REGULARITIES OF THE DRYING OF LACTULOSE SOLUTIONS

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Abstract: The existing patented technologies of the production of lactulose were analyzed, and leading producers were defined. Crystalline lactulose was produced via the spray drying of lactulose solutions with various mass fractions of solids. The principal dependences of the finished product output on the drying temperature, the solution flow rate, the air flow rate generated by an aspirator, and the gas spray rate were studied. The results of analyzing the presented dependences allowed us to determine the optimal mass fraction of solids in a solution for spray drying. The results of studying the quantitative parameters of dry lactulose, including hygroscopicity, particle size, moisture content, and finished product solubility index, were presented.

Key words: lactulose, lactose, drying, solution, temperature, solution flow rate, air flow rate, mass fraction, solubility index

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INTRODUCTION

Lactulose is a carbohydrate that belongs to the class of oligosaccharides and the subclass of disaccharides. Its molecule consists of the galactose and fructose moieties. Lactulose is obtained from milk sugar (lactose) via the deep processing of milk. Lactulose is a white odorless sweet crystalline substance, which is well soluble in water [1].

Lactulose is a carbohydrate, which is indigestible in the gastrointestinal tract, but usable by beneficial microflora as a nutritious substrate [2].

The bifidogenic properties of lactulose were first revealed and studied by F. Petuely, a British pediatrician, who has succeeded in separating lactulose from human breast milk and also in establishing its beneficial effect on the destroyed microbiocenosis of the gastrointestinal tract of artificially fed babies and their general health [3].

Lactulose is currently classified as a classic prebiotic. The general principle of its effect consists in reducing the amount of pathogenic bacteria, such as *Escherichia coli* and *Staphylococcus*. Moreover, lactulose stimulates the growth of probiotic bacteria, such as *Bifidobacterium*, which are known to produce a positive effect [4].

According to research data, the world production of lactulose preparations is currently 20 000 t/year, and over 5% of produced lactulose is used for this purpose. The assortment of lactulose preparations includes over ten items. Japan alone produces three types of syrups and two types of crystalline lactulose that satisfy the international quality standard [5].

The leading company in the world market of lactulose is Morinaga Milk Industry (Japan) dealing with the problems of studying the properties of lactulose and the development of methods for its production since 1953, i.e., for more than 60 years.

The market of lactulose in the Russian Federation has generally been represented by the pharmaceutical preparations of foreign production until now. OOO Shekhon-Laktuloze that produces lactulose syrup with a mass fraction of solids of no less than 66% is recognized to be the only large-capacity producer of lactulose in the Russian Federation.

It is considered that dry lactulose powders are more technological in use [5]. However, the problem of the production of crystalline lactulose has not completely been solved in Russia until now. However, a number of technologies have already been patented.

According to patent data, the key problem in the existing technologies of the spray drying of lactulose solutions is the presence of binders, and the amorphous powder obtained at the outlet has high hygroscopicity. The analysis of the existing patented technologies and parameters of the drying of lactulose solutions is given in Table 1.

The analysis of the performed patent studies shows that among the disadvantages of the production of dry lactulose preparations by spray drying is a lowered content of lactulose in a product due to different catalysts and binders that are added to reduce hygroscopicity and accelerate the process. The growth of price due to the incorporation of fillers is also noteworthy.

Table 1. Technologies and parameters of the drying of lactulose solutions with various mass fractions of solids

Patent name	Drying parameters			
	technology	temperature, °C	time, h	auxiliary components
A process for the production of lactulose powder (United States) [6]	Spray drying	80–150	–	0.3-% cOPPUAkY solution
A method for the production of lactulose-containing powder and its application in fodders. A method for the production of lactulose-containing powder (Japan, Great Britain) [7, 8]	Spray drying	95–140	2–3	Potassium hydroxide
A method for the purification of lactulose syrup (Japan) [9]	Lyophilic drying	90–120	4–5	–
A method for the separation of lactulose. A method for the production of non-hygroscopic lactulose-containing powder (Japan, United States) [10, 11]	Vacuum drying	below 80	1	Ethanol
A composition on the basis of plant fibers and lactulose (United States) [12, 13]	Spray drying	60–90	6	Alginates
A method for the production of solid lactulose (United States) [14]	Solution inspissation, milling, crushing	80–100	5–7	–
A method for the production of crystalline lactulose (Unites States) [15]	Vaporization, cooling, crystallization	50–60	100–120	Lactulose trihydrate

The methods of the production of crystalline lactulose forms are very laborious, so the prices of preparations grow by 1–2 orders of magnitude in comparison with syrups. For the reason of the abovesaid, it is topical to study the spray drying of lactulose solutions with the purpose of establishing the optimal parameters, which will help us to obtain a product with high qualitative characteristics without fillers. The fact that import preparations have a rather high price in addition to "nature-identical" ingredients will provide the competitiveness of a product.

