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Studying of Lactulose Hygroscopicity and Microstructure after Spray Dehydration.

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ABSTRACT

The present work investigates the impact of spray dehydration technological parameters upon lactulose yield. It is ascertained that the maximum plant productivity is ensured at dehydrating of solutions with the lactulose mass fraction of 50% at the temperature of 140±1°C and the speed of feeding the solution into the chamber of 6 ml/min. Impact of spray dehydration technological parameters upon the end-product quality characteristics has been studied. It has been ascertained that correspondence of the end-product to the quality characteristics is gained by usage of the dehydration temperature of 140±1°C, the feeding speed of 6 ml/min, the airspeed of 20-25 m^3/h. Lactulose received by hydrating a solution with the mass fraction of 50% meets the requirements laid on food carbohydrates as the solubility index lies within the range from 0,10±0,02 to 0,30±0,02 cm^3 of wet residue. The particles size does not exceed the standardized value of 10 µm. Microstructure of dry lactulose received at different dehydration temperatures has been studied. It is ascertained that the optimum dehydration temperature is 140°C. At the temperatures of 100-120°C dehydrated lactulose is prone to glueing of the particles. Heated over 140°C, the product has a more dispersed structure, but there are signs of caramelization.

Keywords: lactulose, spray dehydration, microstructure.

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INTRODUCTION

Lactulose is a carbohydrate referred to the class of oligosaccharides and subclass of disaccharides, its molecule consists of galactose and fructose units. The bond is effected between the first and the fourth carbonium atoms – 1-4 bond. The chemical name of lactulose in the modern terminology is 4-O-β-D-galactopyranosyl-D-fructose [1]. The lactulose molecular structure is appropriate to be presented as conformational, structural and cyclic formula.

Theoretically, there are five possible lactulose conformations: α- or β-pyranose, α- or β-furanose and acyclic ones. By nuclear magnetic resonance spectroscopy method (NMR-spectroscopy) crystalline lactulose in anhydride form is received in three different ways it was established that in all the samples β-fructofuranose form prevails over α-fructofuranose and β-fructopyranose forms with the following ratio: 0,745:0,100:0,155. However, numerous investigations failed to detect residues of the two other forms.

Lactulose presents a white crystalline substance without smell, well dissolved in water. This prebiotic is sweeter than lactose, but is less sweet compared to sucrose. Lactulose is exposed to recrystallization from a solution with methanol mass fraction of 50% in the form of hexagonal achromatic leaves; anhydride form is thus received. By the present day, with lactulose crystallization in an aqueous solution, a carbohydrate in trihydrate form has been gained that has a whole set of differences with the anhydride form, including even physical and chemical properties. For example, the anhydride form is very hygroscopic: at 30°C and 81% humidity the crystals humidity increases up to 7% in 24 hours already and up to 20% in 48 hours. Lactulose trihydriate form has another important feature: the same conditions preserved, crystals of this form do not alter their main properties, though in case of heating over 37°C lass of crystallization water is possible. That is why they are stored at relatively low temperatures [2,3,4].

Lactulose can be used both as syrup and in crystalline form. The following items can be referred to advantages of drug products dry form: dosing accuracy, compactability, packaging and transportation convenience, duration of storage and possibility of intended application in dissolved form. Lactulose dry forms have a significant advantage in the medicine: the drug is easily digested due to the large actual contacting area in the gastro-intestinal tract [5,6].

Necessity of improving the technique of receiving lactulose as an active bifidogenic factor for functional food products led to the possibility and acute necessity of systemization, improvement and development of physical and chemical patterns of lactulose receiving processes under the present day conditions [7].

Nowadays there are several techniques of receiving crystalline lactulose. The drawbacks of those techniques include reduction of lactulose content in the product as to reduce hygroscopicity and to accelerate the process different catalysts and binding substances are added. One may also note the price increase due to additives usage [8].

In spite of a good number of investigations devoted to receiving crystalline lactulose, there are practically no industrial biotechnologies with significant economical potentials in Russia, while the RF market exhibits certain attractiveness characterized with its potential and expected profitability [9].

Ways of lactulose crystalline forms production are extremely labour-intensive, as a result, the drugs prices increase by 1-2 orders compared to syrups. Taking the above said into account, it is timely to investigate the process of lactulose solutions spray dehydration aimed at determination of optimal parameters the usage of which will help to receive a product with high qualitative characteristics without usage of additives. The fact that imported goods aside from containing “nature-identical” ingredients have quite a high price will guarantee the product competitive ability [10].

Lactulose dry powders are considered to be more effective in usage. However, the problem of crystalline lactulose production in our country has not been solved to the full extent yet. Nevertheless a number of techniques have already been patented.

