Sterols and fatty acids in the seeds of Roselle (*Hibiscus sabdariffa* L.) cultivated in Egypt

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Summary

The comparative study on the composition of fatty acids and sterols in the seed oil of four forms of Roselle (*Hibiscus sabdariffa* L.) cultivated in Egypt was carried out. The total oil content in the seeds of the investigated forms ranged from 15.31% to 18.99%. As a result of the GC analysis five fatty acids were identified: palmitic, stearic, linolic, γ-linoleic, and α-linoleic acid. The forms differed both in respect of the presence and the content of particular fatty acids. The HPLC analysis indicated the presence of four free sterols in the Roselle seed oil: brassicasterol, campesterol, stigmasterol and sitosterol. Sitosterol appeared to be the main sterol and its content depended on the form of Roselle.

Key words: GC, HPLC, linolic acid, linoleic acid, sitosterol

Roselle is a wild growing and cultivated plant in the countries of subtropical and tropical zone. Thick sepals (*Flos Hibisci*) are commonly used as a substrate for herbal teas and refreshing drinks characterised by unique purple-red colour [1]. Sepals are separated from mature fruits (sacs) containing numerous seeds, which are a by-product of this process [2].

Previous studies on chemical composition of Roselle seeds indicated the presence of sterols and fatty acids [3-8]. Unsaturated fatty acids (especially linolic acid) are precursors of tissue hormones [9] and sterols play an important role in the cell metabolism since they are crucial part of the cell membrane [10].

There are different forms, botanical varieties and cultivars of Roselle cultivated in many countries. One of the biggest world producers of Roselle sepals is Egypt.

The aim of this study was to compare the composition of fatty acids and sterols in the seed oil of four Roselle forms cultivated in Egypt.

MATERIALS AND METHODS

The object of the study were the seeds of four cultivated forms of Roselle: Roselle White, Roselle Light Red I and Roselle Dark Red grown in north-eastern Egypt, and Roselle Light Red II grown in Sinai. The seeds were harvested in 2002 from plants chosen at random at selected Roselle plantations.

The phytochemical analyses were carried out at the Department of Vegetable and Medicinal Plants of Warsaw Agricultural University in 2003.

The oil content was determined according to Soxhlet method [11].

Separation and identification of fatty acids

Powdered seeds were extracted for 30 min. with hexane in ultrasound bath at room temperature. After evaporation of solvent, five drops of oil were collected in the ampoule. Saponification with the methanol solution of sodium hydroxide followed by estrification with methanol solution of boric trifluoride were carried out at 75°C.

Gas chromatography was performed on the Anglia Instrument Chromatograph equipped with the capillary column Carbowax 20M (length 25 m, diameter 0.32 mm). The following conditions of analysis were applied: detector temperature 250°C, injector temperature 220°C, carrier gas – helium with the flow rate 1.7 ml/min. Column temperature was programmed as follows: 100°C (2 min), temperature rise 4°C per minute, and finally 220°C (5 min).

Retention times of the standard fatty acids: palmitic, stearic, linolic, γ-linoleic, and α-linoleic acid were: 31.8, 36.3, 37.3, 37.7, and 38.3, respectively.

Separation and identification of free sterols

5 g of powdered seeds were extracted for four hours with hexane. After evaporation of solvent, the residue was dissolved in 2 ml of methanol and subjected to chromatographic analysis. The reversed-phase HPLC was carried out using the Shimadzu Chromatograph equipped with UV detector and C8 column (Phenomenex, Luna 5μ C8 (2) 250 x 4.6 mm). The gradient of acetonitrile in methanol (92%-100%) was applied. The column temperature was 25°C. The detection was carried out at 210 nm. Retention times of the standard sterols: brassicasterol, campesterol, stigmasterol, and sitosterol were: 11.46, 12.14, 13.68, and 14.64 min, respectively. The conditions of chromatographic analysis were elaborated by Wojciechowski et al. at the Department of Biochemistry, Warsaw University.

RESULTS AND DISCUSSION

There were no big differences between the investigated forms of Roselle in respect of oil content in the seeds (Table 1). Only Roselle Light Red I was characterised by a little lower content of this substance. In all forms the oil content was slightly lower in comparison with the results obtained by Samy [4] and Duke and du Cellier [7].

Table 1.

The total oil content in the Roselle seeds (%).

Roselle White	Roselle Light Red I	Roselle Light Red II	Roselle Dark Red
18.99	15.31	18.86	17.33

The chemical analysis of the Roselle seed oil indicated that it is a valuable source of linolic acid – one of the most important unsaturated fatty acids (Fig. 1). The highest content of this acid – comparable to that characteristic for primrose (*Oenothera biennis* L.) seed oil [9] – was observed in the oil obtained from Roselle Dark Red. More unsaturated and more pharmacologically active γ-linoleic acid was found only in the oil of Roselle Light Red II – also in the amount comparable to primrose seed oil. The seed oil of Roselle Light Red I was characterised by the distinct presence of α-linoleic acid. This acid is a precursor of tissue hormones which are responsible for anti-inflammatory and anti-coagulant activity [12]. It is also used for the treatment of man sterility [13]. Apart from unsaturated fatty acids, the oil of all investigated forms contained saturated palmitic acid, whereas another saturated fatty acid – stearic acid – was present only in two forms. Previous studies indicated also the presence of myristic, palmitooleic, oleic, sterculic and malvalic acids in the Roselle seed oil [8].

