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Investigation of the effect of ultrasound-assisted extraction on the yield and tannin content of white oregano extracts

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Oregano and its extracts are rich in a variety of biologically active substances, which makes them suitable for use in food industry, pharmacy, *etc*. The influence of the technological parameters – solvent concentration, temperature and duration of ultrasound-assisted extraction on the yield and tannin content of extracts of white oregano (*Origanum heracleoticum* L.) was studied. The data were compared with those obtained with conventional extraction methods. The resulting ethanol extracts are highly viscous liquids with a dark brown color, characteristic of the plant spice smell and a specific burning taste. The highest yield and highest tannin content in ultrasound-assisted extraction of cultured white oregano was obtained with 70% ethanol at a temperature of 60°C during the process, hydromodule 1:10 and duration 60 min. The use of ultrasound-assisted impact to extract tannins from cultured white oregano allows to achieve higher yields and reduce the duration of the process, compared to conventional extraction methods.

Keywords: white oregano, ultrasound-assisted extraction, ethanol extracts, tannins.

INTRODUCTION

The plants produce diverse groups of secondary metabolites that may find application as additives or antioxidants in food production and in the pharmaceutical industry [1]. The extraction and purification of these metabolites from plant-based raw materials is a challenge for scientists to propose suitable methods that are relatively simple, safe and inexpensive [2].

In recent years, unconventional techniques have been used to obtain enriched plant extracts, such as ultrasound-assisted extraction, enzyme extraction and microwave extraction [3]. According to Michalaki *et al.* [4], the interest in ultrasound-assisted extraction is due to its positive impact on the extraction process of biologically active compounds (high yield of extract, low costs and short duration of the process). Ultrasound-assisted extraction has been used to extract valuable compounds such as proteins [5], carbohydrates [6], polysaccharide-protein complex [7], oils [8, 9], polyphenolic compounds [10], isoflavones [11], lycopene [12] and others.

White oregano (*Origanum heracleoticum* L.) is a herbaceous perennial of *Lamiaceae* family. In natural habitats in Bulgaria, it is widespread in the eastern rocky slopes of the Rhodopes, as well as in Belasitsa, Struma Valley and Kresna Gorge; the cultivated oregano is grown in Ruse, Pleven, Momchilgrad and Plovdiv regions [13]. White oregano and the extracts derived from it have proven antioxidant and antimicrobial properties due to the biologically active substances contained in them. The purpose of the study is to investigate the effect of ultrasound on the yield and composition of extracts of white oregano (*Origanum heracleoticum L.)*, as well as to establish the most appropriate parameters of the process, namely temperature and duration, affecting the degree of extraction of tanning agents [14–18].

The purpose of this study is to investigate the effect of ultrasound on the yield and content of tannins in extracts of white oregano (*Origanum heracleoticum* L.), as well as to establish the most appropriate parameters of the process - temperature and duration, affecting the degree of extraction of tannins.

EXPERIMENTAL

Plant material

The above-ground mass of Bulgarian cultivated white oregano (*Origanum heracleoticum* L.), purchased from the commercial network, 2021 harvest accompanied by a certificate for the main physical, chemical and microbiological indicators, ensuring the quality of the raw material, was used. Prior to analysis, the raw material was milled on a laboratory mill with a particle size of the 0.006 m predominant fraction, determined by sieve analysis [19].

Methods

The moisture content of the raw material (%) was determined by azeotropic distillation in a Dean and

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Stark laboratory apparatus [19]. The content of tanning agents in the raw material was determined by exhaustive extraction with hot water at boiling and titration of the extract obtained with 0.1 N $KMnO_4$ with an indigo carmine indicator, % [20]. Ethanol with concentrations of 50 and 70% was used as a solvent.

The extraction was carried out in an ultrasonic bath of model ELMA, Elmasonic P30H - Germany, with a frequency of 37 kHz at a hydromodule of 1:10, at temperatures of 30, 40, 50 and 60 °C and duration of 15, 30 and 60 min. After filtration, the solvent was separated from the solutions by its evaporation on a rotary vacuum evaporator at a water bath temperature of 60-65°C. Extracts were stored at 4 - 6°C until analysis.

