

Ecophysiological Studies on Saudi Wild Safflower (*Carthamus Oxyacantha* Bieb) Seed Ecotypes

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Abstract: *Carthamus oxyacantha* is a widespread wild safflower species in Middle Eastern countries and northwestern India, including Kazakhstan, Turkmenistan and Uzbekistan and the Kingdom of Saudi Arabia. This study was conducted to evaluate and compare four *C. oxyacantha* ecotypes collected from different regions in the Kingdom of Saudi Arabia, Al-Hassa, Al-Kharaj, Najran and Al- Jouf. Analysis of variance showed significant effects of ecotype on physiological traits (number of heads per plant, heads diameter (cm), number of seeds per head, thousand seed weight (g) and seed color). The ecotypes also significantly differed in their moisture, crude fat, crude protein, crude fiber, ash and carbohydrates seeds contents. Moisture varied from 5.5 to 6.3%, crude fat varied from 25.3 to 27.3%, crude protein from 9.6 to 12.4%, crude fiber varied from 14.6 to 17.9%, ash from 3.2 to 4.2% and carbohydrates varied from 37.3 to 39.3%. The main fatty acids of linoleic, oleic, palmitic and stearic acids composed 89.7-97.2% of the total fatty acids in all ecotypes. The sum of linoleic, oleic and palmitic acids fatty acids in seed oil ranged from 88.5 to 92.1%. The tested ecotypes also varied in their amino acid composition of seed proteins. The seeds were rich in four amino acids: Arginine, Glycine, Valine and Leucine. According to the results of the present study, the Saudi *C. oxyacantha* oil seeds can be a potential source of protein and energy supplements in livestock feed.

Keywords: *Carthamus Oxyacantha*, Safflower Ecotypes, Saudi Conditions, Fatty Acids, Amino Acids

Introduction

The genus *Carthamus* from the Compositae family consists of 16 known species (Knowles and Ashri, 1995). *C. tinctorius* is the only cultivated species of this genus, but the others are wild weeds. *C. oxyacantha* is a wild safflower species found in arid and semi-arid environments, including Turkey, Iraq, Iran, northwestern India, Kazakhstan, Turkmenistan and Uzbekistan (Knowles and Ashri, 1995; Agrawal *et al.*, 2013) and it has also been recorded in many parts of the Kingdom of Saudi Arabia. The naturally growing plants in Saudi Arabia show significant genetic differences. Wild safflower, which is closely related to cultivated safflower, is a spiny-leaved annual herb, growing up to 1.5 m tall. Like other spiny plants in the genus *Carthamus*, wild safflower is closely related to safflower is not grazed by livestock, enabling it to spread on range lands. The yellow flowers grow in flower heads approximately 2-3 cm in diameter and the leaves are

covered with spines. Wild safflower can be distinguished from the cultivated safflower by its larger, pointed spines. *C. oxyacantha* is a self-pollinating plant, but has the potential for out-crossing (10%) via pollen transfer by a number of insects (Singh *et al.*, 2007). *C. oxyacantha* has an indeterminate flowering habit, while the cultivated species is determinate in nature. Safflower has been grown for its oil (Weiss, 2000), as pharmaceuticals (McPherson *et al.*, 2004; Ullah *et al.*, 2014), biofuels and as a source of special oil types due to the presence of useful secondary metabolites, such as essential oils (Velasco and Fernandez-Martinez, 2004). Safflower essential oils are gaining increasing interest because of their wide acceptance by consumers and their use in many applications (Burt, 2004).

The study of genetic differences among wild and cultivated plant populations is important for genetic resources conservation (Mohammadi and Prasanna, 2003). Various traits including: Morphological, biochemical and molecular characteristics are used to

assess plant genetic diversity (Golkar *et al.*, 2011). Seed oil fatty acid composition differs significantly between and within species. The genetic differences in fatty acid composition are needed for the genetic improvement of oilseed plant species (Hamdan *et al.*, 2008). The wild safflower species e.g., *C. oxyacantha* can be crossed with the cultivated species to improve their economical characteristics such as seed oil quality. Several reports have been published on safflower oil (Akhtar *et al.*, 2015; Cosge *et al.*, 2007; Carvalho *et al.*, 2006; Weiss, 2000), but, the oil composition of wild species, specifically KSA, has not yet been fully determined.

