Bulgarian Chemical Communications, Volume 56, Special Issue D2 (pp. 71-77) 2024 DOI: 10.34049/bcc.56.D.S2P44

Characterization of by-products from industrial processing of the essential oil crops rose, hyssop and thyme

A. M. Slavov, G. I. Marovska*

University of Food Technologies, Department of Organic Chemistry and Inorganic Chemistry, 26 Maritsa Blvd., 4000 Plovdiv, Bulgaria

Received: November 3, 2023; Revised: April 11, 2024

Bulgaria is rich in medicinal and aromatic plants and has very well developed essential oil industry. Processing of the essential oil-bearing plants results in obtaining several main products: essential oil, water, absolute, concrete and different extracts, having applications in perfumery, cosmetics, medicine, wellness, tourism, etc. The low content of essential oil in the plants is a prerequisite for generation of large volumes of by-products. The aim of the present study is to investigate three by-products: from rose (*Rosa damascena* Mill.) – provided by Old Rose Distillery, Strelcha, Plovdiv, Bulgaria, 2020; hyssop (*Hyssopus officinalis*) – provided by Galen-N Ltd., Zelenikovo, Brezovo, Bulgaria, 2020; and thyme (*Thymus serpillum*) – provided by EKOMAAT Ltd., Mirkovo, Sofia, Bulgaria, 2020. The three solid by-products resulted from industrial steam distillation of the raw materials. It was found that the residues were rich sources of dietary fibers: 66.77±1.08%, 62.28±1.15%, and 79.94±1.11% for rose, hyssop and thyme, respectively. The by-products were subjected to extraction with hot 70% ethanol and ethanolic extracts and alcohol-insoluble parts (AIP) were obtained. The polyuronide content (PU) of the AIP by-products was in the 4.03±0.24 – 8.89±0.14% range and the degree of esterification: 59.41±2.52 – 86.05±1.25%. The AIPs were extracted employing hot 0.1M HCl and pectic polysaccharides with 12.45±0.14%, 7.19±0.19%, and 7.31±0.23% yield were obtained for the rose, hyssop and thyme, respectively. The results from the present study suggest that the by-products from the essential oil industry, namely rose, hyssop and thyme, could be successfully valorized and serve as a source of dietary fibers and pectic polysaccharides.

Keywords: *Rosa Damascena* Mill., *Hyssopus officinalis*, *Thymus serpillum*, dietary fibers, pectic polysaccharides

INTRODUCTION

Bulgaria is among the countries rich in essential oil-bearing medicinal, edible and aromatic plants, having wide application in cosmetic, perfumery, food industry, tourism, medicine, as well as in culinary technology. Among the most famous are rose and lavender, for which the country holds one of the first places in terms of production and quality of the obtained products [1]. Moreover, a large number of other essential oil-bearing and medicinal plants, herbs and spices, such as: melissa, basil, chamomile, hyssop, thyme, lemon balm, yellow horned poppy, yarrow, etc., although industrially processed in a smaller scale, are recently gaining popularity.

The rose has been among the flowering plants most appreciated by humankind since ancient time. Its significance lies in socio-cultural life, decorative usage, symbol of appreciation, but roses are also a rich source of biologically active substances, and they are widely used by the essential oil industry. Important products obtained industrially from oil-bearing roses are aroma products: essential oil, water, absolute, etc. The most traditional processing of roses is steam-water distillation (about 90% of the plant material processed), followed by production of rose concrete and absolute through non-polar solvent extraction (5-6%), and use of the remaining 3-4% for making rose water [1]. A relatively new treatment is supercritical CO_2 extraction [2]. On average, the essential oil amount in fresh *Rosa damascena* flowers is in the 0.030-0.045 % range [1]. For the *Rosa centifolia* and *Rosa alba*, these amounts are even lower: 0.02 % [3] and 0.015-0.030 % [4], respectively. About 3500-4000 kg of fresh *Rosa damascena* flowers are needed for every kilogram of rose oil, and approximately 2 kg of wet wastes are generated from 1 kg of the initial rose mass [5]. The residues generated are usually discarded but they could serve as a starting material for obtaining valuable substances, such as dietary polyphenols, polysaccharides, etc.

