COMPARATIVE ANALYSIS OF SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) GENOTYPES BASED ON SEED AND OIL CHARACTERISTICS

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(Received 25th Apr 2020; accepted 13th Aug 2020)

Abstract. The performance of nine safflower genotypes was estimated for the physical properties of seeds, chemical, and physiochemical characteristics of seed oil. They are with different response to most physical properties, that are considered in machinery designation for handling, harvesting, and oil extraction process. Local and Ardny genotypes had the highest percentage of oleic acid, while linoleic was the highest in Rabiaa, indicating their high usability in food preparation. Al-Shamia is preferable for direct use in food preparation, containing the lowest palmate ratio. Free fatty acid was ranged from 31.59-41.02% for G-2018 and Zaafarani genotypes, respectively. G-2018 had the lowest iodine value at 136.350 g I_2/100 g, providing an oil with a high saturation value. Al-Shamia and Local genotypes had low palmitic and stearic acid (saturated fatty acids) percentages, therefore, a highly stable and healthy edible oil can be extracted from the plants. The principal component analyses attributed high variation among the genotypes. Local and Rabiaa genotypes could be involved in the improvement of oil quality in Kurdistan. Dissimilarity matrix clarified a wide distance between G-2018 and other genotypes based on the traits. Hybridization programs would be reasonable for the oil quality improvement of these genotypes. Thermal stability and frying qualities of oleic and linoleic oils could be studied in the future.
Keywords: *true density, absorption index, protein%, refractive index, fatty acids composition*

Introduction

Safflower (*Carthamus tinctorius* L.) is an annual, highly branched, herbaceous plant, self-pollinated and belongs to the Asteraceae family (Pasandi et al., 2018), has a haploid genome size of about 1.4 GB and 2n = 24 chromosomes (Kumari et al., 2017). Safflower is a minor crop and one of the major oilseed crops worldwide (Gouzy et al., 2016), with a world production of about 627,653 tons in 2018 (FAOSTAT, 2019). There are several global uses of safflower such as dye production, various medicinal utilization, and extraction of edible oil. The extracted oil has a realized effect on reducing blood cholesterol levels (Emongor, 2010), due to containing a higher amount of oleic and linoleic acids than other oilseed crops (Khalid et al., 2017). Safflower seed oil content is ranged between 35 and 50% (Coşge et al., 2007). The oil is flavourless and colourless, and similar in composition to sunflower oil (Kaffka and Kearney, 1998; Han et al., 2009). Considerable attention has been generated in the consumption and development of safflower seed oil as an excellent health care product including the prevention and treatment of hyperlipidemia, arteriosclerosis, and coronary heart disease (Abidi, 2001). Safflower oil is stable and its consistency does not change at low temperatures, making the oil particularly suitable for the preparation of chilled and frozen foods (Ekin, 2005). The high oleic contents of safflower oil make the oil stable during heating and do not give smoke smell during frying (Gyulai, 1996). The oil is not allergenic, making it an ideal material for cosmetics. High polyunsaturated essential fatty acid, linoleic, make the oil to

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

be valuable nutritionally and therapeutically for human consumption (Vosoughkia et al., 2011). There is a growing tendency to study new genotypes of safflower with higher seed oil contents (Bart et al., 2010). The objective of this work is to assess the performance and variability of available safflower genotypes in Kurdistan-Iraq for some physical properties, oil characteristics and physicochemical properties of seed oil, and identifying their relationship for efficient selection to increase the oil yield in the safflower breeding program.

Materials and methods

A laboratory experiment was conducted, in the College of Agricultural Engineering Science, University of Sulaimani. Nine varieties (G-2018, Rabiaa, Zaafarani, Al-Mais, Ardny, Iden, Gilla, Al-Shamia and Local) were used in this study. All the genotypes are registered varieties at the Ministry of Agriculture and water resources of Kurdistan- Iraq. Gilla and Al-Mais genotypes were obtained from Erbil Polytechnic University, while the other genotypes were from Bakrajo Agriculture Research Center in Sulaimani, Kurdistan-Iraq. To erase the environmental effects on the genotypes, the varieties were grown in the field of College of Agricultural Engineering Sciences for 2018-2019 growing season in a Complete Randomized Block Design (CRBD) with three replicates. The varieties were grown under rainfed condition of the semi-arid region of Sulaimani-Iraq. The seeds were sown at 6 cm depth and with a plant density 40000 plant/ha as recommended by Ati and Hassan (2016). The collected seeds from these genotypes were used in the analyses. A complete randomized design was applied to examine the study data, with three replicates. Statistical analyses were performed. The source of variance and comparison between the genotypes were determined. The results were expressed as mean, minimum and maximum values with standard deviations (SD). The comparisons of traits' means were made using Duncan's multiple range test at the probability level of 5%. The data were subjected to statistical analysis by using the statistical program package "XLSTAT 2016 software". The one-way analysis of variance (ANOVA) followed by Duncan multiple range tests was employed. Principal component analysis (PCA) was performed in order to discriminate between different genotypes on the basis of different studied properties. Cluster analysis based on squared Euclidean distance was also performed for the genotypes.

Studied characteristics included physical characteristics of seeds, chemical and physiochemical properties of the extracted safflower seed oil. The examinations were carried out as follows:

A. Physical properties of seeds

Seed volume: a total of 100 seeds were selected randomly from each replicate and spilled into a calibrated cylinder containing a certain amount of water to cover the entire surface of the seeds. For each replication, two volumes of water were recorded; water volume before and after inserting the seeds into the cylinder. The numerical difference between the two readings were recorded as the seed volume (Mohsenin, 1986).

True density: seed weight was divided by its volume and it was expressed based on g/ml (Mohsenin, 1986).

Water absorption capacity (g): a total of 100 seeds from each replicate were weighed and then poured into a flask containing water and adjusted at temperature 22 °C for 12 h until they completely soaked and swelled, then surface water of turgidity seeds was

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

removed by absorbent paper and weighed again, and then the numerical difference between the two readings was divided by 100 (Mohsenin, 1986).

