Bulgarian Chemical Communications, Volume 57, Issue 3 (pp.166-169) 2025 DOI: 10.34049/bcc.57.3.TS-EO

Determination of fatty acids in plant oils by gas chromatography, production and analysis of solid soap from these oils

T. Sabudak*, E. Ozcan

Department of Chemistry, Tekirdag Namik Kemal University, 59030, Tekirdag, Turkey

Accepted: Accepted: August 17, 2024

Historically, humans have utilized various natural ingredients for medicinal and cosmetic purposes, favoring herbal cosmetics over synthetic alternatives. Given the potential for synthetic products to induce significant skin damage, natural herbs are increasingly incorporated into skincare formulations. This study focused on producing and analyzing soap *via* a cold process employing natural plant oils, and characterizing these oils using gas chromatography (GC). GC analysis revealed oleic acid (18:1), linoleic acid (18:2), and palmitic acid (16:0) as the predominant fatty acids in all oil samples. Notably, the produced natural solid soaps exhibited zero total alkali content. Based on total fatty matter values, the soaps were classified as Grade 1 and Grade 2, aligning with international standards.

Keywords: Plant oil, gas chromatography, fatty acid, natural solid soap, soap analysis.

INTRODUCTION

For centuries, plant-based preparations, including aromatic and fixed oils, have been used by various civilizations in religious rituals, for skin care, cleansing, and decoration, to enhance beauty and promote good health. There is a growing interest in cosmetic products derived from natural sources due to their beneficial effects compared to synthetic ingredients enriched with chemicals [1]. Also, the addition of herbal extracts into skincare formulations has numerous benefits, including anti-inflammatory, antioxidant, antiseptic, and antimicrobial activities [2]. Soap is one of the oldest treatment and cleaning agents in the world. The most useful soap for human health is made from natural vegetable oils without any chemicals [3]. The fact that natural soap prepared using natural products is more environmentally friendly and rich in antioxidants has become a promising alternative to soaps produced using synthetic chemicals [4].

The aim of this study is the production of soap using natural plant oils and characterization of the used oils by gas chromatography (GC). For this purpose, a cold saponification reaction was carried out without heating, using olive oil as the main raw material. In addition, some plant oils (coconut oil, castor oil, cocoa butter, shea butter, daphne oil, argan oil, meningic oil) were added to the reaction medium to increase the beneficial use of the soap on the skin. This study falls under the "Valuable Chemicals from Plant Sources" section within the framework of TUBITAK 2022-2023 Priority R&D and Innovation Topics and contributes to the development of more environmentally sensitive products and the production of valuable chemicals from plant sources [5].

EXPERIMENTAL

General experimental procedures

Chemicals used in this study and their suppliers: Daphne oil (MSA cosmetics), argan oil (MSA cosmetics), menengic oil (MSA cosmetics), castor oil (MSA cosmetics), olive oil (Simya), coconut oil (MSA cosmetics), cacao oil (MSA cosmetics), shea butter (MSA cosmetics), NaOH (Aromel Chemistry), NaCl (Aromel Chemistry), methyl orange (Zag Chemistry), Hydrochloric acid (Aromel Chemistry), dietylether (Tekkim-TK.05013001000), sodium sulfate (Sigma-Aldrich), ethanol (Isolab-920.052.5000), barium chloride (Kimyalab-V01719.901), phenolphthalein (Merck). Devices used in this study and their suppliers: Rotary evaporator (Büchi; B-491), distilled water device (Elga, DV25), ultrasonic bath (Wisd, WUC-D06H), gas chromatograph (Agilent 6890), blender (Fakir).

