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Fatty Acid and Amino Acid Composition of *Citrullus Colocynthis* Seeds Growing in Algeria

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Abstract

Citrullus colocynthis is an herbaceous perennial wild species of the Cucurbitaceae family, used in traditional medicine in the treatment of diabetes mellitus. In the present work, *C. colocynthis* seed oils have been assessed for their fatty acids profile and concentration by Gas Chromatography–Mass Spectrometry (GC-MS), while oil and protein extracts were investigated in respect to their amino acid compositions by using Ultra High-Performance Liquid Chromatography-Electrospray Ionization-Triple Quadrupole Mass Spectrometry (UHPLC-ESI-QqQ-MS/MS) instrumentation. Results revealed that the predominant fatty acids in *C. colocynthis* seeds were linoleic (70.7 %), oleic (10.9 %), palmitic (8.3 %), and stearic (7.8 %) acids. On other hand, the analysis of the free amino acids content noticed the presence of threonine (0.32 μgmL^{-1}), valine (0.26 μgmL^{-1}), and tryptophan (0.19 μgmL^{-1}). Besides, the *C. colocynthis* seeds oil contained the non-essential amino acids like serine (0.29 μgmL^{-1}), ethanolamine (0.18 μgmL^{-1}), glycine (0.25 μgmL^{-1}) and aspartic acid (0.12 μgmL^{-1}). In conclusion, the results obtained revealed the valuable nutritional value of *C. colocynthis* and derived co-products, suggesting its suitability to be used as a nutritional ingredient and to be considered in the development of dietary supplements.

Keywords: *Citrullus colocynthis*, Cucurbitaceae; Amino acids; Fatty acids; GC-MS; UHPLC-ESI-QqQ-MS/MS.

1. Introduction

Plants have had a prominent place in human nutrition from ancient times. All civilizations have used wild and domestic plants for food, protection, clothing, or healing. Application of plants has diversified over time to adapt to human needs [1]. In addition, cumulative knowledge has enabled communities to use plants as an essential source of therapeutics [2,3]. The Algerian flora involves almost 3000 species, which belong to several botanical families. However, 15 % of endemic species remain scarcely explored from both phytochemical and pharmacological points of view. Therefore, characterization of genetic resources allows us to evaluate the valorization and application of local medicinal plant alternatives, which can contribute to the Algerian food and pharmaceutical industries, with positive impacts on the socioeconomic structure of the local area [4, 5].

Citrullus colocynthis is a perennial herb from the *Cucurbitaceae* family, which has been extensively considered for its medicinal applications in the treatment of diabetes mellitus. This plant, with seeds rich in lipids and proteins, grows in arid and semi-arid soils, although it can be also found in both humid and moderately dry tropical areas [6]. This species has been found across a large geographic area from North Africa to India and the Mediterranean Basin [7-9]. Studies on this species have revealed that a characteristic bioactive compound of *C. colocynthis*, known as cucurbitacin glucoside [10-12], exhibits anti-inflammatory, antipyretic, and antitumor activities [13], as well as cytotoxic and insecticidal

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effects such as the inhibition of cell adhesion resulting from the cytoskeleton destabilizing in cancer cells exposed to cucurbitacin E [12, 14, 15]. In addition to cucurbitacin glucoside, *C. colocynthis* has been also described as a valuable natural source of alkaloids [16]. Considering the compositional features of *C. colocynthis* as a source of bioactive phytochemicals, assessment of the plant seeds regarding the basic fatty acid and amino acid composition can help clarify their nutritional value and promote their application as food supplements to enrich the protein and fat contents of foods for local consumption and industrial application. The degree of damage or toxicity depends on the amount of plant fruit which is taken. Usual dose of dried fruit as a cathartic agent is 120 mg in adult person and toxic dose is 2-4 g [17]. Therefore, in the present study we aimed to assess Algerian *C. colocynthis* regarding its crude protein, essential amino acid, non-essential amino acid, and fatty acid composition in order to retrieve information about rational valorization procedures of this wild species as a source of nutrients (Figure 1).

2. Materials and Methods

2.1. Plant materials

Citrullus colocynthis was collected in February 2013 from Al-Mansura, located in Ghardaia town in the central region of Northern Sahara (500 km south of Algiers). The collected plants were confirmed by the members of the Laboratory for Process Engineering of the University of Laghouat, Algeria. A voucher specimen number (LGP Cco/02/13) was deposited at Laboratory for Process Engineering of the University of Laghouat, Algeria.

