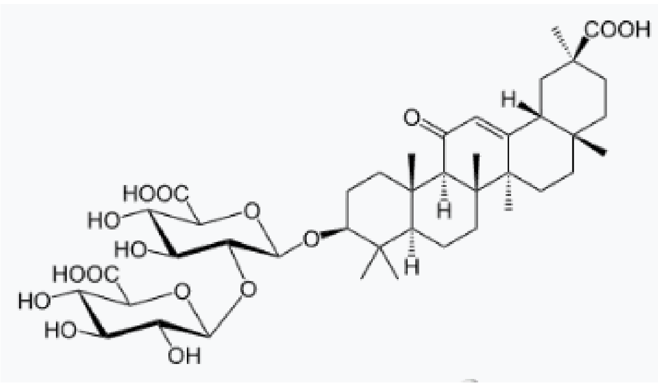
ECAMPUZ - European World Talent Camp for Uzbekistan Scientists in Food Science and Technology

REPORT - Team 5

Title: Developing functional bioactive ingredients based on uzbek traditionally consumed herbs or spices

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ABSTRACT

Nowadays, plant extracts are widely used in the production of biologically active additives, flavouring and colouring preservatives. Liquorice root extract is used in the food industry as a unique flavouring and colouring agent. Studying and introducing into practice the technology of obtaining syrup and preservatives from the local liquorice plant is one of the urgent tasks of our time.

In particular, liquorice syrup contains the food additive E-958 - glycyrrhizic acid, which is a sweetener in food products. It is 50 times stronger than sucrose.

I. MAIN PART

1.1 General characterisation of licorice raw material:

BAS – Biologically active substances

ES – Extractive substance

GA – glycyrrhizinic acid

GcA – glycyrretinoic acid

MSGA – monoammonium salt of glycyrrhizic acid.

More than 570 medicinal plants have been identified in our country. From these 230 species of medicinal plants, various raw materials are procured for pharmaceutical industry. Among them, licorice is one of the widely used plants. Licorice plant belongs to the family of *Fabaceace* (legumes). Licorice root is one of the oldest medicines. There are 13 species of licorice in the world. The most common species are licorice (Glycyhriza glabra), Ural licorice *(Glycyrrhiza uralensis Fisch*) and Korshinsky's licorice *(Glycyrrhizae Korshinskyi Grig)* [7,8]. Among them, common licorice is famous, the roots of which contain the greatest amount of BAS [2, 3]. Many species of Licorice grow in Eurasia.

The raw material is mostly cylindrical in shape with a slightly smaller, sometimes longitudinally cut, root segment diameter.

1-5 cm, length 15-40 cm. Externally, the unpurified root is purplish brown. The purified root is yellow, with coarse hairs, radial inside. Glycyrrhizin is the main component of the root.

1.2. Characteristics of Glycyrrhizinic acid

In appearance, Glycyrrhizin is a colourless crystals or crystalline powder with a pronounced sweet liquorice taste, without a definite odour. The food additive E-958 is about 50 times sweeter than sucrose. In the form of licorice extract, the substance has the appearance of a homogeneous thick mass, coloured dark brown, possessing a cloying, irritating odour. Glycyrrhizin is characterised by a high degree of solubility in hot water and ethyls, in cold water - moderately soluble. In nature, the additive E-958 is found in the juice of plants of the liquorice family (Ural licorice, licorice naked) and the legume family. The sweetener is obtained from the purified juice of these plants. To prepare the extract, the dry root is thoroughly cleaned from the ground, washed and soaked for a day in clean hot water (60-80 °C) to soften. Then it is cut into 3-4 cm pieces and boiled 3-4 times in fresh water. The resulting weak extracts are boiled down. Glycyrrhizin has emulsifying ability and belongs to steroidal saponins. Due to its structural similarity to steroid hormones, it has a number of unique properties: anti-inflammatory, immunomodulatory, antiviral (including herpes simplex virus), anti-allergic, antipruritic.

