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DETERMINING THE QUALITY OF DITERPENE GLYCOSIDES, OBTAINED FROM STEVIA LEAVES

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Aim. To determine the quality of diterpene glycoside powders, produced in Ukraine using the stevia leaves of plants of domestic and foreign origin. **Methods.** Differential scanning calorimetry and thermographic analysis. **Results.** It was demonstrated that the increase in the production of powders of diterpene glycosides in the world results in stepping up the requirements to the selection material of stevia and the quality of powders, obtained from its leaves as a final product. The quality of diterpene glycoside powders, produced in Ukraine using the stevia leaves of plants of domestic and foreign origin, was investigated. **Conclusions.** It was determined that special attention in the analysis of the powder samples of diterpene glycosides should be paid to the sample preparation: increased humidity of the sample promotes a weakening of carbohydrate bonds and rapid decline in their quality. Differential scanning calorimetry allows determining the content of additives, the degree of moisture saturation, and may further be used in the screening of selection samples and forecasting the shelf life of powders of diterpene glycosides.

Keywords: diterpene glycosides, temperature, moisture, glycoside radical.

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INTRODUCTION

The diterpene glycoside substances (DGS), obtained from stevia leaves (*Stevia rebaudiana* Bertoni), are of the highest value in the production of low-calorie products. The increase in the rate of obtaining diterpene glycosides started in 2008 when Food and Drug Administration (USA) gave the status GRAS18 to rebaudioside A (Rebaudioside A, Reb-A) [1−3]. Regardless of the forecast increase in the demand for stevia processing products from 4.67 thousand tons (with the estimated value of USD 336 million) in 2014 to 7.15 thousand tons (for the total amount of USD 578 million) and its strategic relevance, the main problem is insufficient provision of the procession enterprises with leaves as high quality raw material. This fact promotes the development of studies on stevia breeding for the increased content of some specific diterpene glycoside and determining the quality of leaves. The comprehensive characterization of the quality of leaves with the consideration of physical, chemical, and structural-mechanic indices is used to determine the biological activity of some isomers. There are a number of methods, revealing the properties of polysaccharide constituents [4, 5], including the thermographic analysis [6–8], and differential scanning calorimetry (DSC) [8]. The DSC method is more sensitive, thus, it is used to obtain high-quality genetic material [9], to determine the pharmacokinetic specificities of medical preparations [10], structural-functional characteristics of fibers [11], organic and inorganic substances [12, 13], tropic oils, *etc*.

MATERIALS AND METHODS

DGS powders, obtained from dried stevia leaves in the industrial conditions of a processing enterprise,

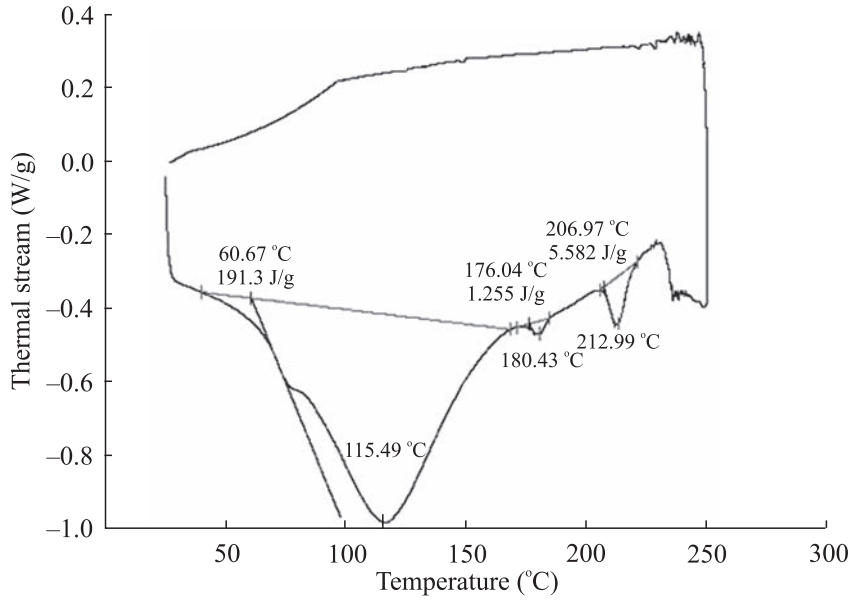

Fig. 1. The curve of DSC of diterpene glycosides (standard sample)

were studied (Kharkiv). Chemically pure samples of diterpene glycosides were used as controls (standard samples).

