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Design of Enzyme Granulation Processes

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Abstract—Results of researches of processes of mixture and granulation by an integration method with preliminary mechanoactivation in relation to fermental preparations are given. Efficiency of use of the granulated enzymes in comparison with a powdery material is shown. Granulation mechanisms for different types of enzymes are revealed. The various equipment of technological lines is tested and recommendations about their regime parameters are made, material characteristics reflecting efficiency of process of a granulation are defined. The technological scheme of receiving the granulated enzymes is developed.

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INTRODUCTION

Enzyme preparations are widely used in different fields. In animal husbandry enzyme preparations are used for enhancing forage assimilation and in the food industry milk-clotting enzyme preparations (MCEs) are widespread. Such enzyme preparations are generally produced as powders. However, their high dispersion adversely affects and environmental situation at workplaces and is the reason of losses of the powder at various stages of its production and transportation. Dusty enzyme prepara-tions are hygroscopic, stick to the surfaces of bunkers and transportation facilities, and cake on storage.

The change to granular MCE preparations will allow intensification of the technological process, improvement of working conditions, and reduction of product losses [1, 2].

At present both natural MCEs and those obtained by microbiological synthesis are widely used [3]. The MCE group includes the milk-clotting preparations obtained from the abdomasum of ruminant animals chymosin young stock (calf and lamb stomachs). Often this term relates to preparations produced from stomachs of adult animals (pepsin), abdomasum of chickens (chicken pepsin), and microbial producers. Rennet includes two enzyme components: chymosin and beef pepsin. Cheese making is based on the coagulation of its principal protein (casein) and milk curd formation. Only one peptide bond (among hundreds of others) in casein is responsible for enzymatic coagulation of this protein. Rupture of the protein molecule by just this bond leads to milk clotting. Chymosin is an enzyme which, by the very its nature (secreted by calf abdomasum for milk clotting), ensures rupture of this specific bond and remains the other bonds intact. Pepsin, vice versa, by its very nature (responsible for protein digestion), ruptures a great number of peptide bonds in casein. The quality of rennet depends on the chymosin/pepsin ratio: the higher the ratio, the higher the rennet quality.

The quality of enzyme is also affected by the insoluble residue. When the content of the latter is high, insoluble particles adsorb pepsin and other enzyme during milk clotting and are entrapped by the milk clot, which may impart a bitter taste to the resulting cheese.

Microbial population of enzyme should not be higher standards specific for this preparation.

The activity of the rennet powder depends on the total content of chymosin and pepsin in it, as weel as on the properties of the milk mixture subject to clotting. The activity of the rennet powders produced in Russia is 100000 arb. units per gram. This means that 1 mL of a 1% aqueous solution of enzyme clots 100 mL of standardized milk within 7.5 min.

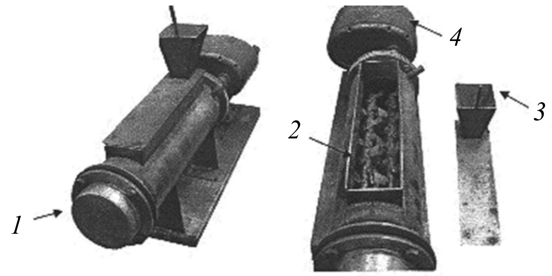

Fig. 1. General view of the pregranulator–mixer: (*1*) housing, (*2*) shaft with blades, (*3*) binder feed fitting, and (*4*) electric motor.

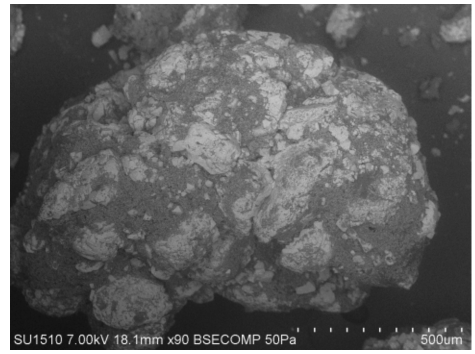

Fig. 2. Structure of an MCE granule obtained by pelletizing. The image obtained on a Hitachi SU1510 scanning electron microscope.

According to technical specifications [for example, those at the Moscow Rennet-Making Plant (MZSF)], the milk-clotting activity of the end product should be no less than 100000 arb. units as measured against an OKO-SF rennet reference standard.

In Russia, a number of rennet and pepsin mixtures differing in composition are produced under different brands: VNIIMS series (FP-VNIIMS, FP-2), SG series (SG-50, SG-25), and Almazim.

The composite powder preparations Bovigrand (SBI, France) and NoVA (Chr.Hansen, Denmark) are also present at the Russian market (see table).

The FP-VNIIMS and Bovigrand are recommended for the production of all types of cheeses, even though they are inferior in quality than rennet powders. The FP-2, SG-25, and NaVO preparations can only be applied for cheeses with a short maturation time (according to TS 10-02-02-52-87).

