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Technology of manufacture concentrate from dried leaves of Stevia

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The purpose. To improve technology of manufacture concentrate from dried leaves of Stevia on the basis of utilization of ecological extractant. **Methods.** Methodical approaches for development of technological features of production of concentrate of high quality. **Results.** Features of extraction of matters of diterpene glycosides by treated water with determination of their optimum ratio, temperature and duration of process are probed. **Conclusions.** Recycling of saturated solution for improving quality of concentrate is justified. Process of purification of extract from ballast matters is probed and instrumental-technological scheme of production is offered.

Key words: *dried leaves of Stevia, concentrate, matters of diterpene glycosides, instrumental-technological scheme.*

Introduction. Despite the problems today, stevia is a promising crop for soil-climatic zones of Ukraine. Stevia enhances immunity and inhibits the development of harmful microflora in food products. In particular, for the human body detects antivirus and antiallergic actions, antioxidant activity, detoxify the action of harmful chemicals and histamine, preventing side effects of the steroid hormone [1, 2]. Leading nutritionists and pharmacologists of the world believe [3] that the use of stevia in food is a significant contribution to the health improvement of populations of their countries. In the manufacture of soft drinks, concentrates from dried stevia leaf, provides a quick satisfy thirst. This led to the ousting from the domestic market of the United States, artificial sweeteners such as aspartame and saccharin, and companies Pepsi-Cola and Coca-Cola completely switched to the use of such a concentrate.

Currently, stevia is used in the production of more than 6000 kinds of products of food and medical supplies chocolate, sweets, biscuits, yogurts, milk drinks and ice cream in products, where the most important factor is the taste of the final product [4]. Patented recipes for food and beauty products, which have received quite high ratings at wine tasting commissions and international exhibitions. The demand is growing for substances of diterpene glycosides and the production of fat-containing products in the Netherlands and Germany. Started production halvarin (half of margarine) and mayonnaise in Ukraine (PJSC "Kyiv margarine factory", trademark "Olkom"). LLC "Horobyna" in Ukraine [5] established the production of special vodka based on flavoured alcohol from stevia leaves dried.

Analysis of recent researches and publications. Concentrate production consists of a sequence of technological operations: preparation of raw material, extraction, purification, concentration, packaging and storage. The choice of optimal conditions of extraction ensures the quality of the finished product. It is for extraction are formed flavor of the concentrate: the presence of bitter taste, intensity of sweetness, and the like.

As extractants are used: methanol, successively methanol and purified water or only purified water. Abou-Arab A. E. [6] the advantage is established of the extraction of the aqueous extractant that provides the ability to get the extract with a large (2.6%) in the stevioside content. In addition to the environmental importance of concentrate production using as the extractant water preference of this method provide for microbiological indicators. The concentrate contains less numbers of strains of *B. subtilis* and *S. Aigai*, and in contrast to the concentrates obtained by the other two methods less strains of microorganisms: *M. luteus, S. marcenscens, P. aeruginosa, B. megaterium, E. coli, P. Valgaris* [7].

In Japan is patented [8, 9] several industrial methods of extraction, purification and use of various degrees of purity substances of diterpene glycosides. Obtaining of glycosides in this way is difficult because

of the imperfection of technology that involves the use of organic solvents and complicated system of waste disposal, the use of special equipment and devices, which getting bigger costs the manufacturing technology of diterpene glycosides on an industrial scale.

Above improvement of the technological conditions of producing a concentrate dedicated works of famous scientists Zubtsova, V. A. [10],Chornoi V.P. and Nikiforuk I. V. [11], Roger G. [8], canadian scientists [12] and scientists of the Taurida National V.I. Vernadsky University Vi in cooperative with private enterprise "Stevia" [13]. Despite on number of studies there are still a number of issues related to the technological features of obtaining a concentrate of stevia leaves dried.