According to patents, the main problem of existing technologies of lactulose solutions spray dehydration is addition of binding components, and the powder received is characterized with high hygroscopicity being in amorphous form [11].

METHODS

The following items were investigation objects at different stages of the work:

– solutions with the lactulose mass fraction of 20-60% (OOO "Shekhon-Lactulose", Russia);
– distilled water corresponding to GOST (= State Standard) 6709-72 "Distilled Water. Technical Specifications";

To get lactulose solutions with different concentration, a solution with the lactulose mass fraction 50% corresponding to TU (= Technical Specification) 9229-003-39185375-2003 was taken and diluted to get solutions with the lactulose mass fraction of 20-40% and condensed to get a solution with the lactulose mass fraction of 60%.

– N-trimethylsilylymidazol (Panreac, Spain);
– hexane (NPK "KRIOCHROME", Russia);
– potato agar (FSZ (= Federal Service of Health Care) 2009/03706);
– beef-extract agar (GOST 20730-75);
– malt agar (FSZ 2009/03709);
– Endo medium (TU 9229-072-00419785-97).

Other used domestic and imported reagents had a grade not less than CP.

When executing the works, generally accepted, standard and original investigation methods were used.

Selection of test samples and their preparation for the analysis were performed in accordance with GOST 9225, GOST 26809, GOST 26929.

The dry substance mass fracture in the lactulose solutions was determined by the refractometric method in accordance with GOST 24908-84.

The mass fraction of lactulose and other carbohydrates in the solutions and dry lactulose was determined with the method of gas-liquid chromatography (GLC) with GCMS-QP2010 Ultra (Shimadzu, Japan) chromatograph.

The method of gas-liquid chromatography is based upon transformation of saccharides into volatile trimethylsilyl derivatives with their subsequent separation at the chromatograph column and determination with a flame ionization detector.

The summary of the method is as follows: the sample under analysis, preliminarly dried and defatted, was treated by N-trimethylsilylymidazol at 60-70 °C during 1-2 hours. Then an exact amount of hexane was added to the mixture, the hexane surplus hydrolyzed with water, after which an aliquot of the hexane phase was injected into the chromatograph. The carbohydrates were separated on the packed column with the polar phase in an isothermal mode. Monosaccharides came out with the solvent front, lactose came out as two peaks corresponding to alpha- and beta-anomers, and lactulose came out as one peak.

During the experiment, the carrier gas from the cylinder, in case of increasing pressure, comes continually into the preparation unit, where it gets additional cleaning. The sample input device is a flow-through the independently thermostated cylinder chamber r. The test sample under analysis (1-10 microlitres) was put into the gas flow at an elevated temperature with an automatic dosing unit through a rubber heat-resistant membrane; the liquid test sample quickly evaporates and is transferred with the gas flow into the chromatograph column placed in the thermostat. The separation was performed at 20-400°C. Sometimes packed columns 0.5-5

m long and 0.2-0.6 cm in diameter are used for analytical separation. The media is a hard sorbent with a developed surface (50-500 м^2/g). The recorder registers the signal change with time.

The quantitative content of lactulose, lactose and other carbohydrates present in the sample was calculated by the method of internal standard, using the preliminarily performed calibration.

Organoleptical properties of the investigated samples were determined in the following order:

– appearance and texture: general visual impression of the product (the surface character, homogeneity, form);
– colour: the colour for the designed product was established as well as colour fluctuations;
– smell: whether the scent is typical for this type of product was determined.
– taste: whether the taste is typical for this type of product was determined.

The end-product quality was estimated by such factors as the particle size, humidity, solubility index and hygroscopicity.

The solubility index was determined in accordance to GOST (=State Standard) 30305.4.95. Two parallel measurements were carried out. Reconstituted product was stirred, then plastic test-tubes Falcon were filled with it up to "10 cm^3" mark and closed with plugs. The test-tubes were centrifugated for 5±1 min. After the centrifuge process, if there was no distinct border, the supernatant was poured out leaving its layer about 5 mm thick above the residue. Then water with the temperature of 25±1 °C was added into the test-tubes up to "10 cm^3" mark, the content of the test-tubes was stirred with a stick, they were closed with plugs and centrifugated for 5±1 min. more. After appearing of a detectable line the lade-down residue amount is estimated.

The microstructure was determined by microscopic examination of a lactulose sample on an upright optical microscope AxioVert.A1 (Carl Zeiss AG, Germany) using lenses with x20, x40 magnifying power.

Moisture mass fraction was determined with an analogue of Chizhova device – Eleks-7 (Eleks-Ulyanovsk, Russia) in accordance with GOST 30305.1.