2.2. Chemicals and reagents

The 6-aminoquinolyl-N-hydroxysccinimidyl carbamate (AQC) reagent was purchased from Chemos GmbH (Regenstauf, Germany). Also, Sigma-Aldrich (Madrid, Spain) provided the Bis-Tris reagent and all authentic amino acid standards, including histidine (His), 1-methyl-histidine (Met-His), 4-hydroxyproline (p-Hyp), asparagine (Asn), phosphoethanolamine (PEA), arginine (Arg), glutamine (Gln), serine (Ser), glycine (Gly), ethanolamine (EA), aspartic acid (Asp), citrulline (Cit), glutamic acid (Glu), threonine (Thr), alanine (Ala), γ-amino-butyric acid (GABA), α-aminoadipic acid (AADA), proline (Pro), ornithine (Orn), β-aminoisobutyric acid (BAIB), α-amino-n-butyric acid (AABA), lysine (Lys), cysteine (Cys-cys), cystathionine (Cysta), tyrosine (Tyr), valine (Val), methionine (Met), homocysteine (Hcys-cys), leucine (Leu), isoleucine (Ile), tryptophan (Trp), and phenylalanine (Phe). In addition, acetonitrile and methanol (both of LC-MS grade), sulfuric acid, nitric acid, perchloric acid, formic acid, ammonium acetate, ascorbic acid, and sodium hydroxide were obtained from Panreac Química S.A. (Barcelona, Spain). Metaphosphoric acid was also purchased from Merck (Darmstadt, Germany), boric acid was purchased from Probus (Badalona, Spain), and calcium disodium EDTA was supplied by Sigma (Steinheim, Germany). Finally, Milli-Q water was produced by an Elix®3 Millipore water purification system (Molsheim, France).

2.3. Determination of fatty acid composition

2.3.1. Lipid extraction

Mature *C. colocynthis* fruits (50 g) were cut to remove the seeds, which were then ground to a fine powder using a microfine grinder (IKA Werke, Germany). Fatty acids from the seed powder were extracted using a Soxhlet extractor (Thermo Fisher Scientific, Waltham, USA), with n-hexane (69 °C) as the extracting solvent. After three hours, the recovered hexane was evaporated under reduced pressure at 50 °C, using a rotatory evaporator (Büchi Rotavapor R-200, Büchi, Flawil, Switzerland). Finally, viscous yellow oil (13.03 g on average) with a foul odor was obtained and stored at -20 ºC; it was protected from light until analysis of protein, fatty acid, and amino acid composition.

2.3.2. Gas chromatography-mass spectrometry (GC/MS) analysis

Assessment of fatty acid methyl ester (FAME) composition was carried out by converting them into methyl esters, according to a method previously described by Christie et al. (1973) [18]. Analysis was performed in a 6800 Plus Chromatograph system with an HP-5ms column (Agilent technologies), coupled with an HP 5973 MSD spectrometer (Agilent Technologies). The chromatograph was equipped with a flame ionization detector (FID) and an HP-5ms chromatographic column (length: 30 m; diameter: 0.25 mm), with a film thickness of 0.25 µm. The temperature of the injector and the detector was set at 250 and 280°C, respectively. The column temperature varied from 90 to 250°C at a flow rate of 4 °C/min. The carrier gas was helium with a flow rate of 1 mL min^{-1}. The peaks obtained by injecting methyl esters were identified by using a mixture of authentic fatty acid standards and comparing the retention times. FAME quantification was performed using standard curves, which were freshly prepared for each day of analysis according to the methodology previously described.

2.4. Determination of amino acid composition

2.4.1. Extraction of proteins

Seed protein extraction was performed using a buffer solution (*p*H = 7.4). After delipidation, 1 g of ground

seeds was dissolved in 25 mL of 0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4 and 2.7 M NaCl (*p*H = 7.4) inside a flask. After stirring for 24 hours, the solution was filtered to a volume of 100 mL