Uses E-958:

Food additive E-958 is used as an elasticiser, flavour and aroma enhancer, and foaming agent. In the food industry, glycyrrhizin is mainly used in the manufacture of diabetic foods and non-cariogenic food products. The specific and persistent liquorice flavour limits the scope of its use to a few items ("spicy" confectionery, bitter tinctures, pastis).

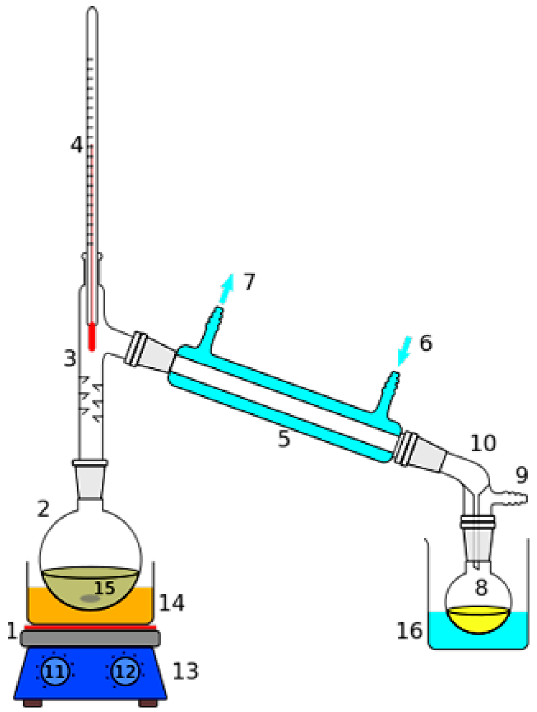
E-958 sweetener in foods: For flavouring tobacco and chewings; in cough medicines, expectorants and anti-inflammatory medicines. The strong liquorice flavour limits the use of glycyrrhizin to "spicy" confectionery, pastis and bitter tinctures. In very low dosage it exhibits properties of a flavour and aroma enhancer.

In Uzbekistan it is most often used as a foaming agent in the production of halva. Caramel mass is beaten with decoction of soap or licorice root or their mixture to form a porous friable mass necessary for obtaining fibrous structure of halva. Decoction of licorice root density of 1120-1150 kg/m3 added to the caramel mass in an amount of up to 2% of the mass and beat at a temperature of 100-115 °C. Duration of beating 15-20 minutes at a boiler load of 100-150 kg. Since the decoction of licorice root is subject to fermentation, it is recommended to prepare it for no more than three days. Foaming agent of the extract is glycyrrhizic acid, so the consumption of the extract is calculated by the content of acid in it, taking into account the content of molasses in the recipe for caramel mass.

II. EXPERIMENTAL RESEARCH PART

Determining the optimal parameters for extracting the root of licorice

Picture 2: Licorice root used in the experiment



Picture 3
Extraction process
1 – heat oven; 2 – flask for extractant; 3 – connecting pipe; 4 – thermometer; 5 – condenser; 6 – cold water inlet; 7 - water outlet; 8 – extract; 9 – a way for a vacuum cleaner; 10 – receiver; 11 – heat adjustment; 12 – magnetic turner adjuster; 13 – furnace chamber; 14 – water bath; 15 – extractant; 16 – cold water bath.

In extractors, 0.2 kg of raw materials with a fineness of 1–4 mm were extracted and concentrated in 50% ethanol at room conditions. By adding water to the thick mass in a ratio of 1:1, extracts were taken 5 times in different solvents from 200 ml, and the yield of extract from licorice root and the yield of foreign substances were analyzed.

