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Evaluation and Chemical Comparison of Triple-Zero Canola Cultivars with Emphasis on the Extracted Oil

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ABSTRACT: Due to insufficient production of edible oil in Iran and its substantial import, much attention should be paid to resources of edible oil in the country. Colza is a crop rich in oil and its cultivation can decrease the dependence on other countries for oil, as well as reducing the required foreign currency for importing it. This research dealt with evaluation and chemical comparison of triple-zero canola cultivars with emphasis on nutritional values of the extracted oil, and introduced superior cultivars with respect to the tested characteristics.

Seven samples were selected completely at random among the improved canola cultivars produced by the Seed and Plant Improvement Institute in Karaj. The oil in each sample was extracted using solvents, and its chemical characteristics were studied separately including fatty acid composition and contents of non-saponifiable compounds, sterols, to copherols, copper, and iron.

GKH-HELENA was the best cultivar with respect to oleic acid content and duration of resistance to oxidation, the cultivar LICORD had the maximum sterol content, and the cultivar WRH-262 possessed the highest tocopherol content.

There were significant differences between the cultivars regarding all the studied characteristics. Oleic acid was the dominant fatty acid in the cultivars, and the oil extracted from the samples had low erucic acid content as the cultivars were from an improved kind.

Keywords: Sterol, fatty acid composition, tocopherol, canola oil, non-saponifiable compounds

INTRODUCTION

Colza (*Brassica napus*), a member of the Cruciferae family, is an amphidiploid species resulting from a cross between certain forms of cabbage and turnip in nature. It is an annual plant and has spring and autumn types. Aside from *B. napus*, other *Brassica* species (*Brassica capestris* or field mustard, *Brassica nigra* or black mustard, and *Brassica carinata* or Ethiopian mustard) are also called colza in international markets (Shariati and Shahnizadeh, 2000).

Canola cultivars have been developed with oil that is rich in oleic acid (60-85%), greatly resembles olive oil, and is very stable and has limited amounts of trans fatty acids (Hui, 1996). Canola oil is a relatively good source of tocopherols and its total to copherol content is close to corn, cottonseed, saffron, and sunflower oils (60-70 mg/100 g). Only soybean oil, with 100-110 mg/100g, contains more tocopherol than canola oil. Total sterol content in canola oil is about 0.53-0.97%. Brassicasterol is one of the sterols that is found only in canola and field mustard and is used to distinguish canola oil from other oils that are falsely sold as canola oil.

The high nutritional value of canola oil is due to its low content of saturated fatty acids (less than 4% of palmitic acid) and its relatively high contents of oleic acid (60%) and alpha-linolenic acid (10%). Canola oil is second to olive oil with respect to oleic acid content among fats and edible vegetable oils, and is effective in reducing total cholesterol and low-density lipoproteins in blood. Moreover, it influences the composition of phospholipids in platelets and is involved in the activity of platelets against blood coagulation (Houboula *et al.*, 2002). Colza is classified in various ways as industrial oil, edible oil, etc. However, the best classification for colza is as follows: Traditional colza cultivars: These contain oil with 26-60% erucic acid and 100-205µ moles of glucosinolates/ 100 g canola meal.

Single-zero cultivars: These Canadian cultivars contain oil with less than 5% erucic acid and 100-205 µ moles of glucosinolates /100 g of canola meal. Double-zero cultivars: These contain oil with less than 2% erucic acid and 0-18 µ moles of glucosinolates/100 g of canola meal.

Triple-zero cultivars: These improved colza cultivars are also called Candle cultivars, and contain oil with minimal amounts of erucic acid. Canola is in fact the shortened form of Canada Oil Low Erucic Acid, and refers to *Brassica napus* and *Brassica campestris*. The oil extracted from canola seed contains 2% erucic acid, and the solid part of the seed contains less than 30 µmoles of glucosinolate/100 g of canola meal.