The methodology of research. For the study of the extraction process were used prepared whole dried stevia leaves content of impurities 3.8% and crushed to particle size of 1-3 mm with a content of impurities, or 0.19%. During researches the content of substances of diterpene glycosides in dried stevia leaves was 10.7 to 11.2 %, flavonoids – 605-625 mg/kg, protein was 7.8%.

As extractant was used purified drinking water for the duration of the process 7 hours. Extraction of substances from dried leaves was carried out for different ratios of raw material and extractive solvent (*experiment 1*) for the integers from 1:1 to 1:7 and crushed to 1:10. The efficiency of the extraction process was determined by the degree of extraction of substances of diterpene glycosides from the whole dried leaves and powdered. The degree of transition of substances of diterpene glycosides, proteins and flavonoids depending on the process temperature was studied (*experiment 2*), °C: 1) 60, 2) 70, 3) 80, 4) 90, 5) 95. The terms of the recirculation of the extract were investigated (*experiment 3*). The meal was separated from the extract and purified by filtration through a caproic filter (*experiment 4*). The technological parameters of the sediment was determined by the filtration rate and volume of sediment formed.

The results of the research. Extraction of substances of diterpene glycosides (SDG) of dried stevia leaves is carried out in accordance with the Fick's law that describes the rate of diffusion of molecules of biologically valuable substances. The size and the layer of raw material is one of the main factors that affects the efficiency of extraction. According to the Fick's law, the extraction efficiency also depends on the ratio of raw material and extractive solvent, process temperature and duration. The kinetics of extraction of substances of diterpene glycosides (*experiment 1)* from the dried leaves is presented in Fig. 1. For a ratio of 1:5 for a whole and 1:7 crushed leaves of dried the maximum value is reached on the content of substances of diterpene glycosides in the extract. From the obtained dependencies it is established that optimal duration of extraction for whole leaves is 6 h, for crushed – 4.5 hours.

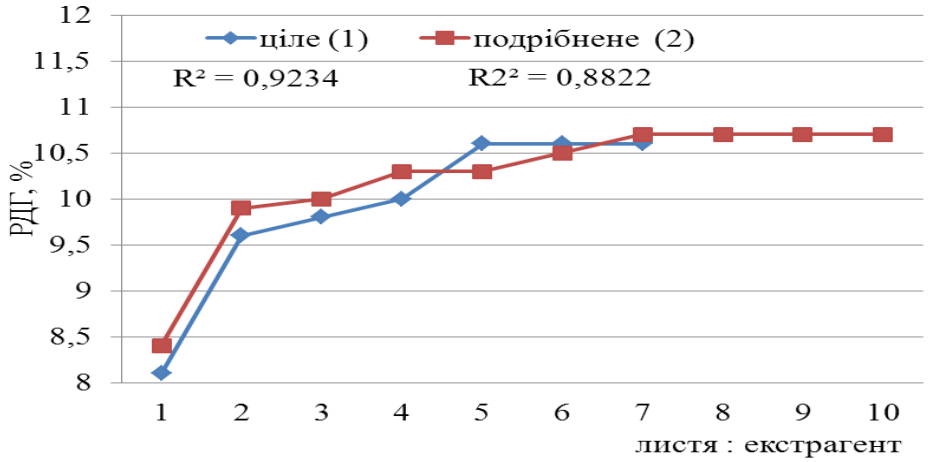

Fig. 1. The dependence of the content of substances of diterpene glycosides from the ratio of raw material to the extractant (*experiment 1*)

The increase in the proportion of water not increases the efficiency of extraction, but only increases the cost of the finished product due to increased electricity costs on its concentration, because the increase in the proportion of water reduces the dry substances content of the extract. In turn, excess moisture extract will lead to lower quality in consequence of oxidation of biologically active compounds and increase the

energy on concentration of the extract. Correlation of the content of the SDG from the ratio of raw material to extractant is represented by the equations:

Whole - $y1= 8,3699x^{0,1347}$

Crushed – $y2 = 0,9036 \ln(x) + 8,8551$

where in SDG; x - the ratio raw-extractant

The correlation coefficient for crushed leaves is 0,92, whole - 0,88. It shows more interaction between the extractant and crushed leaves, which accelerates the process of withdrawal SDG.