Thyme (wild thyme, *Thymus serpyllum*) is well known as a medicinal plant - for making syrups, tinctures, infusions, etc. Besides, the thyme is used for production of essential oil [6]. The main biologically active substances are thymol and carvacrol, but the plant is also rich in 1,8-cineole,

* To whom all correspondence should be sent:
E-mail: gerii_vlaseva@abv.bg

myrcene, β-caryophyllene, germacrene, etc. [7]. It has been found to possess antiseptic, antimicrobial, antitumor and cytotoxic properties, and well-expressed antioxidant activity [8, 9].

Medicinal hyssop (*Hyssopus officinalis*) is a subshrub native to the Mediterranean, North Africa, Middle East, Europe, and North America. The plant is used as a seasoning for salads, meats, soups, and sauces. Hyssop is a component in the production of some liqueurs and absinthe [10]. The plant and its essential oil has pronounced antifungal [11], antimicrobial [12], and antioxidant activity [13].

The economic growth and the increase in the areas of new or forgotten essential oil crops and production of essential oils is also accompanied by an increase in the amount of distilled plant biomass. Essential oil manufacturers are constantly looking for different technological solutions for reduction and utilization of generated plant production waste from the essential oil industry. The aim is, on the one hand, to reduce the cost of the main products of the essential oil industry. Another ecological point of view aims to reduce the impact on the environment by reducing the amount of landfill generated biodegradable waste. This can be achieved by using the distilled biomass as a raw material for obtaining secondary products.

MATERIALS AND METHODS

The solid by-products from essential oil industry were kindly provided by:

1). Old Rose Distillery, Strelcha, Plovdiv, Bulgaria, 2020 harvest; resulting from steam-water distillation of rose (*Rosa damascena* Mill.) – abbreviated as 20RD_SD_S;

2). Galen-N Ltd., Zelenikovo, Brezovo, Bulgaria, 2020 harvest; from steam distillation of hyssop (*Hyssopus officinalis*) – abbreviated as 20HO_SD_Z;

3). ECOMAAT Ltd., Mirkovo, Sofia, Bulgaria, 2020 harvest; from steam distillation of thyme (*Thymus serpillum*) – abbreviated as 20TS_SD_M.

Immediately after the end of distillation and the distillation stills were opened, the solid residues were cooled down, checked for impurities (weeds, insects, minerals, etc.) and collected. The biomass was dried in a laboratory drier at 50°C and stored at ambient temperature until further treatment.

All the reagents used for the analyses were of analytical grade and were provided by the local suppliers.

Water-ethanolic extracts of the solid residues were prepared according to [14]. The alcohol-insoluble parts (AIP) from the extraction were further used for extraction of pectic polysachharides.

The acidic extraction of AIP was performed as follows: 70 g of AIP was extracted with 1.4 L of 0.1 M HCl (pH 1.2) at 90°C for 1 h with constant stirring (100 rpm); the mass was filtered through nylon cloth (250 mesh) and the residue was subjected to a second extraction with 1 L of 0.1 M HCl at 90°C for 1 h. The mass was filtered and both filtrates were combined. The filtrate was precipitated with 3 volumes of 96% ethanol for 24 h at 4°C, and the precipitate was filtered through nylon cloth (250 mesh) and dried. The obtained polysaccharides were dissolved in 100 mL of distilled water and dialyzed (Spectra/Por 1, Breda, the Netherlands, Mr. cut-off 6–8 kDa) for 72 h against deionized water. The remaining solution in the dialysis membrane was freeze-dried and denoted as acid extractable polysaccharides.

