Comparative study of essential oils of onion and garlic by thin-layer methods
chromatography and gas chromatography-mass spectrometry

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SUMMARY

A comparative study of essential oils from raw materials of various varieties of onion and common garlic (by TLC, densitometry and chromatography-mass spectrometry) was carried out in a comparative aspect. It was found that all the studied essential oils of garlic and onion are complex mixtures, consisting of more than 480 compounds, of which 3-5 compounds are present in amounts of more than 5.0 wt.%, About 10 - in amounts of more than 1.0% , about 50 compounds in an amount of more than 0.1%, more than 100 substances in an amount of more than 0.01%, and the content of the rest is in the range of 0.001%. About 20 components of essential oils have been identified, accounting for about 75–85% of the oil mass. All of them belong to sulfur-containing compounds (mono-, di-, tri- and tetrasulfides of linear and cyclic structure, thiophene and its derivatives, etc.). Terpenoids, alcohols, aldehydes,

It is shown that the qualitative and quantitative composition of the studied essential oils significantly differs in individual components, which can be used as one of the criteria for assessing the quality of the initial medicinal plant raw material of onion and garlic.

Introduction

The chemical composition of bulbs of representatives of the genus Allium L. - onion and garlic is well studied [1, 3–5]. Essential oils of onion and garlic differ from essential oils of other plants in that their main components are mainly numerous sulfur-containing compounds of cyclic and acyclic structure, while organic compounds of other groups of biologically active substances (BAS) are poorly represented.

The content of essential oil in onion bulbs ranges from 0.005 to 0.15%, its main components are diallyl sulfide, allylpropyl disulfide and alliin. The maximum amount of essential oil is contained in spicy varieties of onions, in semi-sharp varieties it is 6–7 times less, and in sweet varieties it is almost absent [7]. In the bulbs of common garlic, in comparison with onions, the content of essential oil is higher (according to various sources, from 0.4 to 2.0%), and its main components are diallyl disulfide, diallyltrisulfide, allylpropyl sulfide and alliin.

Analysis of essential oils includes determining the authenticity and goodness by checking organoleptic (color, smell, taste), physical (density, optical rotation, refractive index) and chemical (solubility in alcohol, acid and ether number) indicators, as well as determining the qualitative composition and quantitative content of essential oil components. Specific indicators of the quality of essential oils in different countries are standardized by such official documents and standards bodies as national and international pharmacopoeias, ISO, Essential Oil Association, Merck Index, etc. At the same time, due to very strict criteria for assessing the quality of medicines in pharmacy, only about one and a half dozen essential oils appear in the national pharmacopoeias of various countries.

Thin layer chromatography (TLC) is widely used to standardize and control the quality of medicinal plant raw materials (MPR) and essential oils from it, which has a number of advantages over other methods, in particular, low cost and speed of obtaining results. The use of photodensitometers significantly increases the sensitivity and accuracy of determining the quantitative content of separated substances due to the fact that the area of the adsorption zone and the intensity of its luminescence are taken into account. The use of calibration curves and chromogenic reagents makes it possible to quantitatively determine all separated substances in a fairly short period of time directly on the plate and even compare the shades of the adsorption zones of the substances under study and bystanders. Complete identification of all components of essential oils requires the availability of standard samples (CRM) and the use of gas chromatography-mass spectrometry. The combination of these techniques makes it possible to compose a "chemical portrait" of any essential oil, with the help of which it is distinguished from counterfeits.

The purpose of this work is a comparative study of essential oils from raw onion andsowing garlic.

