Justification of Saponaria officinalis (*S. officinalis*) cultivation in the soil and climatic conditions of the Primorsky region (Russia) and analysis of saponin- containing root extracts

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ABSTRACT

The purpose of this study was to explore the possibility of cultivation of soapwort (*S. officinalis* L.) in its single-flowered and double-flowered (var. flore pleno *hort.*) forms in the soil and climatic conditions of the Primorsky region (Russia); to study the effects of *S. officinalis* L. vegetation time on the composition and micellar properties of saponincontaining root extracts for their use as highly effective natural surfactants. The dynamics of saponin accumulation in the root as a function of cultivation time was studied. The values of surface tension and critical micelle concentration indicators of saponins and water root extracts were established for both *S. officinalis* L. forms and different vegetation times. The effect of various technological factors on the micellar parameters of root saponins for both *S. officinalis* L. types was investigated. It was found that better saponin parameters of double-flowered form of *S. officinalis* L. allow recommending it as a high-potential source of natural surfactants, comparable to commercial *Quillaja* saponins in its micellar parameters. The identified correlation between changes in the hemolytic activity of root extracts of *S. officinalis* L. and its vegetative stage allows expanding the sphere of their application.

Keywords: biological activity, natural surfactants, roots of soapwort, saponins, triterpene glycosides

INTRODUCTION

Today, the problem of replacement of synthetic surface-active agents (surfactants) with natural ones occupies an important place in a variety of industries around the world. In this context, one of the most urgent tasks is to discover natural surfactants and provide a rationale for their wide practical use as foaming agents, emulsifiers and solubilizers.

The most promising in this regard are plant triterpene glycosides, which due to the amphiphilic structure of their molecules belong to the class of high-molecular colloidal surfactants and are able to create strong bilaminar elastic adsorption layers on the phase boundary, to form micelles in an aqueous solution and to dissolve hydrophobic substances (Trapeznikov et al., 1970; Abramzon, 1981, pp. 239-250; Mitra and Dungan, 1997, 2000; Yang et al., 2013).

Moreover, saponins can provide for the functional orientation of food systems through a wide range of biological and physiologic actions (Sparg et al., 2004; Kuznetsova et al., 2014), which makes it possible to consider them as complex food additives (Güçlü– Üstündag and Mazza, 2007; Tamura et al., 2012; Tsybulko et al., 2004).

Numerous *in vitro* and *in vivo* studies have convincingly proved that hypocholesterolemic and anticarcinogenic effects of plant saponins are associated with their ability to form stable complexes with cholesterol and bile acids,

Central European Agriculture 15SN 1332-9049 with subsequent excretion from the body (Rao and Sung, 1995; Lacaille-Dubois and Wagner, 2000; Mitra and Dungan, 2001; Gurfinkel and Rao, 2003; Kim et al., 2003; Güçlü–Üstündag and Mazza, 2007; Man et al., 2010).

Triterpene glycosides are secondary plant metabolites widespread in flora. They have been discovered in more than 90 families, including the pink family (*Caryophyllaceae*), the ginseng family (*Araliaceae Juss.*), legumes (*Leguminosae / Fabaceae*) and the rose family (*Rosaeceae*), with 900 to 2,000 plant species in each. Members of these families are commercially important, as well as of primary interest for searching new efficient sources of triterpene saponins.

At the present moment, *quillaja* and *yucca* saponins derived from the bark of *Quillaia saponaria* and Yucca schidigera have been most intensively studied (Mitra and Dungan, 1997; Cheeke, 2000; Mitra and Dungan, 2000, 2001; Patra et al., 2012), their water extracts having been recognized as safe additives (having the GRAS (Generally Recognized as Safe) status) and used in many industries, including functional food production (Summary of All GRAS Notices, 2005).

To date, the range of domestic natural food surfactants with high micellar parameters in the food industry is limited. One of the reasons impeding the development of the production of saponin-containing food emulsifiers in Russia is the lack of domestic raw material base and consequently of science-based technologies for the production of saponin-containing plant extracts.

In this regards, *Saponaria officinalis* L. is a promising saponin-containing plant, whose roots contain up to 32% of saponins. In addition, it grows in many climatic zones in Russia and is easily cultivated (Motkhin and Sukach, 1970).