To compare the yields and the content of tannins, an exhaustive extraction of the raw material without stirring was carried out with 70% ethanol at a temperature of 60°C (52.23 ± 0.48%). At an interval of 60 min the raw material was treated with pure solvent, the extracts were collected and the solvent was separated on a rotary vacuum evaporator. The content of tannin substances was determined in the extracts according to the methodology described above. The values of the studied technological parameters were chosen based on literature data and preliminary studies. All experiments were performed in triplicate, and the values in the tables and graphs are the arithmetic means presented with their standard deviation. The data obtained from the measurements and calculations were processed in MS Excel 2016 (Microsoft Corp.) at significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The analyzed raw material contains 12.7 ± 0.10 % moisture and 17.12 ± 0.12 % tanning agents. The data point out that the amount of tannins is by about 4% higher than the data published by Baycheva *et al.* [21] (13.84%), which can be explained with the different climatic conditions in different harvest years. The content of tannin substances in cultivated white oregano is lower than the data in the literature for wild white oregano of 20.6 to 22.5 % [21, 22], due to different origins.

The scheme of the experiments and obtained results is presented in Table. 1.

It is seen from the data that the extract yield increases by increase in the duration and temperature of the extraction with both solvents. Its amounts are higher when extracted with 70% ethanol (42.28 ÷ 64.25%).

Table 1. Extract yield of Bulgarian cultivated white oregano.

Duration, min	Temperature, °C	Yield relative to exhaustive extraction, %	
		50% ethanol	70% ethanol
30	15	34.41 ± 0.27	42.28 ± 0.39
	30	42.66 ± 0.36	44.08 ± 0.38
	60	44.32 ± 0.42	45.16 ± 0.33
40	15	40.49 ± 0.35	47.25 ± 0.42
	30	41.99 ± 0.33	47.84 ± 0.43
	60	44.41 ± 0.34	48.94 ± 0.41
50	15	40.14 ± 0.38	46.32 ± 0.43
	30	41.05 ± 0.39	51.48 ± 0.50
	60	43.61 ± 0.35	52.56 ± 0.50
60	15	45.21 ± 0.36	51.94 ± 0.50
	30	48.06 ± 0.42	58.62 ± 0.54
	60	50.01 ± 0.43	64.25 ± 0.61

The conducted studies revealed that lower yields of extract are obtained at different temperatures with duration of the process of 15 min; relatively higher values are found when the temperature is increased to 60 °C and the duration is extended to 60 min. A greater influence on the extract yield has the temperature of the process rather than its duration, confirmed in the literature [21]. According to literature data, the ethanol concentration also affects the extraction of ethanol [23]. Higher concentrations of ethanol (over 70%) get the extraction of biologically active substances (phenols) from oregano more complicated.

The content of tanning agents in the extracts of cultivated white oregano is presented in Table 2, and the degree of their extraction compared to the raw material is presented in Figs. 3 and 4. The content of tanning agents in the extract obtained during exhaustive extraction of cultivated white oregano (14.43 ± 0.01%) was determined - 85.5% compared to that in the initial material.

Table 2. Content of tannins in extracts of cultivated white oregano

No.	Temperature, °C	Duration, min	Tannins, %	
			50% ethanol	70% ethanol
1	30	15	5.4 ± 0.05	6.2 ± 0.05
2	30	30	6.4 ± 0.06	6.8 ± 0.06
3	30	60	7.5 ± 0.07	7.4 ± 0.07
4	40	15	5.9 ± 0.05	7.2 ± 0.06
5	40	30	6.7 ± 0.06	6.3 ± 0.06
6	40	60	7.2 ± 0.06	6.8 ± 0.06
7	50	15	7.4 ± 0.07	7.5 ± 0.07
8	50	30	7.5 ± 0.07	7.6 ± 0.07
9	50	60	8.8 ± 0.08	8.0 ± 0.07
10	60	15	8.3 ± 0.08	8.3 ± 0.08
11	60	30	9.2 ± 0.09	8.9 ± 0.08
12	60	60	9.4 ± 0.09	10.2±0.09

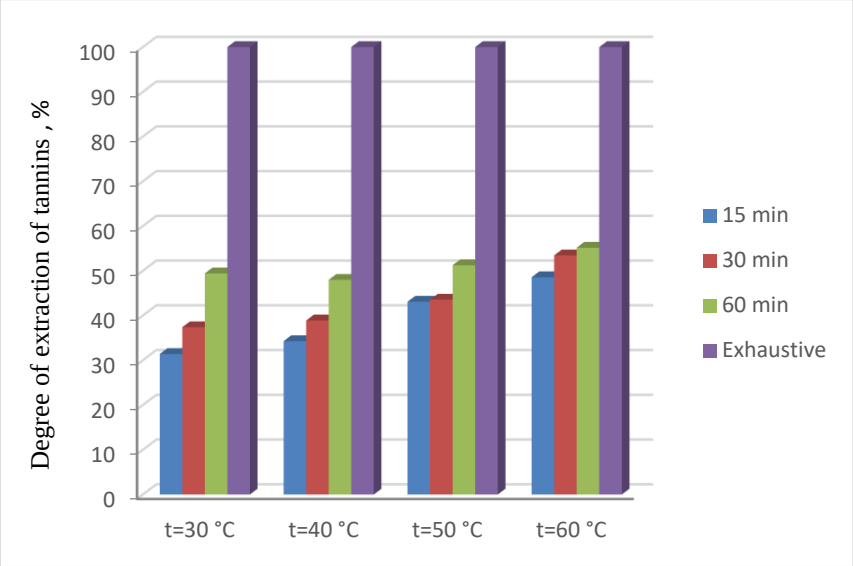

Fig. 1. Extraction rate of tannins at 50% ethanol extraction.