Materials and methods

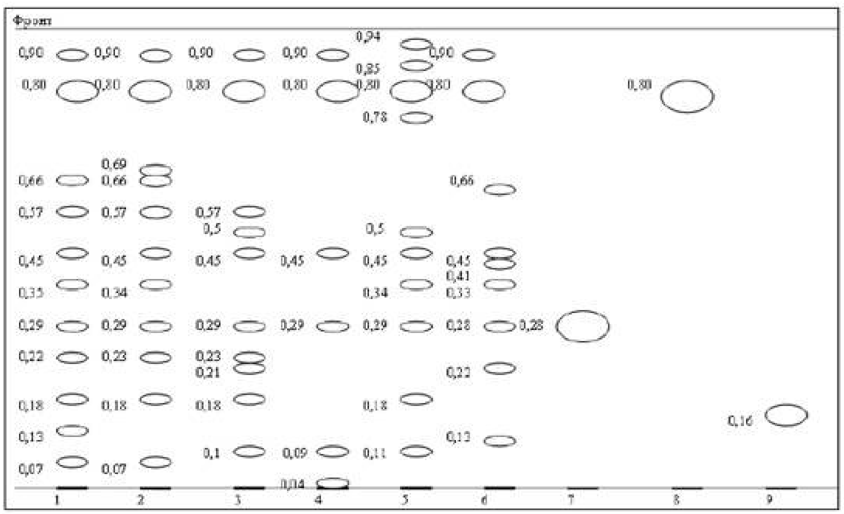
The objects of the study were onion bulbs of the varieties Strigunovsky and Stuttgarten rizen (spicy varieties), Myachkovsky and Red Baron (peninsular varieties) and Gollandsky white (sweet variety) and garlic, purchased in the retail network.

Thin-layer chromatography was performed on Sorbfil plates 5x10 cm in size with PTSKh-AF-F-UF phosphor (Russia) using various mobile phases. To obtain reproducible results, the plate was pre-activated, and the chromatography process was carried out in chambers saturated with vapors of the mobile phase. The best separation was achieved with benzene: ethyl acetate 95: 5.

Samples of menthol, anethole and geraniol were used as witnesses.

Results and its discussion

As a result of TLC analysis in the studied samples of essential oil from onion and garlic bulbs in visible and UV light (wavelength 365 nm), adsorption zones were found that had a red, blue, bluish-violet, yellow and green color. Based on the chromatographic behavior with the use of chromogenic reagents, a number of them were presumably attributed by us to the known groups of biologically active compounds. The general scheme of the distribution of adsorption zones on the chromatogram of the studied samples of onion and garlic essential oil is shown in Fig. 1, and their color in visible and UV light after treatment with anisaldehyde is shown in Table 1.



Rice. 1. Schematic chromatogram of essential oils from onion and garlic bulbs in the system benzene: ethyl acetate 95: 5 in the presence of standards.

Essential oils: 1 - Strigunovsky, 2 - Stuttgarten-Riesen, 3 - Myachkovsky, 4 - Dutch white, 5 - red baron, 6 - sowing garlic. Standards: 7 - menthol, 8 - anethole, 9 - geraniol.

Chromato-mass spectrometric analysis was carried out on a HewlettPackard 5890/11 gas chromatograph with a quadrupole mass spectrometer (HP MSD 5971) using a standard 30-meter glass column HP-5 (copolymer of 5% -biphenyl-95% -dimethylsiloxane) with an internal diameter 0.25 mm and a stationary phase film thickness of 0.25 microns.

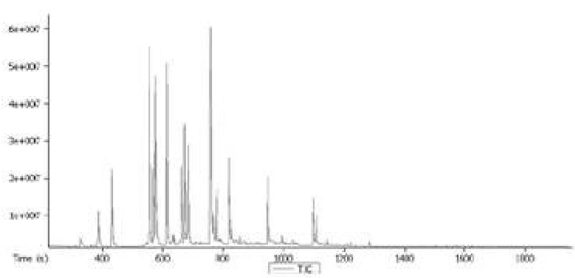
Qualitative analysis of essential oils is based on the comparison of retention times and total mass spectra with the corresponding data from the Wiley 275 mass spectrometric data library and catalogs. The content of individual components was calculated from the peak areas automatically without the use of correcting (correction) factors.

A general view of chromatograms of essential oils of onions and garlic is shown in Fig. 2-7. The similarity of the spectra of the samples of essential oils, which correspond to the semi-sharp and spicy varieties of onions, is clearly manifested. It was found that all the essential oils of garlic and onions studied by us are complex mixtures consisting of more than 480 compounds, of which only 3-5 compounds are present in amounts of more than 5.0 wt.%, About 10 - in amounts of more than 1, 0%, about 50 compounds in an amount of more than 0.1%, more than 100 substances in an amount of more than 0.01%, and the content of the rest hardly exceeds 0.001%.