The structural changes in DGS were studied using the differential scanning calorimeter QDSC-20 Thermo Fisher SCIENTIFIC (Intertech Corporation, USA), in accordance with the common method of thermographic analysis [10], adjusted to DGS [14]. The investigated samples were heated with the interval of 2 °C per 1 min, starting with the room temperature of 19 °C. The results obtained were calculated using STAR^e software.

RESULTS AND DISCUSSION

DGSs are organic substances, containing a carbohydrate (glycon) and non-carbohydrate (aglycon) parts. The glycon part contains rhamnose, glucose, fructose, xylose, arabinose, *etc*., the aglycon part contains sterol, tannin, carotinoid, *etc*. [15]. Stevioside and rebaudioside A are the most common in the production of DGS powders of different purification degree [16]. The authors of [17, 18] established that stevioside is stable at the temperature of 45–140 °C and gradually decomposes (to 95 %) at the temperature of 140−200 °C. Here rebaudioside A is more stable to the effect of high temperatures (melting temperature of 242−244 °C). Other DGSs, present in stevia leaves in a considerably smaller amount, compared to stevioside or rebaudioside A, also have high resistance to high temperatures: rebaudioside B – 193−195 °C, rebaudioside C – 235−238 °C, dulcoside A – 283−286 °C [19, 20].

The study of the DGS standard sample is presented in Fig. 1. According to the character of received endopeaks on the thermogram curve, this process may be conditionally divided into two stages:

– preliminary drying: extraction of hygroscopic and bound water (46−168 °C);

– melting: partial (that of glucose radicals of steviolglycoside) at the temperatures of 176−188.5 °C, and complete (that of stevioside and rebaudioside B, as well as the prevailing number of rebaudiosides A and C) at the temperatures of 205−225 °C.

An insignificant deviation of the curve at the temperature of 60 °C demonstrates the presence of additives (up to 5 %) in the sample. The weight of the standard sample decreased by 79.4 %, 60 % of which was lost at the first stage.

The activation energy, calculated according to the method of *A. Broido*, is expressed by the difference of energies of the transitory and initial stages of the melting reaction [20]. The value of the energy of the standard activation is at the level of 29.4 kilojoule/mol, which demonstrates structural homogeneity and low content of additives. The decrease in the activation energy after the second peak testifies to the increase in the share of additives, which may be presented by the melting products of glucose radicals. The enthalpy value at the beginning of the heating process is high (191.3 joule/g) which shows the stability of DGS at the temperatures of 60−168 °C. After extracting free and bound moisture from the