Mohammadi *et al.* (2007) studied the relationship between fatty acid composition and oil stability in mixtures of sunflower and canola oils. They prepared 7 samples of deodorized oil without synthesized antioxidants at various ratios of sunflower and canola oils and determined their stability at 110°C using the Rancimat method, their fatty acid composition employing gas chromatography measurements, their iodine numbers, their total acidities, and peroxide values. They found that sunflower oil, despite having low amounts of linolenic acid compared to Canola, had much less resistance to oxidation because of its high content of linoleic acid and due to the high content of oleic acid in canola. Therefore, they proposed the 2 formulas of 70% canola oil+ 30% sunflower oil and 50% canola oil + 50% sunflower oil to increase the nutritional value of sunflower oil.

In 2010, Kadivar *et al.* studied 11 different canola cultivars and assessed the chemical characteristics of the extracted oils such as percentages of free fatty acids, non-saponifiable compounds, tocopherols, sterols, and concentrations of phosphorous and phospholipids. Results showed that the cultivar RGS303 had the maximum non-saponifiable compounds, the cultivar ZARFAM had the highest percentage of sterols and tocopherols and the maximum concentrations of phosphorous and phospholipids, the cultivar HEROS had the largest amount of free fatty acids, and the cultivar WILD CAT had the most oil content. Moreover, oleic acid was the dominant fatty acid in canola oil, and beta-sitosterol the dominant sterol in the studied cultivars. Lutfur *et al.* (1987) studied 27 cultivars of rapeseed/mustard with respect to oil content, fatty acid composition, and glucosinolate content. They used the NMR method to determine the oil content of the species based on their dry weight and found it was 33.4%. The species were different in their fatty acid composition, and all of them contained glucosinolates

In 2007, Nalda *et al.* determined the fatty acid composition of canola oil through measuring contents of its methyl esters using gas chromatography and concluded that it contained about 61% monounsaturated fatty acids, the main one being oleic acid with 56%. Moreover, it contained small amounts of saturated fatty acids (8.1%) and moderate quantities of polyunsaturated fatty acids (30.7%), the main ones being linoleic acid (21.5%) and linolenic acid (8%).

Nagola *et al.* (2007) used the high performance liquid chromatography method and fluorescence detection employing the AOCS method (1993) to measure the contents of tocopherols and tocotrienols in canola oil and found their concentrations to be 247 and 348 mg/kg, respectively.

In 2003, Szlyk *et al.* measured phosphorous contents of *Brassica capestris* and canola and used these contents to obtain the quantities of phospholipids through spectrophotometry. The phosphorous content of purified oil was about 2 mg/kg, and the total phospholipid content about 0.82%.

The purpose of this research was the chemical evaluation of 7 canola cultivars in order to identify the best cultivar to be used in future research or to be widely cultivated for further evaluation.

MATERIALS AND METHODS

Seven canola cultivars named GKH-HELENA, GKH-0724, LICORD, GKH-305, GKH-2005, ZARFAM, and WRH-262 were selected. The GKH cultivars were produced in Germany and the rest in Canada (except for ZARFAM that was developed in Iran). The tested cultivars were obtained from the Seed and Plant Improvement Institute in Karaj. They are among the spring type and have low vernalization requirement, but are resistant to cold. Since they are suitable for temperate climates, they may be planted in autumn in temperate regions in Iran such as Karaj. However, if they are to be cultivated in cold regions, they must be grown as autumn crops. None of these cultivars Except for the ZARFAM has been planted in Iran.

Seeds of the 7 cultivars were ground using a grinder, and the resultant powder was kept in glass containers at ambient temperature away from the light. Each ground sample was mixed with twice its volume of petroleum ether, and was put in a shaker for 72 hours. The oil and the solvent were then separated from the meal, transferred into a Falcon tube, and centrifuged. The centrifuged sample was poured in a flask, the solvent was separated from the oil by a rotary evaporator, and the rest of the solvent was removed by using nitrogen gas. The sample was prepared as a methyl ester derivative based on AOCS method 969/33. An Acme 6100 GC (manufactured by the Young Lin Company) equipped with a flame ionization detector (FID) and a 100-meter long CP Sil 88 column was used according to AOCS, cele-91 standard method to determine the composition of fatty acids. The injector, oven, and detector temperatures were 240, 198, and 280, respectively; the carrier gas rate was 14 ml/minute, and the volume of the injected sample 1µl.