It has been established that the main triterpene glycosides of soapwort – *saponariosides* A and B – are highly similar in structure to commercial *quillaja* saponins of the *Q. saponaria* tree bark, which are practically the only ones permitted in the United States, Japan and some European countries for use in the food industry as emulsifiers, foaming agents and solubilizers (Masahiro et al., 1984; Yukio et al., 1985; Ninomiya et al., 1996; Cheeke, 2000; Wen-Teng et al., 2007).

This work therefore aims to study the possibility of cultivation of soapwort *S. officinalis* L. in the soil and climatic conditions of Primorsky region (Russia), to justify its cultivation periods in order to obtain commercial quantities of saponins and to investigate their functional and technological properties to be used as highly effective surfactants in technologies of unsustainable colloidal food systems.

The objectives of the study were:

- to study the dynamics of root mass and saponin accumulation as a function of the cultivation period of *S. officinalis* L. of two types (single-flowered and double-flowered forms);
- to analyze the effect of time periods and vegetative stage of Saponaria officinalis on the chemical composition of root extracts of both types;
- to describe micellar parameters (surface tension and critical concentration for micelle formation) of saponins and water extracts from Saponaria officinalis (*S. officinalis* L.) of different forms and vegetation times;
- to study the effect of different technological factors on micellar parameters of saponins from the roots of Saponaria officinalis of both types;
- to study the chemical structure of double-flowered form (var. flore pleno *hort*.) of Saponaria officinalis of the second vegetation year.

MATERIALS AND METHODS

Saponin extraction

To extract saponins from plant roots, the standard method was used (Dekanosidze et al., 1982, p. 151). It is based on an exhaustive threefold extraction of dry roots with 70% methanol at the solvent's boiling temperature (in a Soxhlet extractor). The saponin content was calculated by root weight.

Low- and high-polar saponin fractionation

Low-polar saponins (LPS), such as mono- and shortchain bidesmosides were fractionated from water extracts by butanol extraction. Subsequent mixing of water extract with acetone made it possible to isolate highpolar bidesmosides (HPB). Related polysaccharides (PS) were pre-precipitated with 70% ethanol (Dekanosidze et al., 1982, p. 151; Ladygina et al., 1983, pp. 41–56).

Quantitative estimation of polysaccharides and saponins in root extracts

To precipitate polysaccharides in the extract, 98% ethanol was added. After formation of the precipitate, it was centrifuged at 5,000 rpm for 10 minutes, washed with 70% ethanol and air-dried to constant weight at 80°C. The supernatant and washing liquid were evaporated using a rotary evaporator until dry. Saponins were precipitated with a fivefold volume of acetone and then left for 4-5 h at -17°C, centrifuged and air-dried to constant weight at 80°C. The content of polysaccharides and saponins was calculated as a percentage of the mass fraction of soluble solids.

CCM determination by method of fluorescent probes

The method is based on imbedding 8-anilino-1naphthalenesulfonic acid (ANS, Sigma) into the formed micelles (Horowits, 1977). An aliquot (0.5 ml) of ANS (1.6 μ g^{*}ml⁻¹) was added to the equal volume of serial dilution of the studied extracts (concentration of 0.03–90 mg^{*}ml⁻¹), and the mixture was incubated in darkness for 1 h at 25°C. The fluorescence of the resulting solutions was measured at 360 nm at excitation 540 nm. The value of fluorescence for each concentration was calculated as the average of three parallel measurements.

Capillary electrophoresis

The method is based on the ability of monosaccharides containing 1,2-*cis*-hydroxyl groups to form complexes with borate (Tava et al., 2000). The electrophoretic mobility of saponins depended on total positive charge conditional upon the size of the carbohydrate chains,

quantity of 1,2-*cis*-OH groups and free carboxyl groups in the molecule. Electrophoresis was performed in a 150 mM borate buffer (pH 9.3) containing 15% methanol, at 40°C and 20 kV, on a Capillary Electrophoresis System 270A (Applied Biosystem), using a glass capillary 40 cm*50 μ m.

Study of the hemolytic activity of saponins in vitro

Hemolytic activity was investigated on human erythrocytes (O blood groups) (Anisimov and Chirva, 1980). Erythrocytes were washed with phosphatebuffered saline. Then saponin solution of the calculated concentration (0.05-7.0%) was added; the mixture was shaken and incubated for 20 min at 37°C. Unlysed erythrocytes were centrifuged for 10 min at 3,000 rpm. The color intensity of lysed erythrocytes was determined at 540 nm by a Specol-11 spectrophotometer (Carl Zeiss-Jena, Germany). Hemolysis percentage was determined by the following formula:

$$X(\%) = (A \times 100) / k_1, \tag{1}$$

where A - optical density of the studied extract;

 k_1 – optical density of lysed erythrocytes.