Contents of chymosin in composite powdered preparations at Russian market

Preparation	Chymosin content, % (no less than)
VNIIMS series	–
FP- VNIIMS	50
FP-2	25
SF	70
SG-50	50
SG-25	25
Altazim	13
Bovigrand, calf rennet	50
NoVA, calf rennet	20

EXPERIMENTAL

We performed complex research on the granulation of the MCEs listed in the table, including the stages of mixing, aggregation, and drying. In this experimental series we used SG-50 and KG-50 rennet powders. It should be mentioned that NaCl was introduced to control the activity characteristics of the substances.

At the first stage the starting components, namely enzyme and salt, are fed to a granulation chamber equipped with moving actuators (shaft with blades). A binder is also fed to the apparatus, it wets the mixed powder particles, and they agglomerate under agitation to form wet granules (Fig. 1).

As binders we used solutions of starch, poly(vinyl alcohol) (PVA), and poly(vinylpyrrolidone) (PVP) derivatives.

The resulting wet granules were dried in “mild” conditions and then transferred to an apparatus with a moving surface (drum, vibratory mixer) or a mixer with moving actuators.

Therewith, agitation and spiral motion of the material leads to hardening of the granules and grinding of large granules to a commercial-scale size (retour). The granulation process involving agitation of the mixture both at the preparatory stage and during operation with already formed granules results in mechanical activation of the mixture, increasing the specific surface and affecting the structure of the surface layer of the particles (Fig. 2). The structural defects that form change the reactivity of the activated particles.

The key factors controlling granulation on drum apparatuses are the type and amount of the binder, fraction of NaCl in the MCE, particle size of the main

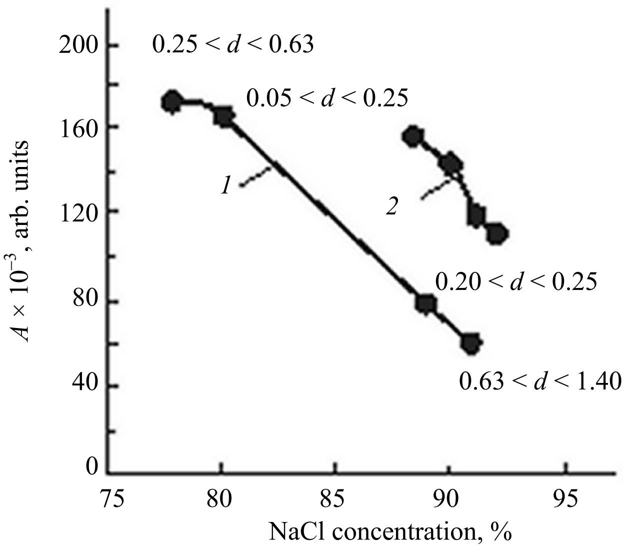

Fig. 3. Dependence of the activity (A) of a granular enzyme preparation on salt concentration: (*1*) fraction with the particle size (d) 0.05–1.4 mm and (*2*) fraction with the particle size 2.0–4.5 mm.

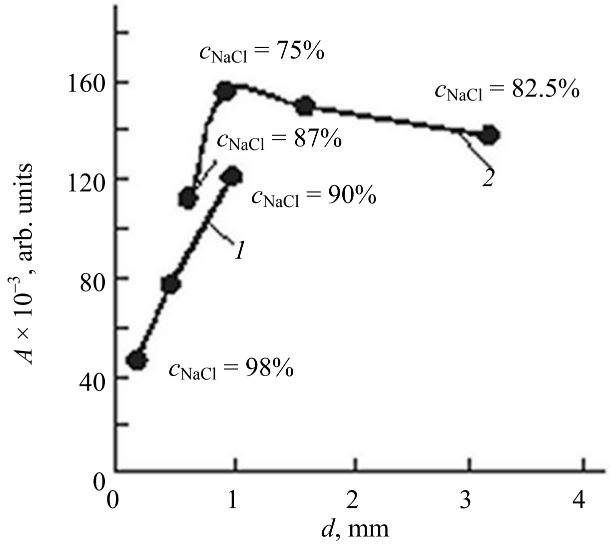

Fig. 4. Dependence of the activity (A) of a granular enzyme preparation on salt particle size (d) and concentration (c_{NaCl}). Binder: (*1*) PVP and (*2*) starch glue.

product, and intensity of agitation of the moving granular material.

As the starting MCE powder, primarily due to the presence of salt, had a polydisperse composition (particle size 0.05–0.63 mm, the resulting granules were, too, varied in size.

RESULTS AND DISCUSSION

The fact that the amount of salt affects the activity of granular MCE preparations at various granulometric compositions of different fractions in a complicated fashion is explained by salt and enzyme redistribution inside granules in the course of the granulation process (Fig. 3).

As seen from curve *1* (Fig. 3), the activity of small granules (0.05–1.4 mm) reaches 170000 arb. units at decreasing salt content and falls to 60000 arb. units as the salt content increases. The same trends are characteristic of the fraction with the particles size 2–4.5 mm (curve *2*).

The study of the influence of the type of binder showed that PVP allows the starting power to be granulated to smaller granules than starch glue (Fig. 4). Thus, with the particle diameter in the starting powder of 0.05–0.65 mm, the resulting granule size is 0.2–1.0 mm (Fig. 4, curve *1*). With starch glue, larger granules (0.6–3.2 mm) are obtained (Fig. 4, curve *2*). Increasing salt content (c_{NaCl}) decreases the activity of the granular preparation, whatever the binder.

