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# PRODUCTION AND ANALYSIS OF ESSENTIAL OIL OF THYME (*THYMUS VULGARIS* L.) OBTAINED BY PRESSURIZED HOT WATER EXTRACTION

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## ABSTRACT

Thyme herb (*Thymus vulgaris* L.) was processed by pressurized hot water extraction under the identical conditions- temperature of 170 ℃ and pressure. The aim is to study the dynamics of extracting the essential oil from the raw material. One experiment is on the raw material subjected to a single extraction, lasting up to 30 min, and in the other, thyme (*Thymus vulgaris* L.) was processed through a sixfold extraction, lasting 60 min. For comparison, a control sample was made using a Clevinger apparatus in order to determine the total content of essential oil in the raw material, as well as an analysis of the processed raw material to determine the unextracted essential oil. The essential oil extracted from the miscellas was also derived. The obtained essential oils were analysed on a gas chromatograph with a mass spectrometer (GC- FID- MS). The results show that with a single extraction for 30 min, the yield of dry matter is 26 %, while with a triple extraction with the same total extraction time, the integral yield of dry matter is 31 %. The analyses of the essential oils showed similar or higher extraction of the alcohols thymol and carvacrol in the oils isolated from the miscella after extraction with superheated water under pressure than those obtained by water distillation.

**KEYWORDS:** Green extraction, pressurized hot water extraction, Thyme, garden Thyme, essential oil.

## INTRODUCTION

Pressurized hot water extraction is an efficient, environmentally friendly method that produces chemically rich extracts.[17] According to the research of[11] the method is suitable for the extraction of alkaloids. Increasing the extraction temperature increases the yield, but has a negative effect on the thermolabile components. 140 ℃ for 15 min are sufficient to extract alkaloids, while a temperature of 180 ℃ for 10 min is required to obtain aflatoxins.[10] Pressurized hot water extraction is used to extract essential oils and polyphenols, and[18] reflecting the increased content of oxygen derivatives in the final products. According to[15] this method is suitable for extracting not only polar but also semi polar compounds.

Thyme (*Thymus vulgaris* L.) is used both as a spice in food processing industry and as a herb in medicine. In conventional medicine, it is used against microbial infections and gastronomic problems.[9] Several chemotypes are known, divided depending on the content of components in the essential oil: linalool, borneol, geraniol, sabinene hydrate, thymol and carvacrol. The yield of essential oil, investigated by water distillation is 1.25 % according to[2,16] found 45 components of the essential oil of thyme (*Thymus vulgaris* L.), which represent 96.75 % of the composition of the oil, while [7] found 33 substances in the essential oil, claiming that there was no difference in the composition of the oil and the extract. Main components are thymol (36.81 %) and p- Cymene (30.90 %),[5] while according to[13] the thymol content is 40.02 % and the second significant component is carvacrol (18.31 %).[1] compares the yields of essential oil from standard water distillation and microwave- assisted distillation. As a result, for 20 min. the assisted distillation gives a similar yield to that from the ordinary distillation for 3.5 h. With microwave- assisted water distillation, the yield of oxygen derivatives in the essential oil is higher than that obtained with standard methods.

The essential oil of thyme (*Thymus vulgaris* L.) has good biological activities [6]. Both alone and in combination with essential oils from other plants, such as cinnamon *(Cinnamomum zeylonicum),* juniper *(Juniperus communis),* spruce *(Picea abies),* lemon balm *(Melissa officinalis),* show strong antibacterial activity against Staphylococcus aureus and Escherichia coli.[8] Another significant importance of the oil is its antioxidant activity.[13] The essential oil of thyme (*Thymus vulgaris* L.) and its main component thymol shows a strong antifungal effect.[12] Its effect was investigated against Fusarium, Penicillium and Aspergillus,[4] as well as

against F. Oxysporum and D. Spicifera. At a concentration of 1600 µL/ L, fungal growth is completely neutralized.[5,3] processes thyme (*Thymus vulgaris* L.) with pressurized hot water. Compared to organic solvent extraction methods and traditional steam distillation, the modern eco- friendly extraction method provides an opportunity to obtain essential oil.

## MATERIALS AND METHODS

The entire aerial part of the thyme plant (*Thymus vulgaris* L.) was used for the experiments. The raw material was grown in Bulgaria, dried and packaged by Botanical EU Ltd., village of Cheshnegirovo, Plovdiv, Bulgaria. Using a Lanphan DHG9070A dryer, China, by drying to constant mass at 105 ℃, the moisture content of the raw material was determined by weight, as well as the total dry matter content of the miscella. In this study, the mass of the raw material, as well as all other samples of up to 200 g were weighed using a Kern ABS 220- 4N analytical balance, Germany, with an accuracy of 0.0001 g. Chemicals and reagents used were of analytical grade and were delivered by Merck. The analysis of the obtained essential oils was performed on a gas chromatograph (GC) with a flame ionization detector (FID) Agilent 6890 N, Agilent Technologies, USA Separation of the components was performed using an Agilent HP- 5 capillary column. Determination of the essential oil composition was performed by mass spectrometry (GC- MS) and using a NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library.