Determination of fatty acids in plant oils by gas chromatography

Gas chromatography method was used to determine the fatty acids in olive oil, daphne oil,

* To whom all correspondence should be sent:
E-mail: *tsabudak@nku.edu.tr*

argan oil and menengic oil used in soap making. First, the conversion of fats to methyl ester derivatives was performed. Fatty acid methyl esters were prepared as in [6]. The instrument Agilent 6890 series gas chromatograph was used for chromatographic analysis. HP-88 ((88% cyanopropyl) aryl polysiloxane, 100 m × 0.25 mm × 250 µm) capillary column was used. Operation conditions were as follows: the injector and detector temperatures were set at 250 and 280 °C, respectively. Manual injections were performed with a split ratio of 50:1. The temperature gradient was programmed as follows: an initial 11-min hold at 120°C, followed by a ramp to 175°C at 10°C/min, maintained for 10 min. Subsequent ramps included an increase to 210°C at 5°C/min (5-min hold) and a final ramp to 230°C at 5°C/min (5-min hold). Helium served as the carrier gas, delivered at 30 ml/min, with a 1 µl injection volume and a column head pressure of 54.6 kPa. The total run time was 50 min. Supelco 37 component FAME mix (10.000 mg/mL) was used as an internal standard. The fatty acids were identified by comparing their retention times to those of pure fatty acid methyl ester standards, and their amounts were determined as a percentage of the total fatty acid area, which includes all minor components.

Saponification reaction and soap formulations

In this study, natural solid soaps were synthesized, and formulations were developed to align properties like bubbling, cleansing, and lathering with public needs. For this purpose, a dedicated software (soapcalc.net) was utilized to formulate the final product. The software operates through a trial-and-error method. Oil types and mass percentages are input (Figure 1), and it calculates the corresponding soap's properties (bubbling, hardness, cleansing, conditioning, lathering, etc.) (Figure 1). Furthermore, the software program calculates the required alkali amount for the soap-making process by considering the SAP (saponification) values of oils, fats, and waxes. Figure 1 shows the software, which was used during this study. In solid soap production, the saponification reaction was carried out without heating in order not to impair the activity of the oils used in this method. Oils (olive oil, coconut oil, castor oil, cocoa butter, shea butter oil / argan oil / daphne oil / bıttım (menengic) oil) were melted by bain-marie method and treated with sodium hydroxide solution. After stirring, the mixture was poured into silicone molds and removed from the molds after 1 day. The resulting soap was kept on the shelf for about 4 weeks. In this part of the study, olive oil soap, daphne soap, argan soap and bıttım (menengic) soap were made (Figure 2).

SoapCalc © Recipe Name: E-A-KS-D:153 New INCI Names Print Recipe

Total oil weight	500 g	Sat : Unsat Ratio	42 : 58
Water as percent of oil weight	35.00 %	Iodine	56
Super Fat/Discount	6 %	INS	152
Lye Concentration	28.456 %	Fragrance Ratio	0
Water : Lye Ratio	2.5142:1	Fragrance Weight	0.00 g

	Pounds	Ounces	Grams
Water	0.386	6.17	175.00
Lye - NaOH	0.153	2.46	69.60
Oils	1.102	17.64	500.00
Fragrance	0.000	0.00	0.00
Soap weight before CP cure or HP cook	1.642	26.27	744.60

#	√	Oil/Fat	%	Pounds	Ounces	Grams
1	☐	Castor Oil	5.00	0.055	0.88	25.00
2	☐	Cocoa Butter	9.00	0.099	1.59	45.00
3	☐	Coconut Oil, 92 deg	28.00	0.309	4.94	140.00
4	☐	Olive Oil	42.00	0.463	7.41	210.00
5	☐	Shea Butter	9.00	0.099	1.59	45.00
6	☐	Argan Oil	7.00	0.077	1.23	35.00
		Totals	100.00	1.102	17.64	500.00

Soap Bar Quality	Range	Your Recipe		
Hardness	29 - 54	40	Lauric	13
Cleansing	12 - 22	19	Myristic	5
Conditioning	44 - 69	56	Palmitic	12
Bubbly	14 - 46	23	Stearic	9
Creamy	16 - 48	26	Ricinoleic	5
Iodine	41 - 70	56	Oleic	42
INS	136 - 165	152	Linoleic	9
			Linolenic	0