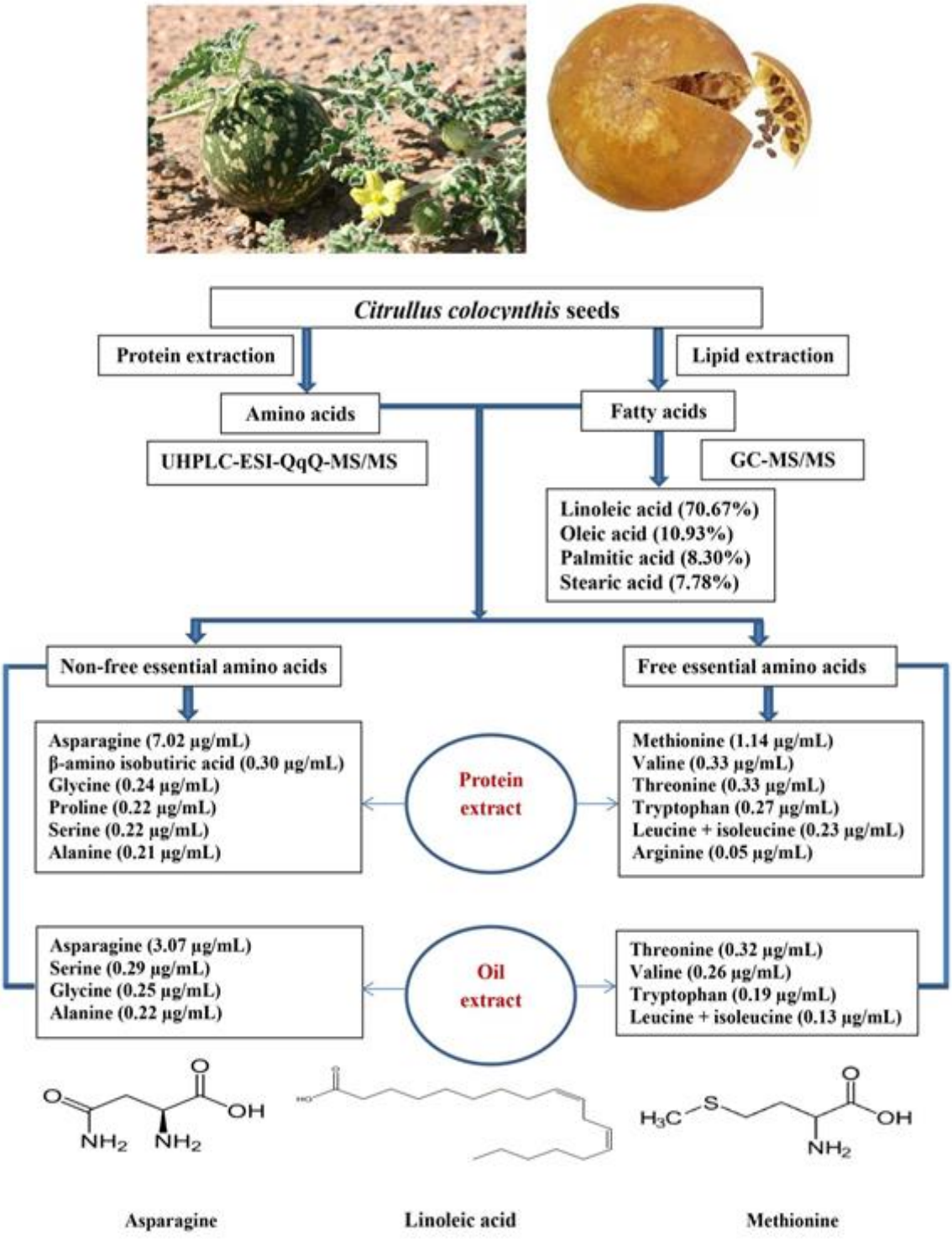

Fig. 1. Extraction, GC-MS/MS and UHPLC-ESI-QqQ-MS/MS analyses of *C. colocynthis* seeds

2.4.2. Processing of samples and standards of amino acid content analysis

Individual amino acids (authentic standards) were dissolved in Bis-Tris (*p*H = 6.5), and calibration standards were obtained by diluting the stock solutions to 1.00, 0.50, 0.25, 0.12, 0.06, and 0.03 mM. For amino thiols, dilutions were prepared in the range of 2.00, 1.00, 0.50, 0.25, 0.12, and 0.06 µM in Milli-Q water. Amino acids and amino thiols in the samples (1 mL) were derivatized, based on the Waters AccQTagTM Ultra UHPLC amino acid analysis procedure, as previously described in the literature [19, 20], with minor modifications. Briefly, 350 µL of borate buffer (0.2 M sodium borate (*p*H = 8.8) and 5 mM calcium disodium EDTA), 50 µL of amino acid standard or the liquid sample, and 100 µL of reconstituted AQC (10 mM AQC dry powder in acetonitrile) were added to a 2-mL propylene vial. The obtained solution was vortexed and allowed to rest for one minute at room temperature. Afterwards, derivatization reactions were developed in a heating block for 10 minutes at 55°C. After removing the vial from the heating block, the samples were injected into a UHPLC-ESI-QqQ-MS/MS system for analytical purposes.