In this connection, we have studied the spray drying of lactulose solutions.

OBJECTS AND METHODS OF STUDY

At different stages of our work, the objects of study were (1) solutions with a mass fraction of lactulose of 20–60%; and (2) dry lactulose with a mass fraction of lactulose of 40%.

They were prepared from a solution with a mass fraction of lactulose of 50% via its dilution to 20–40% and additional inspissation to obtain a solution with a mass fraction of lactulose of 60%.

The solutions were dried on a Mini Spray Dryer B-290 setup (BUCHI Labortechnick AG) with the possibility of adjusting the flow rate of a working solution and the velocity of a spraying flow. The setup allows the production of a finished product with a particle size of 1–25 μm. The materials that come into contact with a product are acid-proof stainless steel, borosilicate glass, and silicone. The technical characteristics of the drier are given in Table 2.

The directions of the sprayed sample and the drying air coincide in the standard operational mode of the dryer. The pressure in the system is subatmospheric to prevent the contamination of a product in the case of leaks.

The spray drying setup is schematized in Fig. 1. The heating of the supplied gas and two-flow nozzle *1* are controlled by Fuzzy-Logic microprocessor automatic device *2* with a digital display and a PT-100 temperature detector, which provides a high accuracy of temperature adjustment. The solution is passed through the nozzle spraying it into smallest drops and enters chamber *3*, in which the process of drying occurs. The particles entrained by the gas flow move into cyclone *4*, where they are separated under gravity. The setup is equipped by filter *5* that rejects small particles and aspirator *6* that creates the air flow. The view of the setup is shown in Fig. 2.

Table 2. Technical characteristics of the experimental spray drying setup

Characteristic	Value
Voltage	200/230 V; 50–60 Hz
Maximum air flow rate	35 m^3/h
Motor control	Frequency transducer
Maximum inlet temperature	220°C
Heating power	2300 W
Heating control	PT-100; Fuzzy Logic; temperature control accuracy, ±3°C
Serial interface	RS-232 for the transmission of all parameters
Sprayed gas	Compressed air, 200–1000 dm^3/h, 5–8 bar
Nozzle hole diameter	0.7 mm
Nozzle cap diameter	1.4 and 1.5 mm
Average residence time	1.0–1.5 s
Dimensions, width × length × height	60 × 50 × 110 cm
Weight	48 kg

The mass fraction of solids in lactulose solutions was determined refractometrically according to GOST (State Standard) 24908-84.

The mass fraction of lactulose and other carbohydrates in solutions and dry lactulose was determined by gas-liquid chromatography (GLC), as it is an available and well-proven technique of the analysis for carbohydrates. It is based on the conversion of saccharides into volatile trimethylsilyl derivatives and their subsequent

separation on a GLC column and analysis with a flame ionization detector.

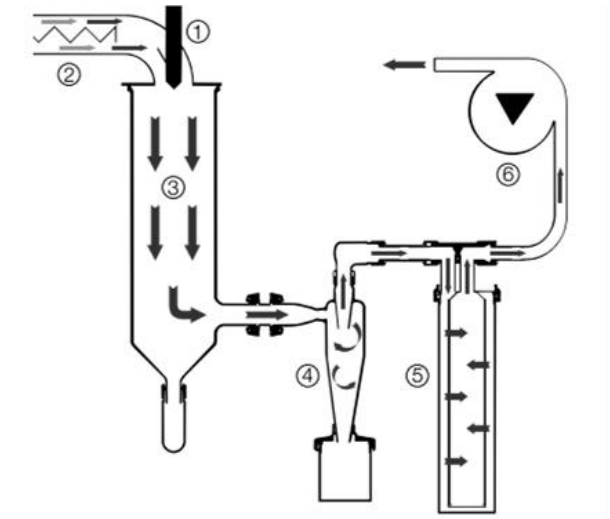


Fig. 1. Scheme of the spray drying setup: (*1*) nozzle, (*2*) heater, (*3*) spray chamber (cylinder), (*4*) cyclone, (*5*) filter, (*6*) aspirator.

Fig. 2. Spray drying setup.

The essence of the methods consists in the following. An analyzed sample after preliminary drying and skimming is treated with *N*-trimethylsilylimidazole at a temperature of 60–70°C for 1–2 h. A precisely dosed volume of hexane, the excess of which is hydrolyzed with water, is then added to the mixture, thereupon a hexane phase aliquot is injected into a chromatograph. Carbohydrates are separated on a packed column with a polar phase in the isothermal regime. Monosaccharides go out with solvent background, lactose goes out in the form of two peaks corresponding to α- and β-anomers, and lactulose goes out in the form of a single peak.

The content of lactulose and lactose was calculated by the inner standard method with precalibration.

The quality of the finished product was estimated by such characteristics as particle size, moisture content, solubility index, and hygroscopicity.

The solubility index was determined in compliance with GOST (State Standard) 30305.4.95.