Fig. 1. Software used in soap formulations

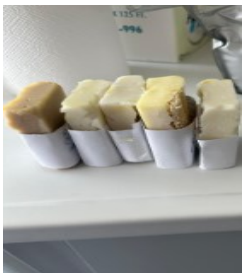

Fig. 2. Produced natural solid soaps

Analysis of produced natural solid soaps

- *Total free alkali:* 10 g soap was dissolved in 100 mL of ethanol and boiled in a water bath for 15 min. The flask was removed from the water bath and 10 % $BaCl_2$ solution was added slowly until no more precipitate was formed. Then 0.5 mL of 1 % phenolphthalein indicator was added to this solution and titrated with 0.1 N HCl acid solution. The total alkali content was calculated using the following formula [7].

$$\% \text{ Total free alkali} = [(V \times N) / m] \times (40/1000) \times 100$$

where V = volume of HCl, N = concentration of HCl, m = weight of soap.

- *Total fatty matter:* Approximately 10 g of powdered soap was diluted in 50 mL of distilled water and heated. Then, the soap solution was allowed to cool until it became clear. Two drops of methylorange (0.1 %) were added to the solution and titrated with 20 % HCl solution until the color turned red. The mixture was taken into an extraction flask and diethyl ether was added. The ether phase was washed with 10 % NaCl solution and dried over anhydrous Na_2SO_4. After removing Na_2SO_4 by

filtration, the ether phase was evaporated in a rotary evaporator and the fatty acids were dried in an oven at 70°C and weighed. Total fatty matter was calculated using the following formula [7].

$$\% \text{ Total fatty matter (TFM)} = (a \times b) \times 100 / m$$

where a=weight of the balloon with TFM, b= weight of the balloon, m= weight of soap.

RESULTS AND DISCUSSION

Determination of fatty acids in plant oils by gas chroatography

Gas chromatography was used to determine the fatty acids in olive, daphne, argan and menengic oils used in soap making. The obtained results are shown in Table 1. A total of 10 fatty acids were determined as a result of GC analysis of olive oil (Table 1).

Table 1. GC analysis results of olive oil, daphne oil, argan oil and menengic oil

No	Fatty acids	Peak area (%)			
		Olive oil	Daphne oil	Argan oil	Menengic oil
1	Lauric acid (12:0)	-	0.32	-	-
2	Myristic acid (14:0)	0.30	0.35	0.15	0.16
3	Palmitic acid (16:0)	14.78	18.50	13.46	11.58
4	Palmitoleic acid (16:1)	0.27	0.18	0.08	0.79
5	Stearic acid (18:0)	2.93	3.48	5.86	2.42
6	Oleic acid (18:1)	23.82	43.86	46.73	56.06
7	Linoleic acid (18:2)	54.44	29.64	32.66	27.91
8	Arachidic acid (20:0)	0.48	0.69	0.38	0.23
9	Linolenic acid (18:3)	2.29	0.29	0.06	0.22
10	Eicosenoic acid (20:1)	0.32	0.51	0.43	0.24
11	Behenic acid (22:0)	0.16	1.11	0.12	0.17
12	Lignoseric acid (24:0)	-	0.52	-	0.16
	Total saturated fatty acids (SFA)	18.65	25.47	19.97	14.78
	Total unsaturated fatty acids (UFA)	81.14	74.48	79.96	85.22

According to Table 1, the most abundant fatty acids are linoleic acid (54.44 %), oleic acid (23.83 %) and palmitic acid (14.78 %). A total of 12 fatty acids were determined by GC analysis of daphne oil (Table 1), the most abundant being oleic acid (43.86 %), linoleic acid (29.65%) and palmitic acid (18.50%). A total of 10 fatty acids were determined by GC analysis of argan oil (Table 1), the most abundant being oleic acid (46.74 %), linoleic acid (32.67 %), palmitic acid (13.46 %) and stearic acid (5.86 %). As a result of GC analysis of menengic oil, a total of 11 fatty acids were determined (Table 1), the most abundant being oleic acid (56.06 %), linoleic acid (27.91 %) and palmitic acid (11.59 %). According to these data, the highest unsaturated fatty acid content was determined in menengic oil (85.22 %) and the lowest was determined in daphne oil (74.48 %). The highest saturated fatty acid content was determined in daphne oil (25.47 %) and the lowest content in menengic oil (14.72 %). Oleic acid (18:1), linoleic acid (18:2) and palmitic acid (16:0) were found to be the main fatty acids with high levels in all oil varieties.