To establish the optimal duration was studied the kinetics of extraction of substances of diterpene glycosides (Fig. 2) from the dried leaves depending on the duration of the process (*experiment 2*).

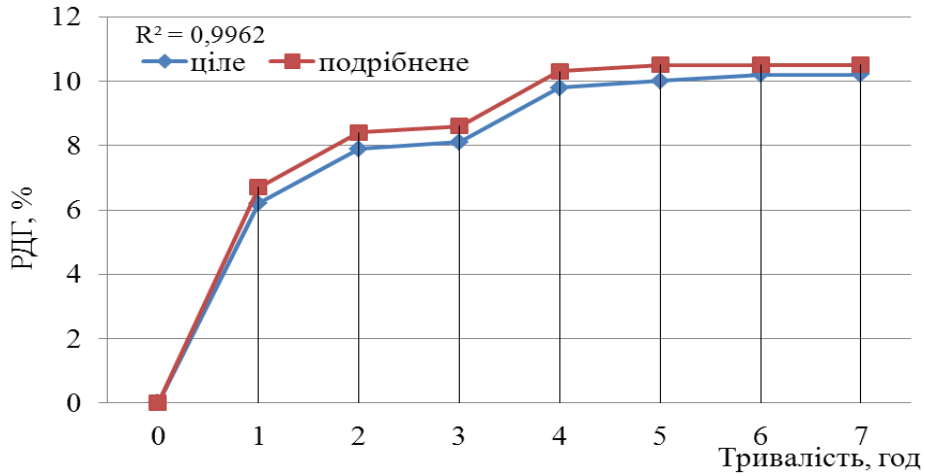

Fig. 2. The content of substances of diterpene glycosides in the extract depending on the duration of the process (*experiment 2*)

From obtained dependences it is seen that after 3 hrs of extraction is the so-called "threshold", after which the speed of the extraction process increases. This indicates the two-phase process. So, within 3 hours of extraction, there is an intensive transfer into solution is the main part of the SDG and in the future, the rate of internal diffusion slows down. More efficient extraction takes place with the use of dried crushed leaves for 4,5–5,0 hrs. This enables the production of extract containing the SDG to 10.65%. Herewith the loss of raw materials SDG will be 0.25% for the crushed leaves and 0.32% for the whole leaves. The intensity of the transition SDG for the extraction is performed in two stages:

I stage – during the first hour of extraction is the transfer of the maximum possible number of SDG. Herewith the terpenes evaporate quickly, are easily oxidized and decompose. The internal diffusion coefficients is 2.4 10^{-4} m^2/s;

II stage – after three hours of extraction, the transition SDG in solution increased by 1.1-fold and decreased the coefficient of internal diffusion – 2.7-fold.

The effect of duration of extraction on the extraction SDG from dried leaf is represented by the equations for the leaves dried:

- whole

$y=-0,0001x^6 + 0,017x^5 - 0,36x^4 + 3,303x^3 - 14,581x^2 + 31,867 x - 20,25$

- crushed

$y = 7E-05x^6 + 0,013x^5 - 0,356x^4 + 3,439x^3 - 15,617x^2 + 34,474 x - 21,963$

where in SDG; x - duration of the extraction.

The correlation coefficient in both cases is of 0.99.

Determined the effect of temperature on efficiency of extraction from the dried leaves (*experiment 3*): the SDG (Fig. 3), flavonoids (Fig. 4) and protein (Fig. 5) [14].

