Prospects of naked licorice cultivation in the caspian region for the creation of foam licorice baths (based on the drug glytsrfit)

Liudmila Sukhenko(0000-0001-5841-655X)[1], **Elsayed Zeitar**(0000-0003-2106-556X)[1,2 (1)], **Anna Fedotova**(0000-0003-0241-1797)[1] **Mikhail Egorov**(0000-0001-5788-6242)[1,3]

[1] Astrakhan State University, Astrakhan, Russia
[2] Damanhour University, Damanhour, Egypt
[3] Moscow State University of Food Production, Moscow, Russia

Abstract. The prospects of cultivation of naked licorice in the territory of the Caspian region and the possibility of using extractive saponin-containing foaming components of licorice root to create foam licorice baths with foam content stability were studied. The latitude, longitude, and height of licorice root collection sites with row furrows are presented only in rows. A new approach to preserving the hydrophilic-lipophilic balance in a licorice bath is proposed by embedding colloidal surfactants into the micelles of licorice root saponins, which increases the stability of licorice foam in the bath. The data of the critical concentration of micelle formation are presented. The possibility of restoring the stocks of licorice naked during its cultivation and harvesting by the row method is shown, and methods for preserving the solubilizing ability of foam licorice baths under conditions of preserving licorice foam micelles in the presence of some surfactants are disclosed.

Keywords: Licorice naked root, Glycyrrhiza glabra L., Glytsrfit, micelle formation, solubilization, Caspian region.

1. INTRODUCTION

Currently, preparations from medicinal plants are of particular interest. A wide range of effects from the molecular to the level of the organism, the absence of allergic and toxic reactions, and other complications with prolonged use have gained them great popularity. The most significant use of them is with the help of new delivery systems: foam baths, drinking oxygen cocktails, cosmetics, which expand the scope of application, indications and narrow the range of contraindications in the conditions of sanatorium treatment and domestic conditions. Recently, foam licorice baths have become more and more active in spa therapy. This is a special kind of foam bath in combination with herbal medicine, which ultimately provides a high therapeutic effect. The naked licorice plant Glycyrrhiza glabra L. of the Fabaceae family, a well-known medicinal plant, is cultivated as an agricultural crop in some regions. Licorice components are used as a drug as an expectorant, hypoallergenic agent, there are publications about the hepatoprotective, immunogenic nature of the action of licorice components [1, 13]. Licorice root contains many biologically active components of different groups, but the main component is glycyrrhizic acid and its salts, its isomer is glycyrrhizic

[1] Corresponding author: elsayed.zeitar@agr.dmu.edu.eg

acid, it is an aglycone, many groups of flavonoid compounds contain saponins, terpenoids, resins, carbohydrate compounds, proteins, including lectin proteins, which have the absorption properties of antibodies [2]. The components of licorice extract Glytsrfit have an immunostimulatory effect on the activity of peritoneal macrophages of peripheral blood [3]. Numerous areas of licorice fields are observed in the Caspian region, including the territory of Astrakhan region, Russia [4]. The territory of the Caspian region with an arid climate and alluvial delta soils, the peculiarity of which is the layering of the soil profile and the presence of enriched fertile alluvial sediments [5]. It is an ideal place for the distribution and cultivation of licorice as a crop, and climatic characteristics, such as high annual insolation and temperature allow high levels of biologically active substances to accumulate in the roots and the aboveground part of licorice [14].

