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Determination of Saponin Content in Turkish Tahini Halvah by Using HPLC

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Abstract: The total saponin content, components and fatty acid composition of 14 different Tahini Halvah samples were investigated. Total saponin was determined by HPLC. The quantities of moisture, oil, protein, ash, total sugar and brix were determined with various methods. The fatty acid composition was determined by GC. Moisture, oil, protein, ash, total sugar, brix and total saponin samples from 1.22-2.60, 27.5-35.2, 9.23-15.12, 1.40-1.87, 40.79-49.80, 49-57% and 32-172 mg/kg in Tahini Halvah, repectively. The main components of Tahini Halvah are tahini, sugar and the liquid extract of soapwort. Tahini is obtained by sesame seeds. Soapwort extract is obtained by boiling the roots of the soapwort. Saponin that is active substance of soapwort extract effects positively the colour and consistency of the Tahini Halvah and prevents especially the oozing of the oil from the halvah in time by acting an emülsifier. Many saponins show haemolytic activity, have a bitter taste and are toxic to fish. The research was shown that saponin is a highly significant component for Tahini Halvah.

Key words: HPLC, saponin, soapwort, Tahini Halvah

INTRODUCTION

Tahini Halvah which reflects plate flavour of Turkish, is known specific of Turkish public in the world. Not only it is consumed in Turkey, but also it is consumed in lots of countries such as Germany, England, United States of America and Russian Federation. It is prepared from a mixture of sesame-seed paste (tahini) with inverted sugar syrup. As commonly prepared, halvah has been found to possess hemolytic activity, due to the saponins originated from the liquid extract of saponaria root used as emülsifier. By substituting for the latter a powdered soapwort extract commercially avaliable, we succeeded in preparing a uniform quality product of greater stability, separating less oil and showing no hemolytic activity (Artık, 1997). Soapwort extract contains 11.58-19.58% total saponin which increases importance of soapwort (Battal, 2002).

The classical definition of saponins is based on their surface activity; many saponins have detergent properties, give stable foams in water, show haemolytic activity, have a bitter taste and are toxic to fish (piscicidal). Although toxic, saponins are very poorly absorbed by the body and so tend to pass through without causing harm, they are also destroyed by thorough cooking. Saponins are found in many plants, including several that are often used for food, such as certain beans. It is advisable not to eat large quantities of food that contain saponins. Saponins are much more toxic to some creatures, such as fish, and hunting tribes have traditionally put large quantities of them in streams, lakes etc in order to stupefy or kill the fish (Chevallier, 1996). Such attributes, while not common to all saponins, have frequently been used to characterize this class of natural products (Abe *et al.*,1993; Anonymous, 1990).

Saponins comprise a large family of structurally diverse compounds containing a sterodial or triterpenoid aglycone linked to one or more oligosaccharide moieties. The aglycone or non-saccharide portion of the saponin molecule is called the *genin* or *sapogenin*. Depending on the type of genin present, the saponins can be divided into three major classes: (Hostettmann and Marston, 1995).

- Triterpene glycosides [triterpenoid saponozites (C_{30})]
- Steroid glycosides [stereoidal saponozites (C_{27})]
  - Spirostanol saponin
  - Furastanol saponin
  - Nugatigenin saponin
  - Polipodo saponin
- Steroid alkoloid glycosides (glikoalkoloids). Steroid glycosides saponins are shown in Fig. 1.

The number of known triterpenes is very large and only a small proportion has been characterized in glycosidic form. Triterpenes are frequently isolated only after hydrolysis of plant extracts and it is not always easy to ascertain from published work whether they actually occur in the free or glycosidic forms in the plant itself (Boiteau *et al.,* 1964).