Further we studied the MCE granulation processes in different apparatuses. Therewith, depending on the mode of binder delivery and the applied equipment, the following mechanisms of granule formation are realized. If granulation if performed in a drum apparatus, and binder is dispersed as large droplets, polydisperse agglomerates are formed. If this mechanism is operative, the granulation apparatus is additionally loaded with milling balls to prevent too fast growth of wet granules. In this case, the process parameters that are varied by a preset schedule program provide mechanical activation and grinding of large granules to a commercial-scale size.

If binder is delivered as a solution or fine emulsion, granulation occurs by gradual deposition of thin film on particle surface and is accompanied by rapid hardening. Because enzyme particles are few in number and small in size, they agglomerate on the surface of large salt particles (Fig. 5).

An optimal weight ratio of balls and product particles ensures that granules grow to the target size.

Generally, the mean diameter (D_{mean}) of granules produced from MCE powders can be calculated from the following equation:

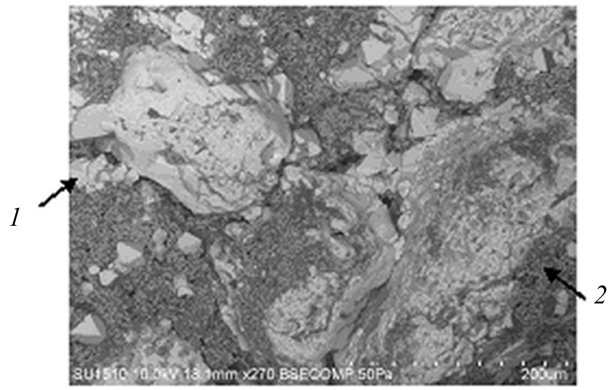

Fig. 5. Structural model of an MCE granule: (*1*) NaCl and (*2*) enzyme particles.

$$D_{\mathrm{mean}} = D_0 e^{\left[m\left(\frac{P}{1 - R_{\mathrm{int}}} R_{\mathrm{int}} \frac{D_0}{D_{\mathrm{r}}}\right) - P_{\mathrm{init}} + U_{\mathrm{r}} R_{\mathrm{int}}\right]^n}, \quad (1)$$

where D_0 is the mean particle diameter in the starting mixture; D_{r}, mean particle size in the internal retour; P, current moisture content in the granules; $R_{\mathrm{int}} = G_{\mathrm{start}}/G_{\mathrm{ball}}$, coefficient of internal retour (G_{start} is the mass of the granular material and G_{ball}, mass of balls); P_{init}, initial moisture content of the starting powder; U_{r}, moisture content of internal retour; and m and n, coefficients relating to the properties of the material and binder. For the media in focus, $m = 237$ and $n = 1.94$ at $U_{\mathrm{r}} = 2–10$; $R_{\mathrm{int}} = 0.33–0.66$.

The final diameter of granules also depends of the delivery time of binder and the residence time of material in the drum.

The experimental results allowed us to design of an industrial production line for granular MCEs. The perspective equipment in this line is a multifunctional vibratory mixer with a toroidal working bearing–set vertical shaft with eccentric masses at both ends. The static moments of the eccentric masses and the phase angle between them are controlled [4]. The rate speed of the shaft of the vibration agitator can be controlled by means of a frequency converter. The vibration pulse created in the toroidal chamber imparts spiral motion to material particles, and this ensures efficient mixing.

The process of mixing and grinding was studied in a SmV-0.005 mixer using a model mixture containing 85% of NaCl and 15% of starch (corresponding to the MCE formulation). The following optimal operation parameters of the vibratory mixer were determined: vertical and horizontal vibration amplitudes at the outer diameter of the chamber 3.6 and 2.3 mm, respectively; vibration frequency 24 Hz; phase angle of eccentric masses 30°; loading factor of the chamber 0.8.

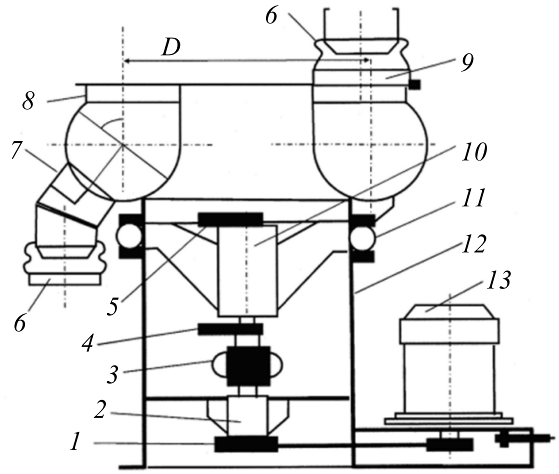

Fig. 6. Schematics of an SmV vibratory mixer: (*1*) vee-belt drive; (*2*) intermediate support; (*3*) elastic joint; (*4, 5*) top and bottom eccentric masses; (*6*) sealer; (*7*) discharge valve; (*8*) chamber; (*9*) feed fitting; (*10*) vibration agitator; (*11*) elastic elements; (*12*) frame; and (*13*) electric motor.

It was found that the required homogeneity factor as measured by the key component (starch) is reached within 30 min.