Thus, the objectives of this study was to evaluate and compare some morphological and physiological characteristics of four *C. oxyacantha* ecotypes collected from different regions in the Kingdom of Saudi Arabia, Al-Hassa, Al-Kharaj, Najran and Al-Jouf. This information is highly necessary for safflower breeding programs.

Materials and Methods

Sampling and Growth Conditions

The heads of four wild safflower (*C. oxyacantha*) ecotypes were collected from different geographical regions in KSA. Each ecotype was composed of seeds from 10 plant heads from each collection site. Al-Hassa (Eastern KSA) seeds were collected from field along Qatar road (Latitude: 25° 24' 0" N, Longitude: 49° 29' 0" E and alt.178 m), while the three others ecotypes were from Central KSA (Kharaj, lat.32° 34' 59" N, long.35° 42' 23" E; alt.503 m), Southern KSA (Najran, lat.17°30'20"N; long.44°11'03"E; alt.1264 m) and Northern KSA (Al-Jouf, lat.29° 34' 48" N, long.40° 32' 28" E; alt.689 m). Seeds from each site were bulked into a single sample. All ecotypes were grown in a randomized complete block design with 4 replicates in a greenhouse at King Faisal University research station, with a temperature of 35/20°C (day/night), photoperiod of 14/10 h (day/night), relative humidity of 70% and a maximum Photon Flux Density (PFD) of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Seeds were sown in plots and each plot consisted of three rows 50 cm apart and 5 m. Fertilizers were uniformly incorporated into the soil before sowing at a rate of 180 kg N ha⁻¹ in the form of urea and 100 kg P₂O₅ ha⁻¹ in the form of triple super phosphate. An additional 100 kg N ha⁻¹ was applied as side dressing at the early flowering stage. Seeds were sown on November 8th, 2013 at a density of 100 plants/m². Pre-irrigation was performed immediately after sowing for uniform seedling emergence and establishment. Irrigation was performed twice per week by a gravity drip irrigation system. The system consisted of a tank and 16 drip tubes (two tubes per plot). The tank (1000 L) was 1 m above the ground. Drip tubes with emitter's spaced 0.25 m apart were placed at the center between

two rows. Irrigation was applied as soon as the potential neared the target value of 75% field capacity, as measured by densitometers. One vacuum gauge densitometer was installed 0.25 m directly underneath one emitter located in the center of the middle row within each plot. The densitometers were checked three times a day (at 8: 00, 12: 00 and 18: 00 h). Weeds were removed by hand when needed. Seeds were harvested in mid-April, 2014. Harvested seeds were air-dried and stored at 4°C until used for further analysis.

Seed Characteristics

The number of heads per plant, head diameter (cm), number of seeds per head, seed color and 1000 seed weight for each ecotype was determined. For seed weight determination, four replicates of 100 seeds per ecotype were used (International Seed Testing Association, 2003) and 1000-seed weights were calculated.

Proximate Analysis

Proximate analysis of the *C. oxyacantha* seeds was performed as described by the Association of Official Analytical Chemists (AOAC, 1995).

Oil Concentration Determination

Seeds were dried at 60°C for 3 h using a ventilated oven to a moisture content of approximately 5% and were ground with a Philips mill and passed through a 16-mesh sieve. The dried seed powder (10 g) was extracted for oil using 300 mL petroleum ether for 6 h in a Soxhlet system according to the AOCS method (AOCS, 2004). Upon completion of oil extraction, the solvent was evaporated at 105°C for 5 h to remove the remaining moisture. The oil content of each sample was calculated based on dry weight (mg g^{-1}).