The moisture content of the by-products was determined with a Kern MLB50-3 moisture analyzer (Kern&Sohn, Germany). The amount of crude protein in the by-products was determined by the Kjeldahl method with an automatic nitrogen analyzer UDK152 (Velp Scientifica, Italy) with a correction factor of 6.25. The ash content was estimated after incineration of the by-products at 605°C in a muffle furnace (MLW 212.11, MLW, Germany) to a constant weight. The degree of esterification (DE, %) and the polyuronide content (PU, %) were determined according to [15].

The amount of total, insoluble and soluble dietary fibers in the by-products were determined using K-TDFR-100A (Megazyme, Ireland), according to AOAC method 991.43 "Total, soluble and insoluble dietary fibers in foods" (First action 1991) and AACC method 32-07.01 "Determination of soluble, insoluble and total dietary fibers in foods and food products" (Final approval 10-16-91) [16].

The polysaccharides' protein content, amount of neutral sugars, and anhydrogalacturonic acid content were determined spectrophotmetrically, employing the Bradford method using AMRESCO E535-KIT (AMRESCO, Solon, Ohio, USA) with bovine gammaglobulin as standard, by the phenol-sulfuric acid method with D-galactose as standard, and by the m-hydroxydiphenyl method using D-galacturonic acid as standard, respectively, as described by Slavov *et al.* [17]. The molecular weght of the isolated polysaccharides was determined using an ELITE LaChrome (Hitachi) HPLC system with a VWR Hitachi Chromaster 5450 refractive index detector, and an OHpak SB-806M (Shodex ®) column. The samples and standards were eluted with 0.1 M $NaNO_3$ at an elution rate of 0.8 mL/min, column temperature 30°C, and detector temperature 35°C. The column was equilibrated with Shodex pullulan (Showa DENKO, Japan) standards (2

mg/mL) with molecular weights of 6.2×10^3, 10.0×10^3, 21.7×10^3, 48.8×10^3, 113.0×10^3, 200.0×10^3, 366.0×10^3, and 805.8×10^3 Da.

The degree of methylesterification (DM) and the degree of acethylation (DAc) of the isolated polysaccharides were determined as described by Slavov *et al.* [17].

The thermal properties of the polysaccharides were investigated employing differential thermal analysis-thermogravimetry (DTA-TG) with TGA Q50 (TA Instruments, Alzenau, Germany). The pectin sample (9.1480 mg, 9.5150 mg and 11.0040 mg for 20RD_SD_S, 20HO_SD_Z and 20TS_SD_M, respectively) was heated in air (air speed 20 mL/min) in a corundum crucible over the 30–550°C range with a rate of 5°C/min. The cooling was performed with 20°C/min rate from 550°C to 30°C.

Statistical analysis. The values were expressed as mean of three replicates ± SD. Statistical significance was detected by analysis of variance (ANOVA, Tukey's test; value of $p < 0.05$ indicated statistical difference).

RESULTS AND DISCUSSION

The general characteristics of the by-products are presented in Table 1. The analys

es carried out to determine the main physicochemical parameters of the by-products (Table 1) suggested that rose had almost twice as high content of mineral substances (similar data were also obtained in the study of other by-products, for example, for lemon balm [18]) in comparison with thyme and hyssop. Wild thyme (20TS_SD_M) had the lowest protein content: 6.82±0.46%, twice less than the other two by-products. The polyuronide content in 20HO_SD_Z and 20TS_SD_M was of the same order: around 5%, and 20RD_SD_S was distinctive with the highest content (8.89±0.14 %). In the following analyses, the dietary fibers content was investigated. Separate analyzes were performed to determine total and soluble and insoluble dietary fibers. From the results it could be concluded (Table 1) that the amounts of insoluble dietary fibers predominated (thyme showed the highest content with 75.79±1.12%), and the soluble dietary fibers amount was comparable with the data for PU (Table 1), which corresponded roughly to the amount of polyuronides (pectin-type polysaccharides). The total dietary fibers content suggested that these by-products from the essential oil industry, also taking into account their polyphenol content [19, 20], could be used to enrich food products (various types of pasta, crackers, snacks, also meat products) with dietary fibers, while also increasing the antioxidant capacity and potentially their shelf life [18].