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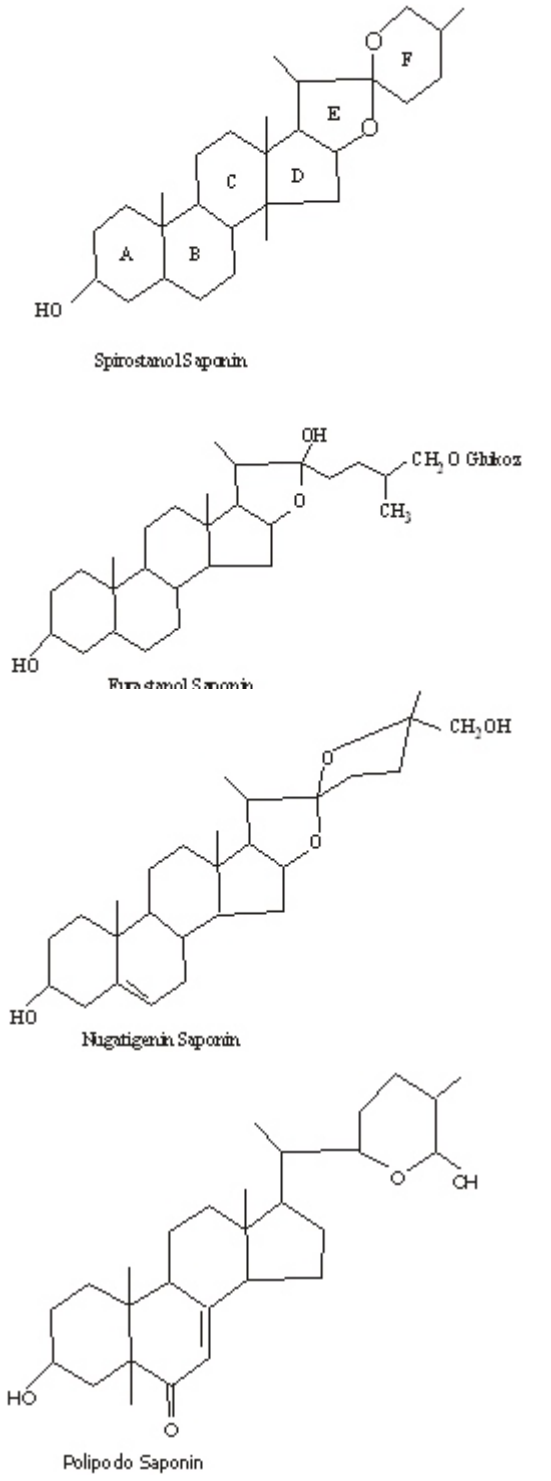

Fig. 1: Steroid glycosides saponins

The agylcones are normally hydroxylated at C-3 and certain methyl groups are frequently oxidized to hydroxmethyl, aldehyde or carboxyl functionalities. When an acid moiety is esterified to the aglycone, the term ester saponin is often used for the respective glycosides. All saponins have in common the attachment of one or more sugar chains to the aglycone. Monodesmosidic saponins have a single sugar chain, normally attached at C-3. Bidesmosidic saponins have two sugar chains, often with one attached through an ether linkage at C-3 and one attached through an ester linkage at C-28 (triterpene saponins) or an ether linkage at C-26 (furostanol saponins). Tridesmosidic saponins have three sugar chains and are seldom found. Bidesmosidic saponins are easily transformed into monodesmosidic saponins by, for example; hydrolysis of the esterified sugar at C-28 in triterpene saponins; they lack many of the characteristic properties and activities of monodesmosidic saponins (Hostettmann and Marston, 1995).

A brief biosynthesis summary of triterpenes and steroids is shown in Fig. 2. They are built up of six isoprene units and have a common biosynthetic origin in that they are all derived from squalene, presumably via ring opening of squalene-2,3-epoxide, followed by a concerted cyclization. It is only recently that the corresponding cyclases have been characterized (Abe *et al.*,1993). While the true triterpenes have 30 carbon atoms, the steroids have only 27 carbons by virtue of the oxidative cleavage of three methyl groups from a C_{30} intermediate (Heftmann, 1968). Saponins are extremely widely distributed in the plant kingdom. It is known that over 90 families contain saponins. A systematic investigation of 1730 central Asian plant species, that 76% of the families contained saponins. Saponins occur in plants which are used as human food; soybeans, chick peas, peanuts, lentils, spinach, oats, garlic, sugar beet, patotoes, green peppers, tomatoes, oins and tea (Birk and Peri, 1980; Oakenfull, 1981; Price *et al.*, 1987).