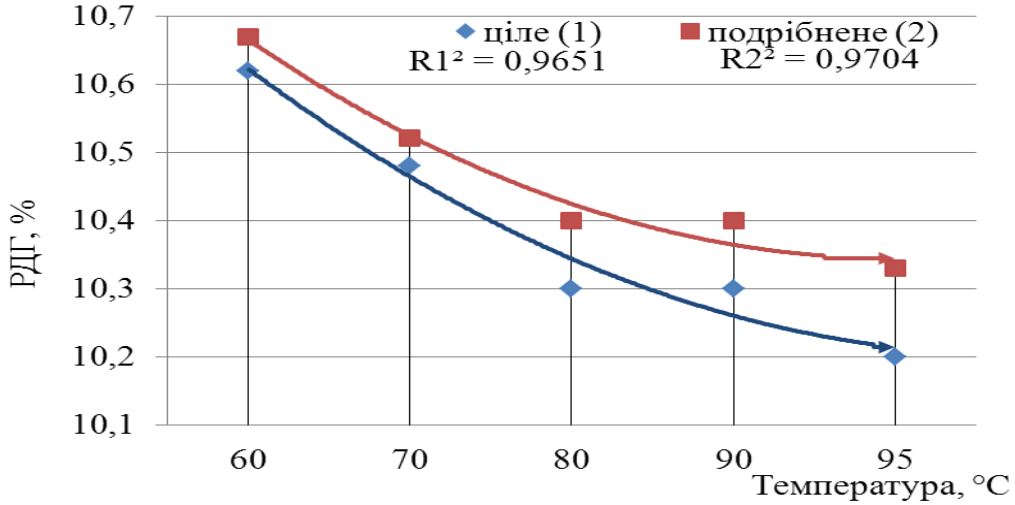

Fig. 3. The dependence of the extractable substances of diterpene glycosides from the process temperature (*experiment 3*)

Noted that from crushed dried leaves transition of these compounds is more intense than the whole leaves. In particular, the largest number of substances diterpene glycosides from dried stevia leaves at the temperature of the extraction 60°C (10,62 and of 10.68%, respectively), the least at 95°C to 10.2 and of 10.32%, respectively. The kinetics of extraction of SDG presented the equations for the leaves of dried stevia:

whole - $y1= 0,019x^2 - 0,214 x+10,82$

crushed - $y2= 0,02x^2 - 0.2 x + 10,84$

where in SDG, %; x - duration of the extraction, hrs.

The correlation connect of the process is for the whole leaves 0,96 and for crushed leaves 0 0,97.

From Fig. 4. noted that it is better extracted flavonoids from dried crushed leaves, the contents of which at the process temperature of 60 °C is 600 mg/kg.