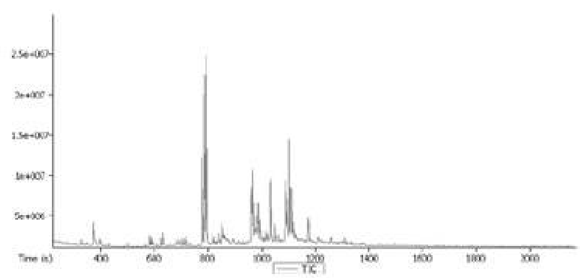
Table 1

TLC results of the study of essential oils of onion and common garlic in the benzene system: ethyl acetate 95: 5 after treatment with anisaldehyde

Интервалы значений R_f	Значения R_f, окраска в видимом / УФ-свете(365 нм)					
	Стригуновский	Штуттгартен-ризен	Мячковский	Ред барон	Белый голландский	Чеснок посевной
0−0,10					0,04 св-корич./желт.	
	0,07 т-сирен./я-желт.	0,07 т-сирен./я-желт.				
					0,09 св-розов./желт.	
0,10−0,20			0,10 св-розов./желт.	0,11 св-сирен./желт.		
	0,13 т-розов./оранж.					0,13 т-сирен./желт.
	0,18 св-розов./желт.	0,18 св-розов./желт.	0,17 св-сирен./желт.	0,18 т-сирен./желт.		
0,20−0,30	0,22 св-розов./желт.		0,21 св-сирен./желт.			0,22 св-сирен./оранж.
		0,23 св-розов./желт.	0,23 св-розов./желт.			
	0,29 св-розов./желт.	0,29 т-розов./желт.	0,29 св-розов./желт.	0,29 св-розов./желт.	0,29 св-розов./желт.	0,28 св-розов./желт.
0,30−0,40		0,34 т-сирен./оранж.		0,34 т-сирен./оранж.		0,33 св-розов./оранж.
	0,35 т-розов./оранж.					
0,40−0,50						0,41 св-малин./желт.
	0,45 св-розов./голуб.	0,45 св-розов./голуб.	0,45 св-розов./голуб.	0,45 св-розов./голуб.	0,45 св-розов./голуб.	
0,50−0,60			0,50 св-корич./голуб.	0,50 св-корич./голуб.		
	0,57 т-розов./голуб.	0,57 т-розов./голуб.	0,57 т-розов./голуб.			
0,60−0,70	0,66 св-корич./голуб.	0,66 св-корич./голуб.				0,66 св-малин./голуб.
		0,69 св-розов./голуб.				
0,70−0,80				0,78 св-корич./голуб.		
0,80−0,90	0,80 т-фиол./розов.	0,80 т-фиол./розов.	0,80 т-фиол./розов.	0,80 т-фиол./розов.	0,80 т-фиол./розов.	0,80 т-фиол./розов.
0,90−1,00	0,90 св-малин./голуб.	0,90 св-малин./голуб.			0,90 св-малин./голуб.	
				0,94 малин./голуб.		



Rice. 2



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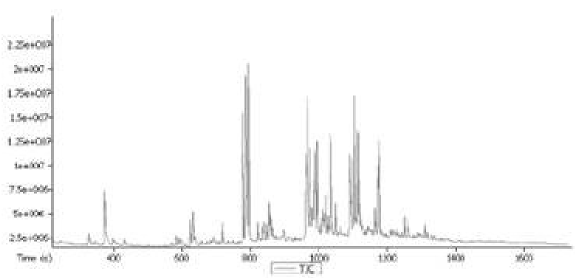

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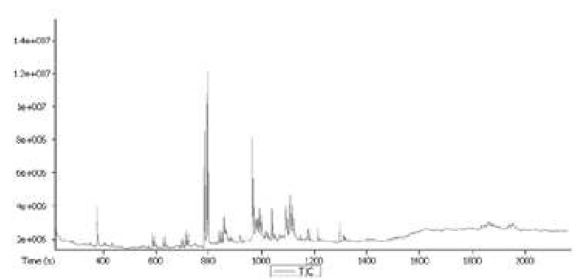