In leaf cells of sugar beet and root cells of the pea plant, the triterpene glycosides are present mainly in a bound from, with the highest concentration occuring in the chloroplasts and mitochondria. The fact that saponins are localized in organells which have a high metabolic turnover rate implies that they are not simply balast material but may be important regulatory substances in the metabolism and development of an organism, i.e. they may be physiologically significant constituents (Anisimov and Chirva, 1980). Generalizations about the solubilites of saponins are hazardous but many are soluble in water (particularly if the water contains small amounts of alkali) and alcohol; some are soluble in ether, chloroform, benzene, ethyl acetate or glacial acetic acid. The amphiphilic nature of saponins dominates their physical properties in solution. They are strongly surface active, form stable foams, act as emulsifying agents and form micelles in much the same way as detergents (Hostettmann and Marston, 1995).

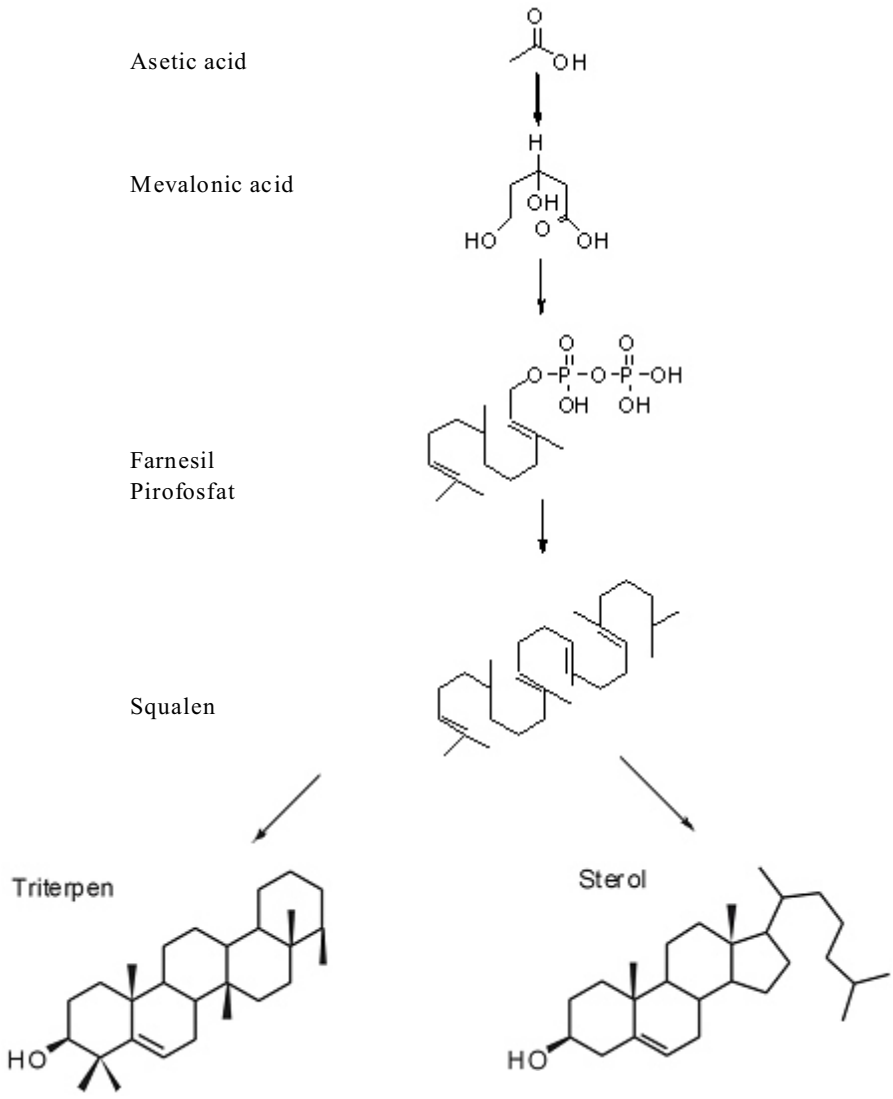

Fig. 2: Biosynthesis of triterpenes and steroids

Legumes, peas and soybeans, also contain small quantities of saponins, which are proving their worthiness as phytochemicals. In the diet, phytochemical saponins have a wide spectrum of activity as antifungal and antibacterial agents, lowering of blood cholesterol and inhibition of cancer cell growth. Recent studies have suggested that the low serum cholesterol levels of Masai tribes in East Africa - who consume a diet very high in animal products, cholesterol, and saturated fat – are probably due to the consumption of saponin - rich herbs. Saponin act by binding with bile acids and cholesterol, so it is thougth that these chemicals "clean" or purge these fatty compounds from the body, lowering the blood cholesterol levels. Saponin content depends on factors such as the cultivar, the age, the physiological state and the geographical location of the plant. There can be considerable variation in composition and quantity of saponins in vegetable material from different places (Kawai *et al*., 1988). Different methods have been employed for the qualitative and quantitative determination of saponins: haemolysis, piscicidal activity, gravimetry, spectrophotometry, TLC, GC, HPLC (Hiller and Voight, 1977; Hiller *et al*., 1966; Price *et al*., 1987).