Water absorption index (WAI): obtained by dividing the water absorption capacity by the main size (g), as followed by Williams et al. (1983).

1000 seed weight (g): one thousand seed weight was obtained using an electronic balance with an accuracy of 0.001 (MODEL: AND,HR-200, Serial no.12317438, Japan)

Geometric mean diameter (mm): The geometric mean diameter values (Gmd) were calculated according to the following formula (Song and Litchfield, 1991):

$$Gmd = (a * b * c *)^{1/3} \quad \text{(Eq.1)}$$

where *a* is the length, *b* is the width and *c* is the thickness of safflower seeds in mm.

To determine the length, width, thickness, weight, 10 seeds were selected randomly for each replicate. Dimension properties were carried out by using a Vernier Calipers with an accuracy of ± 0.01.

Sphericity %: according to Mohsenin (1986) sphericity of safflower seeds was calculated with the following equation by values of length (L), width (W) and thickness (T):

$$S = ((LWT)^{1/3})100 \quad \text{(Eq.2)}$$

B. Chemical properties of safflower seed oil

Protein%: The Kjeldahl method (BUCHI K-424, Germany) was used to determine protein concentration. A conversion factor *(F)* was used to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used for this application, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition (Van Dijk and Houba, 2000).

Oil %: seed oil percentage was determined according to William (2000) using Soxhlet apparatus (BUCHI Extraction System B-811 Buchi Labrotechnik AG, CH-9230 Fkawil, Switzerland).

Fatty acid composition (%): the percentage of saturated and unsaturated fatty acids, namely Oleic %, Linoleic %, Linolenic %, Palmitic % and Stearic % were determined using Gas-Liquid Chromatography (Typ: TS 606/3-I, Ser. Nr.:06410001, Liebherr Kuhlsystem FKS 2600, Bruttogehalt 260, Typ:200051), by the method of the International Union of Pure and Applied Chemistry (Paquot, 2013).

Percentage of free fatty acid (FFA%): it was determined in the oil by standard AOCS method (AOCS, 1980).

C. Physicochemical properties of safflower seeds oil

Refractive index, specific gravity, iodine value, pH and peroxide value for safflower seeds oil were determined by method s described by AOCS (1998).

Results and discussion

The analysed results indicated a highly significant variance among the nine safflower genotypes (at 1% and 5% levels of probability) for all physical properties of the seeds,

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DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

except true density. *Table 1* indicates the reasonable diversity between the studied safflower genotypes.

Table 1. *Analysis of variance for physical properties of safflower seed*

Source	df	Mean square						
		Seed volume (ml)	True density (g/ml)	Water absorption capacity (g)	Water absorption index	1000 kernel weight (g)	Geometric mean diameter (mm)	Sphericity %
Genotype	8	0.001**	5732.944	0.0001**	0.058*	82.051**	0.070**	12.382**
Error	18	0.0001	1802.435	0.0001	0.005	1.126	0.167	16.764
Minimum		0.010	43.889	0.009	0.235	32.800	4.369	57.796
Maximum		0.090	328.000	0.026	0.785	49.100	6.117	75.091
Mean		0.048	103.972	0.018	0.436	42.494	4.977	65.201
Std. deviation		0.018	54.880	0.005	0.145	5.102	0.370	3.926

There were also variable significant effects for chemical properties of safflower oil, as indicated in the result of analyzed variance (*Table 2*). Highly significant differences were recorded for oleic%, palmitic%, oil% and protein%, while significant differences at a 5% level of probability were obtained for stearic%, and non-significant difference for linolenic% was indicated for the seed oil from the nine safflower genotypes. The variance analysis results here indicating the presence of significant genetic variability for seed oil characteristics among different safflower genotypes under study.

Table 2. *Analysis of variance for chemical properties of safflower seed oil*

Source	df	Mean square							
		Oleic %	linoleic %	Palmitic %	Stearic %	Linolenic %	Free fatty acid%	Oil %	Protein %
Genotype	8	0.708**	2.414*	0.981**	0.059*	0.007	26.198**	5.517**	0.204**
Error	18	0.002	0.033	0.022	0.023	0.023	0.007	0.009	0.010
Minimum		8.240	72.490	5.480	2.030	0.120	31.150	30.980	16.010
Maximum		9.810	75.990	7.710	2.650	0.750	41.030	34.610	16.980
Mean		9.227	74.166	6.933	2.321	0.397	33.291	32.805	16.361
Std. deviation		0.468	0.875	0.563	0.185	0.133	2.840	1.305	0.265

A similar pattern was also realized for the analysis of physiochemical characteristics, giving high significant variances for peroxide value and pH, while non-significant difference was identified for refractive index and specific gravity, from data obtained from the seed oil of the nine safflower genotypes, as indicated in the result of analyzed variance in *Table 3*.

Table 3. *Analysis of variance for physiochemical properties of safflower seed oil*

Source	df	Mean square				
		Peroxide value	pH	Iodine value	Refractive index	Specific gravity
Genotype	8	0.035**	0.077**	13.430**	0.006	0.0001
Error	18	0.004	0.002	0.009	0.007	0.001
Minimum		1.990	6.830	136.150	0.530	1.434
Maximum		2.370	7.410	142.510	0.986	1.434
Mean		2.220	7.094	139.885	0.924	1.434
Std. deviation		0.115	0.158	2.034	0.081	1.434

Physical properties

Physical properties measurements of seed are important actions to be considered in the designation of machines and equipment used for the postharvest processing of agricultural products. Seeds from different safflower genotypes had a considerable variable effect on the studied characteristics, except geometric mean diameter and sphericity% (*Table 4*). A maximum seed volume of 0.073 ml was recorded for G-2018, and it was significantly different from all other genotypes. Influencing seed size by genotypes, environment and management practices has been confirmed by researchers (Kaya and Day, 2008; Robinson, 1978). Minimum seed volume was found to be 0.020 ml and recorded for Iden genotype. The seed size has a variable effect on plant growth and postharvest processing. It is realized that smaller seed size to medium has better germination and seedling vigour (Farhoudi and Motamedi, 2010).