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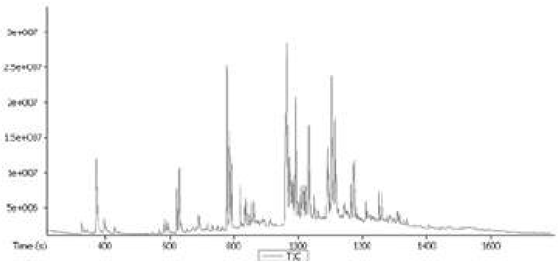

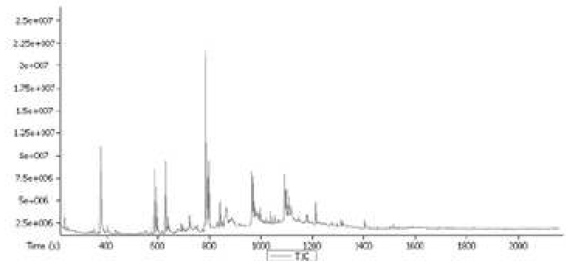

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Rice. 7

Rice. 2. Chromatomass spectrum of essential oil of garlic.
Rice. 3. Chromatomass spectrum of essential oil of onion of the Stuttgarten rizen variety.
Rice. 4. Chromatomass spectrum of the essential oil of the Strigunovsky onion. Rice. 5. The chromatomass spectrum of the essential oil of the Dutch white onion variety. Rice. 6. Chromatomass spectrum of the essential oil of the Red Baron onion.
Rice. 7. Chromatomass spectrum of the essential oil of the onion variety Myachkovsky.

For convenience, they are classified according to groups of similar chemical structure; in some cases, a mixture of individual isomers is presented as one substance. Peak areas are indicated as% of the total area of all peaks. For further targeted research, only those compounds were selected whose content in the mixture exceeds 0.1% (see Table 2).

table 2

The main chemical compounds found in the essential oils of onions and garlic

№ п/п	Группы химических соединений	Чеснок посевной	Луки				
			Ред Барон	Мячковский	Голландский	Штутгартен ризен	Стригуновский
1	**Моносульфиды**						
	Метилаллил-	0,31					
	Аллилпропил -		0,13	0,08	0,02	0,02	0,04
	Дипропил-		0,01	0,17	0,01	0,05	0,09
2	**Дисульфиды**						
	Алкилдисульфид	3,8					
	Диаллилдисульфид	9,57					
	Пропил,изопропил-		0,80	2,9	0,53	0,29	0,20
	Метил,пропил-		0,04	0,29	0,05	0,06	0,10
	2,2-бис-(метилтио)-пропан	0,64	6,0	5,9	6,9	6,8	5,5
	Метил,аллил-	1,7	0,38	0,35	0,20	0,33	0,20
3	**Трисульфиды**						
	Метилаллилтрисульфид	13,38					
	Метилпропилтрисульфид		0,52	2,8	0,34	0,66	0,81
	Диалкилтрисульфиды (пять соединений)		7,22	13,59	3,53	2,88	3,54
	Диаллилилтрисульфид	17,05	0,32	0,54	0,18	0,34	0,15
	Диметилтрисульфид	0,23	0,04	0,11	0,11	0,02	0,04
4	**Тетрасульфиды**						
	Диаллил-	2,08					
5	**Тиофен и его производные**						
	3,4-Диметилтиофен	0,13	2,8	5,4	1,9	2,2	2,3
	2,4-Диметилтиофен		0,60	1,0	0,28	0,56	0,54

