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PARTICLE DISTRIBUTION IN GRANULAR SYNBIOTIC PRODUCTS

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Abstract

This article presents an analysis of the particle distribution of granular lyophilized synbiotic products. The main purpose of the present study is to find a relationship between the particle size distribution and the varying component in the composition of the products. Particle size measurements were performed with an ANALYSETTE 22 NanoTec plus analyzer. The results obtained we then subjected to statistical analysis to study the distribution properties of functional food samples. It has been found that for the preparation of granules with a low degree of dusting it is optimal to use 10% sucrose, 1% locus bean gum and 1% fructooligosaccharides (FOS) for processing the lyophilized mixture. Determining the distribution of granules is directly dependent on their composition and purpose. The natural composition of the granulated "LIO - Milk strawberry" and "LIO - Milk chokeberry" guarantees exceptional uniformity in use, as well as prevention of possible oxidation.

Key words*: granular synbiotic product, locus bean gum, particle size, distribution, fructooligosaccharides.*

INTRODUCTION

In modern everyday life, human society is showing increasing interest in natural remedies that have a positive impact on human health, in the form of probiotics, prebiotics, functional foods, biological stimulants and more. This predisposes to constantly implement and develop new products that meet modern norms and requirements for healthy eating (Mark-Herbert, 2004; Topolska et al., 2021; Ivanov et al., 2021; Terziyska et al., 2015; Valchkov et al., 2016).

Fermented probiotic products, containing live microorganisms, occupy a particularly important place among functional foods because they affect positive on the intestinal microflora, have a health effect greater than that of the main food and affect favorable of human health (Piano et al., 2006; Oliveira et al., 2016; Marco et al., 2021; Zhao et al., 2019; Nacheva et al., 2015).

Probiotics must meet certain conditions in order to be included in some healthy functional product. To this end, it must be clinically established that they show some positive health benefits. For the healthy efects of some probiotic or synbiotic product, it is necessary to have good information to consumers, effective marketing strategies and, above all, the product itself to be of good quality, meeting the expectations of consumers. The most stable and clinically tested bacteria with probiotic properties are available on the commercial network (Zhao et al., 2019; Marinova et al., 2019; Binda et al., 2020).

Kefir is a probiotic dairy product, the result of two fermentations with kefir grains - lactic acid and alcohol. Kefir is a probiotic dairy product, the result of two fermentations with kefir grains - lactic acid and alcohol. Kefir grains contain a mixture of lactose-fermenting lactic acid bacteria - *Lactobacillus*, *Leuconostoc*, *Lactococcus*, acetic acid bacteria and yeast linked in a clustered immobilized system of casein and polysaccharide matrix (Garrote et al., 2010; Seo et al., 2018; Nacheva et al., 2018).

The preservation of viable probiotic strains and biologically active components of a given commercial product during storage are criteria for its healthy characteristics (Rathnayaka, 2013; Marinova et al., 2019).

The technology of freeze-drying (lyophilization) is an established method for obtaining stable food products. The process includes three stages: freezing, primary drying (sublimetion of ice crystals) and secondary drying (desorption of residual moisture). Sublimation drying is used for the preservation and storage of microorganisms, starter cultures, probiotic products and has wide practical application (Lodato et al., 1999; Nowak and Jakubczyk, 2020).

Foods, after undergoing a process of lyophilization, have fully preserved nutritional and biological value, taste, aroma, color, can be preserved for a long period of time, can be avoid difficulties with their transportation. For this reason, more and more manufacturing companies producing functional foods, including probiotics, use lyophilization as the main processing method to obtain high quality products with a long shelf life (Nowak and Jakubczyk, 2020; Bhatta et al., 2020).

This scientific publication presents an analysis of the particle distribution in variations of new granular functional products by means of the ANALYSETTE 22 NanoTecplus laser granulomer. The main purpose of the research done is to determine the influence of a varying component in the composition of the products on the spectrum of distribution of the obtained product granules.

MATERIALS AND METHODS

Experimental setup

ANALYSETTE 22 NanoTec plus measures nanoparticles in an extremely wide measurement range (0.01-2000 pm). The extremely large measuring range of the ANALYSETTE 22 NanoTec plus with a lower measuring limit of approximately 0.01 pm is due to the Triple-laser technology for forward and backward scattering. ANALYSETTE 22 NanoTec plus always uses all 57 measuring channels of the detector. By combining the different measuring positions, it is possible to perform measurements with up to 165 effective channels, which guarantee a particularly high resolution and sensitivity.

ANALYSETTE 22 NanoTec plus is ideal for efficient and reliable particle size distribution in production and quality control, and also in research and development. The device is shown in Figure 1.