Fatty Acid Composition

Total fatty acids from *C. oxyacantha* seeds were transformed to methyl esters using 3% sodium methylate in methanol as previously described (AOCS, 2004). Heptadecanoic acid methyl ester was used as a standard to quantify the fatty acids. The Fatty Acid Methyl Esters (FAME) were analyzed by a gas chromatograph equipped with a flame ionization detector and an electronic pressure control injector. One micro liter of the FAME sample was injected into the chromatograph using a micro liter syringe. Helium was the carrier gas with a head pressure of 18 psi. Injector, detector and oven temperatures were 250, 250 and 200°C, respectively. The FAME samples were positively identified by matching their retention time data and mass spectra with those of a commercial standard FAME mixture. The fatty acid content was determined using a computing integrator and showed as the percentage of the oil yield and the values were means of three injections.

Amino Acid Composition

Amino acid contents the *C. oxyacantha* ecotypes 'seeds were determined by High Performance Liquid Chromatography (HPLC) as previously described by Cserhati and Forgaes (1999) and Kerese (1984). Finely ground *C. oxyacantha* seed samples were hydrolyzed by adding 4.83 g Barium hydroxide and 5 mL boiling water to 500 mg of each sample. The mixture was then heated at 120°C for 8 h. After hydrolysis, the pH was adjusted to 3 with HCl and diluted to 25 mL with distilled water. One mL of each sample was vacuum dried using a flash evaporator and dissolved in citrate buffer (0.1 M, pH = 2.2). Acid hydrolysis was performed with 6N HCl at 110°C for 18-22 h in evacuated and sealed tubes. The hydrolysate was filtered and diluted to 250 mL. A total of 1.0 mL of each sample was vacuum evaporated at 40°C until dry. The samples were then dissolved in citrate buffer (0.1 M; pH2.2). A volume of 20 mL solution was directly injected into the HPLC. Detection was accomplished using the Shimadzu HPLC detector LC-10A with a variable wavelength monitor set at 350-450 nm. The resolution of the amino acid derivatives was accomplished using a binary gradient system. The solvents used were: (A) 58.8 g sodium citrate containing 0.2 N sodium (pH = 3.2), 210 mL 99.5% ethanol and 50 mL (60%) perchloric acid and (B) 58.5 g sodium citrate containing 0.6N sodium (pH = 10), 12.4 g boric acid and 30 mL 4N NaOH solution. The solvent was delivered to the column at a flow rate of 4 ml/min for 7 to 10 min (Ingale and Shrivastava, 2011a; 2011b).

Statistical Analysis

Data were subjected to statistical analysis using the SAS program package (SAS, 2009) and expressed as the mean \pm Standard Deviation (SD). Mean values were compared by using a one-way Analysis Of Variance (ANOVA) followed by Duncan's multiple range tests. The differences between individual means were considered significant at $p < 0.05$.

Results and Discussion

Seed Characteristics

In general, the tested ecotypes displayed significantly different morphological traits (Table 1). The number of heads per plant in the studied Saudi *C. oxyacantha* collection ranged from 27.0 to 37.0. *C. oxyacantha* collected from Najran had the most number of heads per plant (36 ± 3.3). Head diameter ranged from 0.7 to 2.1 cm. *C. oxyacantha* collected from Hassa had the largest head diameter (1.7 ± 0.4 cm). The thousand seed weights ranged from 6.1 to 8.6 g in the Saudi *C. oxyacantha* species. In this study, the Al-Hassa collection had the highest thousand seed weight (Table 1). The seed coat color of Saudi *C. oxyacantha* plant species was varied

from light brown to dark brown (Table 1). These results were similar to those reported by Amini *et al.* (2008) who observed significant differences in morphological traits among safflower genotypes.