In the following experiments, the by-products were subjected to extraction with 70% ethanol. This extractant was chosen in order to ensure maximum extractability of the low-molecular substances, such as: phenolic acids, polyphenolic compounds, terpenes, pigments, sugars, etc., depending on the selectivity of the respective extractant, but also to ensure the preservation of biopolymers (polysaccharides, proteins) in the residue after extraction – the alcohol-insoluble parts (AIP). This methodology has been previously tested with other plant materials and adapted to current by-products [21].

Table 1. General characteristics of rose, hyssop and thyme by-products

By-product / Parameter, %	20RD_SD_S	20HO_SD_Z	20TS_SD_M
Moisture	8.99±0.31^{a}	9.56±0.24^{a}	8.95±0.58^{a}
Ash	8.74±0.36^{a}	5.12±0.86^{b}	4.47±0.25^{b}
Crude protein	13.05±0.38^{a}	12.28±0.62^{a}	6.82±0.46^{b}
PU	8.89±0.14^{a}	5.85±0.12^{b}	4.03±0.24^{c}
DE	62.59±1.28^{b}	59.41±2.52^{b}	86.05±1.25^{a}
SDF	6.98±0.76^{a}	5.21±0.80^{a,b}	4.15±0.69^{b}
IDF	59.79±1.08^{b}	57.07±1.15^{c}	75.79±1.12^{a}
TDF	66.77 ±1.08^{b}	62.28±1.15^{c}	79.94±1.11^{a}

PU - polyuronide content; DE – degree of esterification; SDF – soluble dietary fibers; IDF – insoluble dietary fibers; TDF – total dietary fibers; The results were expressed as mean ± SD (n = 3). ^{a,b,c}Different letters mean statistical difference (Tuckey's HSD test, $p < 0.05$).

Table 2. Yield and physico-chemical parameters of pectic polysaccharides extracted by 0.1 M HCl from AIPs of 20RD_SD_S, 20HO_SD_Z and 20TS_SD_M

	Yield, %	AUAC, µg/mg	DM, %	DAc, %	Neutral sugars, µg/mg	Molecular weight, $\times 10^4$, Da	Proteins, µg/mg
20RD_SD_S	12.45±0.14[a]	760.67±6.33[a]	56.14±1.51[a]	1.02±0.41[c]	881.11±24.87[a]	2.31±0.12[b]	61.15±0.92[c]
20HO_SD_Z	7.19±0.19[b]	729.83±6.98[b]	39.25±1.32[c]	1.83±0.42[b]	853.90±25.10[a]	2.79±0.15[a]	121.65±0.95[a]
20TS_SD_M	7.31±0.23[b]	667.50±5.28[c]	48.93±2.51[b]	2.32±0.11[a]	791.98±19.17[b]	2.55±0.11[b]	71.19±0.81[b]

AUAC – Anhydrouronic acids content; DM – degree of methoxylation; DAc – degree of acetylation; The results were expressed as mean ± SD (n = 3). [a,b,c]Different letters in a column mean statistical difference (Tuckey's HSD test, $p < 0.05$).

Table 3. Uronic acids and monosaccharides composition of pectic polysaccharides extracted by 0.1 M HCl from AIPs of 20RD_SD_S, 20HO_SD_Z and 20TS_SD_M

	GlcA	GalA	Gal	Rha	Ara	Fuc	Xyl	Man
	(µg/mg polysaccharide)							
20RD_SD_S	25.13± 2.14[a]	742.44± 5.89[a]	191.24± 8.37[a]	54.78± 3.47[a]	51.47± 6.31[a]	1.17± 0.10[c]	1.24± 0.47[c]	33.56± 1.23[a]
20HO_SD_Z	9.34±0. 11[c]	710.06± 3.15[b]	110.31± 1.54[b]	39.70± 2.04[b]	43.56± 1.59[b]	1.57± 0.10[b]	11.26± 0.87[b]	25.14± 1.56[b]
20TS_SD_M	12.01± 0.11[b]	639.18± 4.13[c]	181.20± 1.25[a]	51.13± 1.58[a]	36.85± 1.04[c]	1.79± 0.15[a]	15.36± 0.80[a]	31.81± 1.17[a]

GlcA – D-Glucuronic acid; GalA – D-Galacturonic acid; Gal – D-Galactose; Rha – L-Rhamnose; Ara – D-Arabinose; Fuc – L-Fucose; Xyl – D-Xylose; Man – D-Manose. The results were expressed as mean ± SD (n = 3). [a,b,c]Different letters in a column mean statistical difference (Tuckey's HSD test, $p < 0.05$).