Figure 1. ANALYSETTE 22 NanoTec plus

During the experiments, two new lyophilized kefir-based products were evaluated. The technology for obtaining and the composition of the new products are presented in a report (Solakov et al., 2020).

For each of the two synbiotic products "LIO - Milk strawberry" and "LIO - Milk chokeberry" 4 variants of granulating components have been developed.

In order to establish the optimal composition of the granulating mixtures, in which minimal dusting is observed, it is necessary to prepare variants of mixtures with different types and concentrations of substances.

For each of the two synbiotic products "LIO - Milk strawberry" and "LIO - Milk chokeberry" 4 variants of granulating components have been developed.

Variant 1 - lyophilized product with added 30% sucrose.

Variant 2 - lyophilized product with added 10% sucrose and 2% fructooligosaccharides (FOS).

Variant 3 - lyophilized product with added 10% sucrose and 2% locust bean gum.

Variant 4 - lyophilized product with added 10% sucrose, 1% locust bean gum and 1% FOS.

Sucrose solution was used as a component in the technological process of granulation of the product. In order to reduce the sucrose content and in order to preserve the dietary and health qualities of the products, polysaccharide solutions are added in 3 of the variants.

The polysaccharides used have functional properties, which makes them suitable for inclusion in the composition of the granulating mixture, namely: high chemical and biological resistance; hydrophilicity; cryoprotective properties; good permeability for the included material; large relative surface area and porosity after lyophilization; possibility to obtain convenient from a technological and practical point of view forms - powder, granules, tablet, etc.

They are soluble in water, form colloidal solutions, which are influenced by their molecular weight, concentration, temperature and pH, etc. The degree of their hydration depends on the distribution of the particles in the medium, the rate of homogenization, the composition of the hydration medium and the particle size.

The process of production of granular synbiotic products takes place in the following stages:

- Grinding of the obtained lyophilized products;
- Addition of the corresponding granulating solutions and homogenization;
- Granulation of the obtained products in an oscillating granulator "ERWEKA" with a sieve size of 1 mm.

The resulting granulate is dried for 24 hours at 30-40°C, after which 1-3% talc is added to it for better passability of the granules through the particle distribution apparatus.

RESULTS AND DISCUSSIONS

All variant samples were tested with Analysette 22 NanoTec plus. The device works on the basis of laser diffraction and determines the volume, ie. what percentage of the full volume of the sample is filled with particles smaller than x μm.

The results obtained for the three samples are grouped into 102 channels from 0 to 2000 μm.

For particle size distribution (PSD) the most important values are mean (average value - usually average - for example the average of 2.7 and 9 will be 2 + 7 + 9 divided by three, ie - 6), median (the median - the value of the average of the whole aggregate, for example from 7 measured values the average will be the fourth 1, 2, 3, 4, 5, 6, 7) and mode (mode - the most common value in the whole aggregate, for example in the series of numbers 2, 3, 4, 5, 4, 7, 4 the most common is 4).

In symmetric distributions, all these values are equivalent: mean = median = mode. Accordingly, in asymmetric distributions mean, median and mode will have three different values.

The best way to determine the mean volume is by using a histogram (a graphical method of representing the frequency distribution) showing the lower and upper limit of the respective channel and what percentage of the full volume of the sample falls into it.

Some of the most commonly used varieties of the mean volume are the arithmetic mean $\overline{D}10$, the mean diameter $\overline{D}32$, which represents ratio between volume and surface mean and mean particle diameter in volume $\overline{D}43$ (also called the DeBroukere mean), which was chosen for evaluation in the present researches.

Laser diffraction provides information on a quantitative basis so that the mean size can be used to determine the center point, although median is more commonly used than mean when using this technique. The equation for determining the average size is:

$$D[4,3] = \frac{\sum_{i=1}^{n} D_i^4 v_i}{\sum_{i=1}^{n} D_i^3 v_i} \quad (1)$$

where, the value of Di for each channel is the geometric mean value, the square root of the high to small diameters. A geometric mean is a type of mean that shows a major trend or the typical value of a set of numbers using the product of their values (as opposed to the arithmetic mean value that uses their sum). The geometric mean is defined as the n^{th} root of the product of n numbers, ie, for a set of numbers $\{x_i\}_{i=1}^{N}$,, the geometric mean value is determined by the following way:

$$\left(\prod_{i=1}^{N} x_i \right)^{1/N}$$

Thus, for the numerator in equation (1), the geometric value of Di is the product of the fourth power x percent in this channel, summed for all channels. The value of the denominator is the product of the third power of Di x percent in this channel, summed for all channels.

Median values are defined as a value (in microns) at which half of the particles are above this point and the other half is below it. The median for particle size distributions is called D50. Dv50 (or Dv0.5) is the median for volume distribution, DN50 is used for number of distributions and Ds50 is used for surface distributions.