**The raw materials were processed under the following conditions**

Pressurized hot water extraction- dry thyme was subjected to it. The system for the "green" extraction of plant raw materials is fully automated, with a working volume of up to 2 l and a working temperature of up to 175 ℃. The equipment was developed and manufactured by Innosolv Ltd., Plovdiv, Bulgaria and is described in[14] Softened water was used as a solvent. Two experiments were conducted, in the first one a single extraction of the raw material was made, under the same conditions- a mass of 120 g and an extraction temperature of 170 ℃, six times with different durations from 5 min to 30 min. In the second experiment, a raw material with a mass of 120 g was extracted six times, with an extraction duration of 10 min and a temperature of 170 ℃. The miscella obtained from each extraction are designated respectively by the first letter A to F and the second letter O for those obtained from the single extraction and S for those from the sixfold extraction. All miscella were stored in tightly closed PET bottles in a refrigerator at a temperature of 0 to 5 ℃. Each one was analyzed separately for mass and concentration of total dry matter. This data is required for the following calculations:

Hydromodulus, $H_i$- the ratio of the mass of the miscella to the initial mass of the raw material. For the i- th extraction according to the following dependency

$$(1) \qquad H_i = \frac{M_i}{R}.$$

Where

$M_i$- the mass of the i- th miscella, kg.

R- the mass of the raw material, kg.

- Yield of extracted dry matter relative to the absolute dry mass of the raw material $Y_i$ for the i- th extraction is calculated according to the following dependency

$$(2) \quad Y_i = \frac{a_i.M_i}{DM.R}.100, \%.$$

Where

$a_i$- the concentration of dry matter in the i- th miscella, % of the total mass of the miscella. It is determined by weight method through drying.

DM- the content of dry matter in the raw material, % of the total mass of the raw material.

- Integral yield of extracted dry matter $\sum Y_j$ relative to the absolute dry matter in the raw material for the j- th extraction according to the following dependency

$$(3) \qquad \sum Y_j = \sum_{i=1}^{j} Y_i, \%$$

Traditional water distillation to determine essential oil content. The unprocessed thyme, the spent raw material from the extraction (waste) and all the obtained miscellas are subjected to it. The experiments were carried out on a Clevinger- type apparatus with a graduated receiver with an accuracy of 0.01 ml. The duration of the process is until the complete extraction of essential oil from the sample. The content of essential oil, EO, for the i- th sample is determined by the dependency

$$(4) \qquad EM = \frac{V_i}{G}.100, \%.$$

Where

$V_i$- the volume of the obtained essential oil from the i- th sample, ml,

G- the mass of absolute dry raw material, g.

The calculation of the integral yield of the obtained essential oil $\Sigma EO_j$, referred to absolute dry raw material, for the j- th experiment is based on the formula:

$$(5) \qquad \sum EM_j = \sum_{j=1}^{j} EM_j, \%.$$

All obtained results and measurements are presented as the arithmetic mean of triplicate measurements at a probability level of 0.95.

## RESULTS AND DISCUSSION

The dry matter content and the essential oil content of the raw material were measured. The results are as follows: 90.18 % ± 0.11- absolute dry matter in the raw material, 8.87 ml/ kg ± 1.46- the content of essential oil referred to absolute dry raw material.

**Results of a single pressurized hot water extraction.**
Six experiments were made with a raw material mass of 120 g, at a temperature of 170 ℃ and with different durations. In Table 1 summarizes the results.

**Table 1: Results of single extraction of Thyme.**

| № of extraction: | Indication of miscellas: | Duration of extraction, min: | Mass of miscella, g: | Solvent/ raw material ration, kg/ kg | Dry matter concentration in miscella, % |
|---|---|---|---|---|---|
| 1 | AO | 5 | 1400 | 11.7 | 0.780 ± 0.041 |
| 2 | BO | 10 | 1610 | 13.4 | 0.911 ± 0.009 |
| 3 | CO | 15 | 1604 | 13.4 | 1.342 ± 0.060 |
| 4 | DO | 20 | 1640 | 13.7 | 1.626 ± 0.041 |
| 5 | EO | 25 | 1715 | 14.3 | 1.471 ± 0.038 |
| 6 | FO | 30 | 1710 | 14.3 | 1.649 ± 0.028 |

In the Fig. 1., the yield of dry matter, referred to the absolute dry mass of the raw material, is shown graphically.

