleic acid (C18:1), considered to be the healthiest FA in the diet and equally as an indicator of the good nutritional value of this oil [36]. The fatty acid profile of almond oil is comparable to that of olive oil and argan oil with higher values of oleic acid 75.68% and 48.1% respectively [19,37], but it is different compared to non-industrial hemp oil (*Cannabis sativa* L.) with a predominance of linoleic acid (48.26%–55.39%) [31].

The HPLC analysis of triacylglycerols (TAGs) of Beldialmond oil allows the identification of eleven molecular species; four of them are major ones (OOO, OOL, LLO and POO) covering together more than 83% of total TAGs. The other eight TAGs are minor triacylglycerols (LLL, LPL, SOO, LLS+ POL, POP, SLO, and, PPL, together they represent less than 20% of the total TAGs, where P is palmitic acid, O is oleic acid, S is stearic acid and L is linoleic acid (Figure 1).

Table 2: Fatty acids (FA) profile of Beldialmond oil (SFA: Saturated FA; UFA Unsaturated FA; MUFA: Monounsaturated FA; PUFA: Polyunsaturated FA, O/L=Oleic/ Linoleic ratio)

Fatty acids (%)	Mean± Standard deviation
C14:0	0.04 ± 0.01
C16:0	7.23 ± 1.00
C16:1	0.65 ± 0.20
C17:0	0.06 ± 0.02
C17:1	0.09 ± 0.01
C18:0	2.62 ± 0.59
C18:1	64.68 ± 4.00
C18:2	24.37 ± 3.00
C18:3	0.04 ± 0.01
C20:0	0.08 ± 0.02
C20:1	0.06 ± 0.02

SFA	10.03 ± 1.63
MUFA	65.48 ± 4.23
PUFA	24.41 ± 3.02
UFA/SFA	8.96 ± 0.64
O/L	2.65 ± 0.13

The analyzed Beldialmond oil presents two main predominant TAGs, OOO (37.87%) and OOL (23.47%) which in total represent more than 60% of total TAGs. This is in perfect agreement with its richness in oleic acid (64.68%).

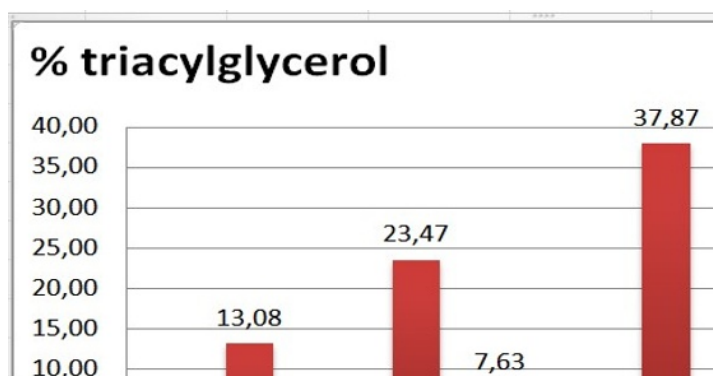


Figure 1: Profile of triacylglycerols (TAGs) of almond oil extracted from defective kernels of almonds Beldiecotype. (Eleven TAGs species were identified, OOO, OOL, LLO and POO as majors ones, LLL, LPL, SOO, LLS+ POL, POP, SLO, PPL as minors and where O = oleic acid, L = linoleic acid, P = palmitic acid and S =stearic acid.

## 4. Conclusion

This work is the first assessment of the quality of Beldialmond oil produced in a rural cooperative located in eastern Morocco. The study concerns the use of defective Beldialmonds (small, split and broken, and malformed kernels) as raw material for the extraction of almond oil by mechanical pressing of defective kernels unfit for human consumption. As shown by chemical analyzes, Beldialmond oil has the required biochemical qualities which make it a product of interesting nutritional quality as edible oil. In addition, its richness in tocopherols and other bioactive molecules gives it other beneficial properties for its cosmetic use. Thus, both for food use but especially for cosmetic applications Beldialmond oil extracted from defective kernels is profitable. Finally, Beldi almond oil generates benefits that could directly improve the small farmers' income, which therefore contributes to the preservation of this culture and its biodiversity as well. In addition to the physico-chemical characterization of almond oil, it is necessary to develop an effective protocol for clonal propagation of interesting "Beldi" almond genotypes carrying the desired agronomic traits

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