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Physico-chemical characteristics of polysaccharides isolated from lavender by-products

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The essential oil industry, in addition to the main aroma products, such as essential oil, water, concrete, etc., results every year large quantities of lignocellulosic under-valorized biomass. Such by-products are obtained from processing of common lavender (*Lavandula angustifolia* Mill.) used industrially in Bulgaria, France, etc. chiefly for essential oil production. The solid biomass is discarded in locations neighboring distilleries. By this way potentially useful biomass is lost, hence the idea behind the present work was utilization of industrial lavender by-products as a source of polysaccharides. Two industrial solid residues were investigated: the first one obtained after supercritical CO_2 extraction (CO2-L), and the second: obtained after steam distillation of lavender (SD-L). The by-products were pretreated with 70% ethanol and alcohol insoluble residue (AIR) was obtained. The AIRs were subjected to a dilute acid extraction whereat the CO2-L and SD-L yielded 6.97±0.14% and 5.95±0.23% acid-soluble polysaccharide, respectively. The monosaccharide profile determined by HPLC revealed presence predominantly of galacturonic acid: 672.44±4.89 µg/mg and 619.17±5.23 µg/mg polysaccharide for CO2-L and SD-L, respectively. The degree of methoxylation: 53.7±1.5% and 48.9±2.5% and degree of acetylation (DAc) 2.7±0.4% and 2.3±0.1% for CO2-L and SD-L, respectively, suggested that the isolated polysaccharides were middle esterified with low DAc. The surface tension of SD-L 58.5±1.2 mN m^{-1} was lower compared to CO2-L (63.2±1.4 mN m^{-1}) which suggests that SD-L polysaccharide has better emulsification properties. The results of the present study suggested that the lavender by-products from essential oil industry could be successfully valorized and serve as a source of pectic type polysaccharides.

Keywords: lavender; by-products; valorization; polysaccharides; CO_2 extraction

INTRODUCTION

The lavender is among the most processed crops by the essential oil manufacturers. The main industrially exploited species are the true lavender (*Lavandula angustifolia* Mill.), lavandin (*Lavandula x intermedia* Emeric ex Loiseleur) and spike lavender (*Lavandula spica* D.C.). Bulgaria, France, UK, China, Ukraine, Spain, and Morocco are the biggest worldwide producers of essential lavender oil. In the last years Bulgaria has overtaken on lavender plantations and lavender oil yield (around 100 tons produced yearly) the long-standing leader in this field France [1]. The main species grown in Bulgaria is the true lavender (*Lavandula angustifolia* Mill.). Due to the fact that the concentration of essential oil in the plant materials (0.8-1.3% / fresh plant) is relatively low, after extraction or distillation of the important biologically active substances large quantities of by-products remain. Throwing simply away or composting are among the very often used procedures to eliminate these by-products. But they could also serve as initial materials for recovery of valuable substances which could be used in the food, cosmetic and perfumery industries. Alternative methods of valorization have also been applied in recent years - fermentation of distilled lavender and biotransformation of terpene compounds into valuable and difficult to chemically synthesize substances [1-5], isolation of substances with strong antioxidant activity – apigenin, rosmarinic acid, luteolin, etc. [1, 6], as well as the use of ethanol extracts from lavandin by-products for potential antifungal activity against *Penicillium verrucosum* (Dierckx), a common microorganism causing loss in cheese production [1]. Pectic polysaccharides recovered from lavender essential oil industry by-products were not reported in the literature. Pectic polysaccharides have two major applications: as texturizing agent in food industry [7] and for medical purposes [8, 9]. When used as gelling agent and viscosity modifier several physicochemical parameters are important, such as surface tension, viscosity, molecular weight, composition, etc. and hence, the hypothesis behind the present work was to explore two lavender essential oil industry by-products as a source of pectic polysaccharides and to compare their properties.

Although lavender was already explored as a source of water-soluble pectic polysaccharides and they have expressed potent immunomodulating activities [8, 9], very few studies are currently

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available on isolation and determination of physico-chemical properties of polysaccharides of true lavender (*Lavandula angustifolia* Mill.) by-products. Additionally, in the literature are missing data on attempts for valorization of by-products after supercritical CO_2 extraction of lavender. These observations determined the aim of the present work – to explore the possibility of utilization of spent lavender biomass (obtained by traditional steam distillation and supercritical CO_2 extraction), with an emphasis on obtaining polysaccharides and to investigate their physico-chemical properties.