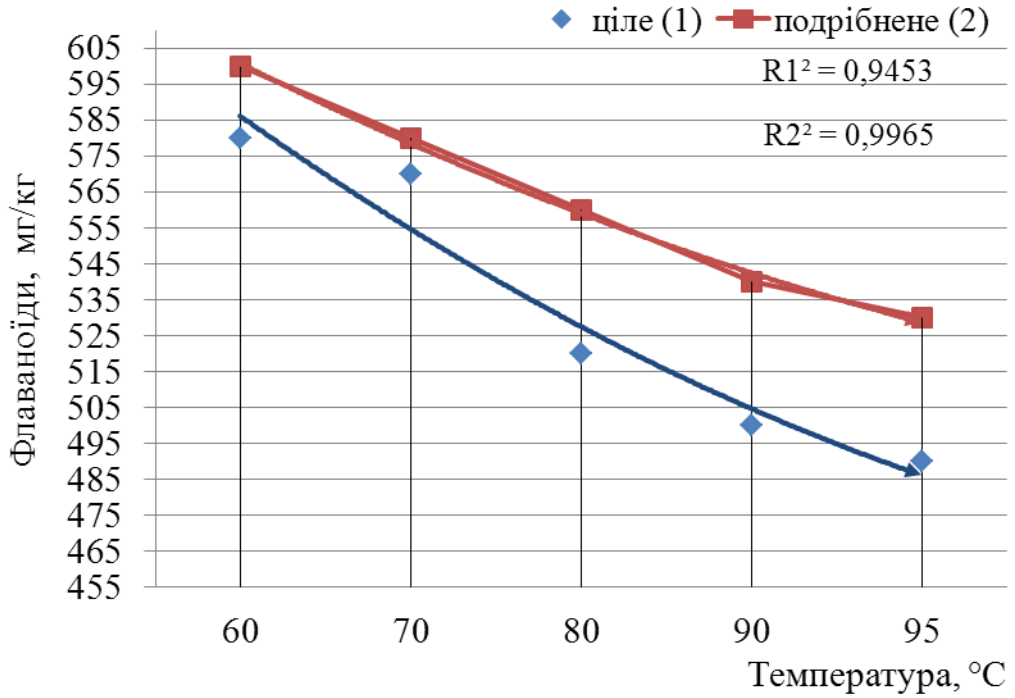

Fig. 4. The dependence of the extraction of flavonoids from the process temperature (*experiment 3*)

With increasing process temperature (95 °C) reduced the content of flavonoids to 530 mg/kg for dried crushed leaves and to 490 mg/kg for whole. The temperature increase leads to splitting of the rutin and to

cyclization of the coloring compounds, removal of which during cleaning is impossible. In the obtained samples of the extract protein content was determined as one of the main criteria for the quality of the finished product that affects the duration of storage (*experiment 3*). The equation of correlation dependence origin of the extraction process of flavonoids have look for stevia leaves dried:

whole - $y1=2,143x^2 - 37,857 x + 622$

crushed - $y2=1,429x^2 -26,57 x + 626$

where: y - content of flavonoids, mg/kg; x - duration of the extraction, hrs.

According to the denotation of correlation coefficients noted that a stronger is connection for applying the crushed leaves (0.99) and less for the whole leaves (0,94).

As shows the obtained dependence (Fig. 5) increase temperature to 60 °C contributes to a better extraction of protein from the extract: for crushed dried leaves – 3% and for whole dried leaves is 2.8%.

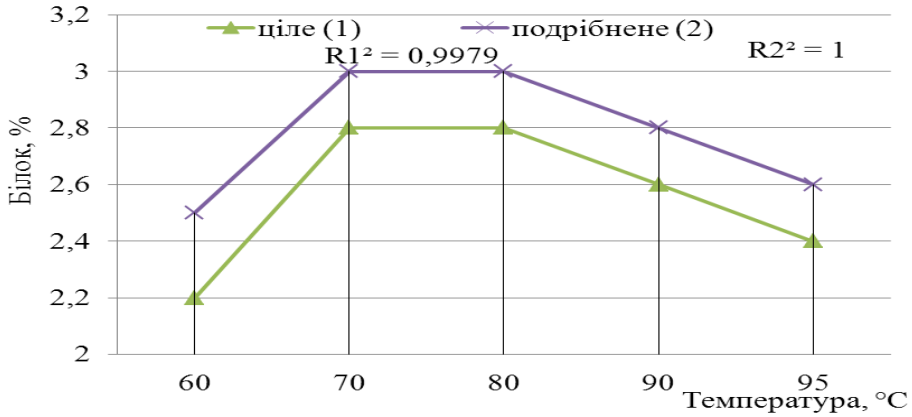

Fig. 5. The dependence of extraction of protein from the process temperature (*experiment 3*)

After 80 °C, the protein content decreases and at 95 °C the process become for the leaves dried crushed - 2.6% and for the whole leaves - 2.4%. This shows that increasing the process temperature leads to decomposition of protein substances with the formation of coloring compounds that gives the finished product an intense dark brown color. The protein removal is described the correlation dependencies for dried stevia leaves:

whole - $y1 = 0,05x^3 - 0,578x^2 + 1,972 x + 0,76$

crushed - $y2 = -0,004x^4 + 0,092x^3 - 0,696x^2 + 2,008 x + 1,1$

where, y - protein content, %; x - duration of the extraction, hrs.

The correlation coefficients are for the whole leaves - 0.99, crushed - 1.00. So,

The receiving of the extract under conditions close to production is achieved by using recirculation of saturated extractant. Determined the content of diterpene glycosides in the extracts in the gradual extraction and with the use of recirculation of the saturated extract (Fig. 6).