Furthermore, the AIPs were subjected to acid extraction employing 0.1 M HCl for one hour at 85°C. This extraction resembles the industrial process for extraction of pectin from citrus and apple pulp (by-products from the fruit juice manufacturing) and serves as rough estimation for the amount of pectic polysaccharides which could be obtained following the industrial protocols [22]. The yield and the physico-chemical characterictics of the obtained pectic polysaccharides are presented in Table 2.

AIPs of rose by-products (20RD_SD_S): 12.45±0.14% showed the highest yield of acid-soluble pectic polysaccharides. The other two by-products yielded approximately two times lower amounts of polysaccharides. The galacturonic acid content was above 650 µg/mg polysaccharide which suggested that these polysaccharides could be regarded from industrial point of view as pectins (Table 3). The DM values suggested that the isolated pectins are middle-esterified and only the 20RD_SD_S polysaccharide had DM above 50% (56.14±1.51%). The degree of acetylation is relatively low (below 2.5%) for all pectins. The molecular weights of the polysaccharides were similar and the highest value had the 20HO_SD_Z pectin ($2.79±0.15 \times 10^4$ Da).

In the next experiments, the monosacchride composition of the pectins was assessed after hydrolysis with 2M trifuoroacetic acid and the data are presented in Table 3. The monosacchride present in the highest concentration was galacturonic acid: 742.44±5.89, 710.06±3.15, and 639.18±4.13 µg/mg in 20RD_SD_S, 20HO_SD_Z, and 20TS_SD_M polysaccharide, respectively. These results are in accordance with the determination of anhydrogalacturonic content (Table 2). The galactose was the next more abundant monosacchride, followed by rhamnose and arabinose. The ratio of GalA to Rha was 13.6, 17.9, and 12.5 for 20RD_SD_S, 20HO_SD_Z and 20TS_SD_M polysaccharide, respectively. These values suggested that this treatment extracted pectic polysaccharides rich in rhamnogalacturonan I macromolecules [23].

Pectic polysaccharides are widely used in the food industry as jellifying agents, stabilizers and thickeners [17, 22]. This application is influenced by the temperature at which different food systems could be subjected to treatments. For this reason, in the next experiments the extracted polysaccharides were investigated for their thermal stability by DTA-TG. The change in the weight of the 20RD_SD_S polysaccharide in the 25–550°C range took place in three stages. When heated from 25°C to 125°C, the weight decreases by 7.5%, which is probably due to the release of bound water. Simlar observations were reported by Einhorn-Stoll *et al.* [24]. The degradation of the material started after 128°C and proceeded in two stages: 128–367°C and 369–500°C. In the first stage, when the temperature rose to 367°C, the sample lost 52.1% of its weight.

Thermal destruction took place in the temperature range 128–367°C, and the maximum speed of the process was at 276.5°C. In the last stage of the process with an increase of temperature to 500°C, the weight was reduced by 30.3%. This stage was associated with oxidative destruction of carbon residues. The peak maximum was observed at 436.2°C (Figure 1). The solid residue above 500°C was 10.1% (by weight).

