Tocopherols and flavonoids of SOS-7 halophyte

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RESUMEN

Tocoferoles y flavonoides de halofito SOS-7

Halofito es una semilla oleaginosa codificada como SOS-7 (semilla oleaginosa *Salicornia,* séptimo año de selección). Los tocoferoles del aceite de halofito SOS-7 fueron determinados directamente en el aceite usando cromatografía líquida de alta presión acoplada a detector fluorescente. Se encontró que el aceite contenía 710 ppm de tocoferoles totales. Los tocoferoles alfa, beta, gamma y delta, se encontraron a niveles de 38.2, 1.0, 58.7 y 2.1%, respectivamente.

Nueve glicósidos flavonoides fueron aislados e identificados de las semillas y se encontró que pertenecen a la clase flavonol dentro de los flavonoides. Estos flavonoles fueron identificados como: quercetina-3,7-diglucosido, quercetina-3-glucosido-7-galactosido, quercetina-3-soforosido, quercetina-3-glucosido, quercetina-3-galactosido, isorannetina-3, 7-di-glucosido, isoranetina-3-glucosido, kampferol-3, 7-diglucosido y kampferol-3-glucosido.

PALABRAS-CLAVE: Aceite de halofito SOS-7 - Flavonoide - Tocoferol.

SUMMARY

Tocopherols and flavonoids of SOS-7 halophyte.

Halophyte is an oil seed coded as SOS-7 (*Salicornía* Oil Seed, 7th year of selection). Tocopherol constituents of SOS-7 halophyte oil were determined directly in the oil by using high pressure liquid chromatography coupled to fluorescence detector. It was found that the oil contains 710 ppm total tocopherols. The tocopherol constituents, alpha, beta, gamma and delta, were found at the level of 38.2, 1.0, 58.7 and 2.1% respectively.

Nine flavonoid glycosides were isolated and identified from the seeds and it was found that they belong to the flavonol class of flavonoids. These flavonol compounds were identified as: quercetin-3, 7-diglucoside, quercetin-3-glucoside-7-galactoside, quercetin-3-sophoroside, quercetin-3-glucoside, quercetin-3-galactoside, isorhamnetin-3, 7-di-glucoside, isorhamnetin-3-glucoside, kaempferol-3, 7-diglucoside and kaempferol-3-glucoside.

KEY-WORDS: Flavonoid - SOS-7 halophyte oil - Tocopherol.

1. INTRODUCTION

The world-wide demand for vegetable oils has greatly increased, this led to the search for new seed oils. SOS-7 Halophyte, is a new oil seed crop, as climatic tolerant, has been successfully tried in desert areas and irrigated with sea water (1). In continuation to the previous studies in this laboratory (2) (3) (4) on characterizing halophyte, SOS-7 oil produced from locally cultivated halophyte plant, it was advisable to carry out further studies dealing with tocopherols and flavonoids to complete the picture of the seed constituents.

Tocopherol content of an oil is of equal interest to fatty acid composition in evaluating the quality of the oil. Tocopherols are the best-known and most universally distributed antioxidants, constitute fat-soluble vitamin E. Four forms, alpha, beta, gamma and delta tocopherols (alpha-T, beta-T, gamma-T and delta-T) (Fig. 1) are found in vegetable oils.

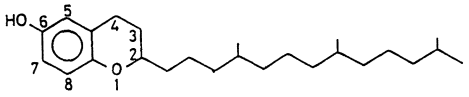

Fig.1

Structure of tocopherols: 5,7,8 trimethyl (alpha-T); 5,8 dimethyl (beta-T); 7,8 dimethyl (gamma-T) and 8 methyl (delta-T).

Gamma-T has been found to be a more effective antioxidant than beta-T which is in turn effective than alpha-T (5).

The term flavonoid is applied to compounds with a structure based on flavone (2-phenyl chromone). Flavone consists of two benzene rings (A and B) joined together by a gamma pyrone ring (Fig. 2). The structures, commonly found in nature, are flavan-3 ols (cathechins), flavonols, flavanones, flavanonols and flavan-3.4-diols.

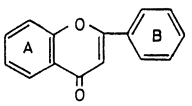

Fig.2

Flavonoid compounds are widely distributed in higher plants. They were isolated from different parts of plants with variations in the types of compounds found according to various anatomical tissues of any plant (6). It has been reported that flavonoids have antioxidant effect on unsaturated fatty acids (7). As far as one knows, nothing has been

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reported about the flavonoids of SOS-7 halophyte in the current literature.

2. MATERIALS AND METHODS

2.1. Tocopherols (T)

The oil was extracted from the ground seeds using $CHCl_3$/MeOH (2:1, v/v) and the solvent was evaporated under vacuum. Standard tocopherols (St.T), alpha-T , beta-T, gamma-T and delta-T were purchased from E. Merk, Germany.