The ability of saponins to cause haemolysis of blood *in vitro*. Low concentrations of saponins are capable of destroying erythrocyte membranes, causing a release of haemoglobin. The phenomenon involves a reduction of surface tension between the aqueous and lipid phases of erythrocyte membrane, causing emulsion of the lipids and their subsequent departure from the membrane. Through these holes Na^+ and water are allowed to enter the cell, while K^+ leaves. This flux occurs until the memrane

ruptures and haemoglobin is shed into the plasma (Hostettmann and Marston, 1995). Haemolytic activity varies considerably with the structure of the glycoside. Monodesmodidic steroids and triterpene saponins are strongly haemolytic but bidesmosidic furostanol saponins and triterpene bidesmosides are virtually inactive (Romussi *et al*., 1980). Many saponins have a reputation for being bitter. The seed-coat of quinoa (*Chenopodium quinoa,* Chenopodiaceae) for example contains bitter saponins which have to be removed before cooking (Mizui *et al*., 1988).

The saponins in the Tahini Halvah and soapwort have been studied by several author (Battal, 2002; Baylan, 1990). Baylan (1990) reported that concentration of saponin ranged between 119-266 mg/kg in Tahini Halvah. Determination of saponin by TLC method. Battal (2002) reported that concentration of saponin 11.58-19.58% in extracts of soapwort. So, aim of the present study was to determine the proximate composition and quantitative of saponin in the Turkish Tahini Halvah.

MATERIALS AND METHODS

This study was led to the Laboratory of Ankara University, Engineering Faculty, Food Engineering Department in Turkey.

Samples: Fourteen different Tahini Halvah samples from producer firm or market were used for determination of total saponin and other components. Tahini Halvah samples were stored in glass jar and kept at 20ºC in laboratory.

Chemicals and reagents: Methanol (HPLC grade), asetic asit (HPLC grade), analytic grade NH_4OH, H_3PO_4, CH_3OH and diastase were purchased from Merck (Darmstadt, Germany) Standart of Gylcyrrhizic acid were purchased from Sigma - Aldrich Co. (St. Louis, MO, USA).