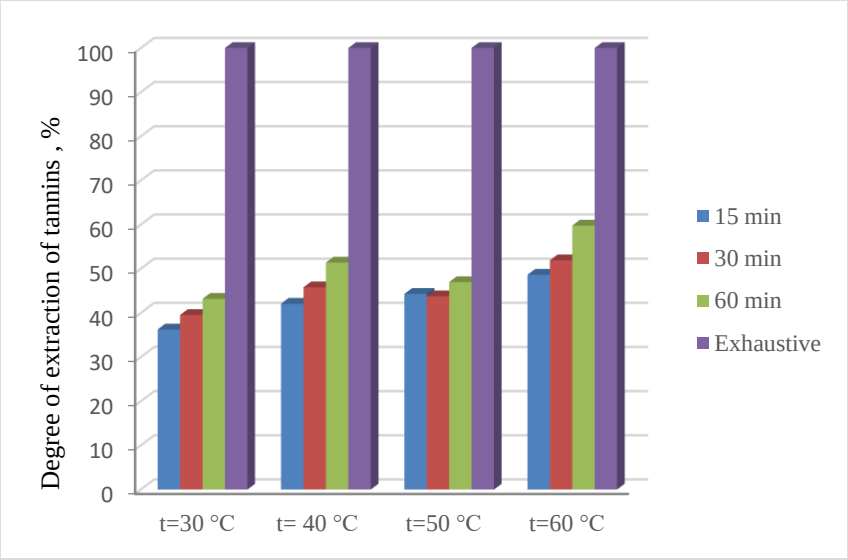

Fig. 2. Extraction rate of tannins at 70% ethanol extraction.

The data show that the content of tannins in the extracts obtained at 30°C and 40°C is relatively low: 5.4 ÷ 7.5% at 50% ethanol and 6.2 ÷ 7.5% at 70% ethanol. Higher values were reported at a temperature of 60°C (8.3 ÷ 9.4% at 50% ethanol and 8.3 ÷ 10.2% at 70% ethanol). The influence of the duration of the process on the content of tannins maintains the same trend as for extract yield. The amount of tannins in the extract obtained for 15 min was significantly lower compared to that at 60 min extraction. Similar values were reported for the content of tannins in the extracts obtained for 30 and 60 min extraction.

From the data presented in Figs. 3 and 4 is evident that at a temperature of 30°C, the degree of extraction of tannin substances from the output raw material is comparatively low (31.4 – 37.4% at 50% ethanol and 36.3 ÷ 43.02% at 70% ethanol). At 60°C, extraction of tannins over 50% was observed, and the highest values are accounted for extraction at 70% ethanol and 60 min process duration (52.1 %).

Similar results were obtained by Baicheva *et al.* [21] with extraction without ultrasound, hydromodule 1:10, temperature 60°C and duration of 6 h (57.88 %).

Similar dependencies for the influence of the temperature and duration of the process on the content of tannins in liquid extracts have also been found with other essential oil raw materials: cape

gooseberry leaves [24], thyme herb [25], rosemary herb [26], sage herb [27].

As a consequence of the cavitation in the ultrasound-assisted extraction, a greater dispersion of the solid phase in the liquid is achieved, resulting in a larger contact surface. In this way, higher yields are obtained in a shorter time with small amounts of solvent [28].

A comparison of the yields and tannin contents in ethanol extracts obtained by conventional extraction (without stirring) [29] and ultrasound-assisted extraction, under the same conditions: solvent concentration, duration, temperature and hydromodulus, indicates 30 – 35 % higher values at ultrasound-assisted extraction.

CONCLUSION

Highest ultrasound-assisted extraction yield of cultivated white oregano with 70% ethanol with the highest content of tannins is obtained at temperature of 60°C during the process, hydro module 1:10 and duration 60 min. The use of ultrasound to extract tannins from cultivated white oregano (*Origanum heracleoticum* L .) allows to achieve higher yields and reduce the process duration.

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