The device work principle consists in dehydration of a raw material sample by way of evaporation due to its heating at a required temperature during a specified time. The samples deaquation is carried out in special bags of weakly stuck paper like rotator or newsprint paper.

A sample of dry lactulose with the weight of 4-6 g was put into a paper bag, dried and weighed on an analytical balance with the accuracy up to 0.01 g; the sample was spread evenly through the whole bag area. Paper filters with the diameter of 11-12.5 mm were used to make the bags.

The bag with its content was weighed and placed between the device plates. Drying at the temperature of 80±0,5 °C was carried out during 5±1 min. The dried bag with lactulose was cooled in an exsiccator to the room temperature, and then weighed.

The investigation was carried out under the conditions of correct average values: the samples were investigated in three replications. The arithmetic average with the error not exceeding 0.1% was calculated by the results of the three measurings.

To determine radiological safety indexes – specific (volume) activity of caesium-137 and strontium-90 – MUK (=methodological instructive regulations) 2.6.1.7171-98 "Radiation control. Strontium-90 and Caesium-137. Food stuffs. Sampling, analysis and hygienic estimation".

Toxic elements, pesticides, antibiotics and radionuclides content determination:

- lead – in accordance to GOST R 51301 "Food stuffs and food staples. Stripping voltammetric methods of determining the content of toxic elements (cadmium, lead, copper and zink)", GOST 26932 "Food staples and products. Lead determination methods", GOST 30178 "Food staples and products. Atomic absorption method of toxic elements determination", GOST 30538 "Food stuffs. Methodology of determining

toxic elements by atomic emission method" and MUK 4.1.986 "Methodology of measuring mass fraction of lead and cadmium in food products and food staples by the method of electrothermal atomic absorption spectrometry. Methodological instructive regulations";

- arsenic – in accordance to GOST R 51766 "Food staples and products. Atomic absorption method of arsenic determination";
- cadmium – in accordance to GOST R 51301 "Food stuffs and food staples. Stripping voltammetric methods of determining the content of toxic elements (cadmium, lead, copper and zink)", GOST 26933 "Food staples and products. Cadmium determination methods", GOST 30178 "Food staples and products. Atomic absorption method of toxic elements determination", GOST 30538 "Food stuffs. Methodology of determining toxic elements by atomic emission method" and MUK 4.1.986 "Methodology of measuring mass fraction of lead and cadmium in food products and food staples by the method of electrothermal atomic absorption spectrometry. Methodological instructive regulations";
- mercury – in accordance to GOST 26927 "Food staples and products. Mercury determination methods" and MU 5178 "Methodological instructive regulations on determining mercury in food stuffs".

Microbiological indicators were determined by way of counting the number of colonies appearing in Petri dishes with breeding grounds of potato, beef-extract and malt agars. To determine general microbial content the investigated sample was diluted with distilled water in the following ratios: 1:100, 1:1000, 1:10000. Inoculations were made from each sample solution into 6 Petri dishes onto agar surface with a dropper sterilized in a burner flame, the test sample amount made 0.3 ml. After that the Petri dishes were incubated at the temperature of 37±1 °C during 48±1 hours.

The following items were determined in the investigated samples: general microbial content, yeast fungi and mould fungi amount, bacteria of collibacillus group and salmonella.

The amount of aerobic and optional anaerobic mesophilic microorganisms was determined by counting the average number of microorganisms colonies per 1 g of a sample in all the Petri dishes.

The general amount of yeast fungi and mould fungi was determined by a sample inoculation into Petri dishes with wort agar in accordance with GOST 10444.12-88 "Food stuffs. Yeast fungi and mould fungi determination methods".

Determination of bacteria of collibacillus group was carried out in accordance with GOST R 52816-2007 "Methods of discovering and determining bacteria amount. Collibacillus (coliform bacteria) groups".

To determine pathogenic microorganisms, an inoculation was made to Kaufman cumulative medium with the following inoculation onto Endo medium in accordance with GOST 50480-93 "Food stuffs. Method of discovering bacteria of Salmonella genus".

The investigations results were processed by mathematical statistics method; to analyze the data, dispersion analysis, regression lines, design of the experiment were used.