MATERIALS AND METHODS

Materials

The lavender by-products were provided by ECOMAAT distillery (Mirkovo, Bulgaria, 2018). The first one was residue from supercritical CO_2-extracted lavender (CO2-L), and the second one – after steam distillation of fresh lavender (SD-L). After treatment the SD-L waste was cooled down, inspected for elimination of impurities and dried at 50°C. The CO2-L waste was removed from the extraction cylinder and checked for impurities. Both wastes were stored at -18 °C until further treatment. All the solvents used were of analytical grade and purchased from local distributors.

Methods

Alcohol-insoluble residue (AIR) was prepared according to Slavov *et al.* [10]. Lavender AIR (70 g) was treated with 1.4 L of 0.1M HCl (pH=1.2) at 85ºC for 1 h while stirring (120 min^{-1}). The mixture was filtered through a nylon cloth (250 mesh), the solid residue was returned in the extraction vessel, 1 L of 0.1M HCl was added and extraction was allowed to proceed at the same conditions. The mass was filtered again and both filtrates were collected. The combined filtrate was precipitated with 3 volumes of 96% C_2H_5OH overnight at 4ºC, and the resulting precipitate was centrifuged (9660 RCF, 4ºC, 25 min). The supernatant was eliminated, the precipitate was dissolved in 150 mL of deionized water and extensively dialyzed (Spectra/Por 1, Breda, the Netherlands, Mr. cut-off 6000-8000 Da) for 72 h against deionized water. The mass remaining in the dialysis tube was lyophilized and represented acid-soluble polysaccharides from lavender by-products. The anhydrogalacturonic acid content (AUAC) was investigated using the *m*-hydroxydiphenyl method with D-galacturonic acid as standard, the degree of methylesterification (DM): according to Slavov *et al.* [7] and the degree of acetylation (DAc) was investigated spectrophotometrically using hydroxamic acid and β-D-glucose pentaacetate (Sigma-Aldrich Chemie Gmbh, Germany) as a standard [11]. The monosaccharide composition of the isolated polysaccharides was ddetermined as follows: 10 mg of polysaccharide was hydrolyzed with 15 mL of 2M trifluoroacetic acid (Sigma-Aldrich, Germany) for 3 h at 120 ºC. The trifluoroacetic acid was removed by evaporation to dryness under vacuum and to the dry mixture 10 mL deionized water was added and evaporated again (repeated three times). The residue from the last evaporation was dissolved in 1 mL of deionized water. The quantities of galactose, rhamnose, arabinose and fucose, galacturonic acid and glucuronic acid were determined on a chromatographic system ELITE LaChrome (Hitachi) HPLC with a VWR Hitachi Chromaster 5450 refractive index detector using Aminex HPX-85H column. The samples and standards were eluted with 5 mM H_2SO_4 (Sigma-Aldrich, Germany) at an elution rate of 0.5 mL min^{-1}, column temperature 50ºC, and detector temperature 35ºC. The amounts of xylose and mannose were determined separately with the same chromatographic system using Sugar SP0810 (Shodex®) column. The samples and standards were eluted with ultrapure water at an elution rate of 1.0 mL min^{-1}, column temperature 85ºC, and detector temperature 35ºC.

The protein content and molecular weight of polysaccharides were determined as described by Slavov *et al.* [12]. The viscosimetric measurements were performed according to Slavov *et al.* [13]. The polysaccharides foam-forming capabilities, emulsifying activity and emulsion stability of model systems were determined as described by Yancheva *et al.* [14].

Statistical analysis

The analyses were run three times, and the data were given as mean values. Statistical significance was determined by analysis of variance (ANOVA, Tukey's test; value of $p<0.05$ indicated statistical difference).