To assess the use of SmV-0.005 as a grinder, steel balls were loaded into the working chamber. After vibration treatment of the mixture with balls for 40 min the specific particle surface area increased from 54 to 623 cm^2/g and the equivalent diameter changed from 196 to 111 μm. For enzyme preparations, the equivalent diameter of (D_{mean}) of the mixture after grinding as a function of treatment time (t) is given by the formula:

$$D_{\mathrm{mean}} = D_0 e^{(t^2 - 100t) \times 10^{-4}}, \quad (2)$$

where D_0 is the mean particle diameter in the starting mixture.

Our research allowed us to determine the granulation process parameters. A batch of a granular MCE preparation with the activity of about 100000 arb. units was obtained. The obtained granules do not form dust, are not adhesive, and their mechanical characteristics (strength, friability, resistance to caking) are sufficient to allow their transportation and dosing and meet customer's requirements. The granular MCE preparation was tested in cheese production at "Staritskii Syr" OAO (Tver Region) and "Yanaul'skii maslosyrkombinat" AO (Bashkortostan).

The cheeses produced using the granular enzyme preparation meet the "Hard Rennet Cheeses" State Standard 7616-85.

Certain MCEs are better produced using a disc granulator. The experiments were performed in a disc granulator 0.35 m in diameter. As binders we used varied-concentration solutions of PVA and PVP with different molecular weights. Qualitative analysis of the obtained granules by principal parameters (milk-clotting activity, salt concentration, insoluble residue) was performed according to the Sectoral Standard 10288-2001.

The technology of the production of powdered MCE of standard activity envisions mixing of a dry enzyme with NaCl. The standard activity of commercial rennet is not less than 100000 arb. units, and the activity of a pure enzyme concentrate is sometimes an order-of-magnitude higher. Thus, to bring the enzyme activity to a standard value, the fraction of salt in the finished product should be 80–90%. Salt and enzyme particles much differ from each other in size, and this leads to MCE segregation on storage. In this connection we developed an engineering process for the MZSF, which includes the following stages:

– grinding NaCl to a preset size or a finished batch of MCE powder to a present degree of mechanical activation;

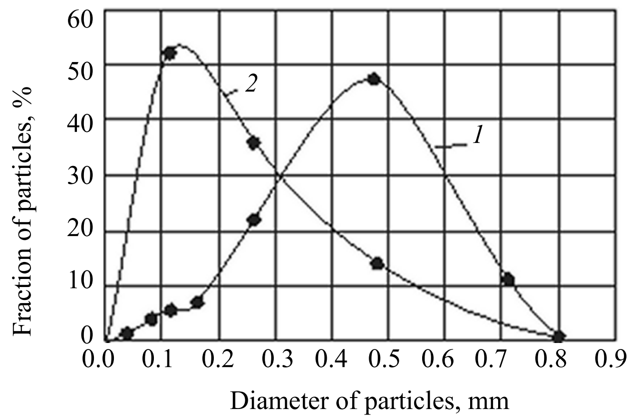

Fig. 7. Granulometric composition of commercial Altazim (*1*) before and (*2*) after grinding in a drum mill (τ_{gr} = 45 min; n = 80 rpm).

– disc granulation;

– drying wet granules in mild conditions at temperatures not higher than 50°C and classification of the product to pick up the commercial fraction.

The effect of the granulometric composition on the mechanism of granulation was studied with different types of MCE preparations. Figures 7 and 8 show the compositions of the MCE preparations produced at the MZSF: Altazim, chicken pepsin, and SG. Curves *1* in these figures show the granulometric composition of commercial MCEs and curves *2*, the compositions of the same samples ground in a ball mill. As seen from Fig. 7, the commercial product has a bimodal particle size distribution and the ground product, unimodal. The particle size of salt in commercial Altazim is 0.1–

