

OPTIMIZATION OF THE STATIC HEADSPACE EXTRACTION OF A CINNAMON BARK: INFLUENCE OF SODIUM CHLORIDE AS A MATRIX MODIFIER

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This study aimed to determine the optimal conditions for static headspace (HS) extraction of the volatile fraction of commercial spice – cinnamon bark. In the applied optimization procedure only one factor was changed, while others were kept constant. The influence of sodium chloride (in different concentrations) as a matrix modifier was investigated. Headspace volatiles (HSVs) were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). According to the results obtained, the content of aroma compounds in the volatile fraction was affected by the changing concentration of NaCl in the aqueous matrix of spice. The principal component of a cinnamon flavor, (*E*)-cinnamaldehyde, increased in content constantly with increments of NaCl concentration in a sample matrix, while the content of 1,8-cineole decreased. Interestingly, benzaldehyde, absent in the sample without the matrix modifier, increased its content in the samples that were salted out. Decrement of monoterpene and sesquiterpene hydrocarbon content was also observed. Overall, this preliminary test on optimization justified some commercial procedures that practice the isolation of cinnamon essential oil from bark macerated in seawater.

Keywords: cinnamon bark, salting out, headspace extraction, optimization

Introduction

Isolation of the plant volatile secondary metabolites, like essential oils, is mainly done by distillation (hydrodistillation, hydrodiffusion, and steam distillation), solvent extraction, extraction with supercritical fluids, and other methods. In addition, low molecular weight volatile compounds could be isolated from plant material by the automated headspace (HS) technique: static, dynamic, and purge/trap headspace [1-4]. Static HS allows the analysis of low molecular weight volatile components without the influence of solvents; the process is automated and allows the screening of a larger number of samples. In addition to good sensitivity, the advantage of static HS is reflected in the low cost of analysis per sample, ease of sample preparation, small sample size, relatively simple monitoring, and elimination of reagents. Whether it is static or dynamic HS, optimization of extraction conditions is a very important step, and it is necessary to keep in mind the influence of sample temperature, matrix solubility, and volatility of the analyte. A common procedure for preparing an HS sample for a static procedure is to place the sample in a specialized vial in which two phases are subsequently formed; gas - headspace and sample phase which can be solid or liquid analyte, to which a

solvent or matrix modifier can be added. Subsequently, the volatiles are sampled from the vapor phase for chromatography and analysis, usually GC-MS or GC-FID [1,2,5]. Sample preparation is a very important factor; thus, proper sample preparation can achieve good sensitivity, i.e., the maximum concentration of volatile components and the minimum concentration of unwanted components from the matrix by calculating the partition coefficient (K , C_s/C_g) and phase ratio (β , V_s/V_g). The partition coefficient can be changed by changing the temperature or composition of the matrix (for example, by adding salt to the aqueous matrix). Derivatization of the analyte can also achieve greater sensitivity; the esterification, alkylation, acetylation, and silylation procedures are most used. Sample size also affects the sensitivity of the technique. Therefore, with optimal extraction conditions and subsequent analysis of volatile compounds, the static headspace method can qualify as a reliable method when evaluating raw materials [1].

The two most important varieties of cinnamon are Ceylon and Chinese cinnamon. In addition, the term Ceylon cinnamon includes the species *C. verum* and *C. zeylanicum*, native to Sri Lanka, assigns as true cinna-

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mon. False cinnamon, which has diverse origins – China, South America, and Indonesia, includes *C. cassia*, *C. aromaticum*, *C. burmannii*, and *C. loureiroi* species. The characteristic chemical composition of the essential oil of true cinnamon affects the organoleptic properties and high market value. Namely, the essential oil of cinnamon bark is rich in cinnamaldehyde, the leaf essential oil in eugenol, while root essential oil contains a notable concentration of camphor. Contrary to the mild and sweet aroma of real cinnamon, false cinnamon has a more astringent taste due to a higher concentration of coumarin in its composition. Certainly, the chemical composition is a feature that makes it possible to distinguish the true cinnamon from the false one [6]. A previous study on GC-MS analyses of essential oil from *C. verum* and *C. cassia* (using hydrodistillation as extraction method) summarized the profile of main volatiles distribution [7]. Evidently, (*E*)-cinnamaldehyde, its analogs (i.e. cinnamic acid, cinnamyl alcohol, and cinnamyl acetate), and benzaldehyde are predominant volatiles in essential oils of cinnamon bark. Our previous study [8] showed an average HS profile of commercial cinnamon bark spice. The most abundant volatiles were: (*E*)-cinnamaldehyde >>> 1,8-cineole >> isobornyl acetate ≈ α -copaene.