RESULTS AND DISCUSSION

The dried biomass obtained after fresh lavender processing: extraction with supercritical CO_2 and steam distillation, was subjected to 70% ethanol treatment and alcohol insoluble residues (AIR), CO2-L-AIR and SD-AIR, respectively, were obtained. From the AIRs polysaccharides were extracted by dilute hydrochloric acid and the yield and characteristics are presented in Table 1. The yield of polysaccharides from CO2-L AIR was higher (6.97±0.14%) and significantly different from the yield of polysaccharides isolated from SD-L AIR

(5.95±0.23%). The influence of the type of industrial treatment of the raw materials on the yield of polysaccharides was observed also in previous works where similar data were obtained for essential rose oil biomass [10], chamomile by-products [15] and pot marigold [12] used as a source of polysaccharides. The neutral sugar content of CO2-L polysaccharide was also slightly higher: 882.93±24.87 µg/mg, but statistically not different compared to SD-L acid-soluble polysaccharide (854.14±19.17 µg/mg). Both polysaccharides had a molecular mass in the 10^4 Da range. The DAc: of 2.7±0.4% and 2.3±0.1% for CO2-L and SD-L acid-soluble polysaccharides, respectively, was substantially lower compared to pectic polysaccharides obtained from chicory roots: 11%, endive: 19%, and sugar beet: 44% [16] and comparable with pectic polysaccharides obtained from pot marigold: 3.3-3.5% [12]. The AUAC content and DM values for both polysaccharides were similar (not statistically different).

Table 1. Extraction of AIRs with dilute HCl – yield and characteristics of the polysaccharides

AIR	Yield, %	AUAC, µg/mg	DM, %	DAc, %	Neutral sugars, µg/mg	Molecular weight × 10^4, Da	Proteins, µg/mg
CO2-L	6.97 ± 0.14^a	653.35 ± 5.28^a	53.7 ± 1.5^a	2.7 ± 0.4^a	882.93 ± 24.87^a	26.6 ± 0.2^a	8.6 ± 0.8^a
SD-L	5.95 ± 0.23^b	642.94 ± 5.21^a	48.9 ± 2.5^b	2.3 ± 0.1^a	854.14 ± 19.17^a	20.7 ± 0.1^b	16.4 ± 0.9^b

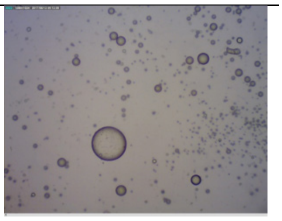
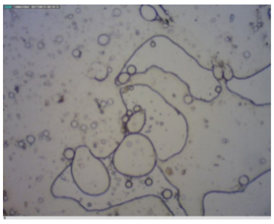
The results are expressed as mean from 3 repetitions ±SD; $^{a,\,b}$ Different letters after the values in a column mean statistical difference (Tukey's HSD test, $p < 0.05$).

Table 2. Monosaccharide composition of lavender by-products polysaccharides

	GlcA	GalA	Gal	Rha	Ara	Xyl	Man
	(µg/mg polysaccharide)						
CO2-L	11.91 ± 0.19^a	672.44 ± 4.89^a	172.32 ± 1.56^a	49.21 ± 1.17^a	44.28 ± 1.06^a	14.47 ± 0.95^b	29.19 ± 1.05^a
SD-L	10.25 ± 0.18^a	619.17 ± 5.23^b	150.35 ± 1.48^b	51.07 ± 1.24^a	37.64 ± 1.25^b	19.63 ± 1.14^a	28.36 ± 1.24^a

$^{a,\,b}$ Different letters in a column indicate significant differences (one-way ANOVA – Tukey's HSD test, $p < 0.05$)

Table 3. Physico-chemical properties of lavender by-products polysaccharides

Poly-saccharide	FF, %	σ, mN m^{-1}	ν, mm^2 s	Stability of emulsion		Microphotograms of emulsions
				EAI, $m^2\ g^{-1}$	ESI, min	
SD-L	26.7 ± 0.5^b	58.5 ± 1.2^b	1.65 ± 0.10^a	8.6 ± 0.5^b	8.5 ± 0.2^b	
CO2-L	16.7 ± 0.8^a	63.2 ± 1.4^a	0.79 ± 0.01^b	11.3 ± 0.6^a	34.0 ± 0.8^a	

The results are expressed as mean from 3 repetitions ±SD; $^{a,\,b}$ Different letters after the values in a column mean statistical difference (Tukey's HSD test, $p < 0.05$).