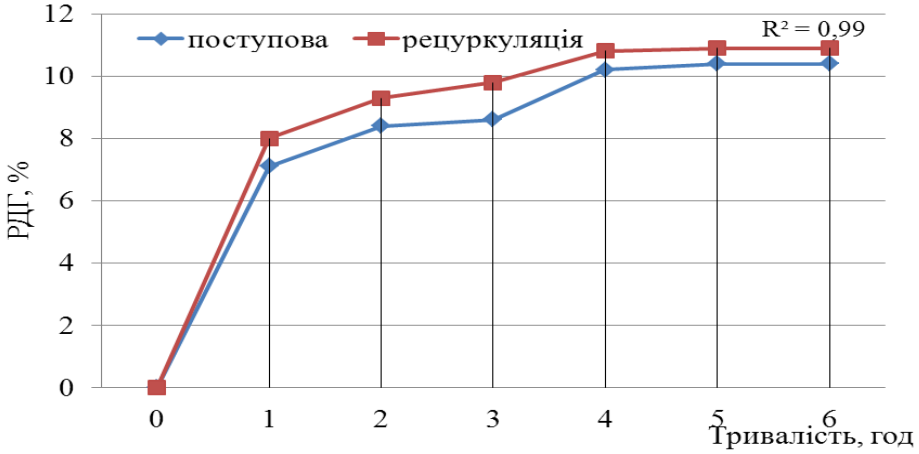

Fig. 6. The kinetics of extraction of crushed dried leaves for different method of extraction (*experiment 4*)

In both cases there is a "shelf" after 3 hours of extraction. However, for the greater speed of extraction by the recirculation method is noted by the more complete saturation of the extract of SDG. The researches shows expediency of application in the manufacture of processing the dried leaves and the extraction of crushed leaves of a dispersion of 1.0-2.5 mm with recirculation for 5 hours at a temperature of 60-70 °C. Upon completion of the extraction the losses of SDG with the meal amount for the gradual extraction – 5% for the recirculation – 3%. The process is described correlating dependence with correlation coefficient of 0.99 for extraction:

gradual - $y = 0{,}026x^5 - 0{,}597x^4 + 5{,}258x^3 - 22{,}046x^2 + 44{,}674\,x - 27{,}329$

with recirculation -

$$y = 0{,}028x^5 - 0{,}645x^4 + 5{,}638x^3 - 23{,}646x^2 + 48{,}293\,x - 29{,}671$$

where, y – SDG, %; x - duration of the extraction, hrs.

After separation of the meal in the extract remain of the particles of fiber and formed flocculated compound (*experiment 5*). Cleaning from resulted sediment is usually carried out by filtration through a disks filters or with application a separator. Technological parameters of the resulting sediment is presented in table.

Table - Technological characteristics of sediment
(*experiment 5*)

№ s/n	Duration, min.	Precipitation rate, cm/min.	The filter coefficient	Sediment volume, cm^3
1	5	1.8	2.0	26.5
2	10	2.2	2.1	27.0
3	15	2.3	2.3	27.4
4	20	2.5	3.2	27.3
5	25	2.8	5.0	27.5
6	30	2.8	5.0	27.5

Full stability with the deposition of sediment the extract gains within 25 min, for gives maximum filtration velocity is 2.8 cm/min while the filtration coefficient is 5. The content of solid phase in the extract is 0.27 and 0.30 g/l, the density of the thickened slurry (sediment) – 1,17-1,24 g/cm^3. Component warehouse of the sediment are presented by fiber content(0.76%), protein (1,36%), SDG (0,11%), fat (0.19%) and flavonoids (0,37 mg/kg), that formed complexes with metals ions. The complete removal of sediment particles occurs within 25 minutes. when filtering at a speed of 2.8 cm/min. The precipitate obtained may be further used as organic fertilizer on fields from growing mother plantation of stevia. The purified extract was concentrated to the content of the mass fraction of dry substances (DS) of 60-65%.