Table 4. *Effect of genotypes on physical properties of safflower seed*

Genotype	Seed volume (ml)	True density (g/ml)	Water absorption capacity (g)	Water absorption index	1000 seed weight (g)	Geometric mean diameter (mm)	Sphericity %
G-2018	0.073 a	58.846 c	0.023 a	0.567 b	40.600 bc	4.772 a	62.571 a
Rabiaa	0.048 bc	96.541 bc	0.019 ab	0.409 c	46.533 a	5.137 a	68.505 a
Zaafarani	0.040 cd	96.323 bc	0.020 ab	0.529 b	37.060 d	4.886 a	66.948 a
Al-Mais	0.057 abc	85.238 bc	0.017 b	0.372 c	47.200 a	5.027 a	66.113 a
Ardny	0.060 ab	79.333 bc	0.018 b	0.370 c	47.600 a	5.024 a	66.753 a
Iden	0.020 e	204.33 a	0.024 a	0.704 a	33.800 e	5.117 a	64.923 a
Gilla	0.053 bc	91.211 bc	0.017 b	0.348 cd	48.333 a	4.743 a	62.643 a
Al-Shamia	0.030 de	142.100 ab	0.009 c	0.241 d	39.120 c	5.147 a	64.479 a
Local	0.052 bc	81.818 bc	0.016 b	0.382 c	42.200 b	4.936 a	63.878 a

Different letters for the traits data indicated significant differences according to comparison analysis of Dunkin's for the traits data at a 95% confidence interval

Seeds from Iden genotype had maximum values for true density, water absorption capacity and water absorption index, while for both traits of seed volume and 1000 seed weight this genotype recorded minimum values. True density is negatively associated with a range of moisture content while positively correlated with the increase in porosity of a grain bed (Baümler et al., 2006). The result obtained here for 1000 seed weight is important and in general, it surpassed results from other studies. Pasandi et al. (2018) obtained a range of 28.29-30.33 g when Esfahan cultivar is grown in Iran under full irrigation, while Aktas et al. (2006) identified a higher 1000 seed weight of safflower seed compared to this research. Sphericity% ranged from 62.571 to 68.505%, however, no significant difference was observed between the studied genotypes. A similar trend was observed for geometric mean diameter. Comparable results of 66.03 to 64.43% of sphericity for safflower seed was obtained by Martins et al. (2017).

Chemical properties

In all the genotypes safflower seed oil varied in chemical properties, except for Linolenic acid (*Table 5*). The highest oleic% and palmitic% were recorded for the Local genotype. A high percent of linoleic acid (75.69) was referred to Rabiaa genotype, while Zaafarani had the maximum amount of free fatty acid%, however, this is slightly lower than the percentages obtained by Mihaela et al. (2013).

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DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

Table 5. *Effect of genotypes on chemical properties of safflower seed oil*

Genotype	Oleic%	Linoleic%	Palmitic%	Stearic%	Linolenic%	FFA%	Oil%	Protein%
G-2018	8.260 h	74.480 b	5.680 d	2.190 b	0.320 a	31.590 f	33.690 bc	16.250 cd
Rabiaa	9.120 e	75.690 a	6.990 b	2.350 ab	0.450 a	32.960 b	32.360 d	16.350 bc
Zaafarani	8.880 g	73.580 cd	6.790 b	2.440 ab	0.350 a	41.020 a	31.250 e	16.020 e
Al-Mais	9.230 d	72.590 e	7.410 a	2.393 ab	0.410 a	32.130 e	33.580 c	16.410 bc
Ardny	9.760 a	73.450 d	7.350 a	2.590 a	0.360 a	31.250 g	31.000 f	16.130 de
Iden	8.980 f	74.580 b	6.903 b	2.330 ab	0.380 a	32.480 d	33.690 bc	16.470 b
Gilla	9.560 b	74.550 b	7.340 a	2.170 b	0.450 a	32.740 c	33.850 b	16.350 bc
Al-Shamia	9.470 c	74.690 b	6.490 c	2.250 b	0.410 a	32.770 c	34.580 a	16.950 a
Local	9.780 a	73.880 c	7.440 a	2.180 b	0.440 a	32.680 c	31.247 e	16.320 bc

Different letters for the traits data indicated significant differences according to comparison analysis of Dunkin's for the traits data at a 95% confidence interval

Higher amount of oleic and linoleic acid in the safflower oil under study, compared to other results, indicate their high usability in food preparation (Khalid et al., 2017), causing the decrease of fat accumulation rate and the diminishing of bodyweight (Norris et al., 2009). A high level of linoleic acid, an essential fatty acid, makes the oil a premium edible oil, because of its nutritional advantages and potential therapeutic properties in the prevention of coronary heart disease and cancer, however the presence of the large amounts of linoleic acid makes the oil quite sensitive to oxidation (Oomah et al., 2000). Palmitic acid was the major saturated fatty acid ranged from 5.680-7.440%, followed by stearic acid (2170.2.590%). Linoleic acid is the principal fatty acid having the range of 72.590-75.690%, followed by oleic acid as the second main fatty acid (8.260-9.770%). Al-Shamia recoded the minimum ratio of palmate compared to all other genotypes, which is considered to be preferable for direct using in food preparation, as low intakes of saturated fatty acids have been associated with decreased blood cholesterol levels. High blood cholesterol level is one of the factors associated with heart diseases (Al Surmi et al., 2016). There is no significant difference between the genotypes for linolenic acid, their ranges varied from 0.32-0.45%.