2.4.3. Free amino acid analysis by UHPLC-ESI-QqQ-MS/MS

The samples were analyzed regarding the content of amino acids and amino thiols by reversed-phase UHPLC, based on the methods reported by Nagumo et al. (2009) and Salazar et al. (2012) [20, 21], with slight modifications. Chromatographic separation was also carried out in an AccQ Tag Ultra BEH column (2.1×100 mm; particle size=1.7 µm) (Waters Corp., Ireland). The mobile phase A consisted of 50 mL of acetonitrile/formic acid/5 mM ammonium acetate in water (10:6:84, v/v/v), diluted in 950 mL of Milli-Q water; the mobile phase B consisted of acetonitrile/formic acid (99.9:0.1, v/v). The injection volume and the flow rate were 20 µL and 0.5 mL/min, respectively. The target compounds were chromatographically resolved upon the gradient. The required time (minutes) for solvent B (%) was follows: 0.00 (0.1 %); 0.50 (99.9 %); 5.70 (9.1 %); 7.70 (21.2 %); 8.00 (59.6 %); 10.00 (59.6 %); 10.01 (90.0 %); 12.00 (90.0 %); and 12.01 (0.1 %) Amino acids and amino thiols were identified in a UHPLC system coupled to a 6460 tandem mass spectrometer (Agilent Technologies, Waldbronn, Germany). Data acquisition and processing were performed using MassHunter version B.04.00 (Agilent Technologies). MS analysis was performed using multiple reaction monitoring (MRM) in the positive ionization mode, based on MS parameters (ion optics and capillary exit or fragmentor voltage); collision energy was optimized for each analyte, according to the method proposed by Collado-González et al. (2015) [22]. The MRM transition used for each derivatized amino acid or amino thiol corresponded, in most cases, to the aminoquinoline (AMQ) moiety (171+), resulting from the collision-induced cleavage at the ureide bond of AMQ adduct of each amino acid/thiol. The working conditions for MS parameters of the electrospray source were as follows: gas flow, 9 L/min; nebulizer, 40 psi; capillary voltage, 4000 V; nozzle voltage, 1000 V; gas temperature, 325 °C; sheath gas temperature, 390 °C; and JetStream gas flow, 11 L/min.

2.5. Statistical analysis

The results are presented as mean±SD. significant differences were evaluated using the analysis of variance (ANOVA) and Turkey's multiple range tests in XLSTAT-2013 (Addinsoft, New York, USA).

3. Results and Discussion

Broadly, forest plant foods can be categorized as leaves, seeds, nuts, fruits, tubers, roots, fungi, gum, and sap. Collectively, they provide protein, energy, vitamins, and essential minerals for the human diet, especially in areas where limited access to balanced diets may constitute a serious drawback to the normal development of human physiological processes. For this reason, characterization of wild species may provide useful information about further development of dietary supplementations, which contribute to correct nutritional changes in some areas.

3.1. Fatty acid content

Assessment of *C. colocynthis* regarding the fatty acid profile provided accurate information about the most abundant compounds of this family (Table 1, Figure 2). According to the collected data, *C. colocynthis* seeds yielded vegetable oil (26.1 %), which exceeded the values reported in the literature (17.0-19.0 %) [23]. The oil was further characterized regarding the relative abundance of diverse fatty acids in *C. colocynthis* seeds. When profiling the content of fatty acids and quantifying their concentration in *C. colocynthis* seed oil, the presence of an array of compounds, varying from 14 to 23 carbons, was confirmed (Table 1). The most abundant fatty acids were linolenic, palmitic, stearic, oleic, and linoleic acids. Detailed analysis of the relative contribution of each fatty acid to the complete profile revealed that linoleic acid (C18:2n-6) was the most abundant fatty acid, constituting 70.7 % of the total fatty acids, followed by oleic acid (C18:1n-9), which represented 10.9 % of the total fatty acid content; other mono- and polyunsaturated fatty acids were found at much lower concentrations. With respect to saturated fatty acids, palmitic (C16:0) and stearic (C18:0) acids accounted for 8.3 % and 7.8 % of the total content, respectively. In addition, it was found that unsaturated and saturated fatty acids, described in Table 1, accounted for 83.5 % and 16.5 % of the total content, respectively. In unsaturated fatty acids,

monounsaturated compounds represented only 11.2 % of the total content, while polyunsaturated fatty acids were the most abundant type from this family (72.3 %) (Table 1).