In the food industry, including in the production of soft drinks, there is wide use of mixture substances that regulate the structure of finished products - emulsifiers, foaming agents, vegetable hydrocolloids, etc. Due to the synergistic effect, they contribute to the production of a new unique texture, such as fluid gels, emulsion drinks, solubilizates, etc., and expand the range and quality of multicomponent liquid food systems [15]. The formation of micelles of surfactants occurs, as a rule, in a narrow concentration range and is characterized by the so-called critical concentration of micelle formation (CCMF), at which a maximum of structure formation is observed in the system. For the roots of licorice smooth (naked) and its other species, the value of the critical concentration of micelle formation is usually determined by the method of incorporation and the characteristic of colloidal surfactants is the hydrophilic-lipophilic balance (HLB), which determines the ratio of the sizes of the polar and non-polar parts of the molecule. High values of HLB and CCMF of plant licorice saponins determine their numerous physicochemical properties - moisturizing, foaming, emulsifying, solubilizing, instantizing, detergent, etc. [6]. The main characteristics of foam systems are 1) foaming capacity - the amount of foam (in ml), or the height of the column (in mm), which is formed from a constant volume of the solution during a given time; 2) the rate of foam - the ratio of the volume of the foam to the volume of the solution; 3) stability of foam - the ability to maintain the total volume, dispersed composition of the liquid; foam dispersion - average bubble size, per unit of foam volume [7]. The main factor affecting the foaming ability of surfactants is the concentration [8]. With an increase in the surfactant concentration, the foaming ability of the solution first increases to a maximum value, then remains practically constant or decreases.

The effect of temperature on foam stability is believed to be due to many competing processes. An increase in temperature leads to an increase in the pressure inside the bubbles, an increase in the solubility of the surfactant, and a decrease in the surface tension. All these factors contribute to increased foam stability [7]. However, as the temperature rises, the thermal vibrations of surfactant molecules adsorbed on the interfacial layer increase and, therefore, the mechanical strength of the formed surface layer weakens. In addition, an increase in temperature leads to a decrease in the viscosity of the solution and a change in the conditions of hydration of the polar groups of the surfactant, which causes an increase in the rate of flow of liquid from the foam and a decrease in the stability of the foam. In this regard, the influence of the temperature of the pH medium on the stability of the foam must be analyzed in each specific case. Most surfactants stabilize foam in an alkaline environment. The foaming ability of nonionic surfactants does not depend on the pH value in the range of values from 3 to 9. Protein solutions exhibit maximum foaming ability at pH values below 7 [8]. An important property of micelles of plant foaming agents is that they have a highly organized internal structure, which is a certain arrangement of surfactant molecules in micelles corresponding to the rule of equalizing polarities. In micelles, in which non-polar parts of the surfactant molecules (usually hydrocarbon chains) are in the inner part of the micelle, they are called straight lines [9]. Some biotechnological preparations were developed based on the studied properties of the plant Glycyrrhiza glabra [10].

Therefore, the purpose of the work is to study the prospects for the cultivation of licorice in the Astrakhan region of the Caspian region and to work out the possibility of using extractive saponin-containing foaming components of licorice root to create foam licorice baths with foam content stability.

2. MATERIALS AND METHODS

To obtain the drug (extract of licorice Glytsrfit), plant roots were taken from different sites of the Astrakhan region (Fig.), including on the territory of the educational and experimental farm of the Astrakhan State University (ASU) Nachala village (sample No. 1). Geo-informational referencing was carried out for sampling sites of licorice root.

Data of latitude, longitude, and altitude of licorice root plots (samples) for studying the chemical composition and the possibility of obtaining licorice foam baths are presented in table 1.

Table 1. Data of latitude, longitude, and altitude of licorice root samples (Astrakhan region)

Plot no.	Latitude	Longitud	Altitude
Sample no. 1	46.3059°	48.24190°	-25 м
Sample no. 2	46.268°	48.40725°	-26 м
Sample no. 3	46.33729°	48.4908°	-25 м
Sample no. 4	46.41903°	48.30067°	-28 м
Sample no. 5	46.38675°	48.20246°	-25 м

After the complete accumulation of all biologically active components of licorice root from September to November 2020, the studied area was plowed with furrows and the root was collected only in rows. Then these furrows were closed with soil for further development of plant specimens and strengt hening of the remaining roots. After harvesting, the roots and rhizomes were cleaned from the soil, dried, weighed, made a visual inspection of the parts of the plant, stored in a paper package.