The ration of linoleic acid, palmitic acid and stearic acids in this study are in accordance with those obtained by Tinctorius (2011) when four common safflower cultivars were studied in Iran, however the oleic acid of the current genotypes ranged less compared to the Iranian cultivars. Similar results of chemical composition were also identified by other researchers who worked on two cultivars of high oleic safflower seeds (Salaberría et al., 2016).

Fatty acid compositions of safflower oils analysed here are similar to those indicated by some other researchers (Rafiquzzaman et al., 2006; Bozan and Temelli, 2008; Yeilaghi et al., 2012), however, oleic acid has less rate compared to 13.75% which obtained by Katkade et al. (2018). The greater amount of linoleic acid increased the oil quality as it can facilitate digestion and blood de-aggregation (Aşkın, 2018).

The fatty acid composition of vegetable oil is the main factor affecting its commercial uses. It is influenced by genotype and environmental conditions (Gecgel et al., 2007). In addition, fatty acid composition affects the taste and chemical quality of the oil (Aşkın, 2018). The free fatty acid percentage of the current study ranged from 31.59% to 41.02 for G-2018 and Zaafarani genotypes, respectively. Fatty acid composition of the oil varies with plant species, cultivar, and growing conditions (Kostik et al., 2013; Sabzalian et al., 2008). Free fatty acid is also a critical acid value to

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

estimate the quality of oil, affecting the oil implication for industrial and domestic uses. It is preferable to be at a low rate, as it indicates the extent of triglyceride hydrolysis to produce mono and di-glyceride (Khalid et al., 2017). The ratios obtained here for different genotypes are lower compared to the ration indicated by other researchers (Ben Moumen et al., 2013). The functionality of oilseeds in industrial, pharmaceutical and food products depends upon their fatty acid composition. In the current study, the highest oil percent of 34.58 refers to Al-Shamia. This could be due to decreasing the shell ration in this genotype making the oil content increase (Applewhite, 1966). The observed values for oil contents in this study were in close range to those reported previously (Çamaş et al., 2007; Esendal et al., 2008; Ashrafi and Razmjoo, 2010; Pasandi et al., 2018), however obtained oil content value for the safflower genotypes were much less than 61.50% that obtained by Salazar Zazueta and Price (1989). Oil percent is ranging from medium to high percent (20-45%) based on the genotype and environmental condition (Liu et al., 2016).

Al-Shamia recorded the highest protein ratio (16.950). Protein ratio was also reasonable in other genotypes such as Iden, Al-Mais, Rabiaa genotypes. In general protein percent of the current study is reasonable for safflower genotypes and it is in accordance to what was obtained by others ranging from 14.70% to 16.21% (Al Surmi et al., 2016).

Physiochemical properties

Peroxide values (PV) for different safflower seed oil samples ranged from 2.00 and 2.360 meq O_2/Kg (*Table 6*). The highest value was 2.360, which was recorded for genotype G-2018. Peroxide value is a crude indicator of the amount of primary oxidation of lipids (Vossen, 2007). The PV is, in fact, a measure of the amount of the hydroperoxide formed through oxidation during storage (Cosio et al., 2006). Peroxide determination is important to set the degradability of raw material for biofuel production (de Oliveira et al., 2018). It is a measure of oxidation during storage and the freshness of the lipid matrix. High peroxide value is an indicator of oxidation level, the greater the peroxide value, the more oxidized oil is present (Atinafu and Bedemo, 2011).

Table 6. *Effect of varieties on physicochemical properties of safflower seeds oil*

Varieties	Peroxide value	pH	Iodine value	Refractive index	Specific gravity
G-2018	2.360 a	6.980 de	136.350 h	0.984 a	1.466 a
Rabiaa	2.237 c	7.023 d	137.560 g	0.924 ab	1.465 a
Zaafarani	2.000 d	6.870 f	141.360 c	0.928 ab	1.467 a
Al-Mais	2.140 c	7.120 c	142.480 a	0.927 ab	1.467 a
Ardny	2.350 ab	7.210 b	139.690 d	0.931 ab	1.455 a
Iden	2.250 bc	7.220 b	139.470 e	0.942 ab	1.458 a
Gilla	2.213 c	7.360 a	141.407 c	0.949 ab	1.459 a
Al-Shamia	2.230 c	7.150 bc	138.580 f	0.815 b	1.469 a
Local	2.200 c	6.913 ef	142.070 b	0.912 ab	1.454 a

Different letters for the traits data indicated significant differences according to comparison analysis of Dunkin’s for the traits data at a 95% confidence interval

The pH of the seed oil is also varied significantly for the different safflower genotypes, Gilla oil had the highest pH value, while Zaafarani had the lowest record. There is an

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DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

effect of pH on the hydrolysis of safflower oil, that is considered during oil extraction. The increased pH value will certainly decrease the degree of hydrolysis (Aziz et al., 2015), by breaking down the substrate (Goswami et al., 2009), through modifying the ionization state of enzyme and altering the activity of an enzyme (Serri et al., 2008).

Iodine value ranged from 136-142 g I_2/100 g oil). The highest iodine value was recorded for Al-Mais (142.480), whereas the lowest iodine value (136.350) was recorded for the genotype G-2018. The obtained results were agreed with the range of 130-150 g I_2/100 g oil which was reported previously by Nagraj (1995) and the range of 136-148 g I_2/100 g oil that was reported by Alimentarius (2013). Higher iodine value indicates a lower degree of saturation and vice versa. This value could be used to quantify the number of double bonds present in the oil, which signifies the susceptibility of oil to oxidation (Dim et al., 2013).

Refractive index and specific gravity measurements are not providing sufficient information for quantitative detection of a pure analyte, however they are highly useful to check oil contamination and/or adulteration (Bhavsar et al., 2017). The refractive index is used mainly to measure the change in the unsaturation of the oil. The refractive index ranged from 0.984 to 0.815 and specific gravity ranged from 1.454 to 1.469. These results are similar to those obtained by Nagraj (1995) and Alimentarius (2013) for safflower seeds oil, but in the current study the oil has a lower refractive index compared to the ranges obtained by Katkade et al. (2018).