The particle size was determined via the microscopy of a lactulose sample on an AxioVert.A1 inverted microscope (Carl Zeiss AG) with a magnification of X40.

The moisture content was determined using a Chizhova instrument.

The operating principle of the instrument consists in exsiccation via the vaporization of a raw material sample by its heating at a required temperature for a specified period of time. The exsiccation of samples is performed in special packages of loosely glued duplicator or newsprint paper.

A precisely weighed 4–6-g portion of dry lactulose was placed into a paper package, which was dried and weighed on an analytical balance with an accuracy of up to 0.01 g, and uniformly spread over the entire surface of the package. Paper filters of 11–12.5 mm in diameter were used to manufacture the packages.

The package with its content was weighed and placed between the plates of the drier. Drying at a temperature of 80 ± 0.5°C lasted for 5 min. The dried package with lactulose was cooled to room temperature in a desiccator and then weighed.

The moisture content in dry lactulose was determined by the formula (1):

$$W = \frac{M_1 - M_2}{M} \times 100, \quad (1)$$

where M_1 is the mass of the package with a lactulose portion before drying, g, M_2 is the mass of the package with a lactulose portion after drying, g, and M is the mass of a lactulose portion taken for drying, g.

The hygroscopicity of dry lactulose powders was determined using a desiccator with 3 cm^3 of water poured on its bottom.

The precisely weighed portion of lactulose in a weighting bottle was placed into a desiccator. No more than 6 weighting bottles were uniformly arranged over the entire surface of the desiccator insertion. The weighting bottles placed into the desiccator were opened, and their stoppers were placed near them. The desiccator was closed with a cover.

The weighting bottles were allowed to stand in the desiccator for 20 h at a temperature of 25 ± 2°C. Then they were closed with stoppers, taken out of the desiccator, and weighed.

The hygroscopicity of dry lactulose was determined by the formula (2)

$$X = \frac{M_1 - M_2}{M} \times 100, \quad (2)$$

where M_1 is the mass of the weighing bottle with a lactulose portion after moistening, g, M_2 is the mass of the weighing bottle with a lactulose portion before weighing, g, and M is the mass of a lactulose portion, g.

We performed two parallel estimations, the results of which were used to calculate the arithmetic mean with an error of less than 0.1%.

RESULTS AND DISCUSSION

Temperature is a principal factor governing the process of drying. The dependences of the product output on the drying temperature are plotted in Fig. 3. The product output is the quantity of the finished product in percents of the mass fraction of solids in an initial solution.

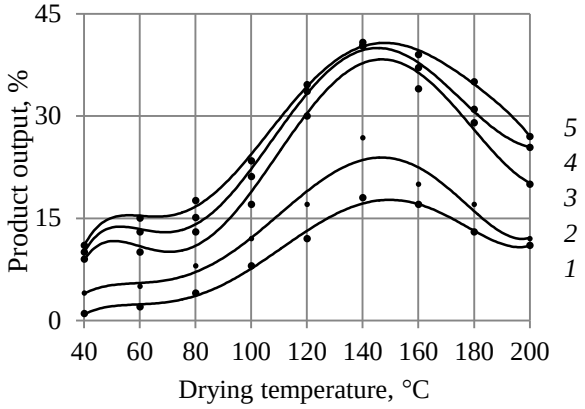


Fig. 3. Product output versus drying temperature at a mass fraction of lactulose of (*1*) 20, (*2*) 30, (*3*) 40, (*4*) 50, and (*5*) 60%.

The analysis of the curves plotted in Fig. 3 shows that the product output grows within a temperature range of 40–140°C. The product output decreases at a heating temperature above 140°C due to such a property of lactulose as the ability to caramelize. At temperatures of 150°C and above, dried crystals adhere to the walls of the drier, thus forming the layer that prevents the accumulation of the finished product.

It has been established that the finished product output increases twice during the drying of solutions with a mass fraction of lactulose of 40–60% in comparison with the drying of solutions with a mass fraction of lactulose of 20 and 30%. However, no appreciable difference between the product outputs in the drying of 40-, 50-, and 60-% solutions has been revealed also due to the effect of caramelization.

Another factor that has an effect on the product output is the solution flow rate. The dependences of the finished product output on the solution flow rate are plotted in Fig. 4.

The dependences plotted in Fig. 4 show that the finished product output grows with an increase in the solution flow rate up to 5–7 ml/min. The flow rate of 1–3 ml/min is optimal for solutions with a mass fraction of lactulose of 20 and 30%, but the product output in this case is only 21 ± 2 and 27 ± 2%, respectively, being twice lower than for solutions with a mass fraction of lactulose of 40–60%. The optimal flow rate for them is 5–7 ml/min. The higher is the solution flow rate, the less is the time spent on the process of drying, so the solution flow rate of 5–7 ml/min and the mass fraction of lactulose in a solution of 40–60% are an optimal proportion.