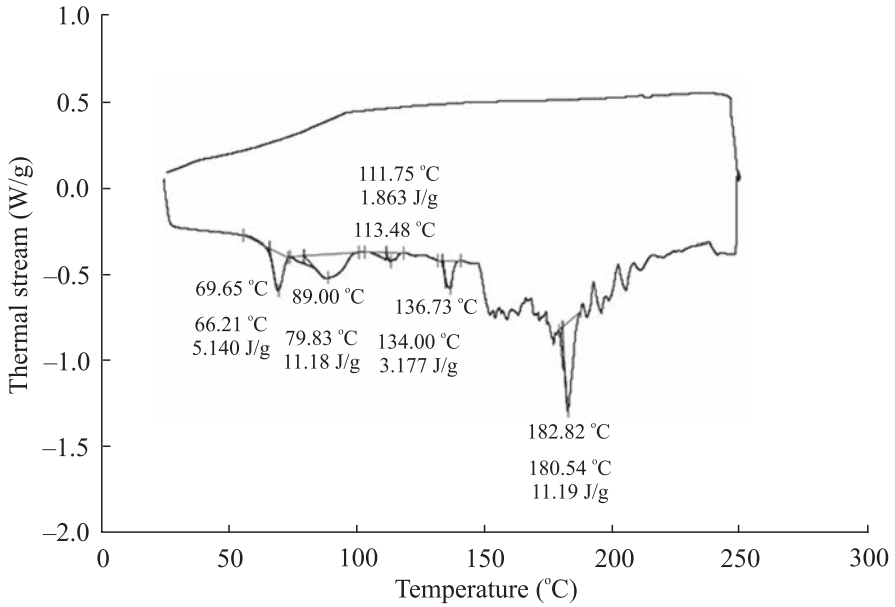

Fig. 2. The curve of DSC of diterpene glycosides (sample I)

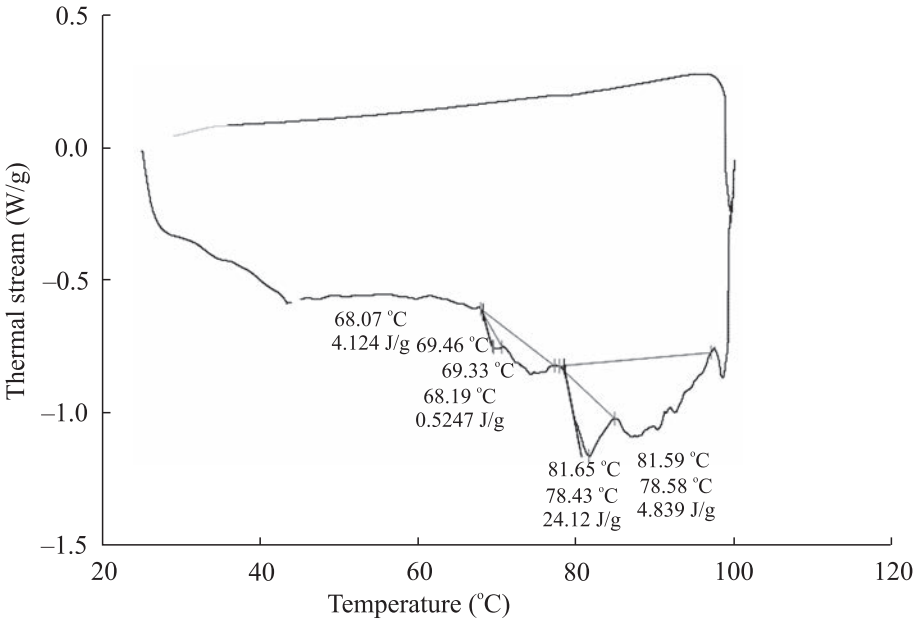

Fig. 3. The curve of DSC of diterpene glycosides (sample II)

standard sample the enthalpy value decreases down to 1.255 joule/g, which demonstrates the decrease in the DGS stability level to the effect of higher temperatures and leads to gradual melting of glucose radicals of steviol-glycoside.

The efficiency of using the method for DGS may be estimated by the results of the analysis of samples from different batches. There was a study of industrial samples of DGS powders, obtained in the industrial conditions from the plant leaves of different origin:

– imported from Paraguay: sample I − 25−250 °C (Fig. 2) and sample II − 25−100 °C (Fig. 3);

– domestic samples: sample III (Fig. 4) − 25−140 °C and sample IV (Fig. 5) – 25−100 °C.

The analysis of Fig. 2–5 shows that the endopeaks, remarkable for temperatures of 69−95 °C, demonstrate the decomposition of coloring agents and the extraction of hygroscopic water. At the temperature of 136 °C (Fig. 2 and 4) there is the complete extraction of the bound water. The incongruent character of the curves