№ п/п	Группы химических соединений	Чеснок посев-ной	Луки				
			Ред Барон	Мяч-ковс-кий	Гол-ланд-ский	Штут-гартен ризен	Стригу-новский
	3-метокситиофен		0,13	0,42	0,33	0,20	0,37
	Диметилтиофен	0,08	0,29	0,37	0,26	0,15	0,19
	9-амино-12-тиофен-2-ил-10,11- диоксатрицикло -[6.2.2.0(1,6)]-додекан-7,7,8-трикарбонитрил		0,89	0,95			
6	**1,3-Дитианы**						
	2-винил-	5,8					
	2-пропил-		0,56	2,1	0,37	0,21	0,30
	2-этил-		0,06	0,18	0,02	0,2	0,2
	2-этилиден-		0,11	0,12	0,03	0,05	0,08
7	**1,3,5-Тритианы**						
	1,3,5-тритиан	0,08	2,2	0,80	3,4	2,9	2,6
	4,6-диметил-		0,84	0,13	0,33	0,07	0,04
8	**Дитиоланы**						
	1,1-диоксид	0,39	0,63	0,92		0,18	
	1,1-диоксид 3,5-диметил-		0,71	0,10	0,29	0,07	0,01
9	**Тритиоланы**						
	Алкилтритиоланы (три соединения в сумме)		15,92	8,5	17,0	10,11	6,6
	Изомеры цис--3,5-диэтил -1,2,4-тритиолана		3,67	1,56	3,74	6,07	4,16
	Диметил-		0,17	-	-	-	-
10	**Тетратиоланы**						
	Тетратиолан	2,7					
11	**Пентатиепан**		1,0	0,73	1,96	3,6	2,4
12	Тиабицикло[3.2.1]октаны						
	6-тиа-		0,14	0,16	0,03	0,02	0,01
	8-тиа-		0,41	0,44	0,41	0,22	0,32
13	**Органические кислоты и амиды**						
	3-аллилтиопропионовая кислота	2,8					
	N,N-диметиламид тиоуксусной кислоты	3,2					
14	**Углеводороды**						
	3-винил-1,2-дитиациклогексен-4	5,9					
	4-метоксиметокси-2-метилоктен-4	1,0					
	Энейкозан		1,0	0,19	-	-	-
	Додекан		0,03	0,06	0,05	0,04	0,03
	Тетрадекан	0,04	0,44	0,26	0,11	0,16	0,06
15	**Спирты и сложные эфиры**						
	Алкилтритиоацетаты		0,57	0,28	0,12	0,11	0,14
	6-этилдекандиол-4.5		0,93	0,20	0,66	0,22	0,47
	Эфир додекановой кислоты	0,01	1,6	0,56	0,50	0,14	0,50
	3-изопропил--4-метил-децен-1-ол-4	1,8	0,16	0,04	-	-	-
	Диметиловый эфир 2-окси-3-метоксиянтарной кислоты	0,25					
	Дибутилфталат	0,05	0,66	1,0	0,26	0,34	0,37
	Диоктиловый эфир о- фталевой кислоты		0,12	0,25			
16	**Гетероциклы**						
	5-метил-1,2,4-тиадиазол	1,3	0,09	0,11	0,09	0,10	0,08
17	**Серусодержащие соединения неустановленного строения**						
	Соединение 1		0,62	0,91	0,71	0,94	1,6
	Соединение 2		5,3	2,3	2,4	1,4	2,4
	Соединение 3		1,1	0,15	3,7	3,6	5,8
	Соединение 4		0,20	0,44	0,11	0,12	0,14

As a result of the study, about 20 components of essential oils were identified, accounting for about 75–85% of the amount of oil obtained for analysis. All of them belong to sulfur-containing compounds (mono-, di-, tri- and tetrasulfides of linear and cyclic structure, thiophene and its derivatives, etc.). Terpenoids, alcohols, aldehydes, ketones, organic acids and their esters presented more than modestly. Among the unidentified components, there are 5 substances, the total content of which in the mixture was about 10%.

Noteworthy is the significantly higher content of mono-, di- and trisulfides and 1,3-dithianes in the essential oil from garlic bulbs compared to the oil from bulbs of various onion varieties. A higher content of thiophene derivatives and 1,3,5-tritianes was also revealed in the composition of essential oil of various onion varieties compared to the essential oil from garlic bulbs. Methylallyltrisulfide is found only in the essential oil of garlic; the content of dimethyltrisulfide in oil from garlic bulbs is 2–5 times higher, and diallyltrisulfide content is 30–100 times higher than in essential oil of various onion varieties. Higher dialkyltrisulfides are present only in essential oils of onions.