Methods:

Extraction procedure for HPLC: All samples were ground in a mill or blender and extracted according to the following procedure. Two grams of Tahini Halvah was weighed to a 100 mL blender cup of a mixer. Approximately 30 mL of water (90-95ºC) and 2 ml of concentrated NH_4OH (37%) were added, and the contents were blended for 3 min. The resulting solution was quantitatively transferred to a 150 mL beaker. The pH of the solution was adjusted to a pH of 7.0 with H_3PO_4 and 1 ml of 10% diastase was added. This was incubated at 37ºC for 30 min, cooled to room temperature, and transferred to a 100 mL volumetric flask with CH_3OH. The final extract was diluted to volume with additional CH_3OH and filtered through Whatman No. 42 paper or

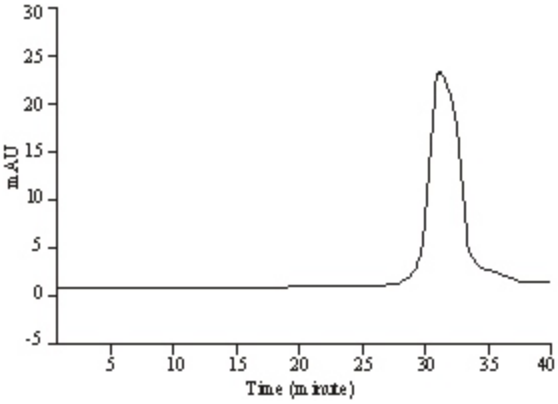

Fig. 3: A typical HPLC chromatogram of standart saponin (Gylcyrrhizic acid)

equivalent prior to analyses (Hurst *et al*., 1983). Before enjection to HPLC column, extracts were filtered though a 0.45 µm membrane fitler (Millipore, Bedford, USA) and analyzed by HPLC.

Standard: Five milligrams of glycyrrhizic acid ammonium salt was weighed into a 100 mL volumetric flask and diluted to volume with LC water for final concentration of 0.05 mg/ml for using saponin standard. The chromatogram of standart saponin (Gylcyrrhizic acid) is shown in Fig. 3.

HPLC analysis: Chromatographic analyses were carried out on an the HPLC system (Shimadzu, Kyoto, Japan) that cosisted of a LC-10 AD-VP gradient pump, a Rheodyne 7125 i valve furnished with 20 µL loop, a SPD-M10A photodiode array detector, CTO-10AS column oven, DGU-14A degasser and a SCL-10A system controller. Seperation of saponins was carried out using an Nucleosil Macherey-Nagel C18 (250 x 4.6 mm ID, particle size 5 µm) column (Barcelona, Spain) at 1.5 ml min^{-1} flow rate. Detection was made at 254 nm and 25ºC.

The HPLC mobile phase consisted of methanol, water and acetic acid in the ratio of 60/34/6 (v/v/v). The mobile was filtered and degassed prior to use. The compounds appering in chromotograms were identified on retention times and spectral data by comparasion with standards. All analyses were done on duplicate in each sample.

Moisture: Moisture content was determination in vacuum at 70ºC temperature of etuve (Regnell, 1976).

Crude Fat: Soxhelet method was used for determination of crude fat content in Tahini Halvah (James, 1995).

Protein: Total protein was determined by Kjeldahl method. The factor of 6.25 is used for estimation of total protein (Anonymous, 1997).

Table 1: Levels of moisture, oil, protein, ash, brix and total sugar in 14 individual samples of Tahini Halvah

No. of smples	Moisture %)	Crude fat (%)	Protein (%)	Ash (%)	Brix (%)	Total sugar (%)
1	1.72	33.26	9.23	1.44	49	43.47
2	2.04	31.46	9.49	1.48	57	49.77
3	1.48	32.25	12.47	1.42	55	48.80
4	1.32	30.45	12.51	1.41	57	49.80
5	1.22	33.15	15.05	1.87	49	40.79
6	1.35	31.30	12.25	1.40	50	42.16
7	1.41	34.50	12.43	1.85	53	47.57
8	2.15	31.85	11.03	1.65	53	47.50
9	2.60	27.50	15.12	1.83	54	48.98
10	1.24	35.20	11.25	1.72	52	46.39
11	1.40	32.40	12.25	1.72	56	49.60
12	1.38	31.90	12.99	1.73	54	48.80
13	1.54	32.90	12.95	1.69	52	46.02
14	1.61	33.00	13.22	1.68	52	47.51