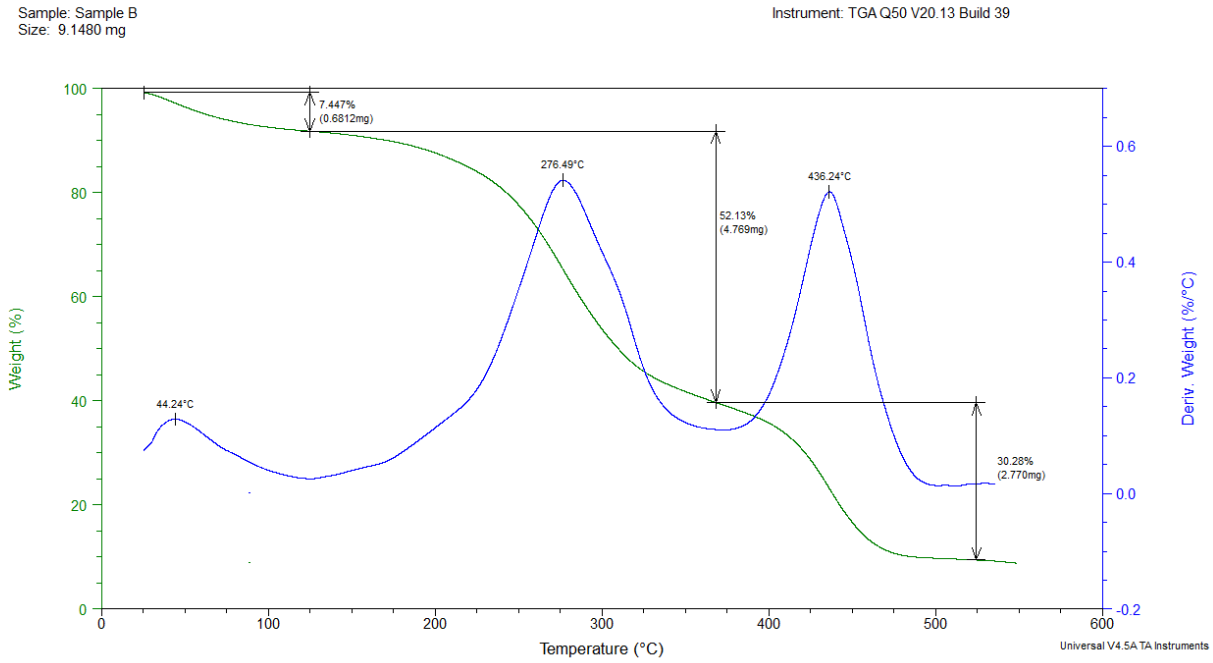

Figure 1. Thermogram of 20RD_SD_S polysaccharide

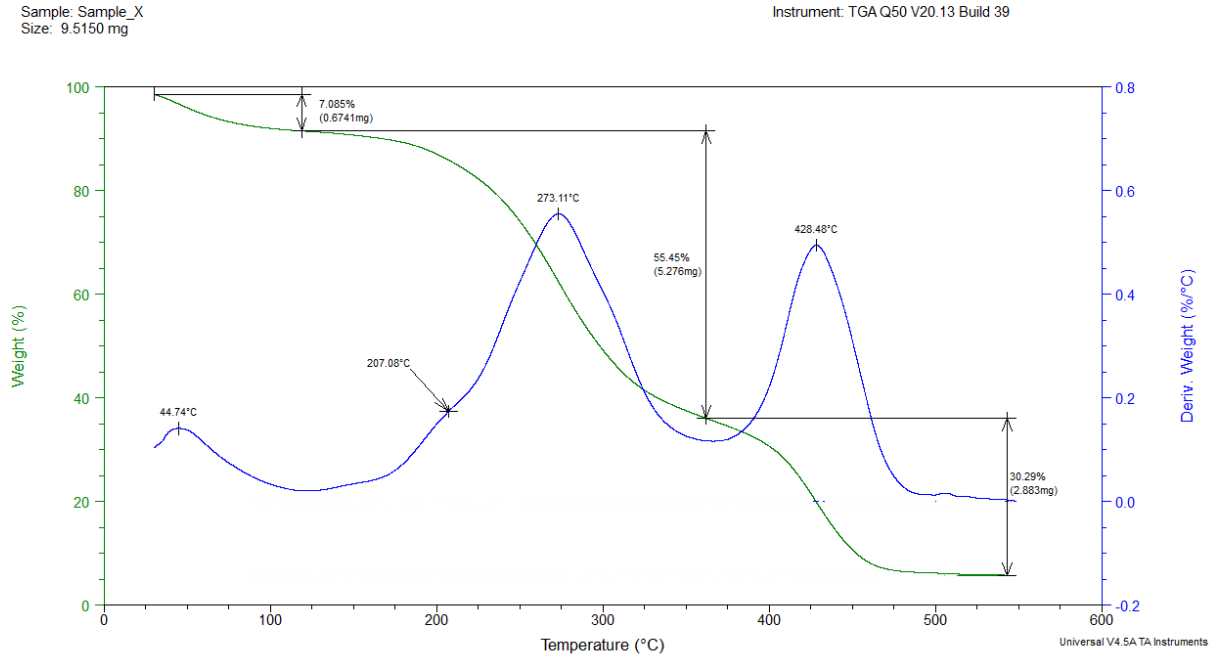

Figure 2. Thermogram of 20HO_SD_Z polysaccharide

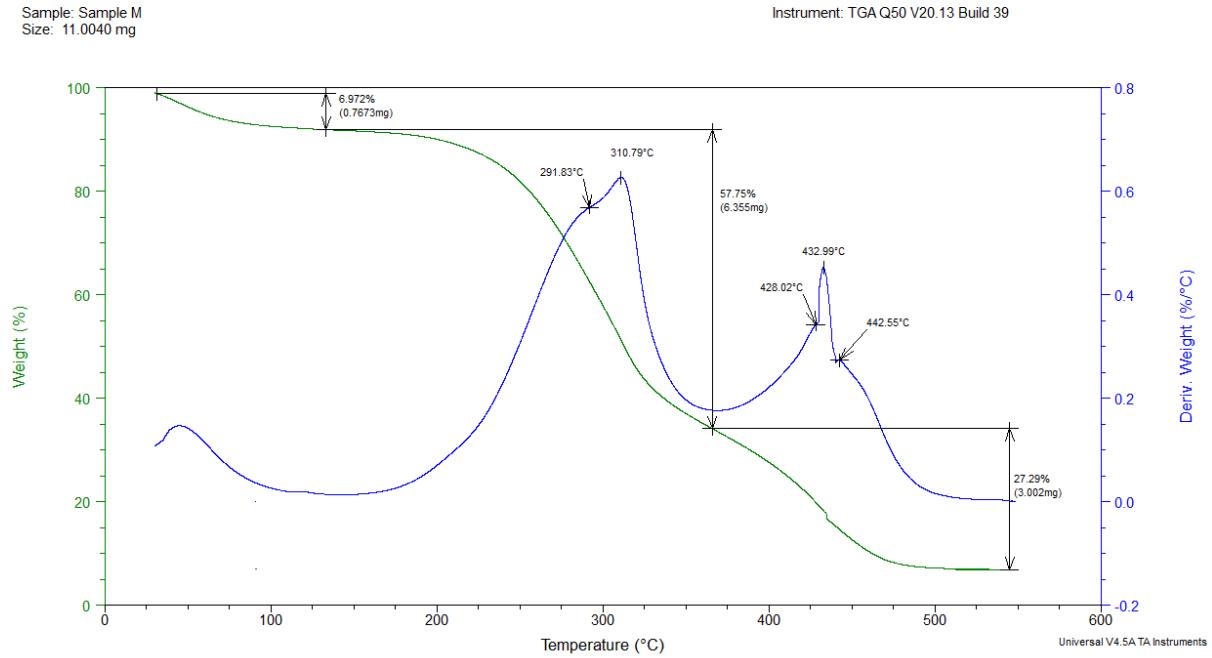

Figure 3. Thermogram of 20TS_SD_M polysaccharide

The change in the weight of the 20HO_SD_Z polysaccharide in the temperature range of 25–550°C ,took place in three stages. When heated from 25°C to 119°C, the weight decreased by 7.1%, which tentatively was due to the release of water. Degradation of the material began after 124°C and proceeded in two stages: first stage in the temperature range 153-362°C and second stage 370-550°C. In the first stage, the highest rate of degradation was observed at 273.1°C, the sample lost 55.5% of its weight, and probably as a result of breaking of chemical bonds with close energy, products with a mass different from that of the main ones for the stage were released, expressed by the appearance of a second peak with a maximum at 207.1°C, which overlaps with the main one. In this stage, thermal destruction of the material took place. In the third stage of the process, with a rise in temperature to 550°C, the weight was reduced by 30.3%, with a maximum rate of decomposition at 428.5°C. In this stage, oxidative destruction took place (Figure 2). The solid residue at 550°C was 7.1% (by weight).

The change in the weight of the sample 20TS_SD_M in the temperature range of 25–550°C took place in three stages. When heated from 25°C to 133°C, the weight decreased by 7.0%, which was probably due to the release of water. The degradation of