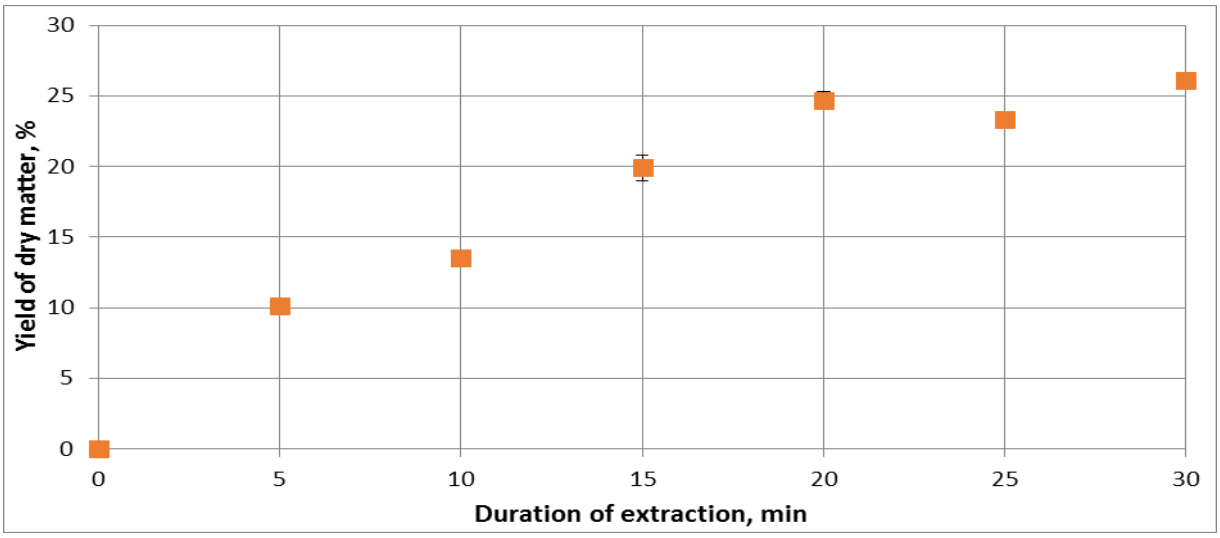


**Figure 1: Dry matter yield % related to totally dry raw material of one time extracted Thyme shown for every single extraction.**

**Results of a sixfold pressurized hot water extraction**
Six experiments were made on the same raw material having a weight of 120 g and the following parameters: a temperature of extraction of 170 ℃ and a duration of each extraction of 10 min. The results are presented in Table 2.

**Table 2: Results of sixfold extraction of Thyme.**

| № of extraction: | Indication of miscellas: | Duration of extraction, min: | Mass of miscella, g: | Solvent/ raw material ration, kg/ kg | Dry matter concentration in miscella, % |
|---|---|---|---|---|---|
| 1 | AS | 10 | 1055 | 8.8 | 1.482 ± 0.009 |
| 2 | BS | 10 | 1130 | 9.4 | 0.940 ± 0.014 |
| 3 | CS | 10 | 1160 | 9.7 | 0.617 ± 0.020 |
| 4 | DS | 10 | 1130 | 9.4 | 0.463 ± 0.020 |
| 5 | ES | 10 | 1300 | 10.8 | 0.276 ± 0.027 |
| 6 | FS | 10 | 1160 | 9.7 | 0.128 ± 0.031 |
| **Summary:** | | 60 | 6935 | 57.8 | |

The obtained results for the differential and integral yield of dry matter, referred to the absolute dry mass of the raw material and giving a graphic idea of the dynamics of extraction, are presented respectively in Fig. 2. and Fig. 3.