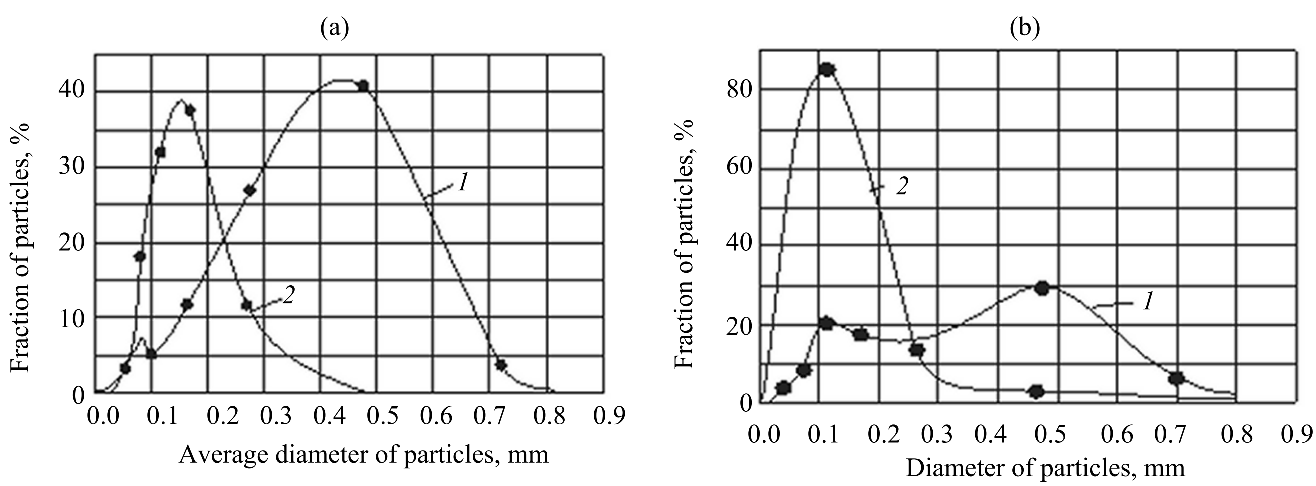

Fig. 8. Fractional composition of (a) chicken pepsin and (b) SG-50 enzyme preparation (*1*) before and (*2*) after grinding.

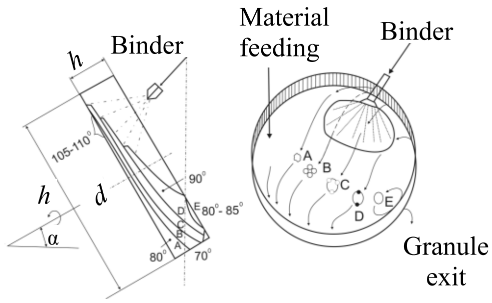

Fig. 9. Schematics of the trajectories of the material over the disc and zones of binder feed and pelletizing: (A) zone of preparation to nuleus formation (powder); (B) zone of nucleus formation; (C) zone of granule growth; (D) zones of pelletizing and hardening; (E) granule exit zone; (*h*) height of the disc rim; (α) disc slope angle; (*d*) disc diameter; and (*n*) disc rotation speed.

0.8 mm and that of enzyme is 0.01–0.2 mm. As seen in curve *1*, the right-hand peak relates exclusively to large salt particles and the left-hand peak, to a mixture of enzyme with smaller salt particles.

It was found that in the course of granulation of such mixtures enzyme particles and a small amount of fine salt form agglomerates which later form a scaffold for deposition of enzyme particles to form a granule. Large salt particles are either scarcely incorporated into granules or form large agglomerates with each other.

As judged from the revealed mechanism of granulation, to avoid a "breakthrough" of large salt particles and obtain structurally uniform granules, salt should be ground to an optimal size commensurate with the size of enzyme particles. The optimal mean equivalent diameter (d_{equiv}) of salt particles to be mixed with enzyme particles was estimated at 0.2 mm.

It was established that salt is better to grind in a mixture with enzyme before the granulation stage. High dynamic loads lead to mechanical activation of the enzyme itself and increase its specific surface area. Grinding and mechanical activation were performed in a drum mill with ceramic balls at the product/ball loading ratios φ of 04–0.6.

Granulation was performed in a pilot disc granulator operated in the batch mode. The starting powdered preparation after grinding was loaded onto the disk mounted at a preset angle and rotated at a certain speed. Specific doses of binder were sprayed at certain time intervals over the bed by means of a pneumatic nozzle. The total amount of binder was varied from 5 to 13 wt %. The granulation time was varied from 10 to 30 min. Powder particles freely moved over the bottom and rose to the highest mark of the disc, thus occupying the maximum possible pelletizing area. Fine binder dispersion was sprayed at a large cone angle. The trajectories of the materials over the disc and zones of powder feed, agglomeration, and granule growth and rolling are shown in Fig. 9.

The mechanism of continuous granulation was established for a wide range of MCE preparations. The powder fed to zone A represents a polydisperse, structureless, and flowable, under dynamic conditions, system. In spray zone B, capillary bridges are formed in the sites where the particles contact with each other. Groups of such particles form loose aggregates. When these aggregates, moving upwards in the rotating disc, again come to spray zone C, they get larger due to adhesive layering of enzyme particles over their surface. As this takes place, a coagulation structure is being finished to form inside granules. In the course of spraying the powdered mixture of MCE and NaCl

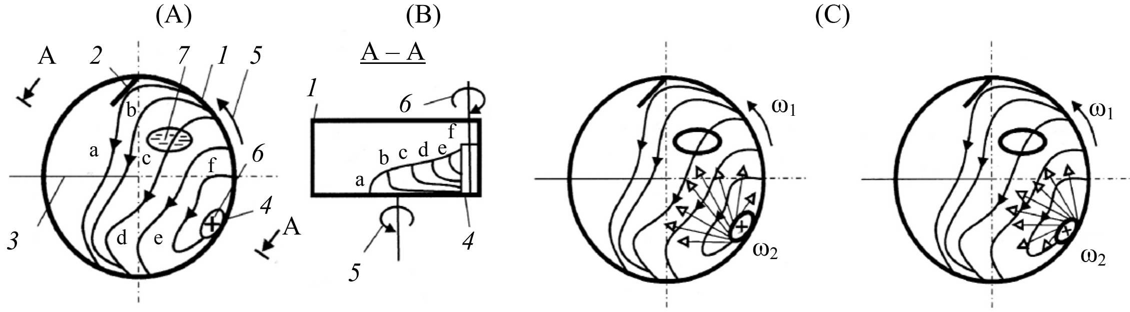

Fig. 10. (A) Schematics of modernized disc granulator and (B, C) trajectory the material in it: (*1*) disc; (*2*) mote knife, (*3*) bottom knife, (*4*) activator capture zone, (*5*) disc rotation direction, (*6*) activator rotation direction, (*7*) binder spray zone, (a, f) trajectories of powder and large granules, and (b–e) trajectories of growing granules; disk and activator move in (B) opposite directions and (C) the same direction.