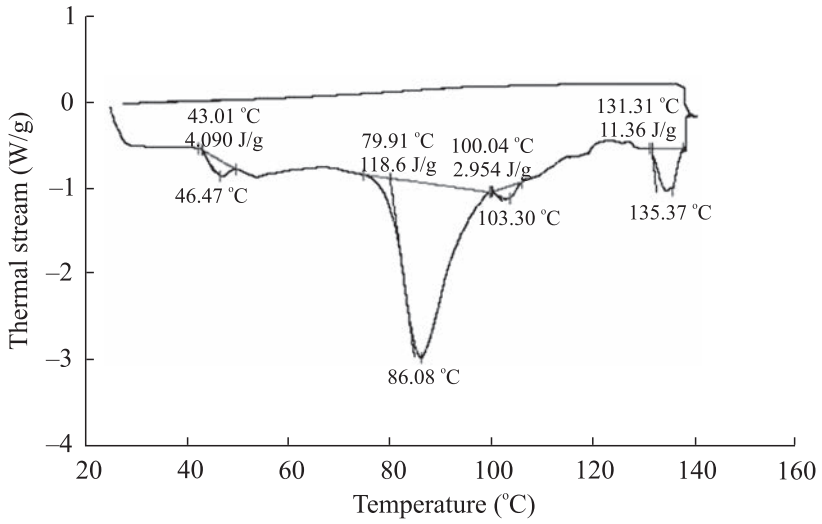

Fig. 4. The curve of DSC of diterpene glycosides (sample III)

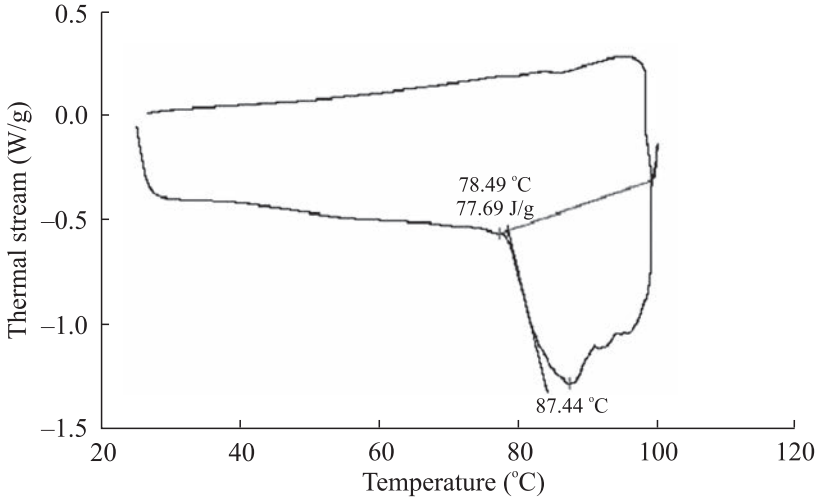

Fig. 5. The curve of DSC of diterpene glycosides (sample IV)

Thermal analysis of diterpene glycosides

N	Interval of effect, °C	Peak, °C					Activation energy, kilojoule/mol				
		S	I	II	III	IV	S	I	II	III	IV
1	40–70	–	69.65	68.19	46.47	–	–	21.2	16.95	43.1	–
	70–100	–	89	78.43	86.08	87.44	–	9.41	8.04	17.7	15.5
	100–110	–	–	–	103.3	–	–	–	–	14.3	–
	110–170	115.49	136.73	–	135.37	–	29.4	18.1	–	13.8	–
2	175–195	180.43	182.82	–	–	–	21.2	43.1	–	–	–
3	205–230	212.99	–	–	–	–	9.4	–	–	–	–

of samples I (Fig. 2) and II (Fig. 3) testifies to a high content of additives, the saturation with hygroscopic water, and the increased content of coloring agents.

The endopeak at the temperature of 182 °C (Fig. 2 and 4) illustrates the melting of glucose radicals and characterizes the degree of DGS melting. The 1.36 °C difference in the temperature for thermogram curves (Fig. 4 and 5) is explained by the difference between the weight of samples III (6.3 mg) and IV (12.5 mg), taken for the analysis. The increase in the weight by 0.44 mg increases the temperature of moisture extraction by 0.1 degree.

The character of thermogram curves allows for the assumption that samples III and IV are of higher purity degree. The endopeak at the temperature of 46 °C (Fig. 4) which is absent on the thermogram curve (Fig. 5), testifies to the saturation of the air of the sample, taken for the analysis, with moisture.