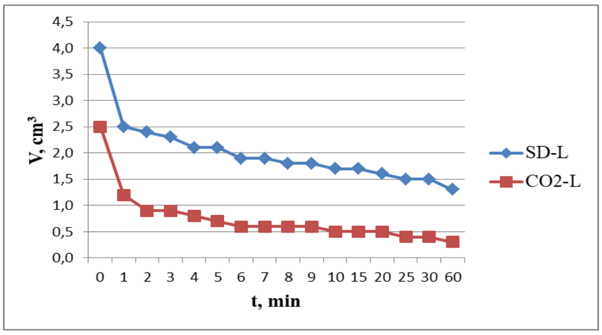


Figure 1. Foam-forming and foam-stabilizing properties of 0.6% polysaccharides solutions

In the next experiments the monosaccharide composition of the isolated acid-soluble polysaccharides was accessed by HPLC after hydrolysis with trifluoroacetic acid. The prevailing monosaccharide building the CO2-L (672.44±4.89 µg/mg polysaccharide) and SD-L (619.17±5.23 µg/mg polysaccharide) polysaccharides were statistically different and along with the other neutral monosaccharides found: galactose, rhamnose and arabinose) suggested that both polysaccharides were pectic type polysaccharides. Furthermore, several important physico-chemical properties of the polysaccharides were investigated. Firstly, the polysaccharides were dissolved in distilled water at 0.6% and foam-forming abilities (FF), surface tension (σ) and kinematic viscosity (ν) were determined (Table 2). The data in Table 3 imply that SD-L polysaccharide has the lowest surface tension (58.5±2.2 mN m^{-1}) of 0.6% aqueous solutions, higher kinematic viscosity: 1.65 mm^2 s and as expected from the data the SD-L solution has better foam-forming capabilities. For food, cosmetic and pharmaceutical applications it is important to utilize polysaccharides capable of increasing the viscosity of a disperse system (emulsion, suspension, foam), thus acting as stabilizers. The experimental results suggested that better foam-forming and foam-stabilizing properties showed 0.6% aqueous solutions of SD-L polysaccharides: 26.7% compared to 16.7% for CO2-L polysaccharides.

From the results it could be concluded that the SD-L acid-extractable polysaccharide showed better foam-forming and foam-stabilizing capabilities compared to CO2-L. This might be due to the higher amounts of proteins in SD-L and to the lower kinematic viscosity. In order to study the emulsifying capabilities of lavender polysaccharides emulsions of type oil in water (50% 0.6% polysaccharides in water and 50% oil) were prepared. The quality of the emulsions was studied by determining the index of emulsifiying activity (EAI, m^2 g^{-1}) and the stability index (ESI, min) – Table 3. The results from the analysis suggested that SD-L showed better emulsifying and emulsion-stability properties than the solution of CO2-L polysaccharide. The emulsions made with CO2-L were non-uniform, diffusive and merged oil droplets.

In the next experiments the emulsions of both polysaccharides were studied for their stability at four different temperatures (Figures 2 and 3).