Effects of solvents on yield of licorice root extract

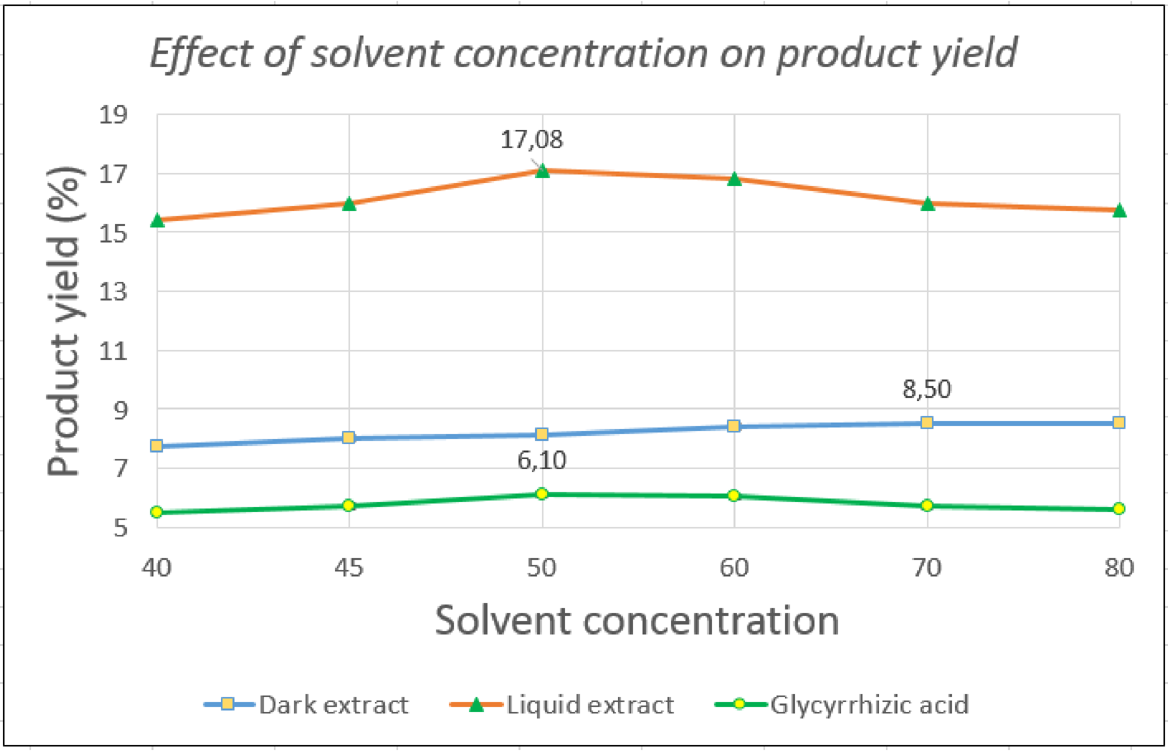
Solvent	Licorice root liquid extract yield, % relative to crude weight
Hexane Extr. gasoline Petrol. air Chloroform Ethyl acetate Ethyl alcohol	10,35 10,39 10,32 12,14 12,21 17,10

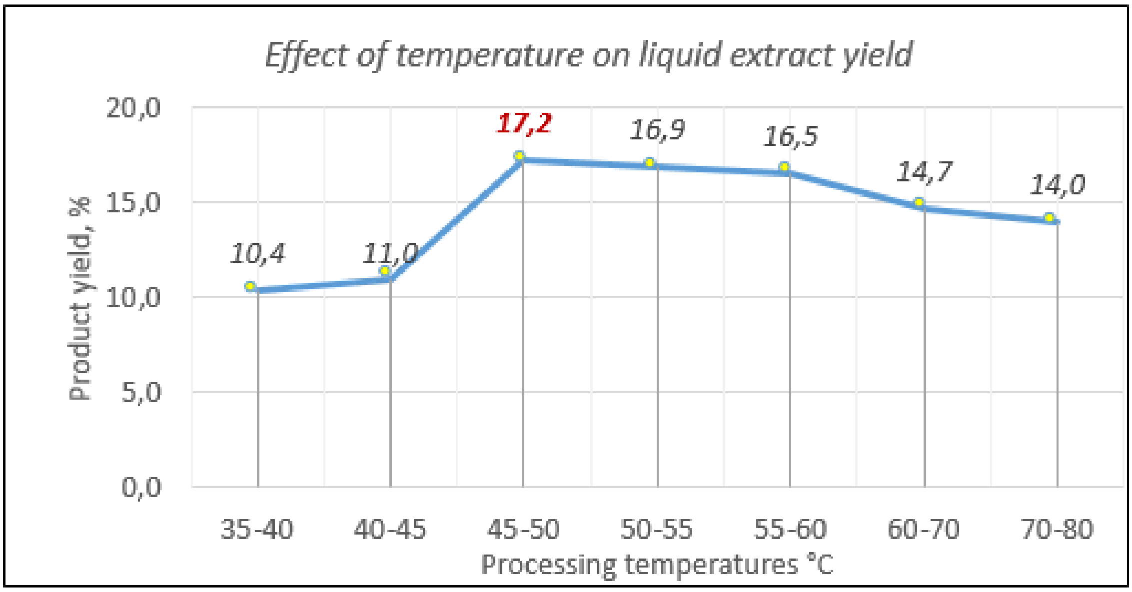
Study of the solvent effect of raw materials on liquid extract yield