Table 2: Fatty Acids composition of 14 individual samples of Tahini Halvah

No. of samples	Palmitic acid (%)	Stearic acid (%)	leic acid (%)	Linoleic acid (%)	Linolenic acid (%)
1	10.425	5.471	43.015	40.668	0.421
2	12.697	6.928	42.673	37.477	0.225
3	11.198	3.841	35.125	49.475	0.361
4	10.009	5.833	42.706	41.214	0.238
5	10.722	4.475	38.231	46.341	0.231
6	12.253	6.983	38.228	42.300	0.236
7	11.447	5.278	40.305	42.701	0.269
8	10.028	5.623	42.324	41.785	0.240
9	9.434	5.544	40.817	43.880	0.325
10	10.843	4.886	39.317	44.725	0.229
11	10.158	5.187	40.203	44.240	0.212
12	10.214	5.662	43.975	39.904	0.245
13	10.234	5.994	40.987	42.542	0.243
14	10.675	3.612	41.921	43.541	0.251

Ash: Tahini halvah samples were set on fire at 550 ± 20ºC in ash oven (Anonymous, 1968).

Total and reducing sugars: Total and reducing sugars were determined by the Lane -Eynon method (Cemeroğlu, 1992).

Soluble solids (Brix): Soluble solids (%) were measured by using a Bausch - Lomb refractometer at 20°C (Regnell, 1976).

Fatty acids composition: GC instrument was used fatty acids composition of Tahini Halvah samples (Anonymous, 1990).

RESULTS AND DISCUSSION

Some analytical properties of 14 different Tahini Halvah samples are given Table 1. For Tahini Halvah; moisture content 1.22-2.60%, oil content 27.5-35.2%, protein content 9.23-15.12%, ash content 1.40-1.87%, total sugar 40.79-49.80% and brix 49-57% were determined in this study.

Fatty acids composition of 14 individual Tahini Halvah samples are given Table 2. Tahini Halvah oil is predominantly made up of linoleic (37.47-49.47%), oleic (35.12-43.97%), palmitic (9.43-12.69%), stearic

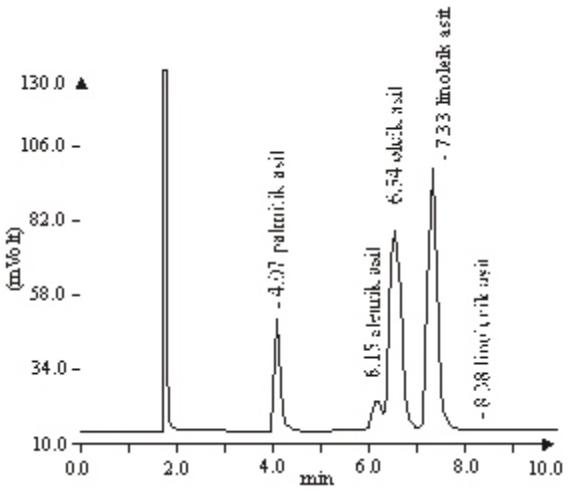

Fig. 4: A typical chromatogram of fatty acids in Tahini Halvah

(3.61-6.98%) and linolenic (0.21-0.42%) acids respectively. The linoleic acid must be min 32%, oleic acid must be min 35%, palmitic acid must be min 7% and linolenic acid must be max 2% in the standart of Tahini Halvah TS 2590 (Anonymous, 1998). The results obtained were in unison with standart of Tahini Halvah TS 2590 in

Turkey. A typical chromatogram of fatty acids in Tahini Halvah is shown in Fig. 4.