High pressure liquid Chromatography (HPLC)

Tocopherol constituents were determined directly in the oil using Toyo Soda-CCPM, high pressure liquid chromatograph, coupled to Hitachi-650-10-S fluorescence detector with an exitation and emmission wave lengths of 290 and 325 nm respectively. The analysis was carried out under the following operating conditions : column, 150 x 4.6 mm packed with silica gel YMC-A-012 SIL; solvent, hexane: isopropyl alcohol (100: 0.5 v/v) at flow rate of 1-2 ml/min; sample size, 20 microlitre (1-1.5% oil in hexane) and chart spead, 5mm/min (8). The amounts of the resolved tocopherol components were estimated from the measured areas under the appropriate peaks (PA) in comparison with those of the standard tocopherol solutions (St.T) according to the following equation (8) (9) (10).

$$\text{T.ppm/Kg oil} = \frac{\text{P.A. of resolved T x g oil/ml x 1000}}{\text{P.A. of St.T. x mg/ml (St.T)}}$$

2.2. Flavonoids

The solvents, n-hexane, chloroform, ethanol, methanol and water were successively used in extracting the different constituents from the ground seeds. The extracts were concentrated under reduced pressure and applied on Whatman paper No. 1 to determine their flavonoid constituents. A two dimensional chromatographic technique was used with solvent systems n-butanol/acetic acid/water (BAW 4:1:5, v/v/v) and 15% acetic acid in the first and second directions respectively. Paper chromatography showed the absence of flavonoids in n-hexane and chloroform extracts (negative results with $AlCl_3$ spray reagent). The other extracts gave positive results with $AlCl_3$ spray reagent and they all had the same flavonoid compounds. The three extracts (ethanol, methanol and water) were combined and concentrated under reduced pressure. The concentrated extract was applied on a cellulose column, and eluted with ethanol containing increasing amounts of water. The collected fractions were further separated into nine single components by applying elution technique on Whatman paper No.3.The isolated compounds were investigated according to the standard methods of identification (11) (12) (13) (14).

All the authentic samples (markers) of the flavonol compounds used in this paper were separated from the egyptian flora and identified by the researchers in the laboratory of Plant Taxonomy and Egyptian Flora, National Research Centre, Cairo. Standard sugars were purchased from BDH Chemicals Ltd., England.

3. RESULTS AND DISCUSSION

3.1. Tocopherols

Tocopherols are important antioxidants which also function as vitamin E. It is known that tocopherols are superior radical chain-breaking antioxidants (15) compared to synthetic ones. Several methods have been developed for the analysis of tocopherols in vegetable oils. It was found that HPLC analysis of tocopherols is a sensitive and selective method. In the present work, HPLC analysis of crude SOS-7 halophyte oil, using a fluorescence detector indicates that this oil is rich in tocopherols and contains as much as 710 ppm total tocopherols . Four tocopherol isomers, alpha-T (38.2%), beta-T (1.0%), gamma-T (58.7%) and delta-T (2.1%) were detected. Their relative retention times were 3.35, 5.65, 6.0 and 10.4 min respectively. From these results it can be seen that SOS-7 halophyte oil is rich in gamma-T which is superior as antioxidant (5).

3.2. Flavonoids

All the nine isolated compounds appeared as brown spots under UV light which changed to yellow on exposure to ammonia vapour. Complete acid hydrolysis of the nine compounds (1-9) gave quercetin, kaempferol and isorhamnetin as aglycones together with glucose and galactose as sugar moities which means that these compounds belong to the O-flavonol glycosides. Acid hydrolysis also showed that compounds 1, 2, 3, 6 and 8 are disaccharide glycosides while compounds 4, 5, 7 and 9 are monosaccharide glycosides . The R_f values, chemical and UV data of the isolated compounds are recorded in Tables I, II and III respectively .

3.2.1. Disaccharide Glycosides (Compounds 1, 2, 3, 6 and 8)

On complete acid hydrolysis compound 1 and 3 gave quercetin and glucose while compound 2 gave quercetin, glucose and galactose. Compound 6 gave isorhamnetin and glucose while compound 8 gave kaempferol and glucose (Table II).

UV data (Table III) show that compounds 1, 2, 6 and 8 were occupied in positions 3 & 7. Occupation of position 3 was confirmed by the stability of the UV spectrum after addition of NaOMe. However, position 7 was confirmed to

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Table I
R_f-values of the isolated flavonol glycosides

Compounds (*)	R_f-values X 100 (**)			
	BAW	H_2O	15%	PhOH
1. Q37G	17	41	45	43
2. Q3G7Gal	13	51	51	35
3. Q3Soph	33	30	43	45
4. Q3G	47	13	31	53
5. Q3Gal	44	13	29	55
6. I37G	23	41	51	76
7. I3G	54	28	35	70
8. K37G	25	54	51	67
9. K3G	61	14	35	60

(*) Q37G = Quercetin-3,7-diglucoside.
Q3G7Gal = Quercetin-3-glucoside-7-galactoside.
Q3Soph = Quercetin-3-sophoroside.
Q3G = Quercetin-3-glucoside.
Q3Gal = Quercetin-3-galactoside.
I37G = Isorhamnetin-3,7-di-glucoside.
I3G = Isorhamnetin-3-glucoside.
K37G = Kaempferol-3,7-diglucoside.
K3G = Kaempferol-3 -glucoside.