These plant stocks were crushed on a special shredder to the size of 0.5-2 cm parts of the dry root, extracted by triple extraction of biologically active components by the method of Sukhenko *et al*. 2009 [11]. Then the concentration, fermentation, pasteurization, and evaporation of ethanol vapors were carried out in a special temperature evaporator. The mass fraction of glycyrrhizin in the obtained extracts was studied by chemical method. To do this, 30 g of samples were taken from each site, an extraction preparation was prepared, and the level of glycyrrhizin was studied. To do this, 200 mg of dry licorice root powder was measured into a centrifuge tube, 20 ml of a solution of 0.4 g/l of potassium hydroxide was added. They were mixed with a piston shaker at 250 rpm for an hour (the stage of primary extraction). This extract was then centrifuged at 10,000 rpm for 5 minutes. The supernatant was separated. 20 ml of potassium hydroxide solution 0.1 g / l was added to a centrifuge tube with sludge residues after collecting the supernatant.

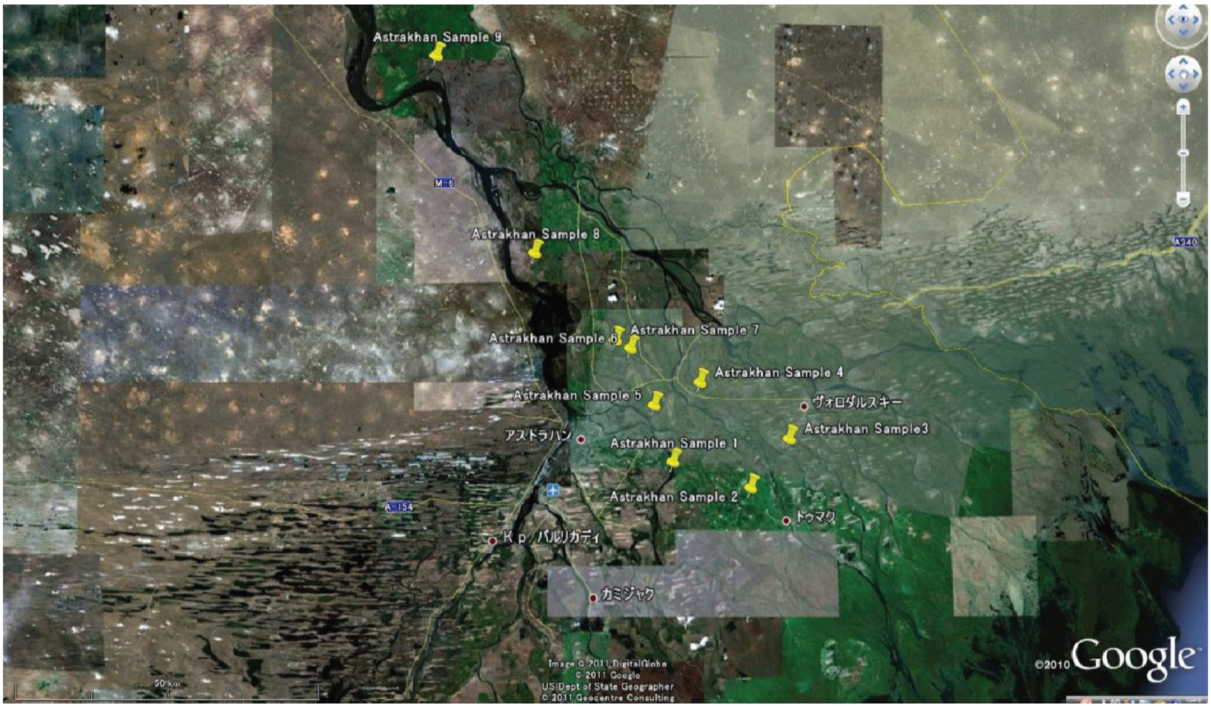

Figure 1. Licorice root collection sites (5 sites) in Astrakhan region (GPS data).