The processes, occurring in DGS samples during heating, are subject to Lavoisier-Laplace's law, according to which the decomposition of a complex compound into simple ones is accompanied with the absorption of the same amount of energy, which is consumed for the formation of the similar compound. The analysis of the investigated DGS samples by the thermal indices is presented in the table, which indicates that the higher purity degree is attributed to the sample, the fewer endopeaks will be on the DSC curve.

The weight of batches of the investigated samples is mostly decreasing at the beginning of the heating process to 100 °C, the loss is 58.2 %, which demonstrates high hygroscopy of samples. The same is proven by low values of activation energy of all the samples of DGS powders (not exceeding 43 kilojoule/mol). It is noteworthy that excessive moisturizing of DGS powders, such as sample I, leads to the weakening of carbohydrate bonds and accelerates the melting of glycosides. This fact proves the necessity of keeping to the norm value of the weight fraction of moisture in DGS powders of different degree of purity while packing, storing, and using them.

Enthalpy, joule/g

S	I	II	III	IV
–	5.14	0.53	4.09	–
–	11.18	24.12	118.6	77.69
–	–	–	2.954	–
191.3	3.177	–	11.36	–
1.255	11.19	–	–	–
5.582	–	–	–	–

CONCLUSIONS

The investigations prove the efficiency of the DSC method to determine the quality of DGS powders. The impact of "accidental moisturizing" of a sample of DGS powder on its quality was demonstrated that will allow forecasting its shelf life in future. It was established that the increase in the temperature leads to the decrease in the process activation energy, especially in the samples with excessive moisture. It results in weakening the carbohydrate bonds and accelerating the melting process of glucose radicals. Therefore, the methods of differential scanning calorimetry may be promising in quality estimation and quantitative determination of thermal degradation of DGS.

Визначення якості дитерпенових глікозидів, отриманих з листків стевії

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Мета. Визначення якості порошків дитерпенових глікозидів, вироблених в Україні з листків стевії вітчизняного та іноземного походження. **Методи.** Диференційна сканувальна калориметрія і термографічний аналіз. **Результати.** Показано, що із зростанням виробництва порошків дитерпенових глікозидів у світі підвищуються вимоги до селекційного матеріалу стевії та якості порошків, отриманих з її листків як кінцевої продукції. Досліджено якість порошків дитерпенових глікозидів, які виробляються в Україні з листків стевії вітчизняного та іноземного походження. **Висновки.** Встановлено, що під час аналізу зразків порошків дитерпенових глікозидів особливу увагу необхідно приділяти підготовці зразка: підвищена вологість зразка сприяє послабленню

вуглеводних зв'язків та швидкому погіршенню їхньої якості. Диференційна сканувальна калориметрія дозволяє визначати вміст домішок, ступінь насичення вологою і в подальшому може бути використана для скринінгу селекційних зразків та прогнозування терміну зберігання порошків дитерпенових глікозидів.

Ключові слова: дитерпенові глікозиди, температура, вологість, глікозидний радикал.

Определение качества дитерпеновых гликозидов, полученных из листьев стевии

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Цель. Определение качества порошков дитерпеновых гликозидов, произведенных в Украине из листьев отечественного и иностранного происхождения. **Методы.** Дифференциальная сканирующая калориметрия и термографический анализ. **Результаты.** Показано, что с ростом производства порошков дитерпеновых гликозидов в мире повышаются требования к селекционному материалу стевии и качеству порошков как конечной продукции. Исследовано качество порошков дитерпеновых гликозидов, производимых в Украине из листьев отечественного и иностранного происхождения. **Выводы**. Установлено, что при анализе образцов порошков дитерпеновых гликозидов особенное внимание необходимо уделять их подготовке: повышенная влажность способствует ослаблению углеводных связей и быстрому ухудшению их качества. Дифференциальная сканирующая калориметрия позволяет определять содержание примесей, степень насыщения влагой и в дальнейшем может быть использована для скрининга селекционных образцов и прогнозирования срока хранения порошков дитерпеновых гликозидов.

Ключевые слова: дитерпеновые гликозиды, температура, влажность, гликозидный радикал.