Principal component analysis

In addition to exploring the variation between different genotypes based on different physical, chemical and physiochemical characteristics, principal companion analysis was performed to estimate the relative importance and contribution of each genotype to the total variance and illustrate their relatedness. Superiority of safflower seed from the nine genotypes for various traits was explained in the PCA diagram for some physical properties (*Fig. 1*).

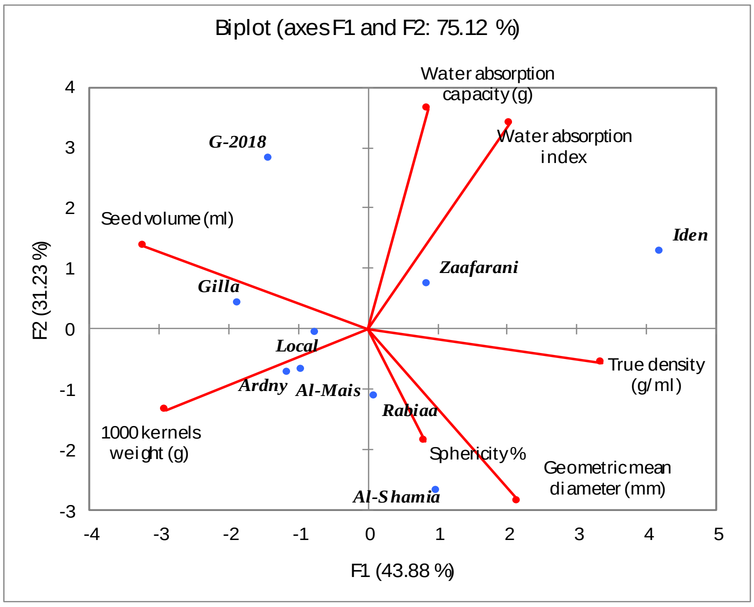

Figure 1. *Biplot diagram of principal component analyses of the first and second components for the distribution of 9 safflower genotypes, based on some physical properties*

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DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

The first two principal components were accounted for 75.12% of the total variations between different genotypes. The biplot indicates the variation derived from the first and second factors (F1 and F2) for seed physical data of all nine safflower genotypes. Al-Shamia and Rabiaa seed oil were superior in sphericity % and geometric mean diameter (mm), while for Gilla and G-2018 seed oil low value was recorded for these two traits, as positioned away from the scatter point of these traits. Iden on the diagram showed a high contribution to true density while it reversely indicates low seed volume, being preferable for the seed germination and manufacturing processes. The attributed variations among these genotypes might partially reflect their different backgrounds at a genetic level (Mohsenin, 1986).

In studying the chemical characteristics of seed oil from different safflower genotypes, the principal component analysis was accounted for 67.1% of the total variation for different genotypes under study (*Fig. 2*). Al-Shamia was found to be superior in protein percent 16.950% followed by the seed oils from Gilla and Rabiaa genotypes. Zaafarani has recorded a minimum value for protein content, however it has the maximum FFA%. In addition, the Local genotype is superior in oleic% and palmitic%, recording maximum values of 9.780% and 7.440%, respectively (*Table 5*). While G-2018 has the minimum value for oleic%, unsaturated fatty acid, and palmitic%, saturated fatty acid. Linoleic acid was superior in Rabia genotype, followed by Al-Shamia, and Iden. Percentage of palmitic and stearic acids in safflower oil are among the factors to determine its quality, however oleic and linoleic acids are significantly affecting oil quality more than the others, due to their direct effects on human health. Fatty acid composition is varied according to genotypes and environmental condition of their growing (Kostik et al., 2013; Pasandi et al., 2018; Oz, 2016; Yorulmaz et al., 2019).

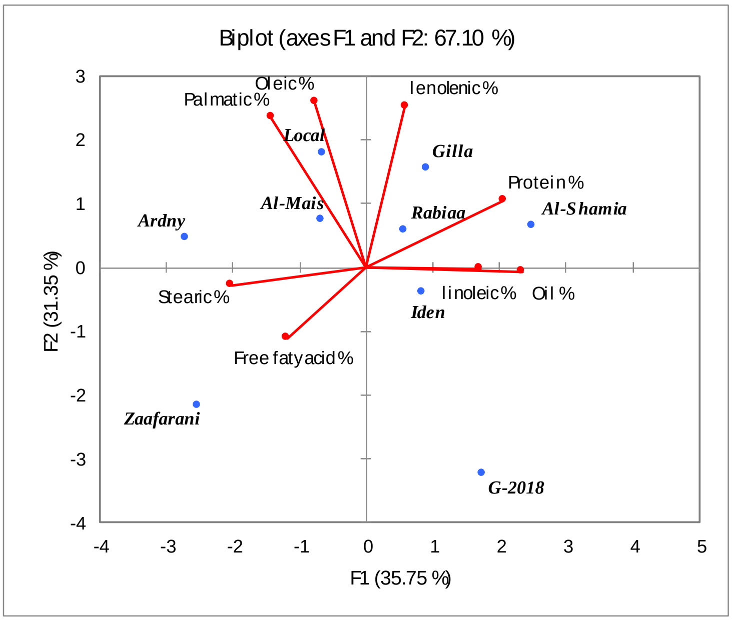

Figure 2. *Biplot diagram of principal component analyses of the first and second components for the distribution of 9 safflower genotypes, based on some chemical properties*

PCA findings, variations in oleic and linoleic acid contents emphasized the possibility of improving oil quality through breeding or cultivation programs for the

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DOI: http://dx.doi.org/10.15666/aeer/1806_75877605

specified genotypes. As fatty acid ratios are fundamental for the market value of safflower, it is reasonable to suppose that genotype with the highest content of oleic and linoleic acids, such as Local and Rabiaa could be considered as valuable genetic material for the improvements in safflower oil quality in Kurdistan-Iraq region.