RESULTS AND DISCUSSION

Plantations of Saponaria officinalis (*S. officinalis* L.) were established at the Primorsky Fruit and Berry Experimental Station (Vladivostok, Russia). Soboles of the roots of *S. officinalis* L. of two types – common soapwort picked in the suburban area of the Primorsky region, and selectively bred double-flowered soapwort (var. flore pleno *hort.*), taken from the botanical collection of the All-Russian Scientific Research Institute of Medicinal and Aromatic Plants (Vladivostok, Russia), recognized for its strong stems, large leaves and double flowers – were used as seeds.

All plants of the first year of planting blossomed in the period of July 20–25 (bud stage); blossom-time continued until frosts. By the end of the first vegetation period in September (fruiting stage), the height of creepers was 20–25 cm; some short above- ground sprigs were formed

in the bushes. One hundred percent overwintering of the plants was observed in spring. In the second year of cultivation, plants took root and proliferated; overgrowth was noticed on April 20–25 (shoot height was 10–15 cm); intensive plant growth was observed starting from the second ten-day period of June; the massive florification began in the period of July 20–25. Plant height reached its maximum in September; also in this period intensive growth of side shoots was observed. The average plant height was 75 cm, the number of stems per bush varied from 35 to 65. Phenological observations and biometric surveys confirmed the possibilities and prospects for the cultivation of two types of *S. officinalis* L. in the soil and climatic conditions of the region.

The weight of the root system and dynamics of saponin accumulation depending on the plant cultivation period were studied to justify the optimal harvest dates (Table 1).

It was found that the root system of both types of Saponaria officinalis was growing most actively during the second year of vegetation. By the end of the third year, a strong expansion of the root system was observed, which made it difficult to prepare raw material – as part of the roots stuck to the ridges, their total weight did not differ significantly from the weight of the roots of the 2nd year of cultivation.

The saponin content in the second-year roots of double-flowered soapwort was higher (by 30%) than in the roots of single-flowered soapwort (23%). Further cultivation up to 3 years showed almost no increase in the saponin content – 35% and 26%, respectively, providing the grounds for reducing the cultivation of *S. officinalis* to

two years. The results of cultivation of *A. gypsophiloides* R. in Turkmenistan showed maximum saponin accumulation in the roots of the plant (up to 20%) was observed only by the fifth year of cultivation, while the yield of roots was almost 2 times lower than the yield of *S. officinalis* (Gladyshev and Mishchenko, 1990, p. 100).

The practicality of reducing the cultivation period was also confirmed by a comparative analysis of the chemical composition of root extracts of cultured single- flowered soapwort (ESFS), double-flowered soapwort (EDFS) and wild forms of the perennial Saponaria (EWS).

The method for preparing the water extract of *A*. *gypsophiloides* roots was taken as a basis for preparing extracts from *S*. *officinalis* L., this plant being officially permitted for use as a foaming agent in the production of halva (root boiling at 100°C at a liquor ratio 1:20 until the content of extractives is equal to 9-11%).

The analysis of water extracts of the roots collected during the main periods of saponin accumulation – at the bud stage (July) and at the fruiting stage (September) – showed that saponins were the dominant components of all analyzed extracts (Table 2).

The content of saponins varied from 55 to 73% depending on the type of Saponaria. Extracts from double-flowered soapwort had higher saponin contents (67–73%) as compared with extracts from the roots of single-flowered soapwort (58–59%) and wild forms of perennial Saponaria (55%).

We found some regularity in changes in the contents of saponins and polysaccharides as the main extract components, which is associated with the phenological

Table 1. Yield and saponin content of S. officinalis roots depending on the cultivation period

Cultivation period	Weight of the root s	system per 1 m², kg	Saponin content, %		
	double- flowered soapwort	single-flowered soapwort	double- flowered soapwort	single- flowered soapwort	
1 st year	0.4	0.4	_	_	
2 nd year	1.0	0.7	30	23	
3 rd year	1.2	0.8	35	26	

Water extract	PS, %	TF, %	PC, %	PC ₅₀ , μg*ml ⁻¹
EWS (September)	15.0	55.0	3.4	250
ESFS (July)	11.9	58.6	1.7	80
ESFS (September)	12.3	57.6	1.0	250
EDFS (July)	4.6	73.1	1.5	50
EDFS (September)	9.1	66.6	1.1	150

Table 2. Effect of vegetation phase of plant on composition and properties of water extracts from the roots of S. officinalis L.