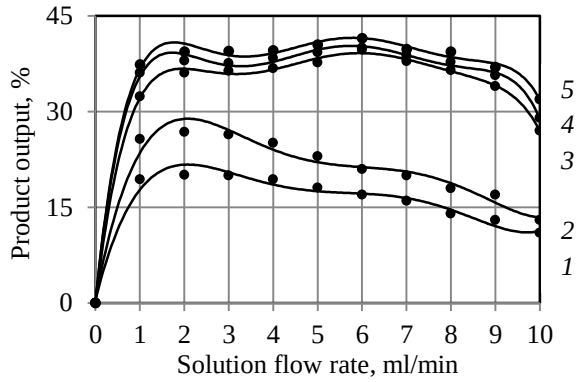


Fig. 4. Product output versus solution flow rate at a mass fraction of lactulose of (*1*) 20, (*2*) 30, (*3*) 40, (*4*) 50, and (*5*) 60%.

Among the principal factors that have an effect on the drying of lactulose is also the air flow rate provided by an aspirator. The aspirator is used to suck air for drying needs in or blow it out through the heater. The dependence of the finished product output on the air flow rate is plotted in Fig. 5.

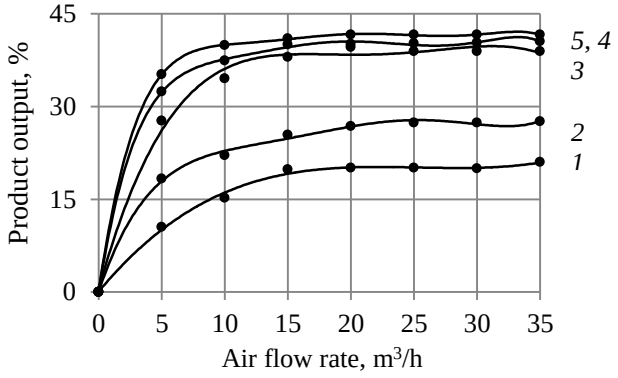


Fig. 5. Product output versus air flow rate at a mass fraction of lactulose of (*1*) 20, (2) 30, (*3*) 40, (*4*) 50, and (*5*) 60%.

The curves plotted in Fig. 5 show the growth of the product output with an increase in the air flow rate up to 15 m^3/h. This is due to the fact that the higher is the air flow rate provided by the aspirator, the faster the particles entrained by this flow will enter the catcher. The air flow rate of no less than 15 m^3/h is optimal for all solutions. The product output considerably grows for the drying of solutions with a mass fraction of lactulose of 40–60% in comparison with 20- and 30-% lactulose solutions (40.3 and 20.1–26.8%, respectively).

Analyzing the data obtained by studying the dependence of the finished product output on the principal drying parameters, we have established that the maximum productivity of the setup is provided for the drying of solutions with a mass fraction of lactulose of 40–60%.

The finished product output and the process time are important factors that govern the drying productivity (Table 3).

Table 3. Output and average duration for the drying of solutions with different mass fraction of lactulose

Mass fraction of lactulose in a solution, %	Maximum product output (theoretical), %	Experimental product output (in % of theoretical)	Specific drying time, ml/min
20	24.8	20.1	0.45 ± 5
30	37.2	26.8	0.55 ± 5
40	49.6	40.3	0.65 ± 5
50	62.0	40.3	0.75 ± 5
60	74.4	40.3	0.85 ± 5

The data given in Table 3 show the growth of the finished product output with an increase in the mass fraction of lactulose in solutions, and the drying time also grows.

The analysis of the data given in Table 3 shows that it is more reasonable to dry a 40-% lactulose solution, as the process time is minimal and the output of crystalline lactulose is maximal.

The quality of the finished product was estimated by such parameters as particle size, moisture content, solubility index, and hygroscopicity.

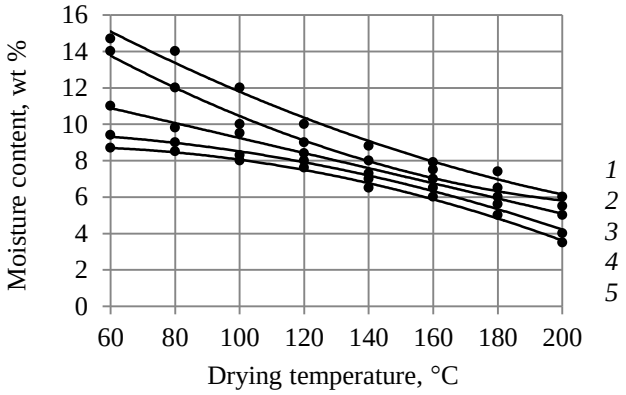


Fig. 6. Moisture content versus drying temperature at a mass fraction of lactulose of (*1*) 20, (*2*) 30, (*3*) 40, (*4*) 50, and (*5*) 60%.