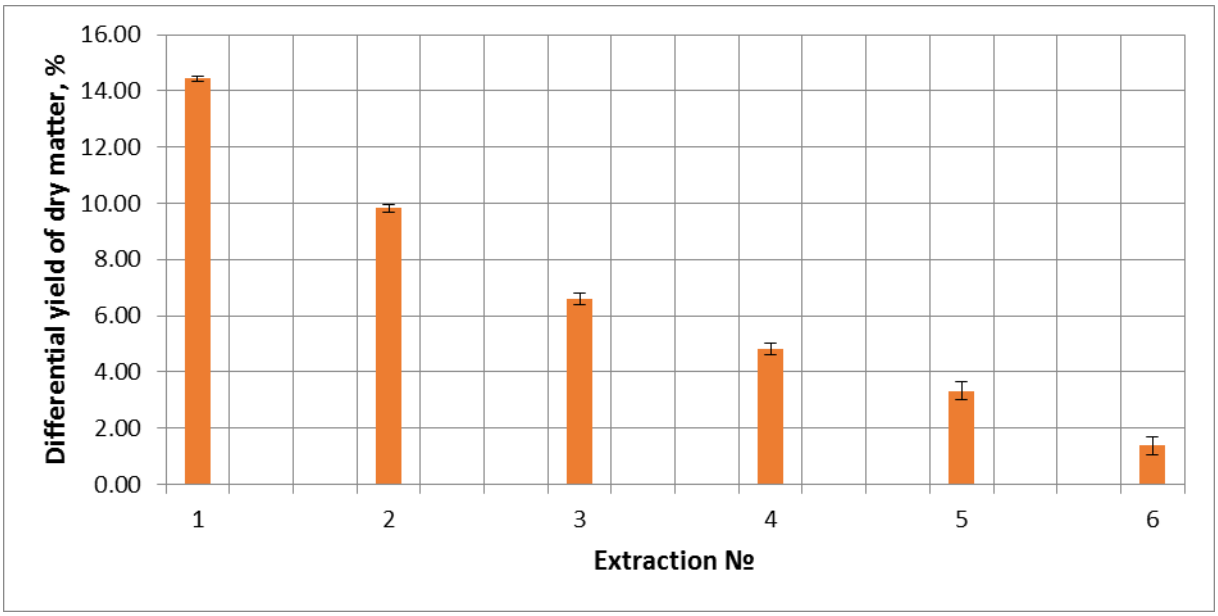


**Figure 2: Differential yield of dry matter % related to totally dry raw material of six time extracted Thyme.**

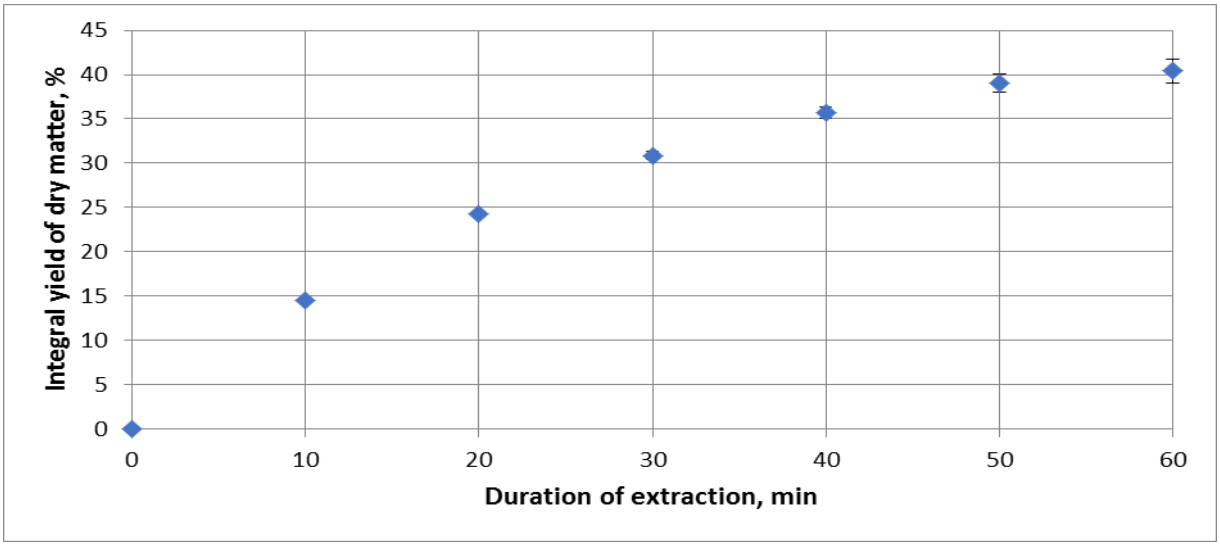


**Figure 3: Integral yield of dry matter % related to totally dry raw material of six time extracted Thyme.**

**Results for the obtained essential oil (EO) from a single extraction.**

The six miscellas were examined for extracted essential oil and the waste from each extraction for residual quantity of oil in the spent raw material. The essential oil yields are referred to the absolute dry matter in the raw material. The results are shown in Table 3. and Table 4.

**Table 3: Results of extracted essential oil from miscellas of single extraction.**

| Miscella № | Extracted dry raw material, g: | Mass of miscella, g: | Extracted essential oil, ml: | Yield of essential oil, ml/ kg: | Extraction degree, %: |
|---|---|---|---|---|---|
| AO | 108.2 | 1400 | 0.28 | 2.59 ± 0.65 | 29.2 |
| BO | 108.2 | 1610 | 0.48 | 4.46 ± 0.74 | 50.3 |
| CO | 108.2 | 1604 | 0.42 | 3.85 ± 0.00 | 43.4 |
| DO | 108.2 | 1640 | 0.39 | 3.64 ± 0.76 | 41.0 |
| EO | 108.2 | 1715 | 0.34 | 3.17 ± 0.00 | 35.7 |
| FO | 108.2 | 1710 | 0.48 | 4.42 ± 0.79 | 49.9 |

**Table 4: Results of unextracted essential oil left in the waste.**

| Waste № | Extracted dry raw material, g: | Mass of waste, g: | Extracted essential oil, ml: | Yield of essential oil, ml/ kg: | Undetermined losses, %: |
|---|---|---|---|---|---|
| AO | 108.2 | 370 | 0.287 | 2.66 ± 0.86 | 40.9 |
| BO | 108.2 | 375 | 0.209 | 1.93 ± 0.00 | 27.9 |
| CO | 108.2 | 325 | 0.220 | 2.03 ± 0.78 | 33.7 |
| DO | 108.2 | 385 | 0.140 | 1.29 ± 0.00 | 44.4 |

In the Fig. 4. and Fig. 5., the results for the yield of essential oil, referred to absolute dry raw material, respectively from the miscellas and the waste, are graphically presented.