 $PS - polysaccharides; TF - total fraction of saponins; PC - phenolic compounds; <math>PC_{50}$ - Hemolytic activity - the concentration required to achieve 50% lysis of erythrocytes

cycle of a plant. For instance, the percentage of saponins decreased and that of polysaccharides increased with increasing length of the vegetation period (Table 2). This trend was more clearly visible in the extract of the roots of wild plants, which can be considered the roots of the perennial soapwort of common type, as they served as seeds for the latter. The polysaccharide content in them increased up to 15%, and the saponin content decreased down to 55% as compared with extracts of the roots of the common type plants of the second year of cultivation which were harvested during the same phenological phase. A similar but less pronounced tendency in polysaccharide accumulation depending on vegetative growth phase was also observed - the extracts of the roots of both species harvested in fall showed a higher percentage of polysaccharides and a lower percentage of saponins. Most probably, the increase in the content of polysaccharides is caused by the accumulation of reserve polysaccharides in the roots during plant preparation for winter, as well as by a decrease in the relative weight of plant bark, which is known to be the main place for the accumulation of saponins, as a result of age-related root lignification.

The vegetative phase did not have a material effect on the saponin content in the extracts, because the total content of saponins as a function of harvesting period varied insignificantly (Table 2). However, a significant change in the hemolytic activity of the extracts was observed – the extracts of summer-harvested roots had higher activity (50–80 μ g*ml⁻¹) as compared with fall-harvested roots (150–250 μ g*ml⁻¹), which may be indicative of the high content of more toxic low-polar saponins in the roots of summer plants (Oda et al., 2000).

Consequently, the high saponin content in the root extracts of the second and third years of cultivation and the observed trend of decrease in saponin content in the perennial root extracts make it possible to reduce the cultivation to two years.

As a final justification of the periods for root harvesting, we conducted a comparative study of the physical and chemical properties of the root extracts of Saponaria of various forms and vegetation times.

The physical and chemical properties of the extracts were estimated by the main aspects that characterize the functional and technological efficiency of an emulsifier, such as: surface (interfacial) activity, which determines the ability of the extracts to be adsorbed on the interfacial surface and to reduce the surface tension at the interface; micellar parameters, which include critical micelle concentration being a quantitative characteristic of the efficiency of an emulsifier, and the degree of hydrophobicity of the formed micelles. These parameters of the extracts were estimated as compared with the same parameters of saponins extracted from the roots of *S. officinalis* L. using the standard method (Dekanosidze et al., 1982, p. 151).

Interfacial tension is one of the main parameters which determine the dispersibility of a two-phase system – low surface tension contributes to the formation of

stable finely dispersed emulsion systems. The surface tension isotherm of saponins (Figure 1A) is indicative of their high interfacial activity, as surface tension decreases at the interface to the minimum value of 41–43 mN*m⁻¹ (depending on the type of the roots) with increase in concentration.



Figure 1. Isotherms of surface tension: A – saponins of the roots of single- flowered (1) and double-flowered (2) type; B – extracts of the roots of single- flowered (1) and double- flowered *S. officinalis* (2) (water, 22°C), determined by drop counting method

Interfacial activity of saponins of the roots of *S*. *officinalis* is comparable with the same of commercial quillaja saponin (by Sigma company), for which this value is 36– 37 mN*m⁻¹ (Mitra and Dungan, 1997).

Root extracts showed lower interfacial activity while the isotherm pattern was almost identical to the same of saponins (Figure 1B). The increase in the minimum surface tension up to 55–58 mN*m⁻¹ seems to be caused by the presence of related plant components.

The observed pattern of isotherm curves – passing through a minimum – is related to high heterogeneity of analyzed saponins. According to the results of capillary electrophoresis, saponins from the roots of double-flowered *S. officinalis* L. are a mixture of at least seven saponins with different polarity (Figure 2) and different degree of the hydrophilic-lipophilic balance, and, as a result, different adsorption rate at the phase interface.