Presence of a higher concentration of polyunsaturated fatty acids indicates the nutritional value of *C. colocynthis* seed oil, because when unsaturation increases, the lipid content increases, as well due to its capacity to reduce the plasma concentration of low-density lipoprotein-cholesterol (LDL-C). Therefore, the incidence and severity of chronic diseases are affected, especially with respect to the specific cardiovascular pathophysiological disturbances [24, 25]. In this regard, Ewadh et al. (2016) recently reported that the cortex of *C. colocynthis* contains 17 % fixed oil, with a high ratio of mono- and polyunsaturated fatty acids, namely linoleic acid (60-70 %), oleic acid (11.7-15 %), and n-3 poly-unsaturated acid (0.5 %) [26]. Furthermore, Igwenyi (2015) reported that *C. colocynthis* seeds grown in Nigeria are mainly composed of unsaturated fatty acids (63 %) [27]. Also, a recent analysis of the fatty acid composition of four varieties of *C. lanatus* seed oil revealed that the most abundant fatty acid was oleic acid (monounsaturated fatty acid), followed by other saturated, long-chain, fatty acids, such as stearic acid and palmitic acid [28]. Moreover, Gurudeeban *et al*. reported that the seeds of *C. colocynthis* are rich in fatty acids, such as myristic, palmitic, stearic, oleic, linoleic, and linolenic acids [29]. In addition, methyl esters of fatty acids, including palmitic, linolenic, linoleic, and stearic acids, are the most abundant components of Algerian *C. colocynthis* [30]. Furthermore, caproic (C6:0), capric (C8:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), heptadecanoic (C17:0), stearic (C18:0), heneicosanoic (C21:0), and tricosanoic (C23:0) acids were detected in *C. colocynthis* from Togo [31]. In light of differences regarding the fatty acid profile of plants collected from different regions, it seems that two major reasons account for such discrepancies, namely the genetic background of species characterized in separate studies and the agro-environmental conditions, which can trigger abiotic stress, and influence the fatty acid profile. Indeed, abiotic stress is linked to non-enzymatic reactions of fatty acids towards the synthesis of oxidative derivatives (oxylipins), mainly represented by phytoprostanes and phytofurans, which modify the fatty acid profile in the plant materials and manufactured products (seed oils) [32-35]. Discussions about the relative merits of different wild plants are frequently dulled by the pronounced differences in their quality as a dietary source of essential and non-essential nutrients, depending on the species, ecotypes, and origins. This seems to be the case for *C. colocynthis* with respect to the fatty acid composition, which is closely dependent on an array of variables that should be further explored in the near future.

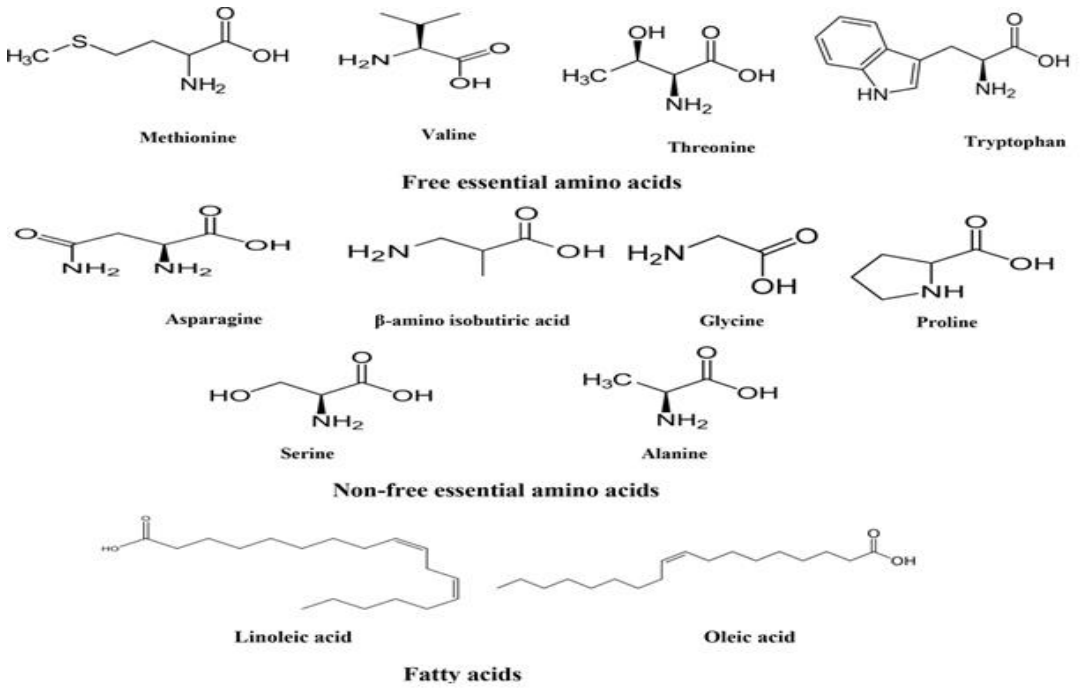

Fig. 2. Chemical structures of some identified free essential amino acids, non-free essential amino acids, and fatty acids in *C. colocynthis* seeds