To identify the non-saponifiable compounds in the oil sample, it was saponified by alcoholic potassium hydroxide; its non-saponifiable compounds were extracted by diethyl ether and, finally, the diethyl ether was evaporated; non-saponifiable compounds were weighed, and their percentages in the oil sample were calculated. Then, thin-layer chromatography was employed and Rhodamine 6G detector was sprayed at the concentration of 0.01% in ethanol to separate the various non-saponifiable compounds from each other (Ghavami *et al.*, 2008). This experiment was conducted according to AOCS method 933.08 with 3 replications. After extracting the non-saponifiable compounds from the oil sample, thin-layer chromatography was employed to separate these compounds from each other in order to determine the sterol compounds. The gas chromatography method with flame ionization detector and capillary column was used to identify and determine the percentages of sterol compounds (Ghavami *et al.*, 2008). The sterol bands removed from the thin layer chromatography plate were extracted by diethyl ether. The sterols extracted from the oil sample were identified according to AOCS standard method 970/51 using a gas chromatograph equipped with flame ionization detector TRB-5 and a capillary column (60 m* 0.32 mm* 0.5 µm) at 265°C. The injector and detector temperatures were 300 and 320°C, respectively; 1µl of the ample was injected, and the carrier gas flow rate was 1 ml/min. The gas chromatography method was used to determine the percentages of the sterol compounds. The unknown sample and the standard sterol compounds were injected separately and under identical conditions into the gas chromatograph. Sterol peaks were identified by comparing RRT (relative retention time) of the unknown sample with the RRT standards of sterol compounds, and the quantities of the sterol compounds were determined by calculating the areas under the peaks.

The high performance liquid chromatography (HPLC) method was used according to AOCS method 8-89Ce to identify and determine the tocopherols (Ghavami, *et al.*, 2008). The column type was TRACER EXEL 120 ODS-A5µ15*0.46 NF-01445 (Part No:TR-016338). Five g of the oil sample was poured into a crucible that was put in an oven at 550°C for 24 hours for incineration. After incinerating the sample, it was cooled and the obtained ash was dissolved in a two-ml solution of 65% nitric acid and 35% hydrochloric acid (at a ratio of 2 to 3) and passed through Whatman filter no 4. The crucible was washed several times with deionized water and the volume of the solution was raised to 50 ml by adding deionized water. The sample was then prepared for the atomic absorption spectrophotometer. After plotting the absorption curve for the concentrations of the standard solutions and comparing these curves with the absorption curve of the sample, the concentrations of copper and iron in the sample were determined.

Oil oxidative resistance, or the period of oxidative stability, is the period between the time the sample reaches the temperature of interest and the time when the production of secondary products resulting from oxidation of the fats increases rapidly. This period was determined by using a Metrohm 743 model Rancimat at 110°C. using 2.5 g of the sample at air current rate of 20l/hr., and was hourly reported (Ghavami *et al.*, 2008).

RESULTS

Oleic and linoleic acids are the dominant fatty acids in canola oil. Therefore, like olive oil, canola oil can be put in the oleic acid-linoleic acid group of oils.

Table 1: Fatty acid composition of oil in various canola cultivars (percentage).

Fatty Acid / Sample	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C18:1 C	C18:2 T	C18:2 C	C18:3 T	C18:3 c	C20:0	C22:1
GKH-HELENA	0.00	0.07	0.03	5.14	0.40	2.45	67.26	0.01	14.28	1.98	7.88	0.05	0.39
GKH-0724	0.21	0.08	0.38	5.56	0.38	2.51	63.03	0.02	18.59	1.62	7.48	0.03	0.39
LICORD	0.01	0.07	0.03	5.55	0.42	2.48	61.66	0.02	19.60	1.75	7.87	0.06	0.42
GKH-305	0.01	0.06	0.02	5.61	0.38	2.38	62.37	0.02	19.04	1.68	7.90	0.05	0.41
GKH-2005	0.01	0.08	0.05	5.67	0.43	2.25	59.37	0.03	21.43	1.64	8.51	0.07	0.41
ZARFAM	0.01	0.07	0.08	5.39	0.43	2.33	60.96	0.02	20.11	1.53	8.55	0.06	0.38
WRH-262		0.08	0.03	5.81	0.45	2.43	60.77	0.02	20.30	1.59	8.01	0.00	0.45