Temperature °C	Solvent		
	Water	Ethyl alcohol 50%	Ethyl alcohol 70%
50	15,58 %	17,1 %	12,5 %

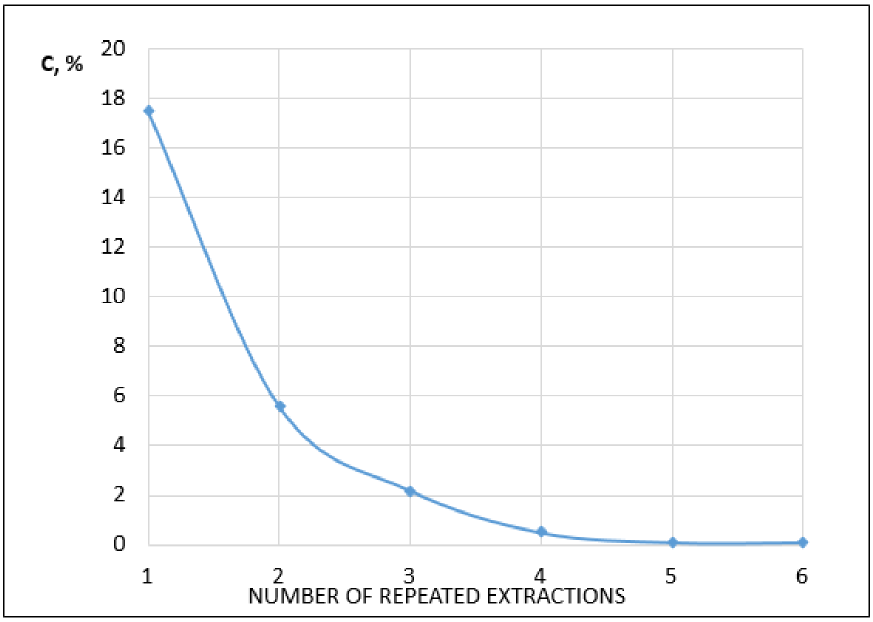
The degree of fineness of the raw material to the product output study the effect

Raw material size (mm)	Deep extract yield (%)
Powdered root	3,0±0,5
1–3	8,0±0,3
2–4	5,7±0,1
3–5	4,8±0,1