Figure 2. Results of capillary electrophoresis of saponins from the roots of double- flowered *S. officinalis* L. (150 mM borate buffer with pH 9.3, 15% methanol; Capillary Electrophoresis System 270A, Applied Biosystem)

A similar pattern of isotherms caused by the complexity of saponins has been shown earlier by Trapeznikov et al. (1970).

Since the emulsifying ability of surfactants depends on their micellar characteristics, the micellar properties of the root extracts of two types of cultivated *S. officinalis* L. are a crucial characteristic when they are used as food emulsifiers (additives). Colloidal surfactants demonstrate their emulsifying properties at concentrations higher than CCM, therefore, this value can serve as a quantitative parameter describing the efficiency of emulsifiers (Abramzon et al., 1984).

CCM was determined using the fluorescence probe method. Figure 3 shows the effect of saponin concentration in the roots of single-flowered and doubleflowered *S. officinalis* L. (summer harvest) on probe incorporation.



Figure 3. Effect of saponin concentration on incorporation of ANS into micelles of saponins from single-flowered (1) and double-flowered (2) types of *S. officinalis* L.

The increase in fluorescence higher than CCM value was indicative of the start of formation of micelles. Saponins from the roots of double-flowered soapwort and single-flowered soapwort had different CCM values, because the linear increase in fluorescence was registered after the critical concentration value of 0.68 and

1.20 mg^{*}ml⁻¹ (in water), respectively, was reached. The slope of fluorescence curves, which was determined by the amount of the incorporated probe, was indicative of a higher level of hydrophobicity of the micelles formed by saponins of double-flowered soapwort.

Micellar characteristics of saponins extracted from double-flowered soapwort were similar to those of commercial samples of *quillaja* saponins (by Sigma and Acros Penco companies), whose CCM value varied from 0.56 to 0.75 mg*ml⁻¹ (Mitra and Dungan, 1997). Any comparison with the properties of traditionally used food emulsifiers, such as lecithin (E322), mono- and diglycerides (E 471), seems incorrect, as they are waterinsoluble and have another mechanism of hydrocarbyl adsorption at the interface. Micellar characteristics of aqueous extracts, as well as characteristics of saponins, depended on the type of plants (Figure 4).



Figure 4. Effect of cultivation time of *S. officinalis* on CCM values of the extracts: 1, 2 – from the roots of double-flowered soapwort, 3, 4 – from the roots of single-flowered soapwort (1, 3 – the third year; 2, 4 – the second year)

An increase in the fluorescence of the root extracts of double-flowered soapwort being indicative of the start of formation of micelles, was also observed at a lower concentration – 1.24 μ g*ml⁻¹, while for the extract of single-flowered soapwort it was observed at the concentration of 3.64 μ g*ml⁻¹ (summer harvest roots). The slope of fluorescence curves also pointed to a higher hydrophobicity of the micelles formed in the extract of double-flowered soapwort. The higher CCM values as compared with the same of saponins, are apparently associated with related plant components (polysaccharides and phenolic compounds) contained in them.

Root age did not have any material effect on the pattern of fluorescence curves. The extracts from the roots of the second and third years of cultivation had a similar slope of fluorescence curves, and formation of micelles started at the same mass fraction of soluble solids (Figure 4).

Micellar characteristics of ionic surfactants are generally known to depend on pH value and ionic strength of water medium. Since carboxyl groups are present in the structure of saponins of *S. officinalis* L. (one in the aglycone, another one – in the carbohydrate chains), it was necessary to identify the effect of these factors on micellar characteristics of saponins (Figure 5).



Figure 5. Effect of pH of the medium (A) and salt concentration (B) on CCM value of saponins of.: 1 – of single-flowered *S. officinalis* L; 2 – of double-flowered *S. officinalis* L (22°C)

The results of our study showed that CCM values of saponins gradually increased with increase in pH values. In an acidic medium (pH 3-5), CCM values of saponins were low as a result of protonization of the charged groups.

lonization of carboxyl groups in an alcaline medium (pH 8) caused an increase in electrostatic repulsive forces, which resulted in micelle formation at a higher saponin concentration.