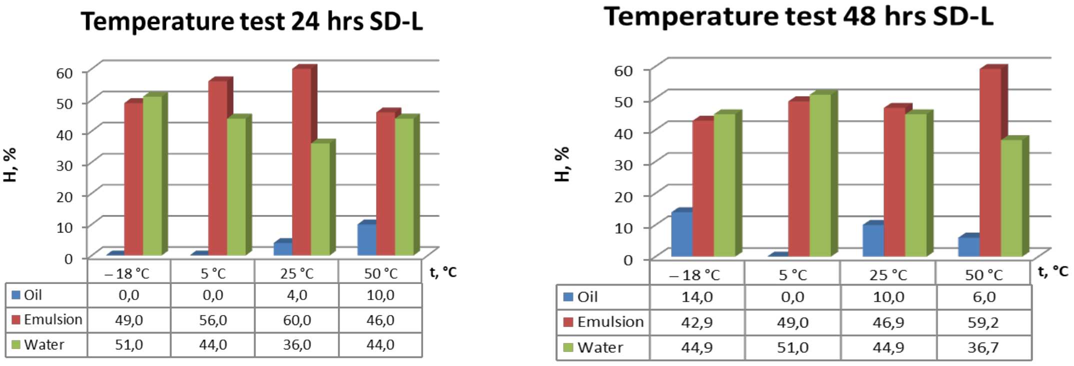


Figure 2. Temperature test for emulsion stability of SD-L at four temperatures

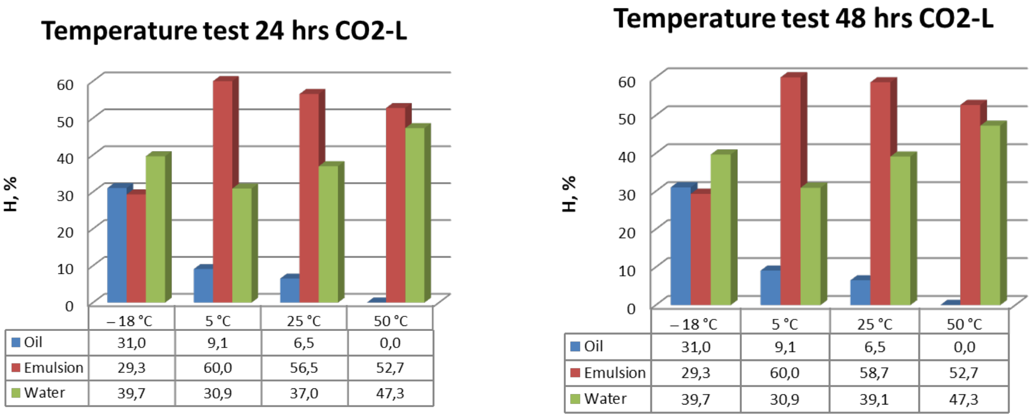


Figure 3. Temperature test for emulsion stability of CO2-L at four temperatures

The temperatures were chosen with regard to imitate the most common regimens of food preservation and keeping. Storage of the emulsions made with both polysaccharides at -18°C (freezing conditions) lead to destruction of the emulsions to a large extent: the amount of water measured was higher than the retained emulsion. At 5°C (storage in a fridge for foodstuffs) the emulsions made with CO2-L were more stable than the emulsions prepared with SD-L. For C2-L after 24- and 48-hour storage the emulsion retained was around 60% from the initial value. Furthermore, the emulsions were investigated for mechanical stability by performing centrifugal test (Figure 4).

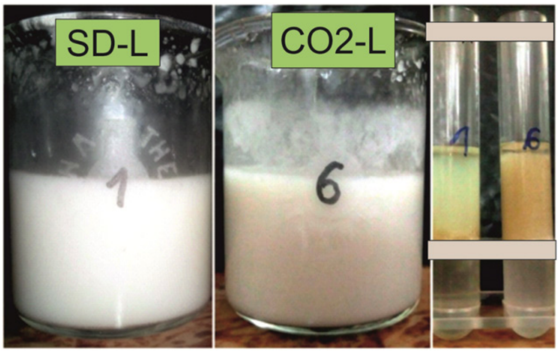


Figure 4. Centrifugal test for emulsion stability of SD-L and CO2-L. Left – 50% oil in water emulsion with 0.6% aqueous solutions of SD-L and CO2-L immediately after emulsification; To the right – after centrifugation at 5000 rpm for 20 min

The results from the centrifugal test (which imitates mechanical destabilization of the emulsions) suggested that both emulsions were destroyed at the tested conditions. The aqueous phases from both CO2-L and SD-L emulsions were comparable. The emulsion phase retained was visually higher for CO2-L emulsion but the conclusion which can be made from the test is that both emulsions were not stable and could be destroyed when mechanically disturbed.

Nevertheless, the polysaccharides extracted by dilute HCl acid from two industrial lavender wastes could be successfully utilized in food systems as viscosity modifiers and for formulation of new functionalized products.