Proximate Analysis

The results of the proximate composition are shown in Table 2. The analysis of variance gave significant F values when we compared the differences of traits between ecotypes. The tested *C. oxyacantha* ecotypes significantly differed in their moisture, crude fat, crude protein, crude fiber, ash and carbohydrate contents. The moisture ($6.3 \pm 0.2\%$), crude protein ($12.4 \pm 1.3\%$) and ash contents ($4.2 \pm 0.1\%$) of Al-Hassa *C. oxyacantha* seeds were higher than in the seeds from other ecotypes, while the crude fat content was higher in Kharaj seeds ($27.3 \pm 1.3\%$) making it a better fat source compared to the others. The high crude fat content in all tested seeds indicates that these ecotypes are good oil sources (Gupta and Shrivastava, 2003).

The moisture content of *C. oxyacantha* ecotypes ranged from $5.5 \pm 0.2\%$ in Kharaj to $6.3 \pm 0.2\%$ in Al-Hassa (Table 2). Mean crude fat content varied from $25.3 \pm 1.6\%$ in Al-Jouf to $27.3 \pm 1.3\%$ in Kharaj (Table 2). The seed oil content of *C. oxyacantha* previously reported by Nagaraj (1994) was much higher than our results. The analysis of variance did not show a significant F-value between Kharaj and Hassa or between Najran and Al-Jouf, indicating the lack of statistically significant differences between each of the two ecotypes when compared to each other, but the differences between the two groups were significant. In other words, the fat content of the Kharaj seed sample ($27.3 \pm 1.3\%$) was higher than that of Al-Hassa seeds ($26.5 \pm 1.2\%$), but the difference was not significant. However, the two kinds of seeds significantly differed from those of Najran ($25.5 \pm 0.9\%$) and Al-Jouf ($25.3 \pm 1.6\%$) in fat content (Table 2). A number of studies have shown that crude protein ranged from 14.9 to 17% and crude fat ranged from 25 to 40% (Pavlov and Tadorov, 1996). The values we obtained in this study fell in the previously reported range.

Crude protein varied from $9.6 \pm 1\%$ in Kharaj to $12.4 \pm 1.3\%$ in Al-Hassa (Table 2). The protein contents of these ecotypes are comparable to those reported for some conventional oil seeds. In a survey of 15 varieties and 9 breeding lines of cultivated safflower, the range of seed protein content was 8.6-16.2%, with a mean of 11.32% (Fernandez-Martinez *et al.*, 1993). We concluded that the wild *Carthamus* species investigated in this study have a higher percentage of seed protein compared to the cultivated species, which were bred for increasing seed oil content for commercial use. However, this increase in oil content inversely correlates with protein content. Therefore, these ecotypes could be used as an alternative source of protein and oil for animal feed.

Table 1. Morphological traits of Saudi *C. oxyacantha* species

Traits	Hassa	Kharaj	Najran	Al-Jouf
Number of heads per plant	33±2.3 ^a	27±1.3 ^b	36±3.3 ^a	28±1.2 ^b
Heads diameter (cm)	1.7±0.4 ^a	1.5±0.8 ^a	1.6±0.9 ^a	1.6±0.5 ^a
Number of seeds per head	16.4±2.3 ^a	12.6±1.2 ^c	13.7±1.3 ^c	15.1±2.0 ^b
Thousand seed weight	8.6±1.5 ^a	6.1±1.1 ^c	6.4±1.0 ^c	7.9±0.9 ^b
Seed colour	Light brown	Light brown	Brown	Dark brown

Means with different letters in the same row symbolize statistically significant differences ($p < 0.05$), according to Duncan's multiple range test