They were mixed with a piston shaker at 250 rpm for an hour (the stage of secondary extraction). The primary extraction supernatant was added to the second extract, shaken on a piston shaker at 250 rpm for 5 minutes. The mixed extract was centrifuged at 10,000 rpm for 5 minutes. The supernatant was filtered through a filter (0.45 microns) and a sample was obtained for quantitative measurement of glycyrrhizin. Based on the quantitative measurement value, the glycyrrhizin content was recalculated by humidity (10%) at complete drying. The finished extracts were mixed with foaming and foam-stabilizing substances to create the stability of licorice foam micelles. Also were used: Decyl Glucoside, a non-ionic surfactant of vegetable origin, used as an additive or a joint surfactant in cosmetic detergents; Lauryl Glucoside, a foaming element of surfactants of detergents obtained in the process of fat rectification, is used for intimate hygiene products and children's shampoos, gels, bath foam, increases the viscosity. Synthesized from natural raw materials such as coconut oil and glucose; Sodium Palmate, obtained by alkaline hydrolysis of palm oil; Cocamidopropyl Hydroxysultaine, a fatty acid from coconut oil; Sodium Cocoamphoacetate, amphoteric surfactant, foam-enhancing surfactant, has a mild cleansing effect (aqueous solution of amphoteric surfactant derived from coconut fatty acids); Potassium Oleate (anionic surfactant); Potassium Cocoate (non-ionic surfactant, the result of the interaction of coconut oil with alkali); Lauryl betaine (amphoteric surfactant, antistatic, has a slight conditioning effect), contains mild detergent components that can be used by people with sensitive skin, the advantage is a mild effect on the skin, since the particles change their charge depending on the environment, has a detergent, bactericidal and foam-stabilizing activity. In addition, a group of amphoteric surfactants, derivatives of betaine (cocoaminopropyl betaine) were used; imidazoline (sodium cocoamphoacetate); cocamidopropylamine oxide 30%; oxides of tertiary amines "OXIPAV" (OXIPAV A14.25; OXIPAV A1416.30; OXIPAV AP.33).

In combination with anionic surfactants, OXIPAS improve foaming ability and increase the harmlessness of formulations. It is known that an important characteristic of the formed foam is the multiplicity of its column. A decrease in the flow rate of liquid from the foam is observed with an increase in the foam rate (K = -0.98), a decrease in the average diameter of an air bubble (K = 0.91) and the viscosity of the solution (K = -0.98) and kinetic stability (%) [12]. The baths with the components of the studied foam baths with the listed surfactants were mixed in a shaker for 3-5 minutes to form abundant foam and left to monitor the foam stability and its decrease for 2 days. Surfactants were added to the Glytsrfit preparation in a ratio of 1: 100. The test of the use of the investigated preparations of foamy licorice baths was carried out at the National Medical Research Center for Rehabilitation and Balneology of the Ministry of Health of the Russian Federation (FSBI "NMIC RK" of the Ministry of Health of Russia).

RESULTS AND DISCUSSION

The level of glycyrrhizin was determined in samples of licorice root taken from different study sites (in two repetitions: analog 1, analog 2) in % of dry weight in terms of humidity. The determination of the level of glycyrrhizin in two samples prepared according to the standard method of the drug Glytsrfit with the studied surfactants is shown in Table 2 at $p \leq 0.5$.

After determining the level of glycyrrhizin content, which turned out to be from 8.0 to 11.4 % by dry residue at different sites of licorice root sampling to prepare the drug Glytsrfit, samples of the drug Glytsrfit with the studied surfactants were studied.

Table 2. The level of glycyrrhizin in samples of licorice root

Plot no.	Glycyrrhizin content (%)			
	General content	analog 1	analog 2	% in dry residue
Sample no. 1	3.39	17.8	6.0	8.9±0.01
Sample no. 2	2.83	18.0	6.5	10.0±0.2
Sample no. 3	3.92	17.1	4.4	8.0±0.01
Sample no. 4	3.26	15.0	7.5	9.5±0.1
Sample no. 5	4.10	17.2	5.8	11.4±0.03

The results of the solubilizing activity of preserving a highly organized internal structure, which is a certain arrangement of surfactant molecules in micelles, corresponding to the rule of equalization of polarities. The results of the study of the foam-preserving properties of the compositions of the drug Glytsrfit and surfactants are presented in Table 3.

As a result of the work, the most stable foaming was found when cocoaminopropyl betaine derivatives (34/120 at t=36 °C), OXYPAV A14.25 (50/480 at t=36 °C); A1416.30 (45/240 at t=36 °C; OXYPAV AP.33 (55/600 at t=36 °C), Lauryl betaine, an amphoteric surfactant (50/45 at t=48 °C) and Sodium Palmate (25/60 at all t). The remaining foaming components in the preparation Glytsrfit formed an abundant foam that enhances the foaming of licorice saponins, however, these mixtures did not have stable micelle formation, preserving the hydrophilic-lipophilic balance in the licorice bath.