Table 1. Fatty acid (%) composition of *C. colocynthis* seeds

Units	Name	M.wt.	M.F.	MS/MS fragments	Retention time [min]	Area [%]
C14:0	Myristic acid	228	$C_{14}H_{28}O_2$	29, 43, 57, 73, 97, 115, 129, 185	19.817	0.0540
C16:0	Palmitic acid	256	$C_{16}H_{32}O_2$	29, 41, 43, 57, 60, 73, 97, 115, 129, 157, 171, 185, 213	23.655	8.3026
C16:1	Palmiteloic acid	254	$C_{16}H_{30}O_2$	96, 117, 129, 145, 183, 194, 229	24.940	0.0316
C17:1	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	29, 43, 57, 60, 71, 73, 85, 115, 129, 143, 171, 185, 227	26.500	0.0049
C18:0	Stearic acid	284	$C_{18}H_{36}O_2$	29, 43, 57, 60, 69, 73, 85, 115, 129, 143, 171, 185, 241	27.380	7.7853
C18:1n-9	Oleic acid	282	$C_{18}H_{34}O_2$	27, 29, 41, 43, 55, 69, 83, 97, 111, 123, 264	28.516	10.9316
C18:2n-6	Linoleic acid	280	$C_{18}H_{32}O_2$	27, 29, 41, 55, 67, 81, 95, 110, 124, 150, 182, 196, 264	30.347	70.6778
C20:0	Arachidic acid	312	$C_{20}H_{40}O_2$	85, 97, 116, 127, 128, 131, 143, 146, 171, 183, 199, 242, 285	30.790	0.2336
C20:1 n-9	*Cis*-11-Eicosanoic acid	310	$C_{20}H_{38}O_2$	29, 41, 55, 69, 83, 97, 111, 125, 151, 208, 221, 250, 292	31.903	0.1402
C18:3n-3	Linolenic acid	278	$C_{18}H_{30}O_2$	29, 41, 55, 67, 79, 93, 95, 108, 121, 135, 149, 222, 249, 263	32.114	0.1218
C22:0	Behenic acid	340	$C_{22}H_{44}O_2$	29, 43, 57, 73, 83, 97, 111, 129, 143, 171, 185, 199, 227, 241, 297	32.604	0.0018
C20:3n-6	*Cis*-8,11,14-Eicosatrienoic acid	306	$C_{20}H_{34}O_2$	41, 55, 67, 74, 81, 87, 95, 109, 121, 141, 150, 163, 177, 222, 289	32.691	0.0325
C22:1n-9	Erucic acid	338	$C_{22}H_{42}O_2$	29, 41, 55, 69, 83, 97, 111, 125, 152, 181, 222, 263, 302, 320	33.159	0.0595
C20:3n-3	*Cis*-8,11,14,17-Eicosatrienoic acid	304	$C_{20}H_{32}O_2$	55, 59, 67, 79, 87, 95, 108, 121, 135, 149, 163, 219, 264, 289	33.456	0.0008
C20:4n-6	Tricosanoic acid	354	$C_{23}H_{46}O_2$	29, 43, 55, 60, 73, 97, 111, 129, 157, 185, 199, 241, 255, 311, 320	33.599	0.0116
C23:0	Arachidonic acid	304	$C_{20}H_{32}O_2$	29, 41, 55, 67, 79, 91, 105, 119, 133, 150, 166, 177, 206, 264	34.192	0.0592
C20:5n-3	*Cis*-5,8,11,14,17-Eicosapentanoic acid	302	$C_{20}H_{30}O_2$	29, 41, 55, 60, 67, 79, 91, 105, 119, 133, 166, 206, 273, 292	35.625	0.0595
C22:6n-3	*Cis*-4,7,10,13,16,19-Docosahexaenoic acid	328	$C_{22}H_{32}O_2$	72, 95, 111, 121, 147, 161, 178, 215, 257, 278	38.131	0.1409
C22:6n-3	*Cis*-4,7,10,13,16,19-Docosahexaenoic acid (isomer)	328	$C_{22}H_{32}O_2$	72, 95, 111, 121, 147, 161, 178, 215, 257, 278	41.453	0.0259
C22:6n-3	*Cis*-4,7,10,13,16,19-Docosahexaenoic acid (isomer)	328	$C_{22}H_{32}O_2$	72, 95, 111, 121, 147, 161, 178, 215, 257, 278	43.367	0.5049
C22:6n-3	*Cis*-4,7,10,13,16,19-Docosahexaenoic acid (isomer)	328	$C_{22}H_{32}O_2$	72, 95, 111, 121, 147, 161, 178, 215, 257, 278	46.611	0.2922
C22:6n-3	*Cis*-4,7,10,13,16,19-Docosahexaenoic acid (isomer)	328	$C_{22}H_{32}O_2$	72, 95, 111, 121, 147, 161, 178, 215, 257, 278	54.792	0.3359

M.wt.: Molecular weight
M.F.: Molecular formula

3.2. Crude protein and amino acid content

Extraction and analysis of the protein content of *C. colocynthis* seeds yielded a crude protein content of 21.8 % on a dry matter basis, indicating the importance of these species as dietary protein sources; this finding further complements the advantages of mono- and polyunsaturated fatty acids. From a nutritional point of view, the level of crude protein in *C. colocynthis* seeds is close to recent reports of minimally processed commercial legumes (faba bean or *Vicia faba* and lupin or *Lupinus angustifolius*), which constitute up to 30 % of the crude protein content [36], these similar values highlight the importance of *C. colocynthis* as a source of dietary protein. Apart from the profiling and quantification of mono- and polyunsaturated fatty acids, protein and lipid extracts of *C. colocynthis* seeds were assessed regarding their essential and non-essential amino acid contents (Tables 2, 3 and Figure 2). Generally, 20 amino acids are involved in the protein metabolism of humans. However, humans can synthesize only 10 out of these 20 molecules. Classically, essential amino acids (tyrosine, asparagine, glutamine, serine, ethanolamine, glycine, aspartic acid, glutamic acid, alanine, α-aminobutyric acid, γ-aminobutyric acid, proline, ornithine, cysteine, and β-amino isobutyric acid), which can be synthesized by human cells and are not essentially provided by dietary proteins, have been distinguished.