This study aimed to determine the optimal conditions for HS extraction of cinnamon bark volatiles. In the applied optimization procedure only one factor was changed, while others were kept constant. The salting out is a useful step that improves separation in

a liquid–liquid extraction procedure of some essential oils after hydrodistillation. By saturating the distillate with ionic compounds e.g. sodium chloride (NaCl), the difference in polarity between the aqueous and organic phases is increased, thus their separation improves. Guided by this fact, the influence of different NaCl concentrations in the aqueous sample matrix on the HSVs content was investigated.

Material and Methods

Plant material. Commercially available milled cinnamon bark (*C. verum*) was used for HS–GC–MS analysis of the volatile fraction.

Sample preparation. Before headspace extraction samples of cinnamon were measured (200 mg) and placed in 20-mL headspace vials. In the next step, vials were filled with distilled water or NaCl solution up to 1 mL, and assigned by the concentration of NaCl, as follows: HS₀ (0%), HS₁₀ (10%), HS₁₅ (15%), HS₂₀ (20%), HS₂₅ (25%), and the highest concentration HS₃₀ (30%), close to the solubility of NaCl in water. Headspace extraction and GC-MS analysis were done in triplicate.

Static headspace extraction and GC-MS analyses. In an automated procedure, the vial was placed in the bath, where the sample was heated at 80 °C for 20 minutes, with alternating mixing (for 5 seconds) and pausing (for 2 seconds). After equilibration (20 minutes), the headspace vapor above the sample (500 μ L,

Table 1. The HSV profiles of cinnamon bark respecting different concentrations of NaCl (%) in the matrix

No.	RI	RA	C	HSV	Content ($\bar{x} \pm \sigma$, %)					
					HS ₀	HS ₁₀	HS ₁₅	HS ₂₀	HS ₂₅	HS ₃₀
1.	932	932	MH	α -Pinene	8.23±0.04	3.11±0.02	1.62±0.02	1.09±0.01	0.00±0.00	0.00±0.00
2.	946	946	MH	Camphene	3.72±0.02	1.69±0.01	0.94±0.01	0.00±0.00	0.00±0.00	0.00±0.00
3.	958	952	A	Benzaldehyde	0.00±0.00	1.22±0.01	1.34±0.01	1.63±0.02	1.60±0.02	1.58±0.02
4.	975	974	MH	β -Pinene	2.35±0.01	0.75±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5.	1023	1020	MH	α -Cymene	1.59±0.01	0.83±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
6.	1026	1024	MH	Limonene	2.67±0.01	1.02±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7.	1029	1026	MO	1,8-Cineole	32.02±0.17	31.09±0.16	26.91±0.26	26.54±0.25	22.01±0.21	19.51±0.19
8.	1096	1095	MO	Linalool	1.21±0.01	1.00±0.01	0.91±0.01	0.95±0.01	0.80±0.01	0.00±0.00
9.	1165	1165	MO	Borneol	0.83±0.00	0.81±0.00	0.00±0.00	0.78±0.01	0.83±0.01	0.00±0.00
10.	1175	1174	MO	Terpinene-4-ol	2.10±0.01	2.41±0.02	2.19±0.02	2.32±0.02	2.45±0.02	2.34±0.02
11.	1188	1186	MO	α -Terpineol	2.11±0.01	2.61±0.02	2.63±0.03	2.79±0.03	3.25±0.03	3.34±0.03
12.	1269	1267	A	(<i>E</i>)-Cinnamaldehyde	34.29±0.18	49.36±0.26	61.59±0.59	62.34±0.59	67.96±0.65	72.1±0.69
13.	1284	1287	MO	Bornyl acetate	3.70±0.01	2.89±0.02	1.87±0.02	1.55±0.01	1.10±0.01	1.13±0.01
14.	1375	1374	SH	α -Copaene	2.18±0.01	0.60±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
15.	1420	1427	SH	(<i>E</i>)-Caryophyllene	1.17±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
16.	1499	1500	SH	α -Muureolene	0.6±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
17.	1523	1522	SH	δ -Cadinene	1.25±1.01	0.61±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
			MH		18.56±0.10	7.4±0.04	2.56±0.02	1.09±0.01	0.00±0.00	0.00±0.00
			MO		41.97±0.22	40.81±0.22	34.51±0.33	34.93±0.33	30.44±0.29	26.32±0.25
			SH		5.2±0.03	1.21±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
			A		34.29±0.18	50.58±0.27	62.93±0.60	63.97±0.61	69.56±0.66	73.68±0.70
			Total		99.60±0.53	99.60±0.53	99.90±0.95	99.90±0.95	99.90±0.95	99.90±0.95