RESULTS

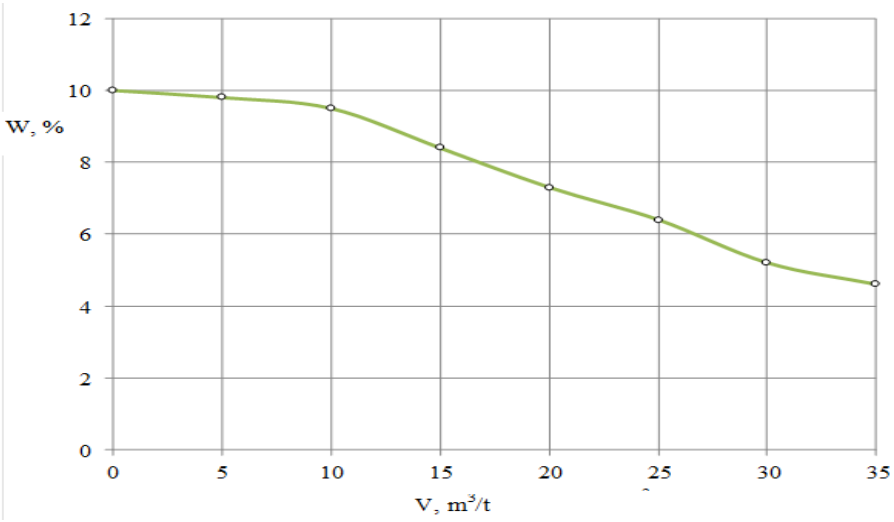
Estimation of the end-product quality indexes is an important stage of investigation that determines efficiency of parameters and methods used to get the end-product. Every product has its own set of indexes that depends on the product purpose, conditions of its production and exploitation and many other factors.

The set of indexes is finally formed at the product designing stage, as they are built into the construction here. Later on, at the production stage, these indexes get their implementation. At the exploitation (consumption) stage the indexes become the product individual characteristics, distinguish it from other types of products (goods), make its consumer properties and, consequently, make it attractive and competitive [12].

From literary sources we know that in case of air flow force increase the dehydration capacity increases too. As a result, the amount of residual moisture in a product reduces and, consequently, the

moisture mass fraction takes place. It means that apart from the dehydration temperature, the airspeed also influences the moisture content in a product.

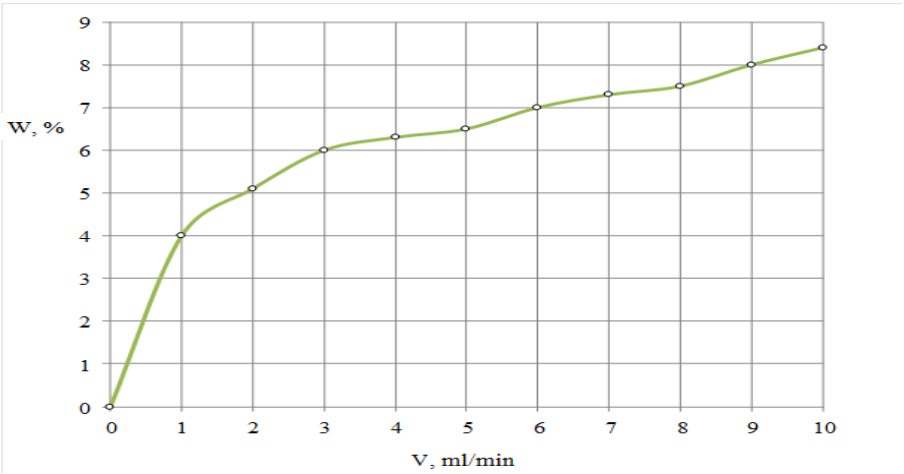
Due to this fact the further investigation is directed to the study of changes moisture mass fraction changes in a product depending on the airspeed (Picture 1). Having analyzed the curve presented in Picture 1, one may conclude that the moisture mass fraction in the end-product is in inverse ratio to the airspeed. It is evident from the graph that at the airspeed over 10 m^3/h sharp reduction of moisture mass fraction from 9.5% to 5.0% takes place. Increase of airspeed contributes to emptying the dehydrating chamber from moisture excess, which reduces partial pressure of the moisture vapour in the chamber and contributes to intensification of evapouration process. For example, with the airspeed of 10 m^3/h moisture mass fraction makes 9.5%. As is said above, optimal moisture mass fraction in dry lactulose must not exceed 7%. This value of moisture mass fraction can be reached by establishing the airspeed over 20 m^3/h for solutions with lactulose mass fraction of 50%. So, the optimal airspeed is makes not less than 20-25 m^3/h.



Picture 1 – Change of moisture mass fraction in the product depending on the airspeed in a solution with lactulose mass fraction of 50 %

Another important factor that has an influence on the moisture mass fraction in the end-product is the solution feeding rate. Partial pressure of moisture vapour increases with the feeding rate increase. Dependence of the moisture mass fraction in the end-product on the solution feeding rate is given in Picture 3.