The highest content of non-saponifiable materials belonged to GHK-0724 and GKH-HELENA with means of 3.02 and 3.075%, and the lowest content was that of ZARFAM with the average of 1.337%. The highest sterol content in the total non-saponifiable compounds belonged to LICORD with the mean of 6469 mg/kg, and the lowest to GKH-305 with the average of 5054 mg/kg. The largest amount of tocopherols in the total non-saponifiable compounds was that of WRH-262 with 393.4 mg/kg, and the minimum that of GKH-0724 with 322.2 mg/kg. The maximum iron content of oil belonged to GKH-2005 with the mean of 101.6 mg/kg, and the minimum to GKH-305 with the average of 79.08 mg/kg. The highest copper content was that of GKH-0724 with the mean of 7.124 mg/kg, and the lowest to GKH-305 with the average of 4.795 mg/kg.

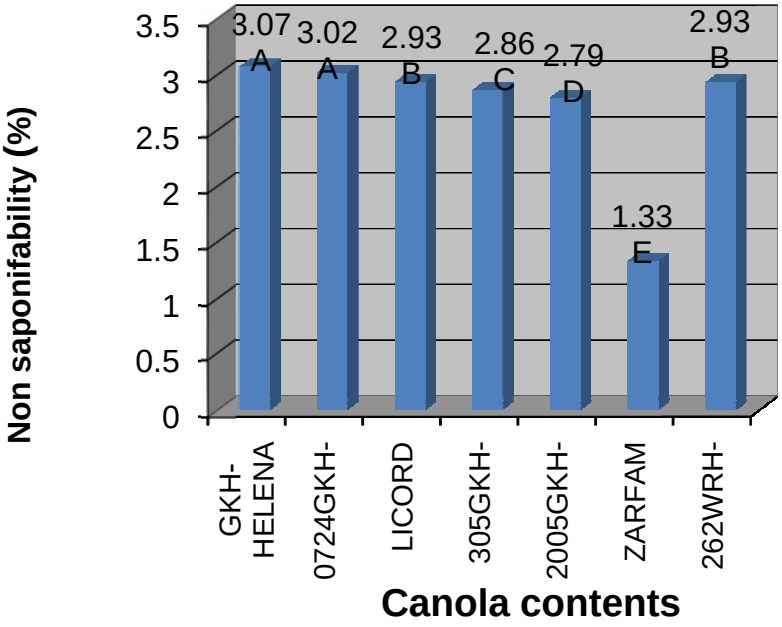

Fig. 1. The quantities of non-saponifiable compounds in the studied oil samples.