RI – Experimental linear retention indices relative to C8–C40 alkanes. RA – Literature indices-Adams' retention indices [9]. C – Class of headspace volatile. HSV – Headspace volatile. HS0–HS30 corresponds to the HS extraction conditions regarding the concentration (%) of NaCl as a matrix modifier: 0%, 10%, 15%, 20%, 25%, 30%. MH – monoterpene hydrocarbons, MO – oxygenated monoterpenes, SH – sesquiterpene hydrocarbons, and A – aldehydes. HS-GC-MS analysis was done in triplicate, mean value (\bar{x})±standard deviation (σ).

split 40:1) was injected from the vial using a hermetically sealed syringe (90 °C) directly into the chromatographic column, via the transfer line (75 °C). Headspace aliquot analysis was performed on a 7890/7000B gas chromatograph (Agilent Technologies, USA) with Combi PAL auto-sampler and HP-5 MS column (5% phenylmethyl siloxane, 30 m x 0.25 mm i.d. and 0.25 µm film thickness) and mass detector (MS). Temperature program: isothermal for the first 2.25 minutes at 50 °C, then a linear increase in temperature range (gradient 4 °C min⁻¹) from 50 to 200 °C. Injector temperature 250 °C, interface 300 °C; carrier gas flow – helium 1.0 mL min⁻¹. Backflash 1.89 min, at 280 °C, with helium at a pressure of 50 psi. MS conditions (EI): ionization –70 eV, mass range 35 – 435 amu (3 scans per second), scan time 0.2 seconds. The percentage composition was recalculated by software salting-out the area of the peaks. Volatile components of the headspace fraction were identified using AMDIS software (Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, DTRA/NIST, 2011), based on linear retention indices (RI), which were determined from the retention times of a standard series of n-alkanes (C8–C40), recorded on the same instrument and under the same conditions as samples. RIs were compared with literature values, RA [9]. After the GC-MS analysis, the obtained HS volatile contents were recalculated in the Microsoft Excel software to obtain the mean values with the corresponding standard deviation (Table 1).

Results and Discussion

Sixteen HSVs are identified in the sample of cinnamon bark HS₀, before adding the solution of NaCl of different concentrations. The results obtained are given in Table 1. The dominant volatile is (*E*)-cinnamaldehyde followed by 1,8-cineole and α-pinene. Benzaldehyde is a volatile that had not been detected before the addition of the NaCl_(aq), but, after salting out, the content of this compound is raised by increasing the ionic strength of the medium. Even more, benzaldehyde content reached the maximum value in sample HS₂₀, while in the next two, HS₂₅ and HS₃₀, it decreased slightly. The content of (*E*)-cinnamaldehyde increases constantly up to 72.1% in the most concentrated sample matrix HS₃₀. Higher concentrations of NaCl decrease the solubility of polar organic volatiles in the sample matrix and promote their transfer into the headspace. Less polar HSVs 1,8-cineole and α-pinene show a tendency to decrease in content with increasing concentration of NaCl in the matrix. Figure 1. shows the graphical dependence of the percentage representation of identified HSVs classes and the concentration of NaCl in the matrix. A fairly sharp decrement in the content of monoterpene and sesquiterpene hydrocarbons, as well as an increment of aldehydes was observed. The decrease in the content of oxygenated monoterpenes is somewhat moderate

going from sample HS₀ to sample HS₃₀. There is a justification for some commercial procedures that macerate cinnamon bark in seawater before hydrodistillation [10]. The volatile fraction isolated from the matrix with the highest concentration of NaCl would be the richest in sweet, balsamic (*E*)-cinnamaldehyde, phenylpropanoid which gives cinnamon and its products characteristic flavor and odor. Furthermore, the content of 1,8-cineole, the component that gives a fresh camphor-like odor and a spicy, cooling taste, would be lower than in the non-salted sample matrix.