Saponins of *S. officinalis* L. demonstrated the same correlation between changes in CCM values and concentration of NaCl, which is typical of all ionic surfactants. For instance, in the solutions with weak ionic force (0.12 M), micelles were formed at the highest

saponin concentration as a result of electrostatic repulsive forces of the carboxyl groups. As salt concentration increased, a significant decrease in CCM values was observed, which is attributed to the partial screening of electrostatic repulsive forces. A sharp decrease in the values was observed at the salt concentration of 0.5 M. Further increase in salt concentration did not have a material effect on the CCM value. In all experiments, the CCM values of saponins from double-flowered soapwort were lower than those from single-flowered soapwort (Table 3).

The morphological characteristics of the structure of *S. officinalis* L. roots, their branched rhizome formed by small offshoots and absence of woody core provide for their high content of extractives. For instance, more than 50% extractives of various nature were extracted from the roots of double-flowered *S. officinalis* L. of the second vegetation year using the standard method, which is widely used for the extraction of secondary metabolites of different classes (Ladygina et al., 1983, pp. 41–56) (Table 4).

The main secondary metabolites of two-year roots were saponins, since their total content was 38% by dry root weight, which is equivalent to 79% of the mass fraction of all extractives. Among them, high-polar saponins were dominant (29%), while the percentage of low-polar saponins was lower (8%). In addition to saponins, the roots contained water-soluble polysaccharides (5.5%) and phenolic glycosides (3%). The content of protein substances and lipids did not exceed 0.9% and 0.3%, respectively, which is typical of plant raw materials. The high content of mineral substances (5%) found in the roots was evidently caused by the ability of the roots to selectively accumulate micro- and macronutrients (Lovkova et al., 2001). The roots of the cultivated S. officinalis L. are "super"-concentrators of some micro- and macronutrients, which are biologically important for any living organism (Table 5).

Their content significantly exceeds the average values typical of the plants of this growing area.

Demulation	pH of medium				NaCl, M, pH 7.0				
Population	рН 3	pH 4	pH 5	pH 7	pH 8	1	0.5	0.25	0.12
Single- flowered soapwort (S. officinalis L.)	0.67	0.82	1.20	1.81	2.14	0.34	1.11	1.51	2.27
Double- flowered soapwort (var. flore pleno <i>hort</i> .)	0.39	0.72	0.95	1.38	1.66	0.19	0.47	0.51	1.01

Table 3. CCM values of saponins of S. officinalis L. as a function of the aqueous phase conditions, mg*ml⁻¹

 Table 4. Chemical composition of roots of double-flowered S. officinalis L. of the second year of vegetation

Content, %								
High- polar saponins (HPS)	Low- polar sa- ponins (LPS)	Water- soluble polysacchari des	Phenolic glyco- sides	Protein substances	Lipids	Minerals		
29	8	5.5	3	0.9	0.3	5		

Table 5. Mineral composition of the roots of cultivated double-flowered S. officinalis L.

Content, μg*g ⁻¹								
К	Ca	Mg	Р	AI	Fe	Mn	Cu	Co
9477	7717	556	2713	444	222	23.1	2.1	0.4

CONCLUSIONS

In summary, the shortest possible cultivation period (2 years), high yields (10 tonnes per 1 ha) of the roots containing 26–35% saponins allow regarding *S. officinalis* L. introduced in the soil and climatic conditions of the Primorsky region as a high- potential commercial source of saponins. The established correlation between the changes in hemolytic activity of the root extracts of *S. officinalis* L. and the vegetative growth stage makes it possible to expand the fields of application of the roots. It is practicable to use roots harvested during autumn (period of their low toxicity) for the production of food emulsifier, and roots harvested during the bud stage (period of the highest toxicity) – for medical and cosmetic purposes.

High surface activity and low CCM values of saponins provide for the functionally technological efficiency of the aqueous root extracts of two types of *S. officinalis* cultivated in the Primorsky region. However, the better parameters of saponins from double-flowered type allow recommending this type as a prospective source of highly effective natural surfactants, which are at least equal to commercial quillaja saponins in terms of their micellar parameters.

A high content of mineral substances (5%) was found in the roots. The roots of cultivated *S. officinalis* L. are "super" - concentrators of some micro- and macronutrients biologically important for living organisms.

A decrease in CCM values under acidic conditions and in the presence of salt makes it possible to modify the emulsion preparation technology using saponins as natural emulsifiers by varying the medium acidity and salt concentration.

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