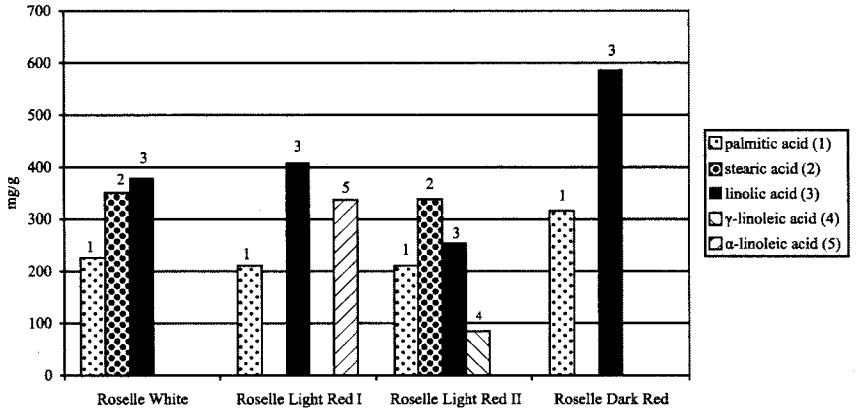

Fig. 1. The content of fatty acids in the Roselle seed oil (mg/g).

Sterols seem to be another important group of biologically active compounds in the Roselle seeds. Salama and Ibrahim [3] found six different free sterols in the Roselle seed oil: cholesterol, ergosterol, campesterol, stigmasterol, sitosterol and spinasterol. Atta and Imaizumi [5] identified only three of them: campesterol, stigmasterol and sitosterol. The studies of Holser et al. [6] indicated the presence in the Roselle seed oil another free sterol – isofucosterol (5-avenasterol). All the above-mentioned authors used GC for identification of these compounds. In the present study HPLC was used for qualitative and quantitative analyses of free sterols in the seed oil of investigated forms of Roselle. Four compounds were found: brassicasterol, campesterol, stigmasterol and sitosterol (Fig. 2 - example). The studied Roselle forms differed in respect of the content of particular sterols in the seed oil (Fig. 3). The seed oil of all forms was characterised by relatively high content of sitosterol, the most interesting sterol from the pharmacological point of view [14]. Campesterol was present in distinctly lower concentration in three investigated forms and in Roselle White it was even not found.

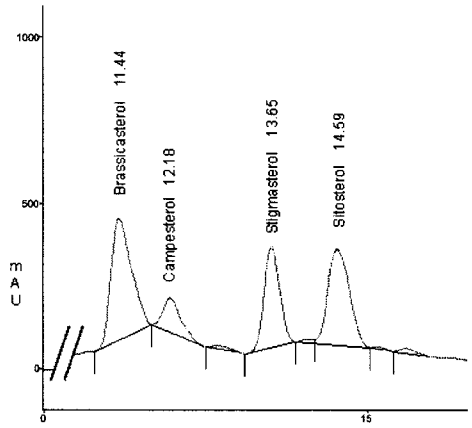

Fig. 2. The HPLC chromatogram of free sterols in the Roselle seed oil.

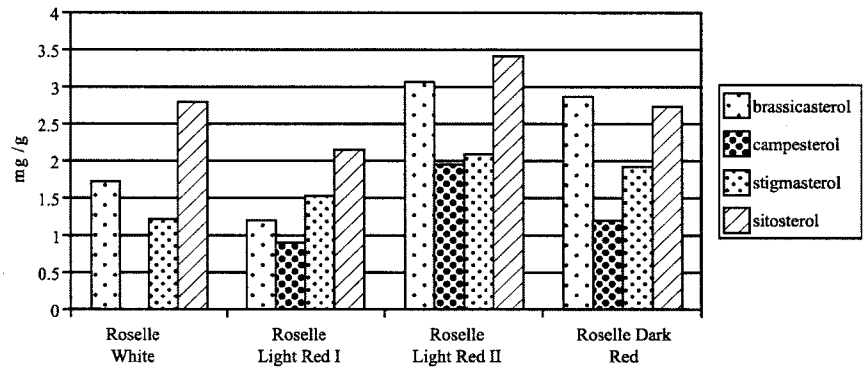

Fig. 3. The content of free sterols in the Roselle seed oil (mg/g).

The obtained results indicate that the Roselle seeds are a rich source of unsaturated fatty acids and sterols. The investigated forms of this plant markedly differ both in the content and composition of these two groups of compounds. As the seeds of Roselle are very easy to obtain and cheap by-product in the production of Roselle sepals, they may be considered as a promising raw material for elaboration of new medicines or dietary supplements.

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STEROLE I KWASY TŁUSZCZOWE W NASIONACH KETMII SZCZAWIOWEJ (*HIBISCUS SABDARIFFA* L.)

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Streszczenie

Porównywano skład kwasów tłuszczowych i steroli w oleju z nasion czterech form ketmii szczawiowej (*Hibiscus sabdariffa* L.) uprawianych w Egipcie. Zawartość oleju w nasionach badanych form wynosiła od 15,31% do 18,99%. W wyniku analizy chromatograficznej (GC) zidentyfikowano pięć kwasów tłuszczowych: palmitynowy, stearynowy, linolowy, γ-linolenowy i α-linolenowy. Badane formy różniły się zarówno pod względem obecności, jak i zawartości poszczególnych kwasów tłuszczowych. Analiza HPLC wykazała obecność w oleju z nasion ketmii szczawiowej czterech wolnych steroli – brasikasterolu, kampesterolu, stigmasterolu i sitosterolu. Sitosterol okazał się głównym sterolem, a jego zawartość w oleju badanych form była różna.

Słowa kluczowe: GC, HPLC, kwas linolowy, kwas linolenowy, sitosterol