Table 2. Proximate chemical composition (%) of *C. oxyacantha* seeds

Trait	Hassa	Kharaj	Najran	Al-Jouf
Moisture	6.3±0.2 ^a	5.5±0.2 ^c	5.9±0.1 ^b	6.0±0.1 ^{ab}
Crude fat	26.5±1.2 ^a	27.3±1.3 ^a	25.5±0.9 ^b	25.3±1.6 ^b
Crude protein	12.4±1.3 ^a	9.6±1.2 ^c	9.7±0.3 ^c	10.1±0.3 ^b
Crude fiber	14.6±0.8 ^c	15.1±0.5 ^c	16.4±0.7 ^b	17.9±0.1 ^a
Ash	4.2±0.1 ^a	3.5±0.4 ^b	3.2±0.1 ^b	3.4±0.3 ^b
*Carbohydrate	36.0±0.6 ^c	39.0±0.4 ^a	39.3±0.1 ^a	37.3±0.4 ^b

All tests were performed in triplicate and values are mean ± standard deviation.

*Carbohydrate = 100- (H₂O + Ash + C p + C fat+ C fiber).

Means with different letters in the same row symbolize statistically significant differences ($p < 0.05$), according to Duncan's multiple range test

There was a significant increase in crude fiber content of Al-Jouf *C. oxyacantha* (17.9±0.1%), while the carbohydrate content (39.3±0.1%) was highest in Najran *C. oxyacantha* seeds. These results are consistent with other oil seed cultivars (Cosge *et al.*, 2007; Amini *et al.*, 2008; Sabzalian *et al.*, 2009). The crude fiber (17.9±0.1%) and ash content (4.2±0.1%) were significantly higher in Al-Jouf and Al-Hassa *C. oxyacantha* seeds, respectively and surpassed all other ecotypes (Table 2).

Fatty Acid composition

The fatty acid composition of the *C. oxyacantha* seeds is shown in Table 3. Four fatty acids were measured in the *C. oxyacantha* ecotypes seeds. The fatty acid profile of the four safflower ecotypes reveals that their lipids are a good source of the nutritionally essential linoleic and oleic acids and linoleic acid (C18: 2) constitutes the major fatty acid among the four ecotypes. Our results are consistent with those of Sabzalian *et al.* (2008) who reported that these four fatty acids were present in safflower seed oil at a proportion of more than 90.2% TFA. Linoleic acid and oleic acid comprised more than 90% of the fatty acids. Similar findings have been previously reported in safflower (Pascual-Villalobos and Albuquerque, 1995; Carvalho *et al.*, 2006; Sabzalian *et al.*, 2008). The Kharaj ecotype displayed a significantly higher linoleic acid levels (C18: 2) (68.7±1.68% TFA) compared to the three other ecotypes (66.3±1.01, 66.0±0.86 and 66.3±0.14% TFA in *C. oxyacantha* seeds of Al-Hassa, Najran and Al-Jouf ecotypes, respectively).

Oleic acid (C18: 1) was the second most abundant fatty acid in the four ecotypes, with levels of 16.2±0.50, 16.5±0.14, 15.7±0.99 and 15.2±1.54% TFA in Al-

Hassa, Kharaj, Najran and Al-Jouf *C. oxyacantha* seeds, respectively. Moreover, its proportion was significantly higher in Kharaj (16.5±0.14% TFA) and Al-Hassa (16.2±0.50% TFA) compared to the two other ecotypes (15.7±0.99 and 15.2±1.54% TFA in Najran and Al-Jouf, respectively).

Palmitic acid (C16: 0) ranged from approximately 6.94±0.79% TFA to 7.11±0.43% TFA. In addition, stearic acid (C18:0) was present at low levels, accounting for 1.15±0.16, 1.19±0.14, 1.15±0.05 and 1.16±0.09% TFA in Al-Hassa, Kharaj, Najran and Al-Jouf, respectively.

In this study, oleic and linoleic acids accounted for more than 88, 86, 85 and 84% of the Total Fatty Acids (TFA) in Kharaj, Al-Hassa, Najran and Al-Jouf ecotypes, respectively, while stearic and palmitic acids comprised more than 8% of the total fatty acid concentration in the studied ecotypes. Palmitic and stearic acids concentration did not significantly change among ecotypes (Table 3). The seed oil obtained from different Saudi wild safflower ecotypes could be an alternative source of edible oil, as they contain several fatty acids that are required for human health. In addition, safflower oil has long been used for industrial purposes, notably for preparing varnish, due to its high linoleic acid content (Gecgel *et al.*, 2007).