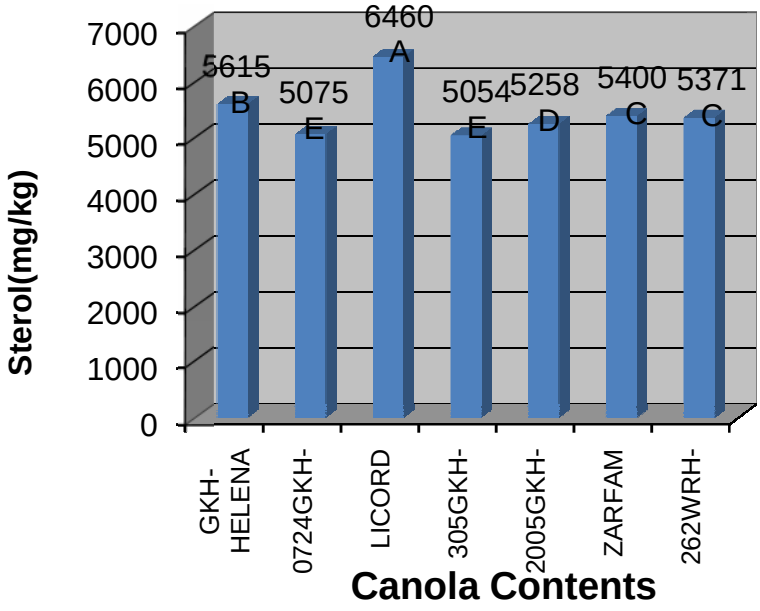

Fig. 2. Sterol contents of oil in various canola cultivars

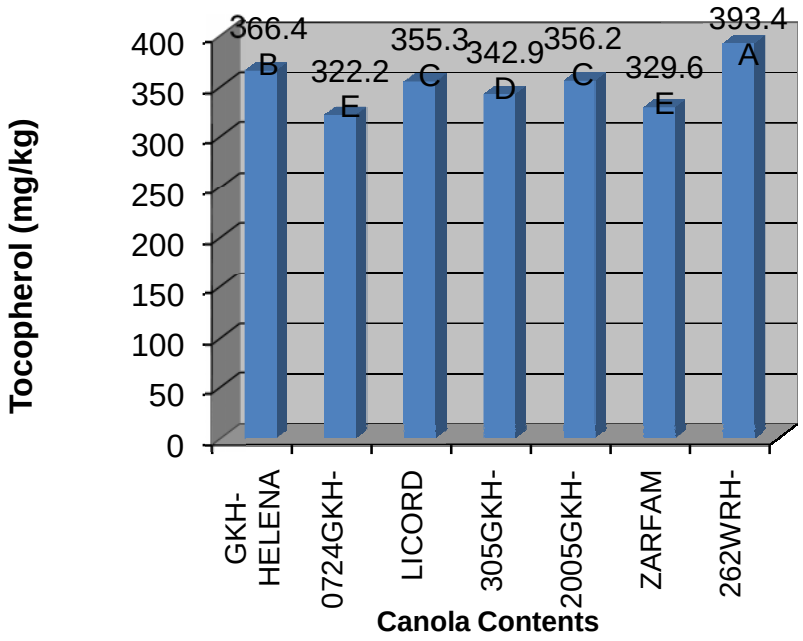

Fig. 3. Tocopherol contents of oil in various canola cultivars.

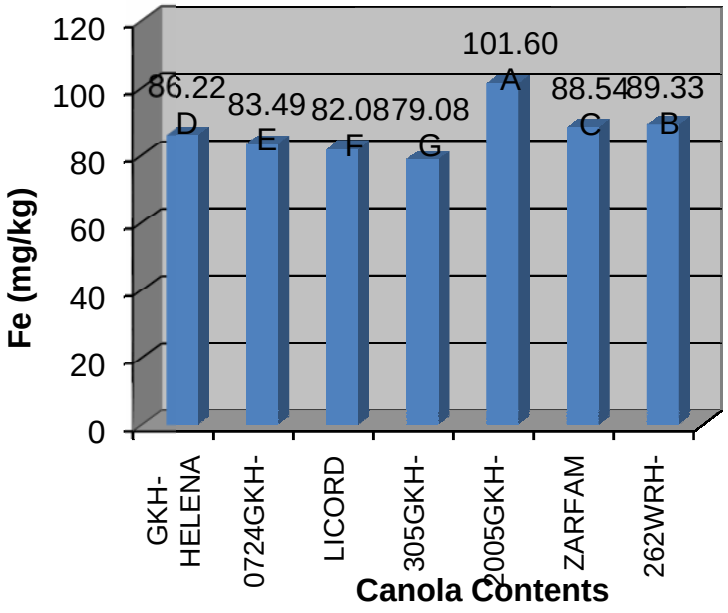

Fig. 4. Iron content of oil in various canola cultivars.