Table 3. The foam preserving ability of the drug Glytsrfit and the studied surfactants in the ratio of 1:100 at different temperature conditions

Surfactants	Foam column height/time, mm / min at temperatures			Kinetic stability of the foam column (%)		
	t = 22 °C	t =36 °C	t = 48 °C	t = 22 °C	t =36 °C	t = 48 °C
cocoaminopropyl betaine	30/120	34/120	32/90	<50	<50	<50
OXIPAV A14.25	45/240	50/480	50/120	50-80	50-80	50-8 0
OXIPAV A1416.30	45/240	45/240	45/60	>80.0	>80.0	>80.0
OXIPAV AP.33	45/120	55/600	45/60	>80.0	>80.0	>80.0
Lauryl betaine	45/60	45/60	50/45	>80.0	>80.0	>80.0
Sodium Palmate	25/60	25/60	25/60	<50	<50	<50

The greatest foaming property was possessed by mixtures of licorice preparation Glytsrfit with OXIPAV AP.33, OXIPAV A14. 25 and cocoaminopropyl betaine, especially at a temperature of t =36 °C. At the same time, the degree of dispersion of the foam-containing phase was 0.4 and 0.5 mm, respectively, and was provided by a mixture of licorice saponins and surfactants. A full and stable foam volume was obtained for a 50 L bath capacity after 2.0-2.5 minutes at a water supply speed of 1.7-2.2 L/Sec.

Our results confirm the opinion of Khadzhieva et al. 2010 [12] on the importance of using foamy licorice baths with anti-inflammatory action in balneological practice.

A new approach is proposed to preserve the hydrophilic-lipophilic balance in a licorice bath by embedding with colloidal surfactants, which increases the stability of licorice foam in the bath. High values of the hydrophilic-lipophilic balance and the critical concentration of micelle formation of licorice plant saponins are achieved as a result of working out mixing under different conditions and exposure of substances with licorice root saponins in a liquid medium. As a result, samples of licorice baths with a foam content (foam stability) from 1.5 hours to 8 hours were obtained.

As a result of the research, a study was developed and conducted on using a new SPA BATH - (a preparation for foam licorice baths). The preparation consists of 95% - saponin-containing root extract and phytohormonal component (glycyrrhizin), flavonoids, tannins, terpenoids, and other dermato-protective and anti-inflammatory components of the plant *Glycyrrhiza glabra* L., 5% - nonionic surfactants - esters of polyglycerin and fatty acids (E 475), esters of sucrose and fatty acids (E 473) used in the food industry for the solubilization of fats, essential and aromatic oils (fragrances) water-based as additional components for obtaining stable micelles (bubbles). The main characteristics of foaming of a spa bath are foam stability, high critical concentration of micelle formation, which has a highly organized internal structure containing useful substances of the licorice root extract in each micelle bubble; neutral acidity (pH 6-6. 5) of the medium; creation of a skincare environment in the bath; inhalation effect.

CONCLUSION

Thus, it was shown that further studies of the agrobiological cultivation of licorice culture on experimental fields would allow us to continue working on the creation of new biotechnological balneological therapeutic rehabilitation and preventive Phyto-preparations with improved numerous Physico-chemical and therapeutic properties (moisturizing, surface foaming, emulsifying, solubilizing properties) during therapeutic, rehabilitation, cosmetic and preventive procedures. The cultivation of wild plants (roots of naked licorice (*Glycyrrhiza glabra* L.) in the conditions of the Arid Zone of the Caspian region of Russia is justified, significant concentrations of broad-spectrum biological active substances have been established in them.

AUTHORS' CONTRIBUTIONS

L. T. Sukhenko: Conceptualization, Methodology, Supervision and Writing Original draft preparation. Elsayed M. Zeitar: Conceptualization, Methodology, Writing, Review and Editing. A. V. Fedotova and M. A. Egorov: Data Curation, Validation and Visualization.