Analysis of total free alkali

Free alkali content is a critical parameter influencing soap abrasiveness. International Standards Organization (ISO) specifications mandate that soaps should contain less than 2% alkali [8, 9]. Free alkali in natural solid soaps was found 0.00 % in this study. This result proved that the sodium hydroxide used during the saponification reaction was used in the correct proportion and all of it reacted. Consumption of all of the sodium hydroxide used in the saponification reaction was considered to be a very good result.

Analysis of total fatty matter

Soap quality is significantly determined by its total fatty matter (TFM), a key metric consistently used in commercial transactions. While higher TFM often correlates with increased hardness, it generally indicates a lower quality product.

Notably, European and certain national standards classify soap based on TFM content, with Grade 1 requiring a minimum of 75% and Grade 2 a minimum of 65%. Soaps exhibiting higher total fatty matter (TFM) are known to produce richer lather, offer extended longevity, and, critically, provide superior and gentler skin cleansing [10, 8]. This study measured the TFM of soap samples and benchmarked these values against established standards (Table 2). The results indicate that the produced solid soaps align with Grade 1 and Grade 2 classifications [10, 8].

Table 2. Results of total fatty matter analysis in solid soap

Solid soaps	Total fatty matter (%)
Olive oil soap	72.53
Daphne soap	65.66
Argan soap	63.34
Menengic soap	81.00

CONCLUSION

In this study, natural solid soaps with high cleaning and foaming properties, free of chemicals and additives, were produced using natural plant oils (daphne oil, argan oil, menengic oil, olive oil, castor oil, coconut oil, cocoa butter, shea butter). Fatty acids of plant oils used in natural solid soap making were determined by gas chromatography (GC). According to the GC results, oleic acid, linoleic acid and palmitic acid were found to be the 3 fatty acids with the highest content in all plant oils. The solid soaps analyzed showed no detectable free alkali, and their total fat content was measured to be between 63% and 81%. These parameters are key indicators of soap quality and its suitability for cleansing purposes. From this, it can be concluded that the soaps produced have lower alkali content and higher TFM value. As a result, the soaps produced are of good quality (Grade 1 /2) soaps suitable for use for health and environment.

Acknowledgement: *The present study was supported by the Scientific Research Project Coordination Center (Project No: NKUBAP.01.YL.24.570).*

REFERENCES

1. K. B. Patkar, *Indian J. Plast Surg.,* **41**, 134 (2008).
2. F. Jahan, A. A. Happy, M. M. H. Chowdhury, M. A. Hossain, *J. Plant Sci.*, **7**, 86 (2019).
3. O. Goktas, B. Gıdık, *Bayburt Un. Fen Bi.l Dergisi,* **2**,136 (2019).
4. K. Rambabu, A. Achazhiyath Edathil, G. S. Nirmala, W. H. Shadi, A. F. Yousef, P. L. Show, F. Banat, *Environ. Tech. Innov.*, **20**, 101153 (2020).
5. Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) (2023). https://tubitak.gov.tr/sites/default/files/18842/arge_ve_yenilik_konu_basliklari_4.08.22.pdf
6. IUPAC Standard Methods for Analysis of Oils, Fats and Derivatives. Seventh ed. Blackwell Scientific Publications, IUPAC method 2, 3001, Report of IUPAC Working Group WG 2/87, 1987.
7. F. Sert, Osmaniye Korkut Ata Ün. Fen Bil. Enst., Osmaniye, 2021.
8. Bureau of Indian standards, DOC: CHD 20 (1861) C, May 2011.
9. The International standard specification for soaps, ISO 685: 1975.
10. K. J. Agents, B. M. Jilu, R. Fathima, J. T. Varkey, *Asian J. Sci. App. Tech.*, **2**, 8 (2013).