(**) BAW = n-Butanol:Acetic acid:Water (4:1:5).
15% = 15% Acetic acid.
PhOH = Phenol:Water (80:20).

Table II
Chemical data of the isolated compounds

Compounds	Complete acid hydrolysis (*)		Mild acid hydrolysis
	Aglycone	Sugar	
1. Q37G	Q	G	Q7G
2. Q3G7Gal	Q	G,Gal	Q7Gal
3. Q3 Soph	Q	G	Q3G
4. Q3G	Q	G	—
5. Q3Gal	Q	Gal	—
6. I37G	I	G	I7G
7. I3G	I	G	—
8. K37G	K	G	K7G
9. K3G	K	G	—

(*) Q = Quercetin. I = Isorhamnetin. K = Kaempferol.
G = Glucose. Gal = Galactose.

be occupied by the absence of the shift in band II with sodium acetate compared with that of methanol and also with the absence of a small peak at 320 nm with NaOMe.

The structures of these compounds were further confirmed by carrying out mild acid hydrolysis which gave intermediates (Table II), namely, quercetin-7-galactoside, quercetin-3-glucoside, isorhamnetin-7-glucoside and kaempferol-7-glucoside. These intermediates were co-chromatographed with authentic samples using different solvent systems namely, n-butanol: acetic acid: water (BAW) (4:1:5); 15% acetic acid and phenol: water (PhOH) (80:20). It was found that the R_f values of the isolated compounds (Table I) were identical with those reported in the literature for the disaccharide flavonoids.

Table III
U.V. data of the isolated compounds

Compounds	MeOH	NaOMe	$AlCl_3$	$AlCl_3$ -HCl	NaOAc	NaOAc -H_3BO_3
1. and 2 Q37G and Q3G7Gal	257 265sh 357	268sh 275 417	267 297sh 362 415	267 297sh 357 400	265 295sh 360 420sh	262 380
3. Q3Soph	255sh 267 278sh 357	280 325sh 420	255sh 270 360 405sh	255sh 270 360 405sh	270 278sh 295sh 365	265 278sh 295sh 375
4. and 5. Q3G and Q3Gal	257 265 357	268sh 282 335sh 425	262 300sh 357sh 420	262 300sh 357sh 400	270 370	262 380
6. I37G	255 270sh 350	250sh 280 430	270 305sh 364 405	269 305sh 364 405	257 270sh 368 420	257 270sh 360
7. I3G	255 267sh 295sh 355	272 327 415	270 300sh 356 405	268 300sh 358 400	275 315 388	258 366sh 305sh 360
8. K37G	265 318sh 350	245 270 295sh 390	274 300 355 400	274 290sh 350 395	265 318sh 360 400sh	265 320sh 350
9. K3G	266 300sh 350	275 325 400	276 305 355 400	276 305 355 400	276 310 390	256 285sh 345

Further investigation was carried out on compound 3 by H_2O_2 oxidation which gave rise to a disaccharide. This disaccharide was confirmed to be sophorose after co-chromatographed with authentic sugar samples.

From the above data it can be concluded that compounds 1, 2, 3, 6 and 8 could be identified as quercetin-3,7-diglucoside, quercetin-3-glucoside-7-galactoside, quercetin-3-sophoroside, isorhamnetin, 3-7-diglucoside and kaempferol-3-7-diglucoside.

3.2.2. Monosaccharide Glycosides (Compounds 4, 5, 7 and 9)

The results of the chemical investigation, complete and mild acid hydrolysis, of compounds 4, 5, 7 and 9 are recorded in Table II. It can be noticed that glycosides 4 and 5 gave quercetin as an aglycone and glucose and galactose, respectively, as sugar moieties on complete acid hydrolysis. However, compounds 7 and 9 gave isorhamnetin and kaempferol as aglycone moities respectively and glucose as sugar moiety. The types of the aglycones and sugars were confirmed by co-chromatographing the products of hydrolysis with authentic samples using different solvent systems (Table II).

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Table III shows the UV data of compounds 4, 5, 7 and 9 and the data are in agreament with those reported for the flavonol monosaccharide glycosides.

It can be concluded that monosaccharide glycosides 4, 5, 7 and 9 can be identified as quercetin-3-glucoside, quercetin-3-galactoside, isorhamnetin-3-glucoside and kaempferol-3-glucoside.

From all the results discussed above, it can be generally concluded that SOS-7 halophyte is rich in natural antioxidants namely, tocopherols and flavonoids.

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