As is seen from the curve presented in Picture 2, the moisture mass fraction in lactulose increases with the increase of the speed of the solution delivery into the chamber.



Picture 2 – Change of moisture mass fraction in the product depending on the solution feeding rate in a solution with lactulose mass fraction of 50 %

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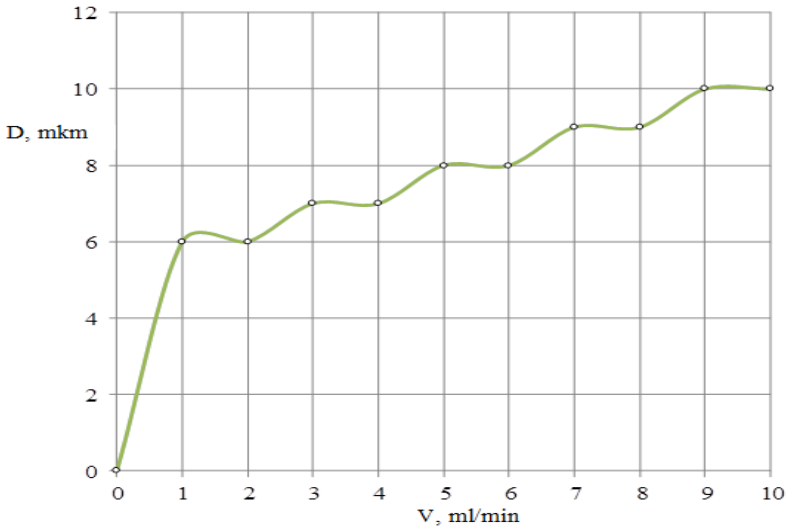
For the required values of moisture mass fraction in the end-product not exceeding 7%, the feeding rate of solutions with lactulose mass fraction of 50% makes 4.5-7 ml/min.

An important characteristic that has an impact on the end-product quality is the particle size, as solubility index and hygroscopicity of the dry lactulose depends on this parameter directly.

The curve of dry lactulose particle size dependence on the solution feeding rate is given in Picture 3.



Picture 3 – Change of dry lactulose particle size depending on the solution feeding rate at lactulose mass fraction of 50 %

According to the required particle size of 7 µm, the optimal solution feeding rate at lactulose mass fraction of 50 % is 5-10 ml/min.

The data given in Table 1 show increase of values of lactulose solubility index as the particle size grows.

Table 1 – Solubility index, particle size, hygroscopicity of the dry lactulose received from solutions with different concentration

Lactulose mass fraction in the solution, %	Particle size, µm	Solubility index, cm^3 of wet residue	Hygroscopicity, %
40,0	8,0±2,0	0,20±0,01	10,0±0,4
50,0	12,0±3,0	0,30±0,03	5,0±0,2
60,0	20,0±5,0	0,30±0,03	1,0±0,1

DISCUSSION

As is seen from the curve presented in Picture 3, the moisture mass fraction in lactulose grows with increasing the speed of the solution feeding into the chamber. A sharp increase of moisture content in dry lactulose from 0% to 4% is observed at solution feeding rate increase from 0 to 1 ml/min.

At further increase of speed of feeding the solution into the chamber, a more gradual increase of moisture mass fraction in the end-product from 4.0% to 8.5% is observed. It is connected with change of the speed balance of the solution new batches inflow and particles outflow in the dehydration chamber. As a result, the balance shifts towards the inflow, which creates moisture excess and slows down the evaporation process. This, in its turn, affects the end-product humidity.

The curve presented in Picture 3 indicates a direct correlation between the end-product particle size and the solution feeding rate. It happens due to increasing the lactulose concentration in the dehydration chamber. Due to a large quantity of lactulose in the chamber, crystallization process intensification and increase of dry lactulose particle size take place.

After a sharp step of the solution feeding rate from 0 to 1 ml/min, slowing down of particle size growth is observed, which is connected with a limited time of the particles staying in the dehydration chamber. That is why further increase of the solution feeding rate does not cause such significant growth of lactulose crystals size.

CONCLUSION

The proved effect is connected with the fact that a big size of the particles makes interaction between molecules of water and lactulose more difficult as well as forming donor and acceptor (hydrogen) bonds, and consequently the solution process. Lactulose received by dehydration of a solution with the mass fraction of 50% meets the requirements put to food carbohydrates as the solubility index lies within the range from 0,10±0,02 to 0,30±0,02 cm^3 of wet residue.

The data given in Table 1 show increase in values of lactulose solubility index as the particle size grows.

So, impact of spray dehydration technological parameters upon lactulose quality characteristics has been proved.

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