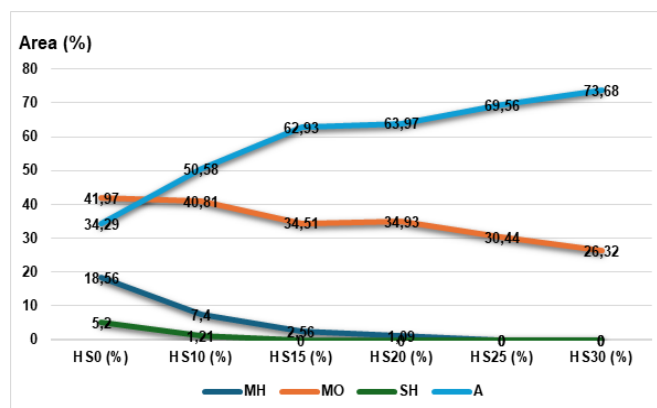


Figure 1. Graphical dependence of the percentage representation of identified HSVs classes and the concentration of NaCl in the matrix: $\text{Area (\%)} = f(\text{cNaCl in matrix})$

Considering the previously published data on the same optimization target (salting out effect), the experiences were quite different. Although the addition of salt facilitates the transfer of VOCs from the matrix to the HS via a salting out effect, the applicability is dictated by the nature of the sample. In the case of honey samples [11] the presence of NaCl resulted in lower peak areas and was therefore rejected for honey analysis. Very similar conclusions emerged from the research of HS-SPME extraction of pineapple fruit samples [12] and static HS extraction of citrus leaf [13]. To the best of our knowledge, no other references have been found that refer to the influence of solvents or salts on static headspace efficiency. However, it is known that the addition of salt was considered as an important variable in the improvement of the volatiles extraction by headspace and stir bar sorptive extraction from some food products: beer [14,15], vinegar [16], and orange juice [17].

Conclusion

The static HS extraction coupled with GC-MS represents the methods of choice in the analysis of the low molecular weight plant volatiles, leaving enough options to adjust better analysis parameters rapidly and efficiently. The aim of the work was achieved due to the verification of the salting out effect: an increase of the ionic strength in the matrix results in greater volatility of certain HSVs and vice versa. Although the focus of this

research was the influence of NaCl on the volatility of the spice constituents, the results can be a guide for the industrial application of obtaining commercial products enriched with a certain component (when targeted profiles are desirable), depending on the salt concentration. From an ecological aspect, in terms of waste management and disposal, the fact that salt can be regenerated from the matrix by extraction with water is also not negligible. The findings could serve as a model for similar studies on other spices or botanicals, as the demonstrated effectiveness of static HS-GC-MS coupled with salting out could be adaptable to other plant matrices rich in volatile compounds.

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Izvod

OPTIMIZACIJA USLOVA EKSTRAKCIJE ISPARLJIVIH JEDINJENJA CIMETA METODOM STATIČKOG HEDSPEJSA: UTICAJ NATRIJUM HLORIDA KAO MATRIKS MODIFIKATORASnežana Č. Jevtović ^{ORCID}, Gordana S. Stojanović ^{ORCID}(ORIGINALNI NAUČNI RAD)
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Ovo istraživanje je imalo za cilj da utvrdi optimalne uslove za ekstrakciju isparljive frakcije komercijalnog začina (kore cimeta) metodom statičkog hedspejsa. U primenjenom postupku optimizacije variran je samo jedan faktor. Istražen je uticaj različitih koncentracija NaCl u matriksu uzorka na sam proces ekstrakcije. Hedspejs isparljive supstance (HSV) su analizirane gasnom hromatografijom u kombinaciji sa mase-
nom spektrometrijom (GC-MS). Prema dobijenim rezultatima, na sadržaj pojedinih jedinjenja u isparljivoj frakciji uticala je promena koncentracije NaCl. Naime, sadržaj glavne komponente arome cimeta, (*E*)-cinamaldehida, konstantno se povećavao sa porastom koncentracije NaCl u matriksu uzorka, dok je sadržaj 1,8-cineola opadao. Zanimljivo je da se sadržaj benzaldehida, koji je inače bio odsutan u uzorku bez modifikatora, povećao u uzorcima koji su bili izoljeni. Takođe je primećeno smanjenje sadržaja monoterpenskih i seskviterpenskih ugljovodonika. Ovaj preliminarni test optimizacije opravdao je neke komercijalne procedure koje praktikuju izolovanje etarskog ulja cimeta iz macerata začina u morskoj vodi.

Ključne reči: cimet, isoljavanje, hedspejs ekstrakcija, optimizacija.

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