the material started after 152°C and took place in two stages: first stage in the temperature interval 153–366°C in which the sample lost 57.7% where the maximum speed of the process was at 310.8°C and second stage 370–550°C, with a weight loss of 27.6% and a maximum speed at 310.8°C. In the first stage of degradation, probably as a result of the breaking of chemical bonds with close energy, products with a mass different from that of the main ones for the stage were released, expressed by the appearance of a second peak with a maximum at 291.2°C, which overlaps with the main one. In this stage, thermal destruction of the material took place. In the third stage of the process with a rise in temperature to 550°C, the weight was reduced by 27.6%, with a maximum rate of decomposition at 433°C. Two secondary peaks appeared with maxima at 428 and 442.5°C, which were due to the release of oxidative degradation products of different masses (Figure 3). Einhorn-Stoll *et al.* [25] investigated the thermal stability of native and modified citrus pectins and concluded that they started to significantly decompose after 200°C. The solid residue at 550°C was 7.7% (by weight).

The DTA-TG analyses suggested that the pectins isolated by acid extraction from 20RD_SD_S, 20HO_SD_Z and 20TS_SD_M had thermal stability up to 200-220°C and above these temperatures substantial degradation of polysaccharide chains was detected. Similar results for marigold pectic polysaccharides were reported by Slavov *et al.* [12].

CONCLUSION