Biplot-PCA for physiochemical properties indicates the presence of high genetic variations among different oil seeds from the nine safflower genotypes (*Fig. 3*). According to the result of principal component analysis, variable components accounted for 65.63% of the total variation for physiochemical properties. Oil from the seed of Iden genotype is superior in refractive index and pH, followed by Ardny and Gilla while Al-Shamia has recorded lower value for both refractive index and pH of the oil. In addition, Al-Shamia shows a maximum value for specific gravity while the minimum value was identified for Gilla. The result of the present study could be exploited in planning and execution of the future breeding programs of safflower genotypes.

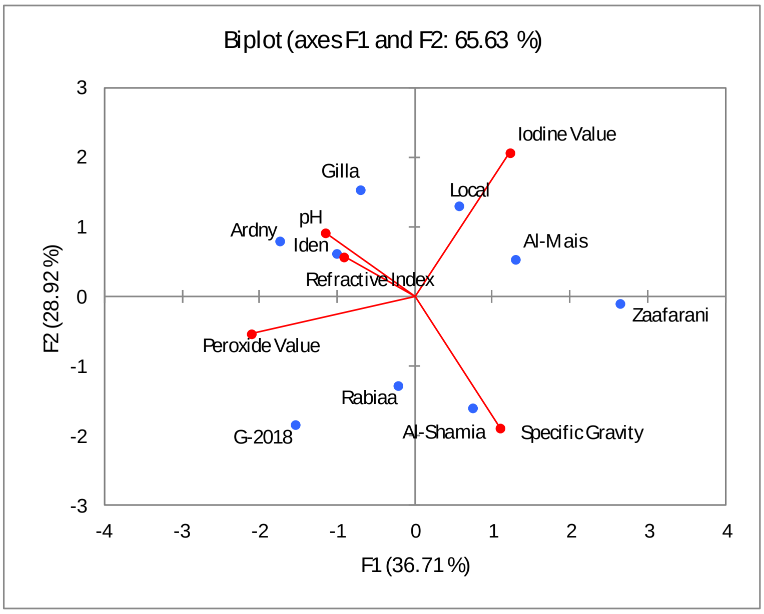

Figure 3. *Biplot diagram of principal component analyses of the first and second components for the distribution of nine safflower genotypes, based on some physiochemical properties of the seed oil*

Agglomerative hierarchical clustering using physical, chemical and physiochemical data

Pairwise comparisons were made between all the nine safflower genotypes and average dissimilarity values were calculated based on the characteristics data. All physical, chemical and physiochemical characteristics data from the safflower genotypes were utilized to evaluate the relationship between the genotypes.

For the physical characteristics of the seed oil, the dissimilarity matrix revealed variable values ranged from 6.604 (between Local and Al-Mais) to 145.665 (between Iden 157 and G-2018), as given in *Table 7*. This wide range of dissimilarity between different genotypes indicates the presence of reasonable variability among the genotypes under study. Hybridization program would be reasonable if conducted between the genotypes with high dissimilarity value.

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Table 7. *Dissimilarity matrix among the seeds of nine safflower genotypes based on physical properties, following Euclidean distance proximity of Ward's method analysis*

	G-2018	Rabiaa	Zaafarani	Al-Mais	Ardny	Iden	Gilla	Al-Shamia	Local
G-2018	0								
Rabiaa	38.619	0							
Zaafarani	37.898	9.607	0						
Al-Mais	27.436	11.573	15.048	0					
Ardny	22.052	17.330	19.996	5.953	0				
Iden	145.665	108.602	108.079	119.853	125.773	0			
Gilla	33.277	8.134	13.107	7.006	12.593	114.076	0		
Al-Shamia	83.290	46.334	45.891	57.457	63.378	62.464	51.750	0	
Local	23.066	16.031	15.693	6.458	6.604	122.808	11.288	60.364	0

For better estimation of the distance between the seed oil of all the safflower genotypes Euclidean distance and unweighted pair-group average were followed to estimate dissimilarity (*Table 8*). The dissimilarity values varied from the lowest value of 0.816 (between Gilla and Iden) to the highest value of 9.87 (between Zaafarani and G-2018).

Table 8. *Dissimilarity matrix among nine safflower genotypes based on chemical properties of safflower seed oil, following Euclidean distance proximity of Ward's method analysis*

	G-2018	Rabiaa	Zaafarani	Al-Mais	Ardny	Iden	Gilla	Al-Shamia	Local
G-2018	0								
Rabiaa	2.760	0							
Zaafarani	9.870	8.419	0						
Al-Mais	2.808	3.462	9.279	0					
Ardny	3.692	3.232	9.831	2.928	0				
Iden	1.699	1.811	8.951	2.103	3.319	0			
Gilla	2.414	1.979	8.787	2.111	3.439	0.816	0		
Al-Shamia	2.202	2.590	9.040	2.652	4.275	1.240	1.285	0	
Local	3.598	2.294	8.429	2.787	1.584	2.734	2.700	3.630	0

The dissimilarity matrix for physiochemical properties shows lower different distances between the safflower genotypes (*Table 9*). The lowest dissimilarity distance value was 0.242 (between Iden and Ardny), while the highest distance value was 6.136 (between Al-Mais and G-2018). It is identified that G-2018 shows a wide distance fromother genotypes based on all physical, chemical and physiochemical properties.

Hierarchical cluster analysis (HCA) is an unsupervised technique utilized to cluster genotypic data. HCA was applied to cluster seed oil from nine safflower genotypes available in Kurdistan Region according to different characteristics of physical, chemical and physiochemical properties. Genetic divergence was investigated successfully in safflower genotypes using cluster analysis based on some agronomic and oil content characteristics (Atole et al., 2018; Shinwari et al., 2014; Sabaghnia et al., 2018).