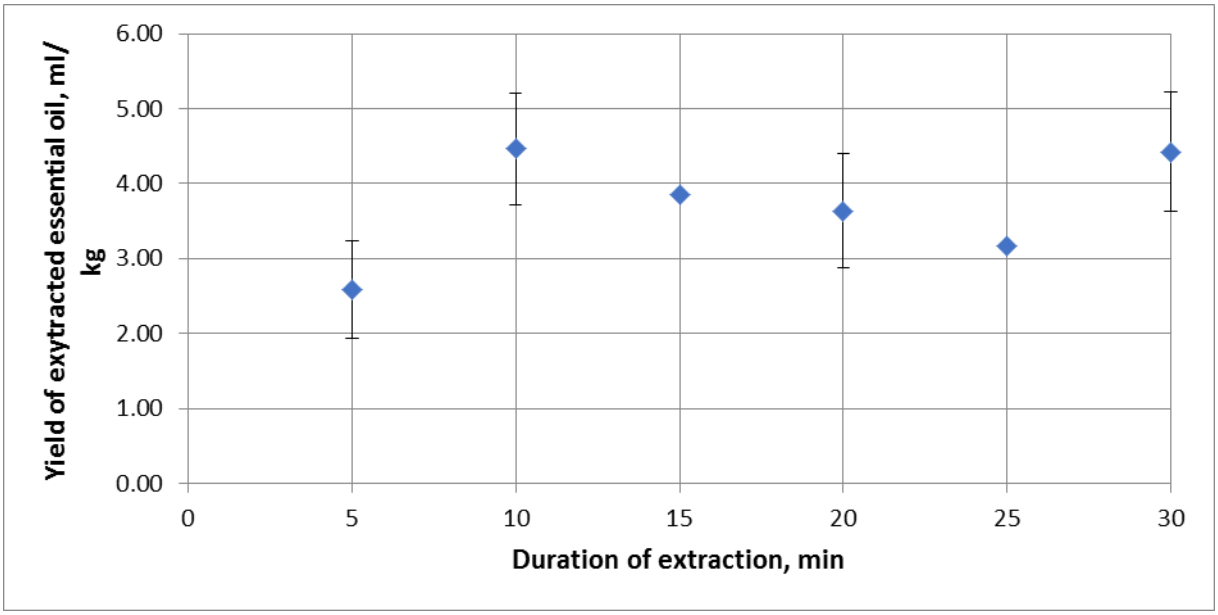


**Figure 4: Yield of extracted essential oil from miscellas of single extraction.**

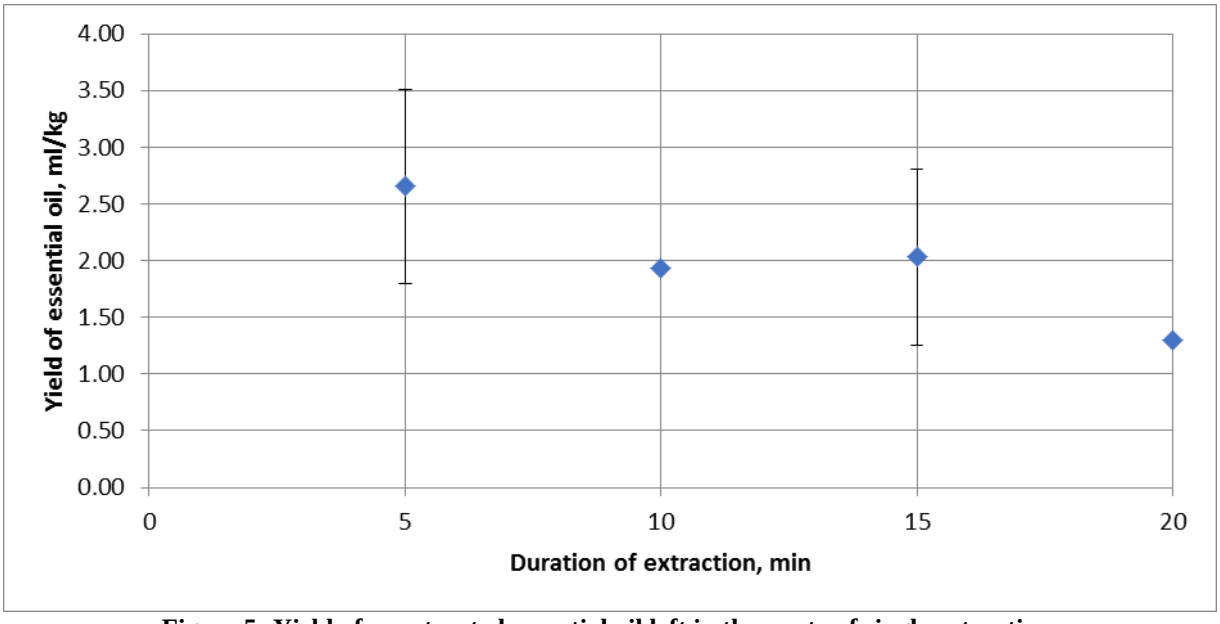


**Figure 5: Yield of unextracted essential oil left in the waste of single extraction.**

**Results for the obtained essential oil (EO) from a sixfold extraction.**

In Table 5., the results of the analysis of the extracted essential oil from each separate miscella by the sixfold extraction are shown.

**Table 5: Results of extracted essential oil from miscellas and waste from sixfold extraction.**

| Miscella № | Extracted dry raw material, g: | Total mass, g: | Extracted essential oil, ml: | Differential yield of EO, ml/ kg: | Extraction degree, %: |
|---|---|---|---|---|---|
| AS | 108.2 | 1055 | 0.250 | 2.34 ± 0.23 | 26.4 |
| BS | 108.2 | 1130 | 0.230 | 2.16 ± 0.13 | 24.3 |
| CS | 108.2 | 1160 | 0.150 | 1.43 ± 0.13 | 16.1 |
| DS | 108.2 | 1130 | 0.080 | 0.77 ± 0.13 | 8.6 |
| ES | 108.2 | 1300 | 0.070 | 0.64 ± 0.13 | 7.3 |
| FS | 108.2 | 1160 | 0.050 | 0.43 ± 0.00 | 4.8 |
| Waste | 108.2 | 360 | 0.139 | 1.28 ± 0.00 | 14.5 |

In the Fig. 6., the differential yield of essential oil from the miscellas of a sixfold extraction of thyme is graphically presented. The integral yield of essential oil is shown in Fig. 7., where the presence of essential oil in the unprocessed raw material is indicated as an upper limit.