On the production of concentrate was developed and approved in The Department of Food of Ministry of Agrarian Policy and Food of Ukraine TI 10.83.14-00.00.2:2014 "The concentrate from the dried leaves of stevia (*Stevia rebaudiana Bertoni*)", which worked on the basis of LLC "Apicosmetic". In Fig. 7 shown technological scheme of production of concentrate from dried leaves of stevia, which includes: preparation to extraction crushed leaves to a particle size of 1-3 mm, the extraction at a temperature of 60-70°C during 4.5 to 5.0 hours, separation of the extract from the meal, cleaning, concentrating, cooling and packaging.

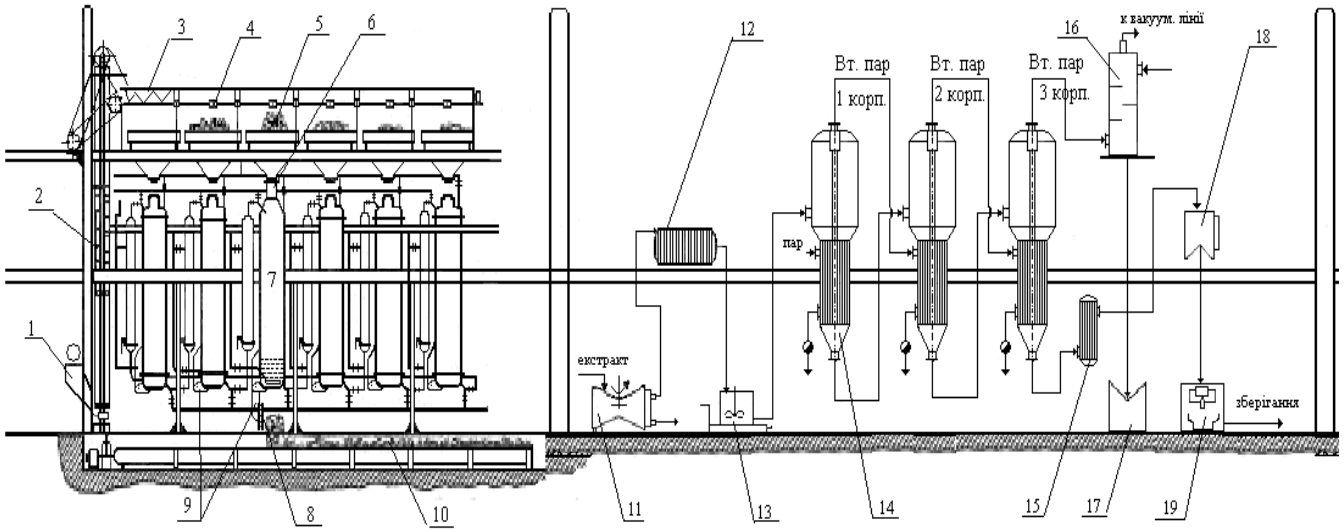

Fig. 7. Hardware-technological scheme of producing a concentrate from dried leaves of stevia: 1 - bunker, 2 – elevator, 3 – auger, 4 – valve, 5 – hopper, 6 – tray, 7 - extractors-percolators, 8 - simulated bottom hatch extractor, 9 - folding hatch cover, 10 – conveyor, 11, 18 – dimensional container, 12 - filter presses, 13 – container scales, 14 – vacuum apparatus, 15 – heat exchanger, 16 - barometric condenser, 17 - container of barometric water, 19 – packer of concentrate.

Conclusion

Substantiated the technological conditions of production of concentrate from dried stevia leaves: the ratio of crushed raw material : the extractant as 1:5 with recycling the purified water extractant for 5 hours at a temperature of 60-70°C; the purification of the extract by filtration from impurities, with followed by concentrating to the content of DS=65%. Developed and coordinated in the Department of Food of Ministry of Agrarian Policy and Food of Ukraine TI 10.83.14-00.00.2:2014 "The concentrates from the dried leaves of stevia (*Stevia rebaudiana Bertoni*)". Improved the elements of technology for processing dried crushed leaves developed on the basis of LLC "Apicosmetic".

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