On the other hand, seven out of ten non-essential amino acids, which cannot be synthesized by human cells (non-essential amino acids; arginine, histidine, threonine, lysine, valine, methionine, leucine, isoleucine, tryptophan, and phenylalanine) [37], were found in the *C. colocynthis* seed oil extract. These non-essential amino acids included histidine, threonine, lysine, valine, leucine+isoleucine (as co-eluting amino acids), and tryptophan at concentrations of 0.01, 0.32, 0.02, 0.26, 0.13, and 0.19 μg/mL, respectively. On the other hand, in the protein extract, nine non-essential amino acids were found, including arginine, histidine, threonine, lysine, valine, methionine, leucine+isoleucine (as co-eluting amino acids), tryptophan, and phenylalanine at concentrations of 0.05, 0.02, 0.33, 0.02, 0.33, 1.14, 0.23, 0.27, and 0.06 μg/mL, respectively, indicating the nutritional value of this plant (Table 2).

According to the results, threonine and valine were essential amino acids found at the highest concentrations in both lipoprotein and protein extracts, followed by tryptophan and leucine+isoleucine as co-eluting amino acids. In addition, arginine, methionine, and phenylalanine were only found in the protein extract. Since human cells cannot store reserves of amino acids for further use, essential amino acids must be ingested daily through food intake. Therefore, lack of even one out of 10 essential amino acids, which need to be ingested by diet, has biological consequences, such as degradation of proteins in cells and tissues; given the structural function of these molecules, subsequent disturbances in tissues and cells are responsible for a range of pathophysiological conditions [38]. Among non-essential amino acids found in *C. colocynthis* seeds, tyrosine and asparagine were the most abundant ones in both lipoprotein and protein extracts, with concentrations of up to 0.11 and 7.02 μg/mL, respectively. On the other hand, additional amino acids identified in this food matrix were present at very lower concentrations. Glutamic acid and glutamine were not detected in the lipid extract. Based on the comparison of amino acid concentrations of *C. colocynthis* seeds between the present study and previous reports, characterization of the amino acid content of *C. colocynthis* seeds grown in Nigeria indicated that the concentrations of arginine, lysine, glutamic acid, methionine, aspartic acid, and histidine were 9.32, 6.35, 5.95, 5.51, 5.02, and 4.79 g/100 g protein, respectively, while amino acids with the lowest concentrations (<2.00 g/100 g protein) included isoleucine, leucine, threonine, cysteine, proline, and tyrosine [27].

Another study carried out by Abudayeh et al. (2016) identified 17 amino acids in different parts of *C. colocynthis* (seeds, pulp, and rind), and arginine was found to be the most abundant essential amino acid in this species (0.210-0.886 mg/100 mg) [39]. According to this study, it was found that glutamic acid is the dominant amino acid in seeds of *C. colocynthis* (0.312-1.488 mg/100 mg), while the lowest concentration of amino acids was attributed to cystine (0.046-0.074 mg/100 mg) [39].

In this regard, a recent analysis of the amino acid composition of *C. colocynthis* grown in Togo revealed the presence of aspartic acid, glutamates, serine+glutamine+histidine,threonine+glycine+argini ne, valine+methionine, and leucine [40]. However, we cannot compare the results reported by Limem *et al*. with those of the present study, since the analytical technique used by Limem et al. resulted in excessive co-elution. The nutritional quality of plants, including crude protein content, fatty acid profile, and amino acid content, seems to largely vary with season, geographic location (closely linked to the availability of soil nutrients), and environmental stress (drought, salinity, and light exposure) [41]. The amino acid content of plant matrices in this study may differ from that described in the literature because of the diverse origins of wild *C. colocynthis*, and consequently, its exposure to specific seasonal, geographical, and environmental factors, which critically influence the level of these compounds. The results of the present study and previous research indicate the valuable nutritional composition of *C. colocynthis* seeds in terms of total protein, fatty acids,

and essential and non-essential amino acids; this suggests the feasible application of this plant material as a nutritional supplement.