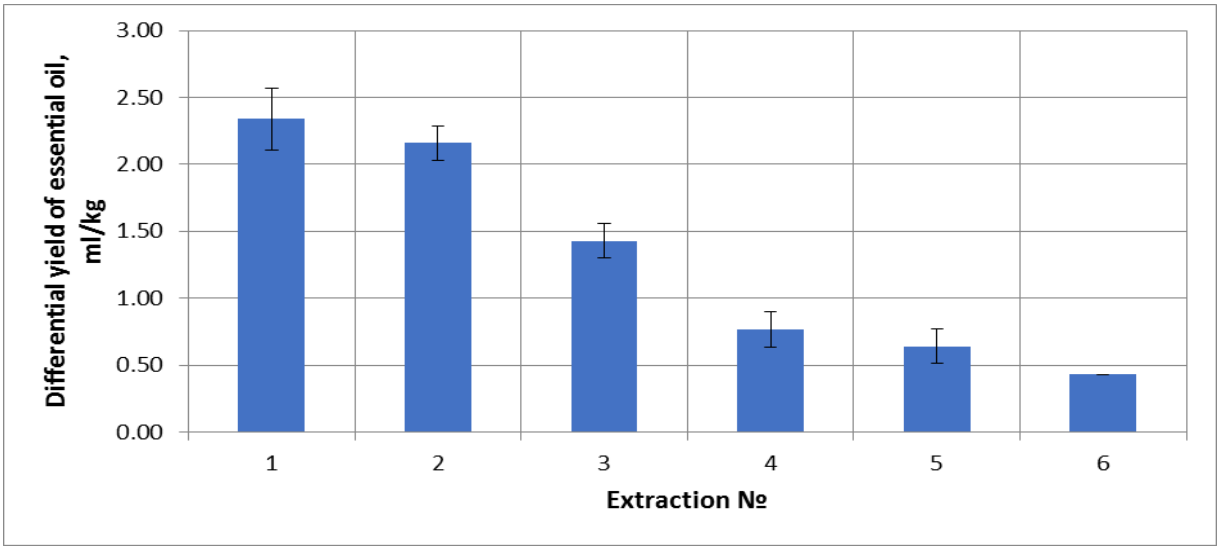


**Figure 6: Differential yield of essential oil from miscellas of sixfold extraction.**

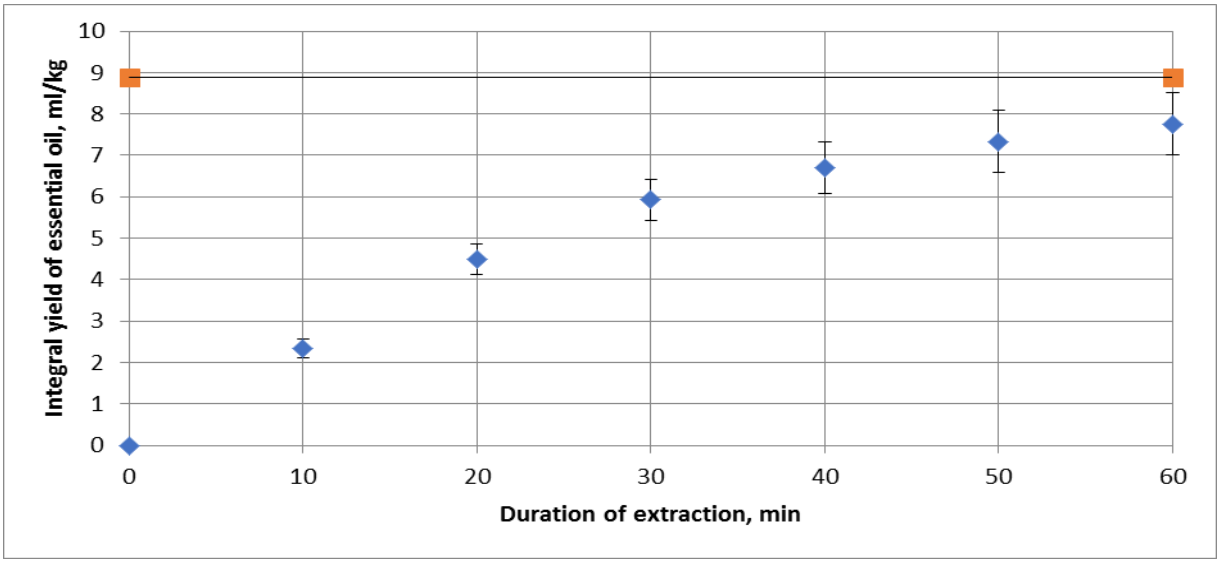


**Figure 7: Integral yield of essential oil from miscellas of sixfold extraction.**

**Results of the analyses of the obtained essential oils.**
A chromatographic analysis of the obtained essential oils was performed. In Table 6., the results of oil components obtained by a single pressurized hot water extraction (OTE), sixfold pressurized hot water extraction (STE) and traditional hydro distillation (HD) are reported.