The change of the moisture content in lactulose depending on the solution drying temperature is illustrated in Fig. 6.

The analysis of the curves plotted in Fig. 6 allows us to conclude that the moisture content in the finished product decreases with an increase in the drying temperature and the mass fraction of lactulose in a solution.

The analysis of literature and patent sources shows that the optimal moisture content in crystalline lactulose is less than 7%. Such moisture content values were obtained for the drying of all solutions at a temperature of 120˚C and higher.

However, the air flow rate also has an effect on the moisture content in addition to the drying temperature. This is explained by the fact that the drying efficiency grows with increasing air flow intensity, thus reducing the residual moisture in a product and, consequently, the moisture content (Fig. 7).

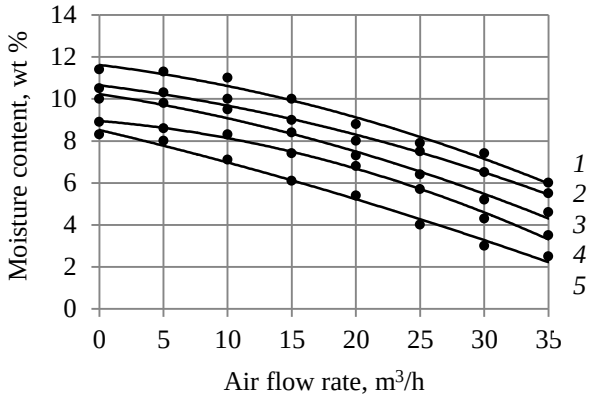


Fig. 7. Moisture content versus air flow rate at a mass fraction of lactulose of (*1*) 20, (*2*) 30, (*3*) 40, (*4*) 50, (*5*) 60%.

From the analysis of the curves plotted in Fig. 7 it can be established that the moisture content in the finished product decreases with an increase in the air flow rate and the mass fraction of lactulose. The optimal air flow rate for the standard moisture content that must not exceed 7% and solutions with a mass fraction of lactulose of 40–60% is no less than 35 m^3/h. The standard parameters for the drying of 20–30-% solutions can be attained only at air flow rates of higher than 25 m^3/h. However, according to Fig. 5, the drying of solutions with mass fractions of 40–60% is most reasonable.

Another factor that has an effect on the moisture content in the finished product is the solution flow rate, as the partial pressure of water vapor also grows with an increase in this parameter. The change of the moisture content in the finished product depending on the solution flow rate is illustrated in Fig. 8.

As shown by the curves plotted in Fig. 8, the moisture content in lactulose grows with an increase in the flow rate of the solution fed to the setup. At required moisture content values of no more than 7%, the flow rate is 1.5–3 ml/min for solutions with a mass fraction of lactulose of 20–30% and 4.5–7 ml/min for 40–60-% solutions. Relying on the earlier obtained data, we can conclude that the product output grows with increasing solution flow rate and, consequently, it is reasonable to dry solutions with a mass fraction of 40–60%.

The following parameter that has an effect on the quality of the finished product is the particle size, as the solubility index and hygroscopicity of dry lactulose directly depend on this parameter.

The dependences of the particle size on the solution flow rate are plotted in Fig. 9.

The curves plotted in Fig. 9 show that the particle size of the finished product grows with an increase in the solution flow rate and the mass fraction of lactulose in a solution. In compliance with the required particle size of 7–10 μm, the optimal flow rate is 5–10, 1–5, and 1 ml/min for a solution with a mass fraction of lactulose of 40, 50, and 60%, respectively. The maximum particle size of the finished product for the drying of solutions with a mass fraction of 20–30% is up to 5 μm.

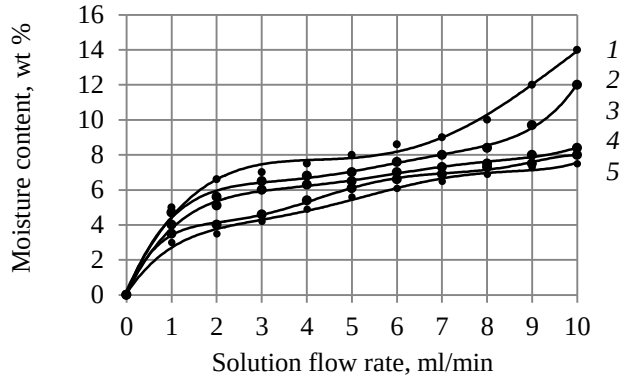


Fig. 8. Moisture content versus flow rate at a mass fraction of lactulose of (*1*) 20, (*2*) 30, (*3*) 40, (*4*) 50, and (*5*) 60%.