CONCLUSIONS

The present study focuses on two plant wastes from essential oil industry: one obtained from steam-distilled lavender (SD-L) and one from subcritical CO_2 extraction of lavender (CO2-L). The lavender wastes were utilized as a source of polysaccharides. The physico-chemical properties of the polysaccharides were investigated and it was suggested that SD-L polysaccharides had better foam-forming abilities than CO2-L. The surface tension of SD-L 58.5±1.2 mN m^{-1} was lower compared to CO2-L (63.2±1.4 mN m^{-1}) which suggests that SD-L polysaccharides have better emulsification properties. The monosaccharide profile determined by HPLC revealed presence predominantly of galacturonic acid: 672.44±4.89 µg/mg and 619.17±5.23 µg/mg polysaccharide for CO2-L and SD-L, respectively. For the first time lavender residues from CO_2-extraction were investigated as a source of acid-extractable polysaccharides, and comparison with SD-L polysaccharides was performed. In general, the results suggested that the lavender waste could be utilized as a source of pectic polysaccharides and has the potential for application in the food industry as additives for foams, emulsions and viscosity modifiers.

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REFERENCES

1. L. Lesage-Meessen, M. Bou, J.C. Sigoillot, C.B. Faulds, A. Lomascolo, *Appl. Microbiol. Biotechnol.,* **99**, 3375 (2015).
2. P. P. Daramwar, R. Rincy, S. Niloferjahan, R. Krithika, A. Gulati, A. Yadav, R. Sharma, H. V. Thulasiram, *Bioresour. Technol.,* **115**, 70 (2012).
3. G. O. Elguea-Culebras, R. Sanchez-Vioque, O. Santana-Meridas, D. Herraiz-Penalver, M. Carmona, M. I. Berruga, *LWT*, **73**, 226 (2016).
4. S. Marumoto, M. Miyazawa, *Tetrahedron*, **67**, 495 (2011).
5. Y. Noma, Y. Asakawa, in: Handbook of essential oils: science, technology and applications, K. H. C. Baser, G. Buchbauer (eds.), Taylor & Francis Group, Boca Raton, 2010, p. 585.
6. I. Méndez-Tovar, B. Herrero, S. Pérez-Magariño, J. A. Pereira, M. C. Asensio-S.-Manzanera, *J. Food Drug Anal.*, **23**, 225 (2015).
7. A. Slavov, M. J. Crépeau, S. Durand, C. Garnier, J.-F. Thibault, E. Bonnin, in: Pectins and Pectinases, H. A. Schols, R. G. F. Visser, A. G. J. Voragen (eds.), Wageningen Academic Publishers, Wageningen, 2009, p. 127.
8. Y. N. Georgiev, M. H. Ognyanov, H. Kiyohara, T. G. Batsalova, B. M. Dzhambazov, M. Ciz, P. N. Denev, H. Yamada, B. S. Paulsen, O. Vasicek, A. Lojek, H. Barsett, D. Antonova, M. G Kratchanova, *Int. J. Biol. Macromol.*, **105**, 730 (2017).
9. Y. N. Georgiev, B. S. Paulsen, H. Kiyohara, M. Ciz, M. H. Ognyanov, O. Vasicek, F. Rise, H. Yamada, P. N. Denev, A. Lojek, V. Kussovski, H. Barsett, A. I. Krastanov, I. Z. Yanakieva, M. G Kratchanova, *Carbohydr. Polym.*, **174**, 948 (2017).
10. A. Slavov, P. Denev, I. Panchev, V. Shikov, N. Nenov, N. Yantcheva, I. Vasileva, *Ind. Crops Prod.,* **100**, 85 (2017).
11. E. A. McComb, R.M. McCready, *Anal. Chem.*, **29(5)**, 819 (1957).
12. A. Slavov, M. Ognyanov, I. Vasileva, *Food Hydrocoll.*, **101**, 105545 (2020).
13. A. Slavov, I. Panchev, D. Kovacheva, I. Vasileva, *Food Hydrocoll.*, **61**, 469 (2016).
14. N. Yancheva, D. Markova, D. Murdzheva, I. Vasileva, A. Slavov, *Acta Sci. Nat.*, **3(1)**, 7 (2016).
15. A. Slavov, N. Yantcheva, I. Vasileva, *Waste Biomass Valor.*, **10(9)**, 2583 (2019).
16. A. Zykwinska, M.H. Boiffard, H. Kontkanen, J. Buchert, J.-F. Thibault, E. Bonnin, *J. Agric. Food Chem.*, **56**, 8926 (2008).