**Table 6: Results of analyses of composition of essential oils.**

| Name of compound | % of TIC | | |
|---|---|---|---|
| | OTE | STE | HD |
| α- Pinene | 0.08 | 0.06 | 0.07 |
| Camphene | 0.13 | 0.18 | 0.27 |
| β- Pinene | 0.35 | 0.30 | 0.09 |
| (3E)- Octen- 2- ol | 0.10 | nd | 0.18 |
| α- Terpinene | 0.26 | 0.15 | 0.47 |
| p- Cymene | 4.18 | 3.81 | 8.61 |
| Limonene | 0.10 | 0.05 | 0.18 |
| Eucalyptol | 0.53 | 0.33 | 0.46 |
| γ- Terpinene | 1.77 | 0.89 | 3.61 |
| trans- Sabinene hydrate | 0.15 | 0.14 | 0.47 |
| β- Linalool | 0.68 | 0.48 | 1.18 |
| Camphor | 0.17 | 0.15 | 0.11 |
| Borneol | 0.56 | 0.46 | 0.78 |
| Terpinen- 4- ol | 0.81 | 0.40 | 0.57 |
| α- Terpieol | 0.11 | 0.13 | 0.43 |
| γ- Terpineol | 0.73 | 0.84 | 0.18 |
| Thymol, methyl ether | 0.66 | 0.55 | 0.89 |
| Carvacrol, methyl ether | 0.52 | 0.40 | 0.63 |
| Carvenone | 0.61 | 0.45 | 0.32 |
| Thymol | 77.88 | 81.82 | 69.65 |
| Carvacrol | 5.36 | 5.16 | 3.99 |
| α- Copaene | nd | nd | 0.08 |
| β- Bourbonene | 0.13 | 0.11 | 0.07 |
| β- Caryophyllene | 1.52 | 1.19 | 2.80 |
| α- Caryophyllene | 0.09 | 0.06 | 0.10 |
| Geranyl propanoate | 0.13 | 0.08 | 0.15 |
| γ- Muurolene | 0.11 | 0.07 | 0.24 |
| α- Muurolene | 0.09 | 0.05 | 0.13 |
| γ- Cadinene | 0.22 | 0.12 | 0.36 |
| δ- Cadinene | 0.33 | 0.16 | 0.49 |
| (Z)- Calamenene | 0.10 | 0.09 | 0.14 |
| Caryophyllene oxide | 0.30 | 0.13 | 0.78 |
| 10- epi- γ- Eudesmol | 0.13 | 0.11 | 0.18 |
| tau.- Cadinol | 0.26 | 0.18 | 0.30 |
| α- Cadinol | 0.15 | 0.10 | 0.11 |
| 14- hydroxy- (Z)- Caryophyllene | 0.12 | 0.08 | 0.14 |

In Fig.8., Fig. 9. and Fig. 10., the chromatograms from the analyses of the obtained essential oils are shown.