Table 3: Levels of saponin in 14 individual samples of Tahini Halvah

No. Of samples	Saponin (ppm)	Saponin (%)
1	32	0.0032
2	76	0.0076
3	68	0.0068
4	96	0.0096
5	44	0.0044
6	48	0.0048
7	65	0.0065
8	84	0.0084
9	36	0.0036
10	163	0.0163
11	172	0.0172
12	148	0.0148
13	112	0.0112
14	104	0.0104

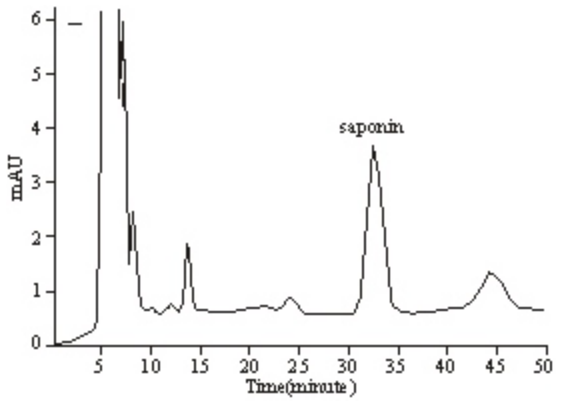

Fig. 5: A typical HPLC chromatogram of saponin in Tahini Halvah

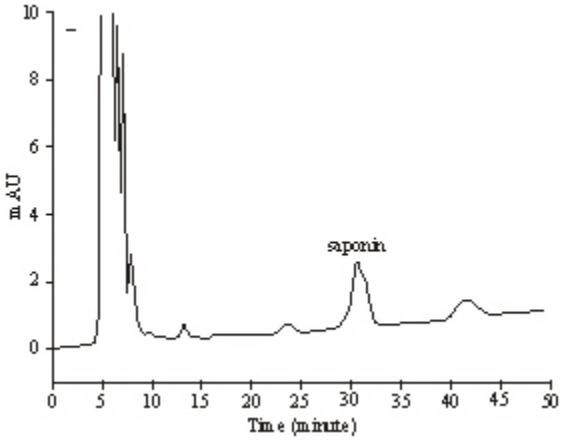

Fig. 6: A typical HPLC chromatogram of saponin in Tahini Halvah

Battal (2002) has reported that total saponin was 11.58-19.58% in soapwort excract. Total saponin content in different soapworts were determined by HPLC. Battal (2002) has also reported regarding saponin yield, optimal extraction time was 8 hours.

We determined that proportion ratio of total saponin renged from 32 to 172 mg/kg. Saponin concentration of Tahini Halvah samples are given Table 3. The chromatograms illustrating saponin in Tahini Halvah are shown in Fig. 5 and 6. Baylan (1990) determined total saponin of Tahini Halvah by TLC and it was reported total saponin content of Tahini Halvah as 119-266 mg/kg. Turkish Standard (TS 2590) is standart of Tahini Halvah in Turkey and total saponin level must be max 0.1% in Tahini Halvah. These studies are harmanious for Turkish Standard (TS 2590) (Anonymous, 1998).

CONCLUSION

The results of present study demonstrated that saponin is a significant component for Tahini Halvah. The liquid extract of soapwort is used as a food additive in the preparation of Tahini Halvah. Saponin that is active substance of soapwort extract effects positively the colour and consistency of the halvah and prevents especially the oozing of the oil from the halvah in time by acting an emülsifier. Sesame and sugar may affect total saponin level of Tahini Halvah. Because both of them contain saponin. Saponin may be carried to Tahini Halvah from sesame or sugar. So this level can be determined differently in samples. Also the halvah producer use different concentration of soapwaort exctract for Tahini Halvah. Thus total saponin level may be determined differently. HPLC technique is rapid, accurate and precise. Additionally, for those laboratories that do not possess an HPLC, the spectrophotometric method, while not as fast, serves as a good alternative for analysis of saponin content in Tahini Halvah.

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