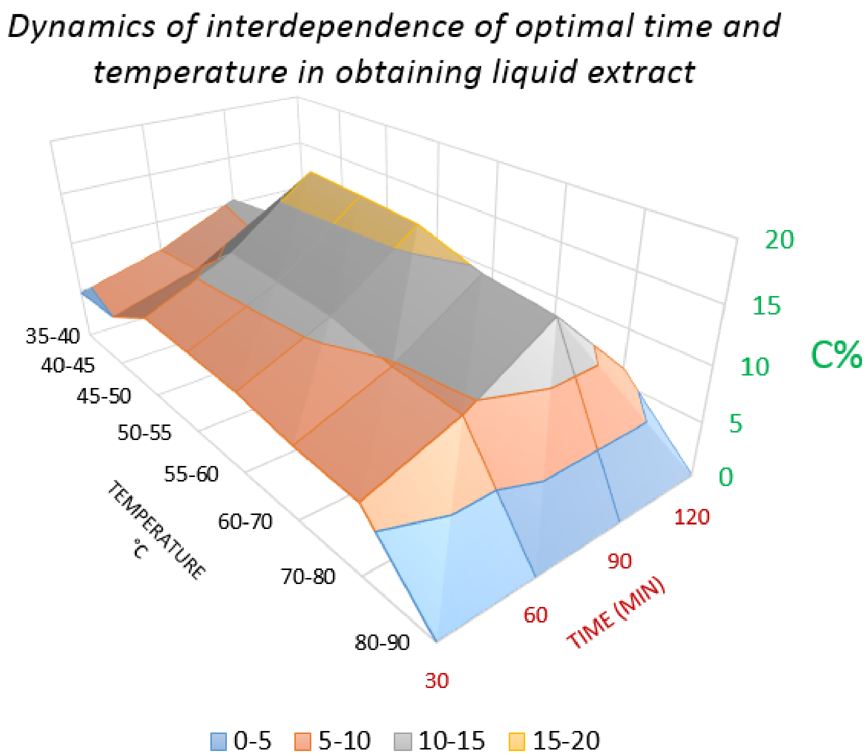




Repeated extraction of licorice root extract extraction dynamics:



As a result of the experiment, a fundamental conclusion was reached: as the processing time increases, the output of the product increases, but its destructibility also increases; in this case, the color of the product changes and darkens, that is, it becomes charred; according to the results of the experiment, it was noted that the optimal processing time is 90 minutes. The dynamics of interdependence of time and temperature during extraction is given in the diagram in below:



A general conclusion can be made as follows:

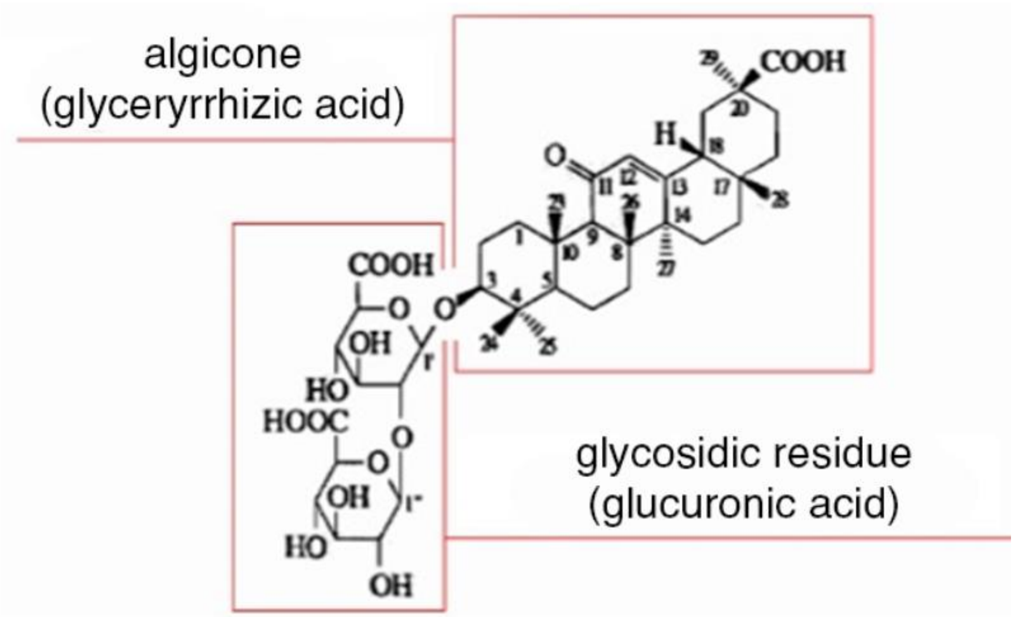
- length of fragments - 1-3 mm;
- ethyl alcohol concentration — 50%;
- processing temperature - 45-50 °C;
- processing time — 90 minutes.