REFERENCES

1. *Alternative* sweeteners in a higher sugar price environment. ISO Study-MECAS(12)04. London. 2012; 60 p.
2. *Savita SM, Sheela K, Sunanda S, Shankar AG, Ramakrishna P. Stevia rebaudiana* – A functional component for food industry. *J Hum Ecol*. 2004; **15**(4): 261−4.
3. *Penner R, Shanks T, Timcke K, Krigbaum J, Uno J*. Stevia from Paraguay: Market perspectives, development and potential, use and international regulations. Asunción, Paraguay Vende. 2004;59 p.
4. *Barton FE 2nd, Akin DE, Morrison WH, Ulrich A, Archibald DD*. Analysis of fiber content in flax stems by near-infrared spectroscopy. *J Agric Food Chem*. 2002; **50**(26):7576−80.
5. *Baley C*. Influence of kink bands on the tensile strength of flax fibers. *J Mater Sci*. 2004;**39**(1):331−4.
6. *Panikarovskiy TL, Britvin SN*. On the use of differential scanning calorimetry to investigate structural specificities of organic and inorganic compounds. Saint Petersburg State University Research Centre for X-ray Diffraction Studies. Saint Petersburg. 2013;4 p.
7. *Yemelina AL*. Differential scanning calorimetry. Moscow, Moscow State University publ. 2009;42 p.
8. *Kharitonova EP*. Fundamentals of differential scanning calorimetry, textbook. Moscow, Moscow State University publ. 2010;17 p.
9. *Titok VV, Shostak LM Laynovskaya IV, Leontiev VN, Khotyleva LV*. Thermogravimetric analysis of flax. *Theses of an international conference in "Cellular kernels and plastids of plants: biochemistry and biotechnology*" (May, 26−28 2004). Minsk, Tekhnoprint. 2004; 267−71.
10. *Smirnova IG, Gildeeva GN, Chistyakov VV*. Analysis of crystalline structure and chirality of drug substances. *Moscow University Chemistry Bulletin*. 2012;**53**(4): 234–40.
11. *Faughey GJ, Sharma SS, McCall D*. Determining fiber fineness in flax using derivative thermogravimetric analysis, scanning electron microscopy and airflow methods. *J Appl Polym Sci*. 2000;**75**(4):508−14.
12. *Stebeleva OP, Kashkina LV, Kulagin VA*. Thermal analysis of cavitationally activated carbonaceous materials. *Journal of Siberian Federal University. Engineering & Technologies*. 2013;**6**(1):56−60.
13. *Thomas GS, Radha AV, Kamath PV, Kannan S*. Thermally induced polytype transformations among the layered double hydroxides (LDHs) of Mg and Zn with Al. *J Phys Chem B*. 2006;**110**(25):12365−71.
14. *Royik MV, Kuznetsova IV, Golodnyak VA, Mazaeva VS*. Application thermographic method for establishing quality diterpene glycosides. *Sugar beets*. 2015; (4):8−9.
15. *Kataev VE, Khaybullin RN, Sharipova RR, Strobykina IY*. Ent-kaurene diterpenoids and glycosides: isolation,

properties, and chemical transformations. *Obzornyi zhurnal po khimii*. 2011;**1**(2):99−167.

16. *Abou-Arab AE, Abou-Arab AA, Abu-Salem MF*. Physicochemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* bertoni plant. *Afr J Food Sci*. 2010;**4**(5):269−81.
17. *Nishiyama P, Alvarez M, Vieira LGE***.** Quantitative analysis of stevioside in the leaves of *Stevia rebaudiana* by near infrared reflectance spectroscopy. *J Sci Food Agric*. 1992;**59**(3):277–81.
18. *Mohamed Diaa ElDin Soliman*. Stevia Plant, Natural Concentrated sweeteners. *Egyptian Society of Sugar Technologists, 28th Annu. Conf.* (Dec. 2−4, 1997).
19. *Steviol* glycosides. *FAO JECFA Monographs 4. Compendium of food additive specifications*. 68th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Geneva, 19–28 June, 2007). Geneva, 2007;61−72.
20. *Van De Verde K, Kiekens P*. Thermal degradation of flax: the determination of kinetic parameters with thermogravimetric analysis. *J Appl Polym Sci*. 2002;**83**(12): 2634−43.