Three solid by-products resulting from industrial steam distillation of rose (20RD_SD_S), hyssop (20HO_SD_Z) and wild thyme (20TS_SD_M) were investigated aiming at their potential valorization. It was found that the residues were rich sources of dietary fibers: 66.77±1.08%, 62.28±1.15%, and 79.94±1.11% for rose, hyssop and thyme,

respectively. The by-products were subjected to extraction with hot 70% ethanol by obtaining ethanolic extracts and alcohol-insoluble parts (AIPs). The polyuronic content of the AIP by-products was in the 4.03±0.24 – 8.89±0.14% range and the degree of esterification: 59.41±2.52% – 86.05±1.24%. The AIPs were extracted by employing 0.1 M HCl and pectic polysaccharides with 12.45±0.14%, 7.19±0.19%, and 7.31±0.23% yield were obtained for rose, hyssop and thyme, respectively,. The DTA-TG analyses suggested that the pectins isolated by acid extraction from 20RD_SD_S, 20HO_SD_Z and 20TS_SD_M had thermal stability up to 200-220°C and above these temperatures substantial degradation of polysaccharide chains was detected. The results from the present study suggested that the by-products from the rose, hyssop and thyme essential oil industry, could be successfully valorized and serve as a source of dietary fibers and pectic polysaccharides.

Acknowledgement: *We acknowledge the financial support from the National Science Fund (Ministry of Education and Science) of Bulgaria, project КП-06-Austria/4. The authors would like to thank Associate Professor Ivelina Vasileva, PhD (Department of Organic and Inorganic Chemistry, University of Food Technologies – Plovdiv, Bulgaria) for the technical support.*

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