III. EXPERIENCE AND METHODOLOGY

3.1. Extraction of Glycyrrhizic Acid from Licorice Root

The simplest method for extracting GA from licorice root is water extraction. The literature describes several methods for extracting GA with water (extraction by mixing, boiling, etc.) [13], but they are not widely used. Despite this, the yield of GA when using water is much higher (4-6%), but maintaining the required temperature requires a long time and high energy consumption. To increase the yield of GA, various methods are used. According to the literature, ammonium hydroxide solutions for obtaining GA, a range of alkaline solutions, organic solvents and acid solutions used [11]. The classic technology for extracting GA from licorice root is the use of aqueous solutions of ammonium hydroxide [15]. Tikhomirova and co-authors [19] showed that when held for 60 minutes at a temperature of 60-120 °C using the method of extraction in an aqueous solution of 1% ammonium hydroxide to obtain hydrocarbons, 7.3% hydrocarbons are formed. The authors of the experiment showed that the use of an ammonia solution converts glycyrrhizin, which is sparingly soluble in water, into a monoammonium salt of glycyrrhizic acid (MSGA), easily soluble in water, and GcA is obtained from this salt. The literature also describes the use of alkaline solutions (NaOH) for the extraction of BAS from licorice root. In particular, according to Denisova's research [8], when extracting GcA from licorice roots, double extraction with 0.5% alkali (NaOH) at a temperature of 20-50 °C for 1 hour allows increasing the volume of GC yield to 17%.

Many experiments have been carried out to study the effect of many organic solvents on the release of GA [13]. In this work, organic solvents were used for the raw material extraction process: chloroform, acetonitrile, methanol and ethanol. The results showed that GcA was more effective than when it was dissolved using methanol or ethanol alone. The GcA content in the ethanol-methanol extract is 0.86 mg/g, which is 2.8 times less than in an aqueous solution under the same conditions. When choosing the optimal temperature, time and alcohol concentration, the best GcA yield indicators are: 30% aqueous ethanol solution; Maintain at 50°C for 60 minutes. However, the content of GA in the extract obtained in this way does not exceed the content of its aqueous extract.



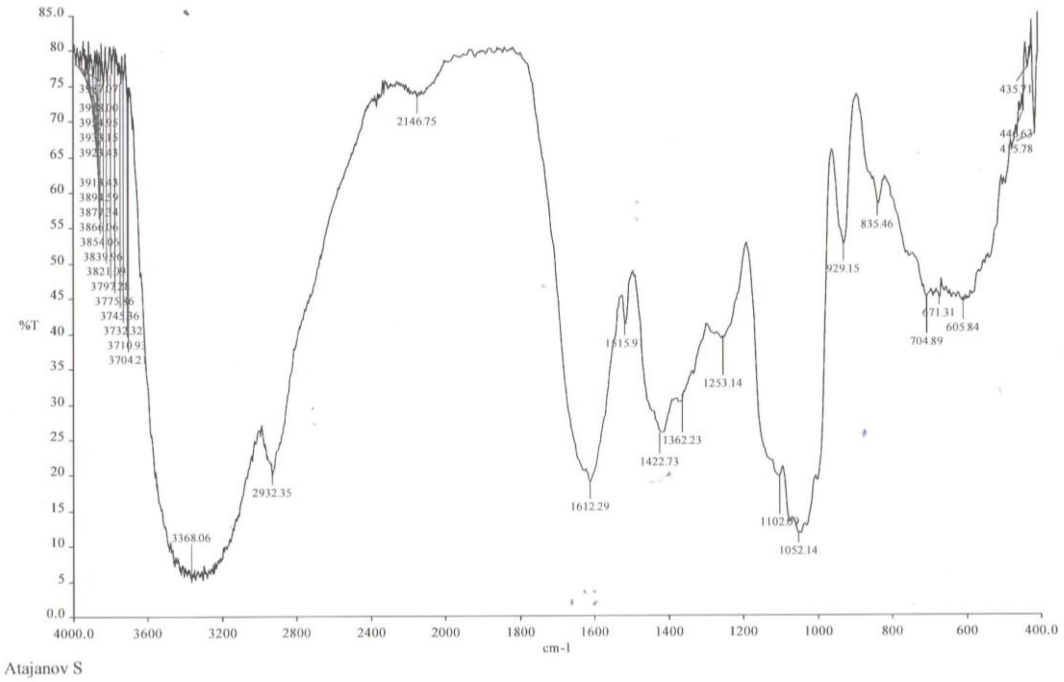
4th picture - Aglycone and glycosidic residues of glycyrrhizic acid