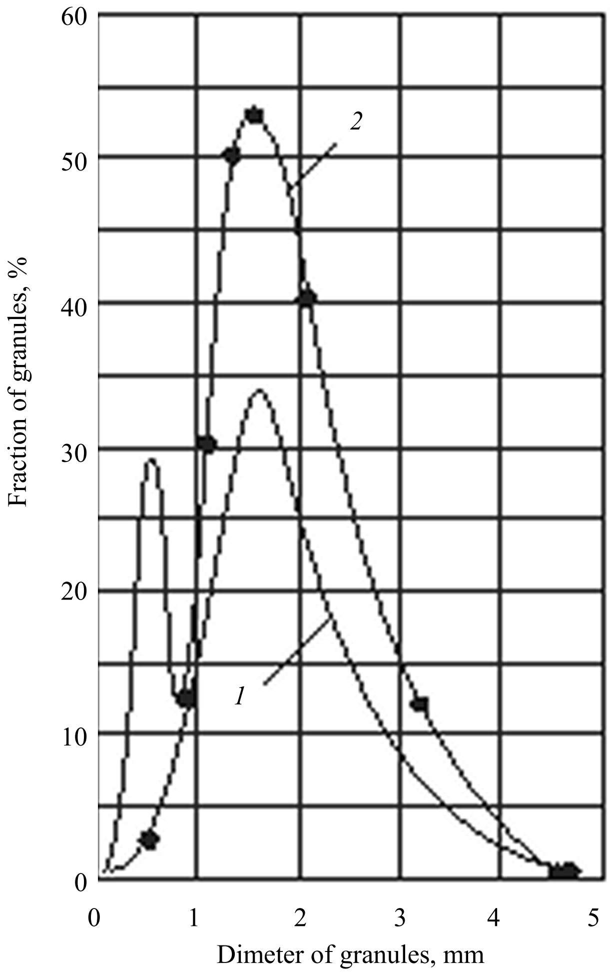

Fig. 11. Granulometric composition of granular Altazim (activity 107000 arb. units; binder 3% PVA; c_{binder} = 12%; τ_{gr} = 15 min) made from (*1*) unground and (*2*) ground Altazim preparation.

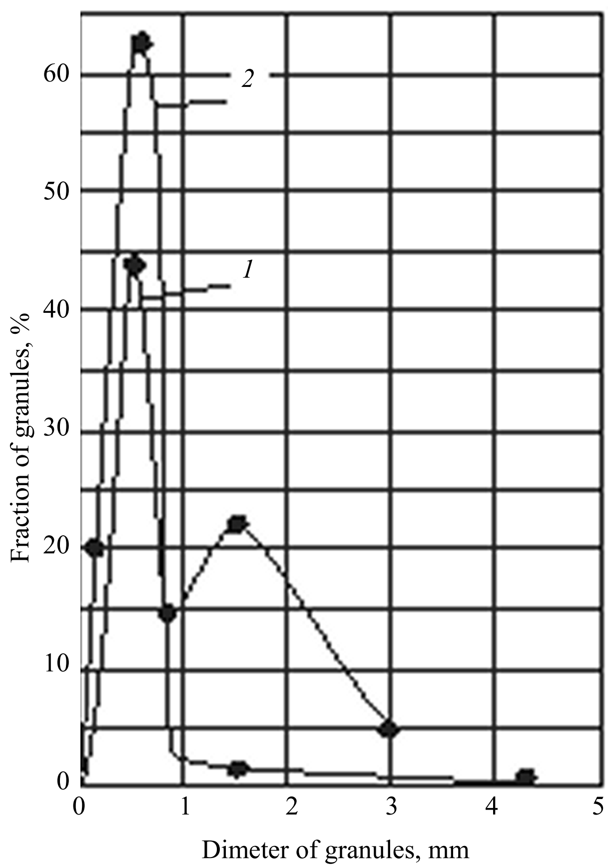

Fig. 12. Granulometric composition of granular KP (chicken pepsin) made from (*1*) unground preparation (A = 98000 arb. units; binder 1% PVA; c_{binder} = 12%; τ_{gr} = 15 min) and (*2*) preparation with ground salt (A = 250000 arb. units; binder 1% PVA; c_{binder} = 13%; τ_{gr} = 12 min).

acquires a certain plastic strength P_m which makes it possible to ball the aggregates in zone D. Therewith, due to the mechanically activated surface, granules entrap excess liquid in surface imperfections, structure defects, and pores The PVA and PVP contained in the binder induce its hardening, which strengthens the granules.