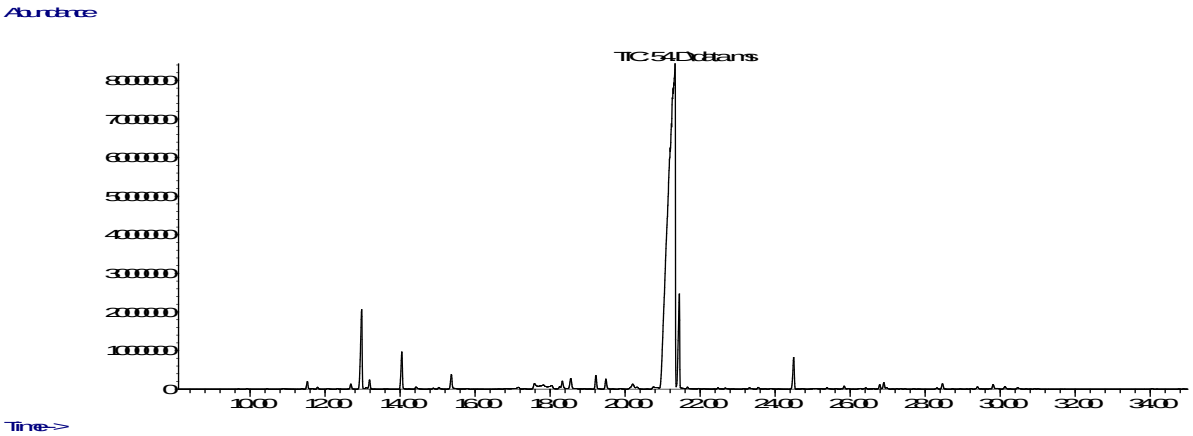


**Figure 8: Result of analysis of essential oil extracted by single extraction.**

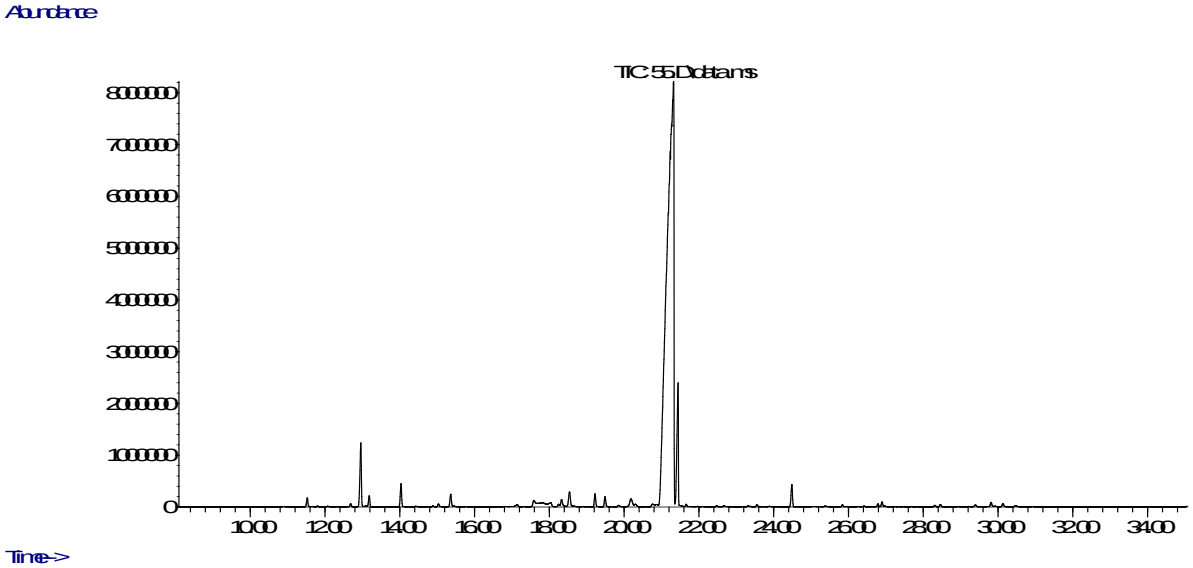


**Figure 9: Result of analysis of essential oil extracted by sixfold extraction.**

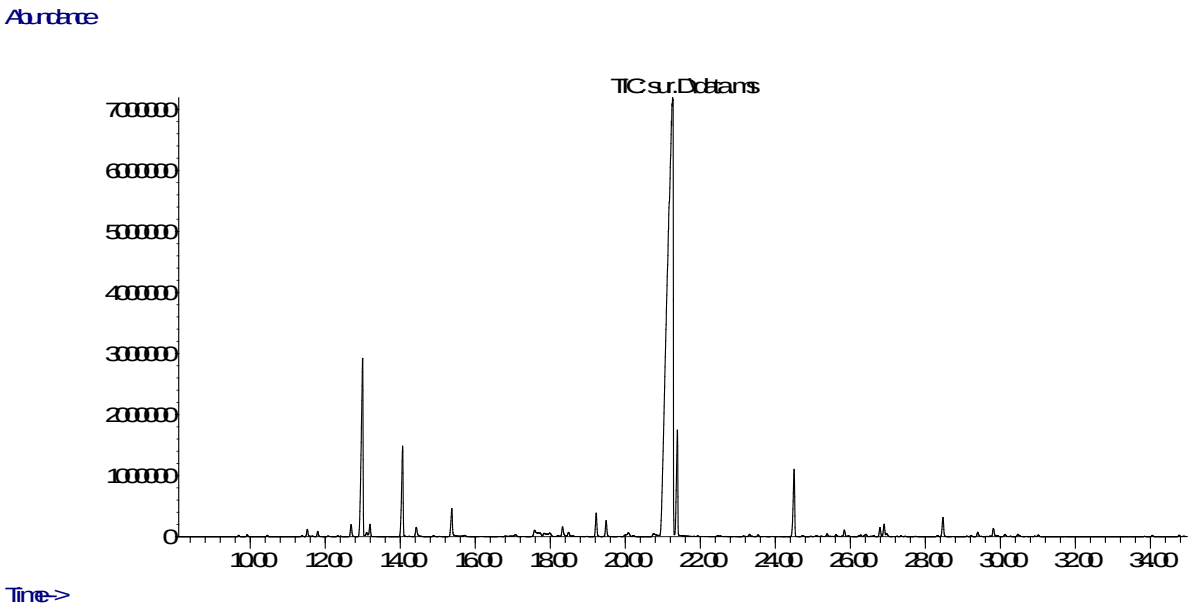


**Figure 10: Result of analysis of essential oil extracted by hydro distillation.**

## DISCUSSION

The obtained results show that when processing thyme (*Thymus Vulgaris* L.) by a single pressurized hot water extraction at a temperature of 170 ℃ and a duration of 30 min, the yield of dry matter, referred to the absolute dry mass of the extracted raw material is 26.05 % (Fig. 1.), while with a sixfold extraction and three changes of the solvent, with a total duration of 30 min, the integral yield of dry matter was 30.88 % (Fig. 3.). With six changes of the solvent and a total duration of 60 min, the total yield increases to 40.40 % of the absolute dry mass of the raw material (Fig. 3.). This is explained by the saturation of the solvent in the single extraction and obeys Fick's law. The investigation of the miscellas for extracted essential oil from a single extraction showed similar results at a duration of 10 and 30 min, respectively 50.3 % and 49.9 % degree of extraction (Table 3., Fig. 4.), compared to the total content of essential oil in the unprocessed raw material (8.87 ml/ kg). The results of the experiments with the waste from the single extraction show a tendency to decrease the content of unextracted essential oil from the raw material (Fig. 5.). With a sixfold extraction, the yield of essential oil tended to decrease with each subsequent miscella (Fig. 6.). The integral yield of oil after six extractions was 7.76 ml/ kg (Fig. 7.), which is 87.5 % of the total content of essential oil in the raw material. The analysis of the oil extracted by water distillation of the fresh raw material shows a high content of Thymol- 69.65 %, p- Cymene- 8.61 %, Carvacrol- 3.99 %, γ- Terpinene- 3.61 %, β- Caryophyllene- 2.80 % (Fig. 10.). The essential oils obtained from the miscellas by pressurized hot water extraction at 170 ℃ have a higher content of Thymol (11.81- 17.47 %) and Carvacrol (29.40- 34.41 %) (Fig. 8. and Fig. 9.). This can be explained by the high extraction temperature of 170 ℃ compared to that of water distillation, which is 100 ℃.

The content of the other macro components in the oil is lower compared to the control sample. The analysis of the oil obtained from a single extraction and compared to that of the oil obtained from a sixfold extraction shows a higher percentage and degree of extraction of the macro components.

## CONCLUSION

Pressurized hot water extraction is a suitable method for extracting essential oils with a high content of oxygen derivatives. The degree of extraction is 87.5 %, the yield of oxygen derivatives is higher compared to that of conventional hydro distillation. The method is faster than the conventional methods for obtaining essential oils.

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