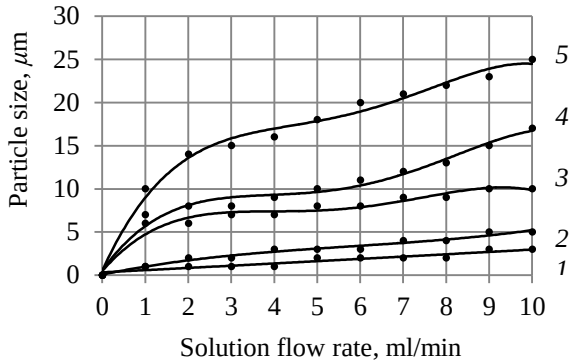


Fig. 9. Particle size versus solution flow rate at a mass fraction of lactulose of (*1*) 20, (*2*) 30, (*3*) 40, (*4*) 50, and (*5*) 60%.

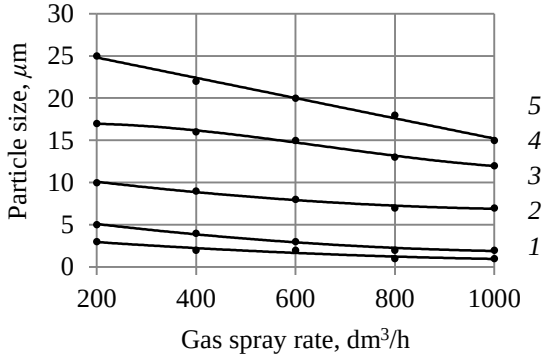


Fig. 10. Particle size versus gas spray rate at a mass fraction of lactulose of (*1*) 20, (*2*) 30, (*3*) 40, (*4*) 50, (*5*) 60%.

Alongside with the solution flow rate, the gas spray rate has a great effect on the particle size. When this parameter is increased, sprayed liquid drops become smaller and, correspondingly, the particle size of the finished product is also reduced (Fig. 10).

According to the curves plotted in Fig. 10, the particle size becomes smaller with increasing gas spray rate. Knowing that the required particle size is 7–10 μm, we have established the optimal gas spray rate range of 500–700 dm^3/h for a solution with a mass fraction of lactulose 40%, as the particle size of the finished product for the drying of solutions with a mass fraction of lactulose of 20, 30, 50, and 60% does not meet requirements. The drying of a 40-% lactulose solution at a specified gas spray rate allows us to obtain a product with a particle size corresponding to the solubility indices (Table 4).

Table 4. Solubility index and hygroscopicity of lactulose with different particle sizes

Mass fraction of lactulose in a solution, %	Particle size, μm	Solubility index, cm^3 of moist precipitate	Hygroscopicity, %
20	1–2	0.10 ± 0.05	25 ± 5
30	3–5	0.15 ± 0.05	20 ± 5
40	7–10	0.20 ± 0.05	10 ± 5
50	11–15	0.30 ± 0.05	5 ± 5
60	16–25	0.30 ± 0.05	1 ± 5

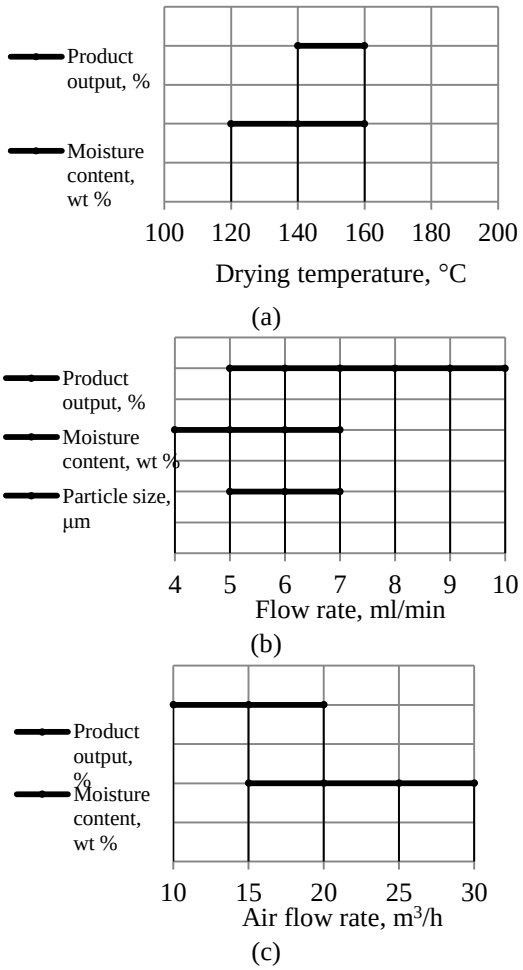


Fig. 11. Optimal parameters for solutions with a lactulose mass fraction of 40%: (a) temperature, (b) flow rate, (c) air flow rate.

The data of Table 4 show that the solubility index grows with increasing particle size and, consequently, the solubility of a product with a greater particle size becomes worse. The obtained lactulose meet the requirements to nutrient carbohydrates, whose solubility index must not exceed 0.4–0.5 cm^3 of moist precipitate. The hygroscopicity of a product decreases with an increase in the mass fraction of lactulose in a solution.