Physicochemical characterisation of biologically active substances of liquorice root: The main active substances of liquorice root are GA, phenolic compounds and carbohydrates. Glycyrrhizic acid: GA is found in more than 10 species of liquorice and exists as a mixture of potassium, calcium and magnesium salts. [6]. The content of GA in licorice roots ranges from 2-24 %. GA is a crystalline colourless substance readily soluble in ethanol and hot water and practically insoluble in cold water [9].

3.2. Analysis of biologically active substances of sweet root

Determination of the content of glycyrrhizic acid: according to the standard methodology of the Pharmacopoeia article, specific weight of pure crushed raw material the flask is poured into bottles, 20 ml of 96% ethyl alcohol is added. A 3% solution of nitric acid in acetone is added to the flask and heated on a water bath for 30 minutes. After that it is filtered and a concentrated solution of ammonia alcohol is added dropwise until heavy precipitation is formed (pH 8.3-9.0). It is dissolved in water and separated into samples A and B. B the optical density of the solution is measured on a spectrophotometer at a wavelength of 258 Nm and water is used as a comparison solution. In parallel, the optical density of CO solution B of glycyram is measured. Glycyrrhizic acid should contain at least 6% glycyrrhizin. To determine the amount of GA in licorice root [11-47 p], a number of samples of licorice root and its derivatives (dry, thick extract) were analysed on a SF-4A spectrophotometer (company number #700322; using neutral optical filters; LOMO) and spectrophotometer SF-26 were investigated by direct spectrophotometry. The optical density of the solutions was measured on a spectrophotometer at a wavelength of 258 nm, in a 10 mm cuvette, using water as a reference solution. The use of standard glycyrrhizin was suggested for accurate determination of GA. From 10.51% to 15.23% by

GA FM in crude licorice, from 15.50% to 17.25% in thick pressed, from 18.35% to 21.80% in dry pressed, in liquid extract should be from 7.08% to 13.91% and in syrup from 0.25% to 0.73%.



Picture 5th : IR spectrophotogramma of Licuorice syrup– *spectrum shows that the absorption peak of GA reaches a maximum around 254 nm*

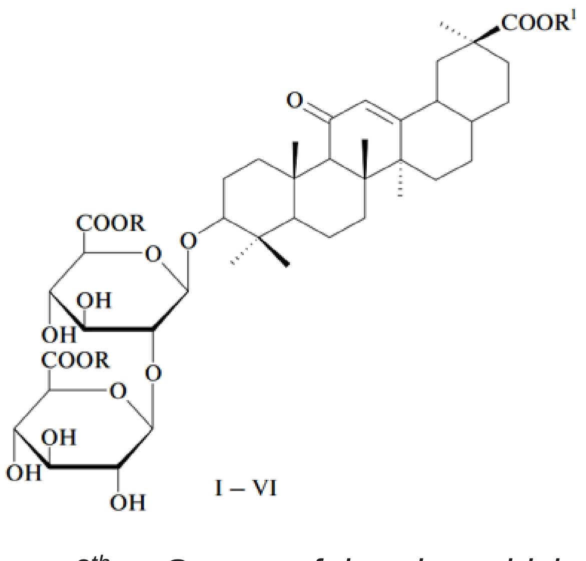
3.3. Obtaining preservative from licorice syrup

Peeled licorice root (3–5 mm) is crushed. Ground licorice root and 1.0 L of hot distilled water were poured into a 2 L (100 g) conical flask. The sample was treated with ultrasound at 40 Hz for 30 minutes. After this, the extract was isolated by decantation. To complete the extraction, add another 1.0 liter of hot distilled water to the sample and repeat the ultrasonic extraction process for 30 minutes. The collected extracts are cooled, filtered through a sieve d=0.2 mm and the solvent is separated at a temperature of 45 °C under vacuum conditions with a pressure of -1.5...-2.0 atm in a rotary evaporator. 15 ml of sulfuric acid with a density of 1.3 g/ml was added to the resulting curd, stirred and cooled. As a result of soaking, the glycosidic precipitate is separated, and it is treated 2-3 times with cold water. The frozen sediment is dried in the open air [13].