Environmental factors, such as, soil and climatic factors often affect fatty acid composition and temperature is the most important factor that affects fatty acid composition (Baydar and Turgut, 1999). We considered environmental effects in this study to be negligible, as the four ecotypes were grown under the same conditions. The deep implication of genetic factor in variations in the seed oil yield of other plant species has been previously reported (Hosni *et al.*, 2010; Bettaieb *et al.*, 2009).

Amino Acid Composition

Amino acid composition is an important factor in the evaluation of protein quality. Human bodies do not synthesize the essential amino acids, but they can be obtained from food products. Eight amino acids are generally regarded as essential for humans: Phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine (Mariod *et al.*, 2012). Six amino acids are considered conditionally essential in the human diet, meaning their synthesis can be limited under special pathophysiological conditions, such as prematurity in the infant or individuals under severe catabolic distress (FAO/WHO/UNU, 2007). These six amino acids are arginine, cysteine, glycine, glutamine, proline and tyrosine. Five amino acids are dispensable in humans, meaning the body can synthesize them. These five amino acids are alanine, aspartic acid, asparagine, glutamic acid and serine (FAO/WHO/UNU, 2007).

Cysteine and tyrosine deficiency can inhibit healing recovery process (Mat *et al.*, 1994). All essential amino acids are present in the tested ecotypes. The amino acid composition of each *C. oxyacantha* ecotypes is presented in Table 4 on the basis of g/100 g protein. The ecotypes

varied in their amino acid composition of seed proteins. Our data are consistent with previous reports (Gupta and Shrivastava, 2006; Singh *et al.*, 2003) and suggested that these ecotypes are good sources for the essential amino acids including arginine, glycine, valine and leucine. The other amino acids were present in low to moderate amounts and serine was the most limiting amino acid. In Saudi Arabia, there is currently fast growing interest for oilseed utilization in animal feeds and for the oil extraction industry. Our work shows that wild safflower seeds are good oil sources.

From the data presented in Table 4, it is clear that the seed protein content in the Al-Hassa ecotype was most highly enriched for arginine (1.566 g/100g protein), while serine was the least abundant (0.025 g/100g protein). The remaining amino acids in ascending order were as follows: proline < alanine < aspartic acid < tyrosine < methionine < tryptophan < cysteine < glutamic acid < histidine < threonine < lysine < isoleucine < phenylalanine < valine < glycine < leucine.

The Kharaj ecotype was rich in arginine (1.579 g/100g), while serine was the least abundant amino acid (0.015 g/100g) (Table 4).

Table 3. Fatty acid composition (% TFA) of Saudi *C. oxyacantha* ecotypes seed

Fatty acids	Hassa	Kharaj	Najran	Al-Jouf
Linoleic acid (C18:2)	66.3 ^b ±1.01	68.7 ^a ±1.28	66.0 ^b ±0.86	66.3±0.14
Oleic acid (C18:1)	16.2 ^a ±0.50	16.5 ^a ±0.14	15.7 ^b ±0.99	15.2 ^b ±1.54
Palmitic acid (C16:0)	7.03±0.16	6.94±0.79	7.11±0.43	7.01±0.36
Stearic acid (C18:0)	1.15±0.16	1.19±0.14	1.15±0.05	1.16±0.09

Means with different letters in the same row symbolize statistically significant differences ($p < 0.05$), according to Duncan's multiple range test.