To enhance the dynamic effect of the disc on granule and ensure a more efficient use of the entire surface, mote knives 2 are mounted above and perpendicularly to the disc (Fig. 10). At high rotation speeds, particles collide with the knives, change trajectory (a), and tumble down the bed. When the knife is positioned in a different way (at an angle to the horizontal), particles form a thin cloud, fly for some time over the bed, and then fall down and tumble down to the discharge zone [5]. Large

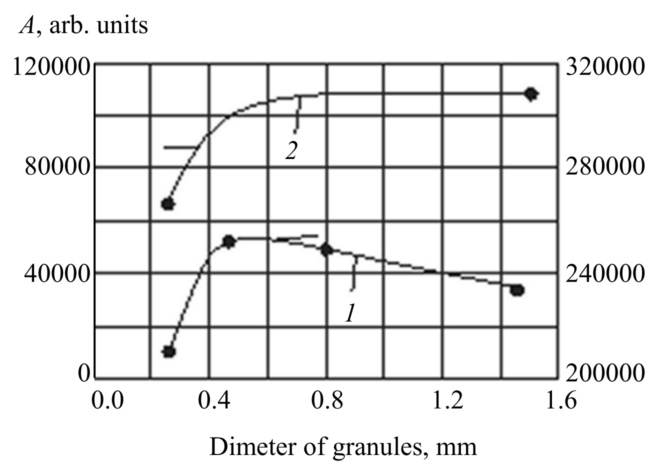

Fig. 13. Dependence of the milk-clotting activity (A) of granular MCE preparations on granule diameter (d_{gr}): (*1*) Altazim (A_{start} = 107000 arb. units; reground preparation, d_{equiv} = 0.13 mm) and (*2*) KP (A_{start} = 248000 arb. units).

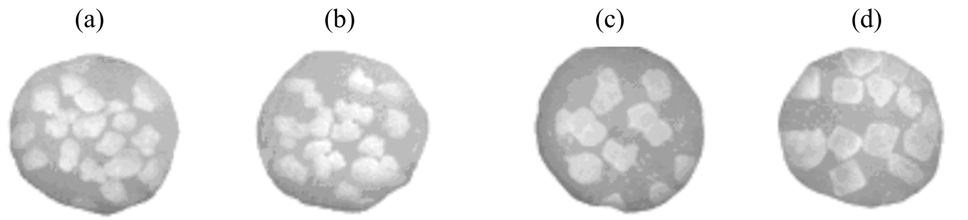

Fig. 14. Microscopic images of MCE preparations obtained by different technologies: (a, b) Altazim, disc pelletizing (MAMI, Russia); (c) crystallization (Chr. Hansen, Denmark), and (d) crystallization (Clerici, Italy).

granules concentrate and circulate in a zone from which they spontaneously discharge over the upper edge of the disc rim (in the case of continuous granulation) to proceed to drying and classification.

To reduce sticking of the powder to the disc surface, bottom knives 3 can also be used.

To prevent granule agglomeration, a vertical agitator with special-shape blades may be mounted in granule collectors. The use of such agitators provides additional dynamic treatment and a shorter and more active particle–particle contact, thereby preventing formation of especially large aggregates.

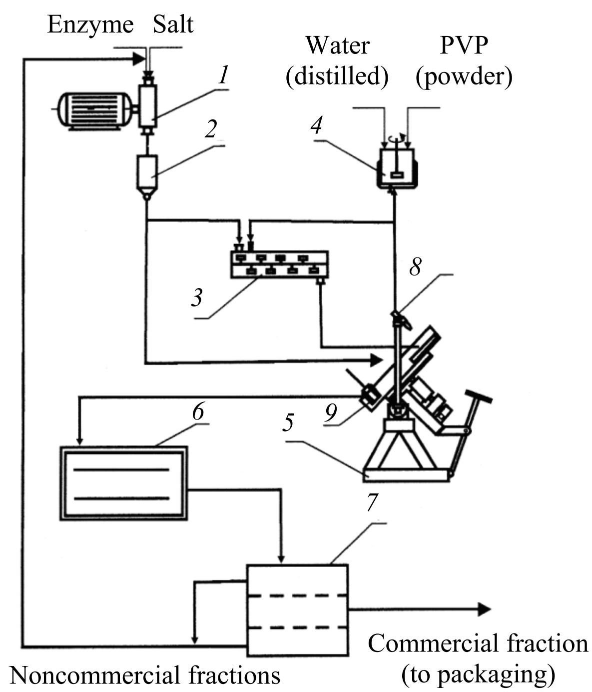

Fig. 15. Process scheme of granulation by disc pelletizing: (*1*) ball mill; (*2*) bunker; (*3*) pregranulator–mixer; (*4*) reactor mixer; (*5*) pelletizing disc; (*6*) dryer; (*7*) shaker; (*8*) pneumatic nozzle; and (*9*) frame mechanical activator.