20.0 g was taken from the crude cooled glycoside, 100 ml of acetone was added to it and left at room temperature with the lid closed for 3 hours. The resulting mixture is heated to 40–45 °C with stirring in a water bath. After cooling, filtered, then added 2 g of sodium alkoxide and made slightly alkaline.

The GA monosodium salt was washed 2–3 times with absolute alcohol and dried at room temperature. The sequence of reactions is presented in Picture 6th. The yield of GA depends on the quality of the roots and averages 6.5% by weight of dry raw materials.

Laboratory experiments allow the creation of an environmentally safe and cost-effective method for isolating glycyrrhizic acid from licorice root. The purity of the obtained GAs is determined by liquid chromatography. To the list of GA preservatives isolated during research (E-958).



I. $R=R^1=H$;

II: $R=R^1=Na^{+3}$;

III: $R=H$; $R^1=Na^+$;

IV: $R=H$ yoki $R^1=H$

Picture 6th – Stages of the glycyrrhizic acid extraction reaction

I – GA in solution; II - trisodium salt of glycoside;

III – monosodium salt of GA; IV – GA crystals.

IV. SAFETY AND QUALITY CONTROL

Organoleptic parameters and qualitative reactions of licorice extract during storage at +25 °C.

Shelf life	Name of the indicator			
	Brown liquid, distinctive odour.	UV-spectrum	Qualitative reaction for glycyrrhizic acid	Qualitative reaction to sugar
Initial state	Appropriate	Appropriate	Appropriate	Appropriate
3rd month				
6th month				
9th month				
12th month				
15th month				

Quantitative parameters during storage of licorice extract at +25 °C

Shelf life	Name of the indicator			
	Density - at 20 °C (*g/sm*^{3})	In a pH 5% solution	Alcohol content (%)	Microbiological cleanliness
	1.237 to 1.256	5.0 to 6.0	Not less than 8.0	Type 3 B
Initial state	1,232	5,30	13,2	Withstands the test
3rd month	1,232	5,31	13,1	
6th month	1,232	5,30	12,9	
9th month	1,232	5,33	11,0	
12th month	1,233	5,34	10,8	
15th month	1,241	5,38	7,8	Test tolerance is questionable

As can be seen from the above research results, at storage period up to 12 months there was no decrease or increase in the studied indicators. But at storage for more than 15 months its density increases due to the reduction of alcohol content in the alcohol extract. Also positive results were obtained in microbiological analyses of samples up to 12 months.

It is recommended to use 8-13% alcoholic solution of licorice extract in food and medicines for up to 1 year. It is unsuitable for use if its shelf life exceeds 15 months.

CONCLUSION

1. The qualitative parameters of the extract from the root of the liquorice plant were analysed. It was found that the results of the analyses in the experimental processes were by the existing standards.

2. It was found that 17-20% liquid extract and 8.5-9% thick extract can be obtained from liquorice plants growing in local areas.

3. It was found that 5.7-6.1% glycyrrhizic acid can be obtained from liquorice plants.

4. The optimum physicochemical parameters were determined in the extraction process. These are: optimum solvent - 50% ethyl alcohol; optimum temperature - 45-50 °C; optimum time - 90 minutes; optimum raw material size - 1-3 mm.

5. With the additional use of the ultrasonic physical method in the extraction process, it was possible to reduce the extraction time from 2-3 days to 30 minutes. The optimum parameters of the ultrasonic process were 40 Hz; the duration was 30 minutes and the temperature was determined as 55°C.

7. For the extraction of preservative E-958 from liquorice roots using ultrasound at 55°C, 1 kg of crude liquorice root of 1-3 mm size was extracted with 50% ethyl alcohol on average to obtain 58 g of GA. GA is a sweetening ingredient in the food industry.

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