With the data obtained from the analysis of the curves shown in Figs. 6–10, it becomes clear that the drying of solutions with a mass fraction of lactulose of

40% is most rational, as confirmed by the results of studying the finished product output (Figs. 3–5).

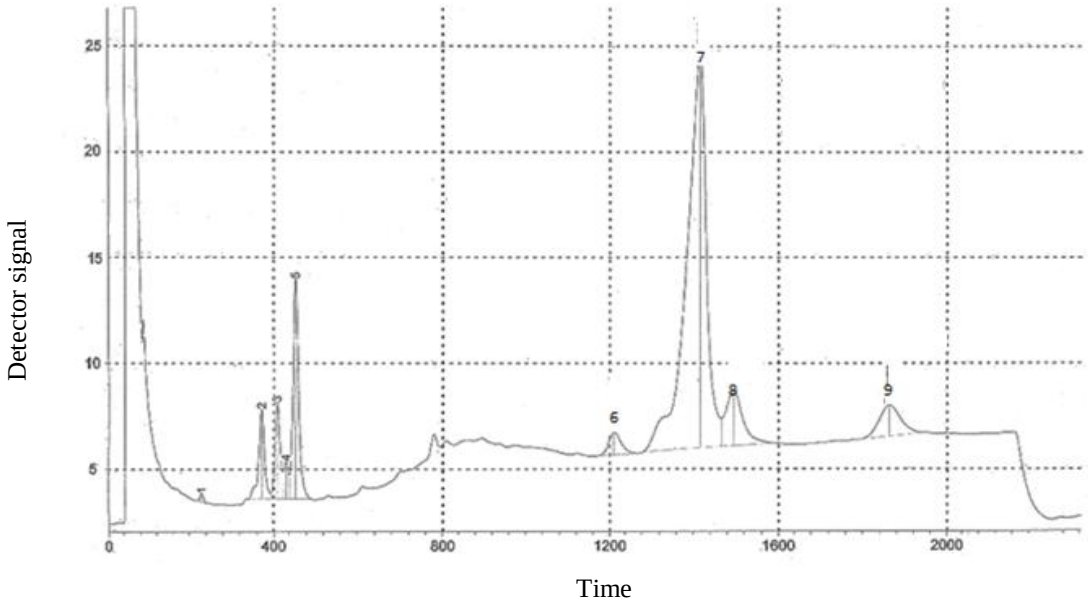
Analyzing the above considered data on the principal factors responsible for the process of drying, let us construct the summary plot of the optimal parameters for the selected solution with a mass fraction of lactulose of 40%. When specifying the drying temperature, we took into account the moisture content in the finished product and its output (Fig. 11).

To determine the solution flow rate values, we considered such parameters as the moisture content in the finished product and its output and particle size (Fig. 11b). It has been established that the flow rate of 5–7 ml/min is optimal.

The optimal air flow rates are plotted in Fig. 11c. The optimal range of air flow rates of 15–25 m^3/h has been established.According to the data plotted in Fig. 11a, the finished product output is maximal at a drying temperature of 140–160°C, and the moisture content meets the requirements at 120–160°C. Hence, the temperatures of 140–160°C are optimal. To confirm the results of studying the qualitative characteristics of dry lactulose, the obtained sample and the initial lactulose solution were subjected to chromatographic analysis. The GLC method has been selected for this purpose, as it has high precision and resolution and allows the quantitative and qualitative analysis for lactulose in the presence of α- and β-lactulose, galactose, glucose, tagatose, fructose, and other carbohydrates (Figs. 12 and 13). The abscissa is the chromatographic run time (mobile phase volume), and the ordinate is the analytical signal, which depends on the concentration of components in the eluent (response).