In what follows we present the results of experiments on MCE granulation from activated preparations. When part of the binder has been sprayed onto a ground mixture on the disc, an internal retour is formed as an agglomerate of enzyme and salt particles. The powder is then deposited onto these granulation nuclei to form homogeneous spherical granules. The granulometric composition depends on the process parameters, primarily, quantity of the sprayed binder and spray dispersion, as well as pelletizing time. The yield of commercial preparation with d_p = 0.3–2.0 mm in our experiments was as high as 70–80 %, and the moisture content of the granules before drying was 12–13 %. The granulometric compositions of the products obtained from standard and mechanically activated Altazim and KP after drying at 50°C are shown in Figs. 11 and 12. As seen from the figures, the particle size distribution in the granules made from an unground powder is bimodal, whereas those made from a mechanically activated power have a normal size distribution.

It should be noted that KP was prepared from a concentrated powder with the milk-clotting activity *A* = 106000 arb. units and ground NaCl. The activity of the final mixture was 250000 arb. units. Analysis of the activity of Altazim and KP granules with different diameters revealed almost no size dependence of activity for commercial fractions of both preparations (Fig.13). The same was found to be true of the fractions containing large particles (d_{equiv} = 1.5–4.0 mm). We also revealed no NaCl redistribution in the commercial and noncommercial fractions, specifically, salt is uniformly distributed between all fractions.

We also studied the effect of process parameters, such as dispersion of the binder spray, amount of the product on the disc, physicochemical characteristics and purity of the MCE, and type of the drying equipment and drying regimes, on the quality of the final product.

When the degree of spray dispersion is low, the resulting granules have a bimodal size distribution with a high fraction of large particles (d_{equiv} = 2.5–5.0 mm).

It was found that if the performance of disc granulator was increased 1.5–2 times, the particle size distribution for granules 0.2–3.0 mm in diameter became bimodal. Therewith, no particles except for granules were found. The granules were irregularly shaped agglomerates comprising salt and enzyme. The noncommercial fraction comprising large salt particles with small enzyme particles on the surface was less than 15%. The moisture content of granules at the exit of the granulator (W_{fin}) was 12.5%, and the residual moisture content after drying (W_{gr}) was 2 %.

It was shown that the intensity of the disc granulation process is primarily dependent of whether the product contains granulation centers. If the material is fed irregularly, such centers are formed fairly slowly. In this connection, to stabilize the process, we introduced an additional, pregranulation stage involving mechanical activation and partial wetting in a frame mixer. According to this protocol, the mixture is first mechanically activated at 400 rpm, after which a binder (7% solution of PVP) is fed in a quantity of 10 wt %; these conditions trigger the formation of granulation centers. The mixture is then transferred into the granulator, where after the moisture content has reached the required level (12–13 %), the pelletizing process is initiated. The resulting granules have a spherical shape, and the yield of the 0.8–3.0-mm fraction was about 85%.

We also studied the effect of the tested granulation technologies on the strength and caking ability of MCE granules. It was found that the strength of the granules obtained with pregranulation and mechanical activation is higher (0.5–1.4 MPa). The strength of the granules obtained in one stage varies from 0.3 to 0.8 MPa. The granules prepared by both technologies and having the above strength characteristics do not show tendency for caking when stored and do not destroy and give up dust when transported. Testing granules with different strength characteristics for solubility showed that they all dissolve at the same rate.

One more quite important characteristic of MCE preparations is their ability to retain activity on storage. It a powdered product has not been consumed within a few days after opening its packaging and stored in inappropriate conditions, it absorbs moisture, cakes, and loses activity. To go around this effect, consumers have to use more MCE preparation, say, to use more than 50 g of the material per 2 tons of milk.

Activity study of granular MCE preparations was performed at the manufacturing and testing laboratory of the MZSF, using pilot batches of Altazim, chicken pepsin, and SG-50. Activity was tested for 6 months by a standard procedure. It was found that all the MCE preparations with d_{equiv} = 0.3–1.0 mm and d_{equiv} = 1–2 mm did not lose activity within 5 months. This finding was explained by the fact that mechanical activation increases the specific surface area of chymosin which activates the process for a long time. It should be noted that granular preparations were stored in open packages, they did not absorb moisture, did not cake, and remained flowable.

The microscopic images of the obtained granules are presented in Fig. 14. As seen, the granules obtained by disc pelletizing have a near-spherical shape. Crystal-shaped granules can also be prepared.

The results of the research allowed us to develop a process design (Fig. 15).

The process occurs in the following way. The starting MCE and salt are fed to ball mill 1. After grinding the mixture with the desired granulometric composition is transferred to bunker 2 equipped with a batcher. Part of the powdered product is fed to pregranulator–mixer 3 to obtain wet agglomerates with W_{start} = 10% (retour). This retour is fed onto the disc together with the other part of the powdered enzyme. In the presence of a binder (7% solution of PVP), granules are formed and pelletized into spherical particles. In the case of continuous granulation, the design of the disc allows granules to be discharged once they have reached a certain size. A wet granular material is dried and conveyed to shaker 7, where it is classified and commercial (0.5–2.0 mm) and noncommercial fractions are separated. The comercial fraction comes to packaging unit 8, whereas non-commercial fractions (small and large granules) are returned to mill 1.

CONCLUSIONS

Different mechanisms of granulation of MCE preparations of different types are revealed.

An influence of mechanical activation on the quality of the end product is established.

Process lines with different equipment are tested and recommendations on process parameters are proposed.

Process lines for Shtykov & Co. (Moscow) and the Moscow Rennet-Making Plant are designed.

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