Clustering analysis, based on physical properties, revealed two major groups at the dissimilarity based on the threshold value for physical properties (*Fig. 4*). These groups had a variance decomposition between the classes and variance within the class. The first cluster comprised of the genotypes; G-2018, Local, Al-Mais, Ardny, Zaafarani, Rabiaa and Gilla). While in the second group Iden and Al-Shamia were

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clustered together. This will clarify the variation between the genotypes for any improving program in the future to select the right genotypes based on the purpose of development.

Table 9. *Dissimilarity matrix among nine safflower genotypes based on physiochemical properties of safflower seed oil, following Euclidean distance proximity of Ward's method analysis*

	G-2018	Rabiaa	Zaafarani	Al-Mais	Ardny	Iden	Gilla	Al-Shamia	Local
G-2018	0								
Rabiaa	1.218	0							
Zaafarani	5.024	3.810	0						
Al-Mais	6.136	4.922	1.156	0					
Ardny	3.348	2.141	1.740	2.799	0				
Iden	3.131	1.920	1.938	3.014	0.242	0			
Gilla	5.074	3.862	0.537	1.102	1.729	1.942	0		
Al-Shamia	2.247	1.034	2.806	3.903	1.124	0.902	2.838	0	
Local	5.723	4.512	0.739	0.464	2.403	2.619	0.801	3.500	0

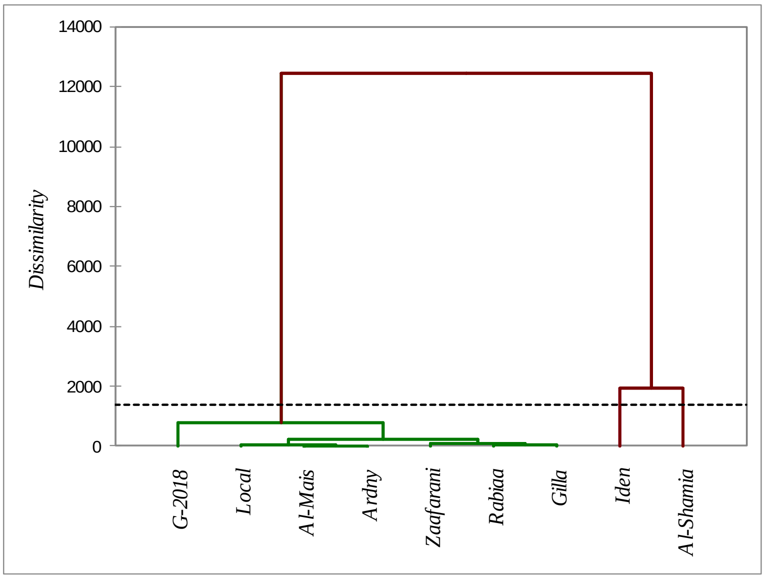

Figure 4. *Dendrogram generated using Unweighted Euclidean distance proximity of Ward's method analysis of dissimilarity, showing the distance between safflower genotypes using physical data of safflower seed. Dissimilarity values are present at the left side of the dendrogram*

The dendrogram was generated by UPGMA clustering pattern of nine safflower genotypes using chemical properties (*Fig. 5*). The dendrogram clearly revealed four clusters based on the threshold value. Zaafarani is different from the others occupying a separate group. Ardny and Local genotypes were clustered together making another group. Al-Mais made a third group, while the fourth cluster comprised of the genotypes; Rabiaa, G-2018, Al-Shamia, Iden and Gilla. Research institutes should take

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consideration of the necessity for the collection, conservation, and utilization of local genotypes. The necessity of such action has been highlighted in this study by giving a reasonable extension to the safflower gene pool through the contribution of indigenous genetic resources such as the Local genotype.

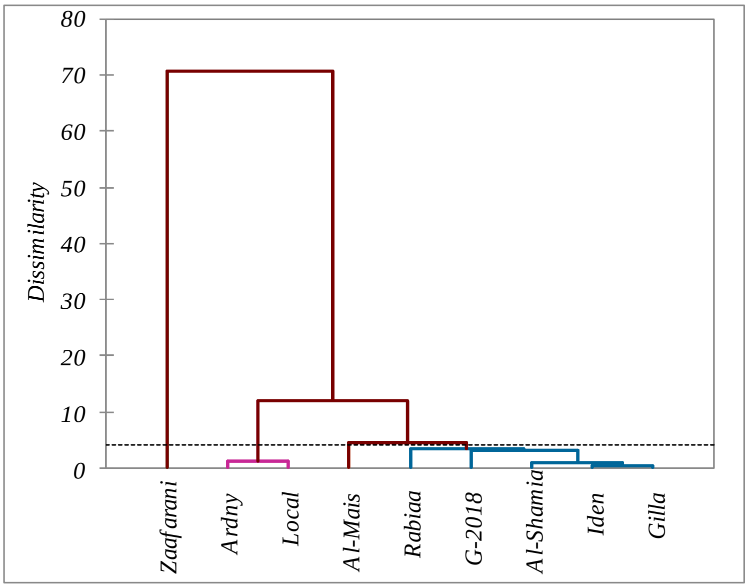

Figure 5. *Dendrogram generated using Unweighted Euclidean distance proximity of Ward's method analysis of dissimilarity, showing the distance between safflower genotypes using chemical data of safflower seed oil. Dissimilarity values are present at the left side of the dendrogram*

Pairwise comparisons were made between all the nine genotypes and the average dissimilarity values were calculated based on the physiochemical properties, three groups in the dendrogram were clustered based on the threshold level (*Fig. 6*). The first group contains Al-Mais, Local, Zaafarani and Gilla, while both Ardny and Iden made the second group, the third cluster consists of the genotypes; G-2018, Rabiaa and Al-Shamia.