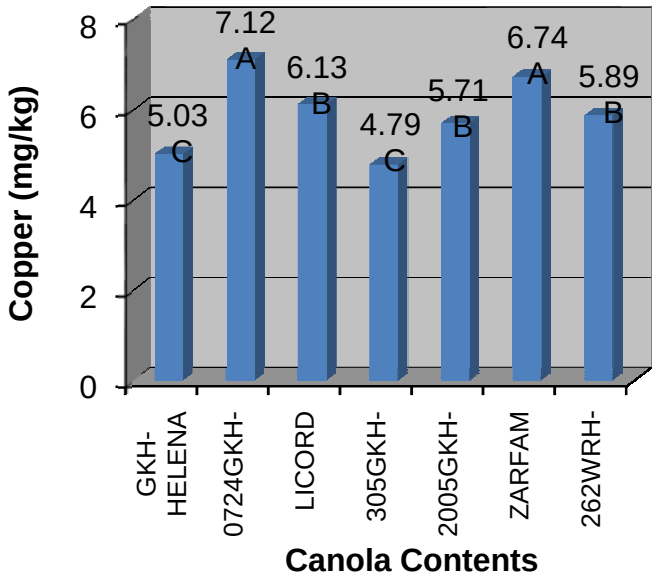

Fig. 5. Copper content of oil in various canola varieties.

The longest period of oxidative stability was observed in GKH-HELENA with the mean of 11.42 hours, and the minimum in GKH-2005 with the average of 7.447 hours.

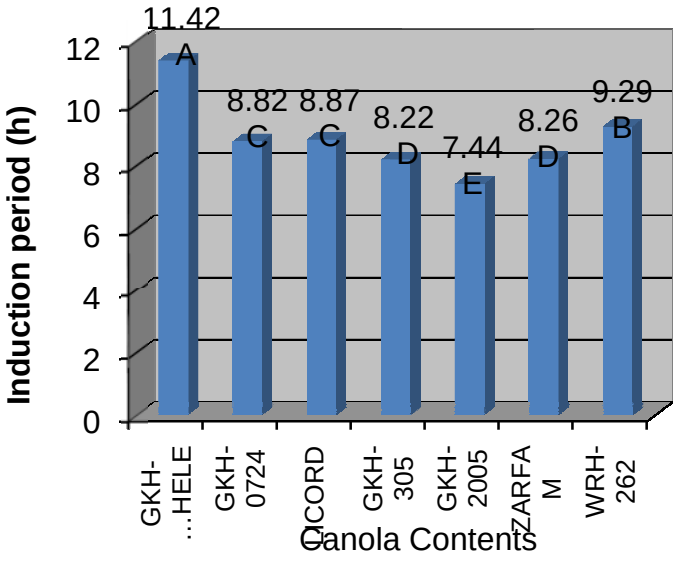

Fig. 6. Periods of oxidative stability in oil extracted from various canola cultivars.

DISCUSSION

Oleic acid is the dominant fatty acid in canola oil, and its content in the studied samples varied from 59.37 to 67.26%, with the maximum observed in GKH-HELENA. The improved cultivars had high contents of non-saponifiable compounds that are destroyed during the purification process, especially in the deodorization stage. Since valuable substances such as sterols and tocopherols that cause oxidative stability of oils are among the non-saponifiable compounds, the high percentages of these compounds in the oil of these cultivars compared to unimproved cultivars and other oil seeds are the reasons for the superiority of these canola cultivars.

Sterols and tocopherols protect oils from oxidation and, in addition, tocopherols have nutritional value because they possess vitamin E activity. Most hydrocarbons in colza consist of pigments such as chlorophylls and carotenoids, and the reason for the intense color of colza oil is that it contains large quantities of carotenoid compounds that completely disintegrate during canola oil processing. Beta sitosterol is the dominant sterol in colza oil, with campesterol and brassicasterol ranking second and third, respectively. Colza oil is the only oil that contains brassicasterol, and this is used to recognize oils that are falsely presented as colza oil.

Table 2: Sterols present in the studied canola cultivars (ppm).

Contents / Sterols	GKH-HELENA	GKH-0724	LICORD	GKH-305	GKH-2005	ZARFAM	WRH-262
Brassicasterol	15.04	10.76	11.72	13.05	11.8	11.25	11.59
Campesterol	31.31	30.57	32.18	31.45	31.28	31.5	31.78
Stigmasterol	0.42	0.59	0.6	0.45	0.55	0.98	1.02
β-sitosterol	50.85	53.59	52.52	49.65	52.22	51.63	41.48
Δ5 Avenasterol	0.89	1.86	1.81	2.35	1.35	1.87	11.73
Δ7 Stigmasterol	0.27	0.52	0.24	0.32	0.53	0.76	0.75
Δ7Avenasterol	0.13	0.25	0.33	0.68	0.49	0.45	0.23
Others	1.09	1.86	0.6	2.1	1.78	1.56	1.33

As shown in table 2, beta sitosterol is the dominant sterol compound in all of the 7 cultivars. Sterol compounds in the studied canola cultivars (ppm). Gamma tocopherol is the best natural antioxidant in canola oil and is present in greater quantities in canola oil compared to the alpha- tocopherol isomer.