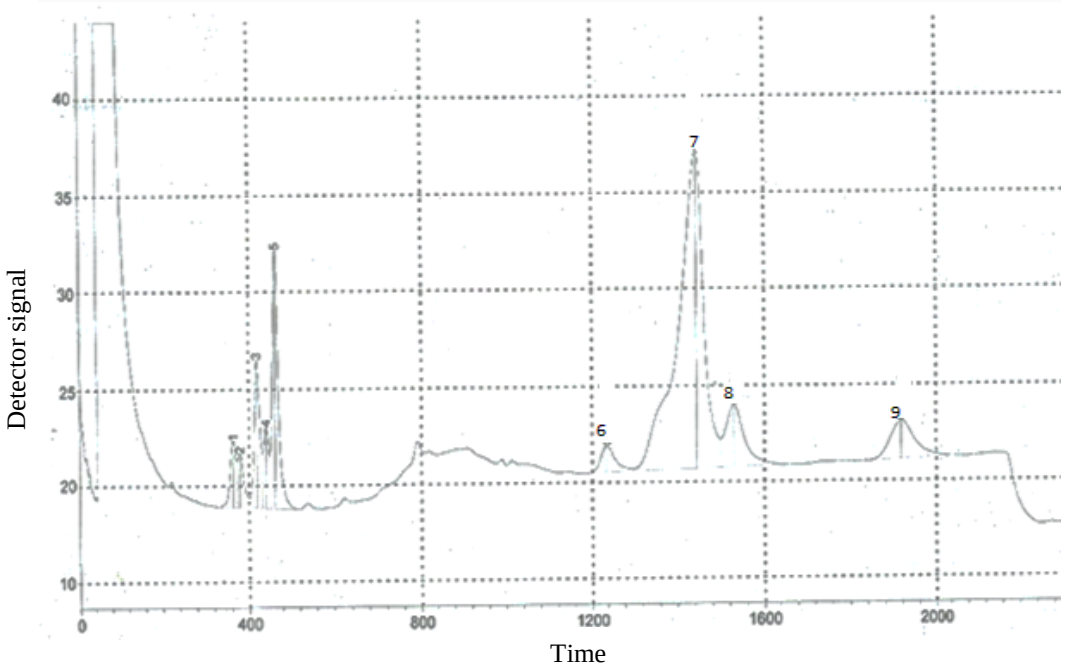
From the data shown in Figs. 12 and 13 it can be established that the concentration of lactulose is 40.4% in the initial syrup and 64.2% in the finished product. Hence, we have managed to increase the concentration of lactulose by more than 20% using the method of spray drying.

Among the advantages of dry lactulose are precise dosing, compactness, packing and transportation convenience, prolonged storage, and the possibility of its target application in the form of a solution. Dry lactulose forms have a considerable advantage in medicine: the preparation is well-digestible due to its large active area of contact in the gastrointestinal tract.



No	Name	Time	Height	Area	Concentration	Dose
1		360	3.5	56.3	8.9	1.0
2		375	2.8	46.8	7.4	1.0
3		415	7.7	117.6	6.0	1.0
4		435	4.2	58.8	3.0	1.0
5		457	13.4	214.6	10.9	1.0
6	ε-lactose	1221	1.4	52.2	2.6	1.0
7	lactulose	1430	16.6	1126.7	40.4	1.0
8	α-lactose	1514	3.3	164.8	11.4	1.0
9	β-lactose	1899	2.0	133.4	6.8	1.0
In sum			54.7	1971.3	100.0	

Fig. 12. GLC pattern of a lactulose solution with a mass fraction of solids of 40%.



No	Name	Time	Height	Area	Concentration	Dose
1		226	0.4	4.0	0.3	1.0
2		368	4.3	70.8	4.5	1.0
3		405	4.6	66.0	4.2	1.0
4		425	1.8	23.0	1.5	1.0
5		448	10.4	159.1	10.1	1.0
6	ε-lactose	1198	1.1	36.8	2.3	1.0
7	lactulose	1401	18.6	1015.1	64.2	1.0
8	α-lactose	1479	2.6	122.1	7.7	1.0
9	β-lactose	1844	1.5	85.4	5.4	1.0
In sum			45.2	1582.3	100.0	

Fig. 13. GLC pattern of dry lactulose.

REFERENCES

1. Gavrilov, G.B., *Tekhnologiya membrannykh protsessov pererabotki molochnoi syvorotki i sozdanie produktov s funktsional'nymi svoistvami* (Technology of Membrane Whey Processing and Creation of Products with Functional Properties), Moscow: Rossel'khozakademiya, 2006.
2. Voskuijl, W, de Lorijn, F, Verwijs, W, et al., PEG 3350 (Transipeg) versus lactulose in the treatment of childhood functional constipation: a double blind, randomised, controlled, multicentre trial, *Gut*, 2004, vol. 53, no. 11, pp. 1590–1594.
3. Petuely, F., Bifidusflora bei Flaschenkindern durch bifidogene Substanzen (Bifidusfactor), *Z. Kinderheimkd*, 1957, vol. 79, no. 2, pp.174–179.
4. Shukla, S., Shukla, A., Mehboob, S., and Guha, S., Meta-analysis: the effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy, *Alimentary Pharmacology & Therapeutics*, 2011, vol. 33, no. 6, pp. 662–671.
5. Khramtsov, A.G., Brykalov, B.A., and Kharitonov, D.V., *Lactuloza: tsennost', ispol'zovanie, marketing i effektivnost' proizvodstva* (Lactulose: Value, Application, Marketing, and Production Efficiency), Stavropol: Argus, 2004.
6. US Patent 41 422 916, 1979.
7. Jpn. Patent 52-21 063, 1972.
8. FRG Patent 2 224 680, 1972.
9. US Patent 3 716 408, 1971.
10. Jpn. Patent 56-39 200, 1981.
11. US Patent 4 605 646, 1986.
12. US Patent 5 644 050, 1993.
13. US Patent 5 415 695, 1988.
14. US Patent 5 480 491, 1993.
15. US Patent 5 688 521, 1994.