Table 2. Concentrations of free essential amino acids (µg/mL) in oil and protein extract of *C. colocynthis* seeds

Amino acids/amino thiols	M.wt.	M.F.	Type of extract	
			Oil extract	Protein extract
Arginine	174	$C_6H_{14}N_4O_2$	N.d.	0.05 ± 0.01
Histidine	155	$C_6H_9N_3O_2$	0.01 ± 0.00	0.02 ± 0.01
Threonine	119	$C_4H_9NO_3$	0.32 ± 0.02	0.33 ± 0.06
Lysine	146	$C_6H_{14}N_2O_2$	0.02 ± 0.01	0.02 ± 0.00
Valine	117	$C_5H_{11}NO_2$	0.26 ± 0.01	0.33 ± 0.11
Methionine	149	$C_5H_{11}NO_2S$	N.d.	1.14 ± 0.55
Leucine + isoleucine	131	$C_6H_{13}NO_2$	0.13 ± 0.01	0.23 ± 0.01
Tryptophan	204	$C_{11}H_{12}N_2O_2$	0.19 ± 0.03	0.27 ± 0.00
Phenylalanine	165	$C_9H_{11}NO_2$	N.d.	0.01 ± 0.00

Description of the statistical treatment. N.d., no detected

Table 3. Concentrations of non-essential free amino acids (µg/mL) in oil and protein extract of *C. colocynthis* seeds

Amino acids/amino thiols	M.wt.	M.F.	Type of extract	
			Oil extract	Protein extract
Asparagine	132	$C_4H_8N_2O_3$	3.07 ± 0.50	7.02 ± 2.60
Tyrosine	181	$C_9H_{11}NO_3$	0.09 ± 0.02	0.11 ± 0.02
Glutamine	146	$C_5H_{10}N_2O_3$	N.d.	0.02 ± 0.00
Serine	105	$C_3H_7NO_3$	0.29 ± 0.04	0.22 ± 0.02
Ethanolamine	61	C_2H_7NO	0.18 ± 0.07	0.04 ± 0.01
Glycine	75	$C_2H_5NO_2$	0.25 ± 0.01	0.24 ± 0.02
Aspartic acid	133	$C_4H_7NO_4$	0.12 ± 0.00	0.13 ± 0.01
Glutamic acid	147	$C_5H_9NO_4$	N.d.	0.04 ± 0.00
Alanine	89	$C_3H_7NO_2$	0.22 ± 0.01	0.21 ± 0.02
α-Aminobutiric acid	103	$C_4H_9NO_2$	0.06 ± 0.01	0.09 ± 0.01
γ-aminobutyric acid	103	$C_4H_9NO_2$	0.01 ± 0.00	0.01 ± 0.00
Proline	115	$C_5H_9NO_2$	0.09 ±0.00	0.22 ±0.07
Ornitine	132	$C_5H_{12}N_2O_2$	0.10 ± 0.00	0.11 ± 0.02
Cistatine	-	-	N.d.	N.d.
β-amino isobutiric acid	103	$C_4H_9NO_2$	0.09 ± 0.01	0.30 ±0.02

Description of the statistical treatment. N.d., no detected

4. Conclusion

In recent years, several attempts have been made to categorize wild plant species (FAO 1982; 1983a; 1983b; 1984; 1986a; 1986b). Although a large number of species have been identified regarding their nutritional value to date, available information on the produced quantities, seasonal variability of production, and year-to-year variability is scarce. Therefore, it is often difficult to assess their relative importance as food sources, and this critical issue should be addressed in the near future among species, such as *C. colocynthis*. The findings of the present study and systematic comparison with the literature regarding the content of crude protein, mono- and polyunsaturated fatty acids, and essential and non-essential amino acids indicate the nutritional value of this species and allows us to determine the application of *C. colocynthis* seeds; this promotes the current use of this plant and allows us to take advantage of its nutritional composition. However, given the discrepancies between of available data in the literature and the present study, further research is needed to determine the specific impact of environmental factors on this wild plant in order to make rational decisions about its valorization as a dietary supplement in different seasons and environmental conditions.

5. Abbreviations

GC-MS: Gas Chromatography–Mass Spectrometry
UHPLC-ESI-QqQ-MS/MS: Ultra High-Performance Liquid Chromatography-Electrospray Ionization-Triple Quadrupole Mass Spectrometry.
AQC: 6-aminoquinolyl-N-hydroxysccinimidyl carbamate.
EDTA: Ethylenediaminetetraacetic acid.
FAME: Fatty acid methyl ester.
FID: Flame ionization detector.
AccQTagTM: Ultra C18 column.
MRM: Multiple reaction monitoring.
AMQ: Aminoquinoline.
SD: Standard deviation.
ANOVA: Analysis of variance.
LDL-C: Low-density lipoprotein-cholesterol.
FAO: Food and Agriculture Organization.

6. Conflict of Interest

The authors declare no conflict of interest.

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