Tri- and tetrathiolans are found only in essential oils from onion bulbs, and their maximum amount was recorded in the varieties Gollandskiy Belyi and Red Baron. Organic acids are present in noticeable quantities only in the essential oil from garlic bulbs. The presence of higher paraffins is typical only for essential oils of onions, and the content of dodecane is similar for all varieties, and the content of tetradecane is maximum in the varieties Red Baron and Myachkovsky.

Of the heterocycles in essential oils from onion and garlic bulbs, only 5-methyl-1,2,4-thiadiazole was found in noticeable amounts, the content of which in the essential oil from garlic bulbs is more than 1%, and in essential oils from bulbs of different onion varieties, almost 10 times less. Dibutyl phthalate in essential oil of garlic is present at a level of 0.05%, and in essential oils from bulbs of various onion varieties it is 5–20 times more (in the variety Myachkovsky it reaches a maximum value of about 1.0%). The fragrant substance 3-isopropyl-4-methydecene-1-ol-4 is absent in the essential oil of onions of the Gollandsky white, Stuttgarten rizen and Strigunovsky varieties, there is an insignificant amount in the Red Baron variety, and in the essential oil from the garlic bulbs it is almost 2%. In essential oils of onions, 5 sulfur-containing compounds of unknown structure were also found in a total amount from 0.11 (Myachkovsky) to 5, 8% (Strigunovsky). This is the first time that polythianic compounds have been identified in the essential oils of onion and garlic.

As can be seen from the data presented, the qualitative and quantitative composition of essential oils differs significantly in individual components, which could be used as one of the criteria for assessing the authenticity and quality. Within the framework of this study, it remains unclear to what extent these differences will be reproduced by other authors using other analytical equipment. A similar issue is widely discussed in the specialized literature in relation to other types of medicinal plant materials.

When standardizing chemical raw materials used as a pharmacopoeial one, it is very important to accurately identify its composition by the parameters of an essential oil. It is known, however, that with a change in the isolation technology, with the transition from oil from fresh raw materials to oil from dried raw materials, and even simply during storage, its composition changes. For example, S.M. Repyakh. and sotr. [8] proposed a generalized mathematical model that takes into account age-related, seasonal and daily changes in the content of essential oil in the greenery of Scots pine.

The role of the former is manifested in the fact that after cutting the plants, the normal processes of biosynthesis are disrupted, but they do not stop immediately, but after a certain time, so that the speed, temperature and light regime of drying affect the composition of the essential oil. The influence of this factor is the less, the higher the speed and the lower the drying temperature. Physical processes (diffusion and evaporation) are determined by the physical properties of the individual components of the oils, of which volatility is the most important property. Finally, the influence of chemical processes manifests itself in a more or less light oxidation of the components of oils under the influence of various environmental factors, especially those that contain a system of conjugated C = C bonds.

In our study, we also found that during long-term storage of essential oil from bulbs of various varieties of onions and garlic, even in the refrigerator, it becomes cloudy, and its composition changes, which we recorded by TLC. The appearance of new adsorption zones on the chromatograms or a decrease in the fluorescence intensity of the previously detected zones only allows us to make some assumptions about which compounds have undergone destruction during the storage of raw materials. In the chromatography-mass spectra of the studied essential oils, terpenoids and other groups of biologically active substances are present only in minor amounts, although on the chromatograms in a thin layer the corresponding adsorption zones are recorded quite definitely. Further research in this area will make it possible to develop the most objective and scientifically substantiated criteria for assessing the quality of medicinal plant raw materials onion and garlic.

conclusions

1. The qualitative composition of essential oils from garlic raw materials was studied by the method of chromatography-mass spectrometry.

sowing and various varieties of onions.

2. It was found that the essential oil from the bulbs of sowing garlic and various varieties of onions for each type of raw material is a complex mixture, consisting of more than 480 compounds.

3.Using the method of gas chromatography-mass spectrometry, 20 sulfur-containing compounds of essential oil from the bulbs of common garlic and various varieties of onions, which in total make up 75–85% of the essential oil taken for analysis.

4. Differences in the qualitative composition and quantitative content of the essential oil of garlic are shown sowing and various varieties of onions, which can later be used as one of the criteria for assessing their quality.

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