Table 4. Amino acid composition (g/100g protein) in Saudi *C. oxyacantha* ecotypes seed proteins

Amino acids	Hassa	Kharaj	Najran	Al-Jouf
Aspartic acid	0.150	0.145	0.147	0.149
Threonine	0.489	0.472	0.494	0.469
Serine	0.025	0.015	0.020	0.017
Glutamic acid	0.350	0.349	0.343	0.351
Proline	0.068	0.065	0.069	0.072
Glycine	0.974	0.992	1.002	0.997
Alanine	0.099	0.108	0.102	0.101
Cysteine	0.327	0.267	0.348	0.314
Valine	0.968	0.998	0.977	1.014
Methionine	0.234	0.236	0.231	0.223
Isoleucine	0.680	0.683	0.667	0.692
Leucine	0.999	0.996	1.003	1.001
Tyrosine	0.191	0.193	0.204	0.177
Phenylalanine	0.724	0.714	0.720	0.718
Histidine	0.458	0.461	0.452	0.457
Lysine	0.652	0.654	0.642	0.623
Arginine	1.566	1.579	1.585	1.575
Tryptophan	0.280	0.276	0.277	0.286

Means with different letters in the same row symbolize statistically significant differences between treatments at probability level $p < 0.05$, according to the Duncan's multiple range test.

The other amino acids are lying in between them in ascending order were as follows: proline < alanine < aspartic acid < tyrosine < methionine < cysteine < tryptophan < glutamic acid < histidine < threonine < lysine < isoleucine < phenylalanine < glycine < leucine < valine.

The Najran ecotype was enriched for arginine content (1.585 g/100g) and serine is the least abundant amino acid (0.020 g/100g) (Table 4). The remaining amino acids in ascending order were as follows: proline < alanine < aspartic acid < tyrosine < methionine < tryptophan < glutamic acid < cysteine < histidine < threonine < lysine < isoleucine < phenylalanine < valine < glycine < leucine.

Similarly, Al-Jouf seed proteins contained high amounts of arginine (1.575 g/100g protein) and low amounts of serine (0.017g/100g protein). The remaining amino acids in ascending order were as follows: proline < alanine < aspartic acid < tyrosine < methionine < tryptophan < cysteine < glutamic acid < histidine < threonine < lysine < isoleucine < phenylalanine < glycine < leucine < valine.

Essential amino acids found in the seeds contribute to good health. Lysine deficiency leads to physical and mental handicaps (Papes *et al.*, 2001). Amino acid antioxidant activity is essential for disease prevention, for example arginine is beneficial for cardiovascular diseases prevention. Arginine is an important factor in maintaining the nitrogen balance in muscles and it can enhance the lean tissue to fat tissue ratio, an important factor in weight management (Amino Acid, 2005). Aspartic acid deficiency decreases cellular energy and may be a factor in chronic fatigue (Amino Acid, 2005). Adequate methionine prevents hair, skin and nail disorder, reduces liver fat and protects the kidney (Amino Acid, 2005).

Conclusion

The results reported here on traits of the four *C. oxyacantha* seeds originating from Al-Hassa, Kharaj, Najran and Al-Jouf revealed variations in the crude protein, crude fat, crude fiber, ash, fatty acid and amino acid contents according to geographical origin which can be attributed to genetic factors. Linoleic, oleic and palmitic were the predominant fatty acids in the tested ecotypes' oil samples. Amino acids analysis showed that the protein contained nutritionally useful quantities of most of the essential amino acids. The arginine, valine, glycine and leucine were the most abundant amino acids in the ecotype seed samples, while they were low in serine. According to the results of the present study, the Saudi *C. oxyacantha* oil seeds can be a potential source of protein and energy supplements in livestock feed.

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Author's Contributions

Sulaiman M. Al Fadal: Writing of the manuscript, Designed the research plan and organized the study, Sampling and Growth Conditions, Fatty Acid Composition, Statistical Analysis.

Mohammed A. Al-Fredan: Writing of the manuscript, Designed the research plan and organized the study, Sampling and Growth Conditions, Seed Characteristics, Proximate Analysis, Oil Concentration Determination, Statistical Analysis.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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