On the TLC plate of colza oil, tocopherols are separated as the delta, gamma, and alpha isomers (from bottom to top, respectively), with gamma tocopherol being the most important and the most abundant of the three (followed by the alpha and delta isomers, respectively. The tocopherol contents in the studied samples were lower than those observed in previous studies, probably because of the longer relative retention times that cause a reduction in the quantities of antioxidant compounds such as tocopherols.

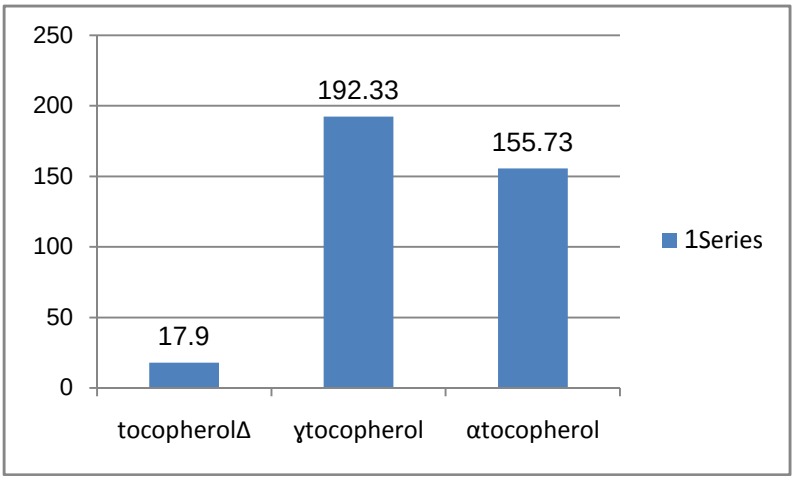

Fig. 7. The quantities of the various tocopherols in the cultivar WRH-262.

Metal ions, including iron and copper ions, play a very effective role in accelerating oil oxidation. They impart their influence through decomposing hydroperoxides. These metal ions may originate from the oil processing equipment, packaging materials, or the food material itself (some enzymes and proteins contain metals). Divalent iron decomposes peroxides 10 times faster than trivalent iron, and it can be continuously produced. Heavy metals may also directly attack fatty acids and convert them into free radicals, or may change oxygen into singlet oxygen (Fatemi, 2002). The maximum permissible contents of these compounds depend on the metal ions and the fatty acid (Zandi, 1994).

In general, stability of a fat or oil is defined as the shelf life of the product until decay becomes clearly visible, although other types of decay may happen simultaneously and complicate this problem. For example, in some cases hydrolytic decay, which causes a change in flavor, is mistaken for oxidation effect, and this may lead to taking ineffective preventive measures.

GKH-HELENA has a high oleic acid content compared to other 6 cultivars; and it also possesses the second highest tocopherol content after WRH-262, which leads to its high level of resistance against oxidation.

Presence of heavy metals such as iron and copper in large quantities increases the risk of oxidation and raises the iodine value. GKH-2005 had the maximum iron content and the third largest copper content after ZARFAM and GKH-0724, which reduces the period its oil can resist against oxidation.

CONCLUSIONS

Due to the insufficient supply of edible oils in the country, and because of the large quantities of imported oil, much attention should be paid to oil resources of the country. Canola is a crop rich in oil, and can reduce the dependence on foreign edible oil, as well as preventing foreign currency expenditure for oil imports. The best canola cultivar with respect to oleic acid content, long period of oxidative resistance, and high content of non-saponifiable compounds was GKH-HELENA, while the maximum sterol content belonged to LICORD, and the highest tocopherol content was observed inWRH-262.

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