The analyses accomplished here indicate the genetic distinction between the safflower genotypes distributed over different clusters, implying high potential of the genotypes in improvement programs, as some genotypes showed superiority in most of the studied parameters. The distance between safflower genotypes using physical, chemical and physiochemical data of safflower seeds oil indicate the extension of genetic bases for the genotypes under study to be manipulated in their utilizing based on the required purposes for the seed oil in safflower. To reduce the risk of bottlenecking the diversity in safflower, adopting further genotypes with wide diversities have to be involved in the future improvement programs of this crop.

No distinct regional grouping patterns of these safflower genotypes were clearly identified group clusters, because the origins of the entire genotypes are not presented herein this investigation. The results obtained from the genotype clustering would be valuable for plant breeders, whereby the most promising genotypes in the population could be selected from different clusters for the improvement of safflower based on the required characteristics.

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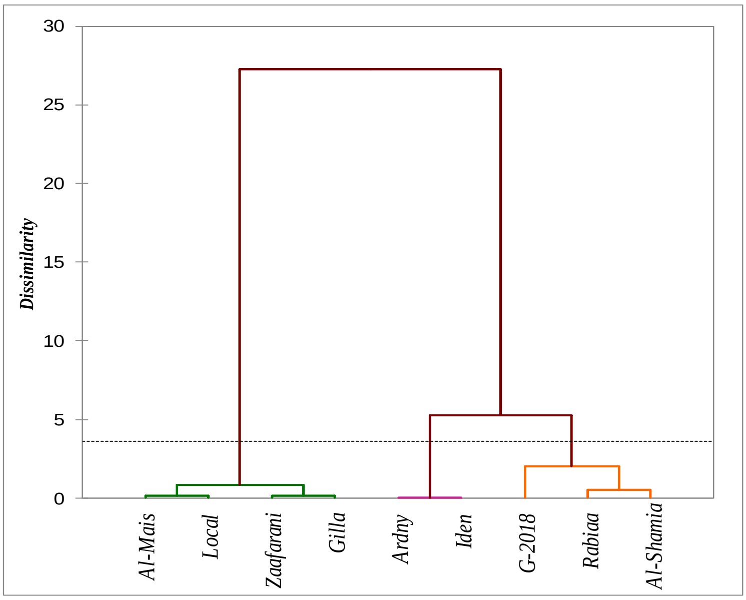

Figure 6. *Dendrogram generated using unweighted Euclidean distance proximity of Ward's method analysis of dissimilarity, showing the distance between safflower genotypes using physiochemical data of safflower seed oil. Dissimilarity values are present at the left side of the dendrogram*

Conclusions

The analyses of variance indicated highly significant variances (at 5% and 1% level of probability for most of the studied traits (physical, chemical and physiochemical). Safflower genotypes are different in their response to physical properties of seed except for geometric mean diameter and sphericity%. A maximum seed volume of 0.073 ml was recorded for G-2018, which was significantly different from all other genotypes. Physical properties of safflower seeds are essential for designing types of equipment for handling, harvesting, storing and oil extraction process. These properties are affected by numerous factors such as genotypes and environmental conditions of growing.

The different safflower genotypes varied in their chemical properties for seed oil, except for linolenic acid. The highest oleic% was identified for Local genotype and Ardny (being non significantly different), while high percent of linoleic acid was referred to Rabiaa genotype, indicating their high usability in food preparation because they can decrease fat accumulation rate and diminish body weight. Al-Shamia is considered to be preferable for direct use in food preparation, due to containing the lowest ratio of palmate. Free fatty acid content was variable and ranged from 31.59% to 41.02 for G-2018 and Zaafarani genotypes, respectively. This is a critical value to estimate the quality of oil for industrial and domestic uses. Protein percentage was reasonable in different genotypes such as; Al-Shamia, Iden, Al-Mais and Rabiaa. Peroxide value as a measure of oxidation during storage ranged from 2.00 and 2.360 meq O_2/Kg. There are also significant differences in pH and iodine values for the different genotypes. G-2018 had the lowest iodine value of 136.350 g I_2/100 g oil, which means that the oil has a high saturation value. Palmitic and stearic acid were identified to be low in Al-Shamia and Local genotypes. The lower saturated fatty acid form in these two genotypes indicates a high stability oil with superior quality for edible

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purpose and commercial applications. The oil obtained from safflower varieties is a relatively rich source of various important functional nutrients.

The presence of variability is important for genetic studies and consequently improvement and selection. Principal companion analysis was performed to estimate the variability of genotypes and their contribution to total variance based on the traits studied. The first two principal components were accounted for 75.12%, 67.1% and 65.63% of the total variation based on physical, chemical and physiochemical properties. The attributed variations among the genotypes based on physical properties might partially reflect their genetic construction. The variation realized via PCA for oleic and linoleic acid content could make a possibility of improving the oil quality through the development programs of safflower. Focusing on Local and Rabiaa genotype, will be reasonable, in the future improvement program of seed oil quality in Kurdistan-Iraq.

Dissimilarity matrix specified G-2018 genotype with a wide distance from other genotypes based on all the physical, chemical and physiochemical properties. Hybridization program would be reasonable for oil quality improvement if conducted between the genotypes with high dissimilarity value. Genetic divergence was investigated successfully in the current safflower genotypes based on hierarchical cluster analysis. With the help of clustering pattern and genetic relationship, breeders could identify the diverse genotype with least similarity from clusters and employ them in future breeding programs of safflower, because despite the nutritional value of safflower seed oil there is an increased potential in medicinal purposes of seed oil at global level. Based on the current study it is recommended to include wider genotypes of safflower in the improvement program of the seed oil and its quality based on the recommended references. Different field trial and agricultural practices are recommended before evaluating the oil quality parameters. In addition, oleic and linoleic oils have to be investigated in the future for thermal stability and frying qualities.

Acknowledgments. The authors are grateful for the College of Agricultural Engineering Sciences, the University of Sulaimani for providing all laboratories facilities and for providing land to conduct the research.

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