ACKNOWLEDGMENTS

The researcher Elsayed M. Zeitar is funded by a scholarship under the joint (executive program between the Arab Republic of Egypt and the Russian Federation)

REFERENCES

1. B. Fu, H. Li, X. Wang, F. S. C. Lee, S. Cui, Isolation and Identification of Flavonoids in Licorice and a Study of Their Inhibitory Effects on Tyrosinase, in: Journal of Agricultural and Food Chemistry, 2005, no. 53 (19), pp. 7408-7414. DOI: 10.1021/jf051258h

2. L. Wang, C.L. Weller, Recent advances in extraction of nutraceuticals from plants, in: Trends in Food Science & Technology, 2006, no. 17 (6), pp. 300-312. DOI: 10.1016/j.tifs.2005.12.004
3. A.K. Maslov, G.N. Nazarova, L.T. Sukhenko, Vliyaniye ekstrakta iz kornya solodki na funktsionalnuyu aktivnost peritonealnykh makrofagov myshey, zarazhennykh vnutribryushinno mikrobakteriyami tuberkuleza, in: Vestnik novykh meditsinskikh tekhnologiy, 2008, no. 15 (4), pp. 212-213.
4. L.T. Sukhenko, Dikorastushchiye rasteniya flory YugaRossii kak istochnik tsennykh fitokomponentov s protivomikrobnymi i bioregulyatornymi svoystvami, KNORUS, Moscow, 2017, 304 p.
5. A.P. Sorokin, S.P. Strelkov, A.V. Fedotova, Sravnitelnyy analiz Agrofizicheskogo sostoyaniya lugovykh pochv Volgo-Akhtubinskoy poymy i delty reki Volgi, in: Agrarnyy vestnik Urala, 2017, no. 9 (163), p. 10.
6. S. Rayne, G. Mazza, Biological Activities of Extracts from Sumac (Rhus spp): A Review, in: Plant Foods for Human Nutrition, 2007, no. 62 (4), pp. 165-175. DOI: 10.1007/s11130-007-0058-4
7. K.R. Lange, Poverkhnostno-aktivnyye veshchestva. Sintez, svoystva, analiz, primeneniye, Professiya, St. Petersburg, 2005, 238 p.
8. V.K. Tikhomirov, Peny. Teoriya i praktika ikh polucheniya i razrusheniya, Khimiya, Moscow, 1983, 264 p.
9. G.A. Fattakhova, Saponiny kak biologicheski aktivnyye veshchestva rastitelnogo proiskhozhdeniya, in: Vesnik Kazanskogo tekhnologicheskogo universiteta, 2014, no. 3, pp. 196-202.
10. L.T. Sukhenko, M.A. Egorov, Biotekhnologiya sozdaniya pishchevoy dobavki "Ekstrakt solodki Glytsrfit" v kachestve fitobiotika dlya normalizatsii kishechnoy mikroflory, in: Kaspiy XXI veka: puti ustoychivogo razvitiya: Materialy Mezhdunarodnogo nauchnogo foruma, 2020, pp. 48-51.
11. L.T. Sukhenko, G.N. Nazarova, N.G. Urlyapova, Ekstrakt solodki goloy, obladayushchiy protivotuberkuleznoy aktivnostyu. Patent RF no. 2362577. 2009, in: Byulleten, 2009, no. 21, pp. 1-7.
12. Z.D. Khadzhiyeva, Tekhnologiya pennykh vann dlya ispolzovaniya v balneologicheskoy praktike, in: Nauchnyye vedomosti. Seriya Meditsina, Farmatsiya, 2010, no. 22 (93), iss. 12/2, pp. 55-57.
13. A. Abramovag, M.V. Palagina. Solodka uralskaya i ee ispolzovaniye v pishchevoy i farmatsevticheskoy promyshlennosti, in: Vestnik TGEU, 2005, no. 1, pp. 77-87.
14. L. Wang, C.L. Weller, Recent advances in extraction of nutra-ceuticals from plants, in: Trends in Food Sciense & Technology, 2006, no. 17, pp. 300-312.
15. E.I. Cherevach, R.K Shchekaleva, 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, in: Journal of Central European Agriculture, 2020, no. 21 (2), pp. 420-430.