Since the main result of laser diffraction is volume distribution, by default the cited D50 is the median of the volume and D50 usually refers to DV50. This value is one of the easiest statistics to understand, and also one of the most significant for the particle size distribution.

Mode is visualized as the highest peak in the chart distribution. The mode most often expresses the particle size (or size range) represented in the distribution. Less attention is paid to determining whether the value is based on the volume, area or number. The mode is

not so often used, but can be descriptive, in particular, if there is more than one peak in the distribution. The modes are useful for describing the midpoint of the various peaks.
Most instruments are used to measure the distribution of the size of particles, which means an interest in the width or breadth of the distribution. In the field of statistics, some calculations are used to describe the width of distributions, and these calculations are sometimes used in the field of particle characterization. The most common calculations are standard deviation and variance. The standard deviation (SD) is the preferred value in our study area.
After the introduction of "model independent" algorithms, many scientists working in the field of particles began to use various calculations to describe the width of the distribution. One of the most common values used for laser diffraction results is the duration of the step (span), defined in the equation below:

$$Span = \frac{D_{v0.9} - D_{v0.1}}{D_{v0.5}} \qquad (2)$$

where: Dv0.5 or D50 is the median defined as the diameter at which half of the particles are below this value. Similarly, 90% of the distribution lies below D90, and 10% of the particles are lower than D10.
According to the results obtained from ANALYSETTE 22 NanoTec plus, it is depicts graphically on the histogram and presents a cumulative frequency distribution in the variant samples.
The results of the granulometric analysis of the three granule variants are presented in Figures 2-9. Analysis of the presented histograms shows that the frequency of distribution is not symmetrical. In Variant 1, consisting of a granular lyophilized product (control) and sucrose powder, the resulting histogram shows that the largest volume of particles have a size between 250 and 1100 µm, and the volumes of particles with size bigger than 1500 µm are practically empty, i.e. there are no particles of this size (Figures 2 and 6).
The results of samples of granular product with FOS and locust bean gum were analyzed in the same way. The results for variants from 2nd to 4th for both products are similar.

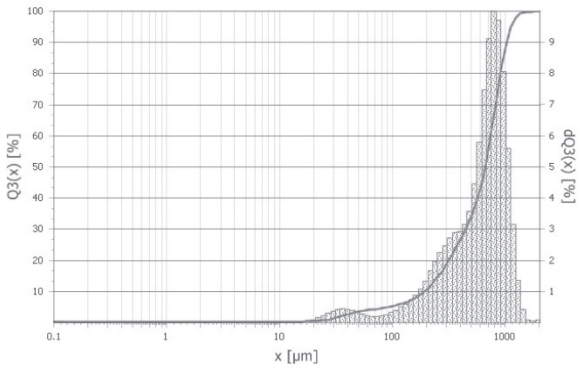

Figure 2. Histogram and cumulative frequency in Variant 1 of granular LIO - Milk strawberry

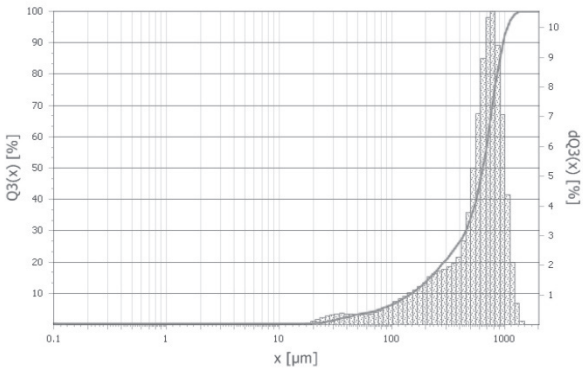

Figure 3. Histogram and cumulative frequency in Variant 2 of granular LIO - Milk strawberry

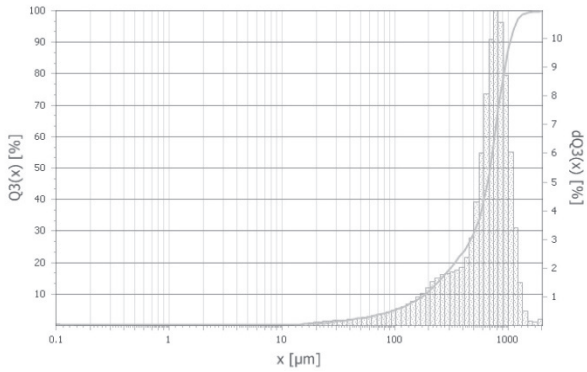

Figure 4. Histogram and cumulative frequency in Variant 3 of granular LIO - Milk strawberry

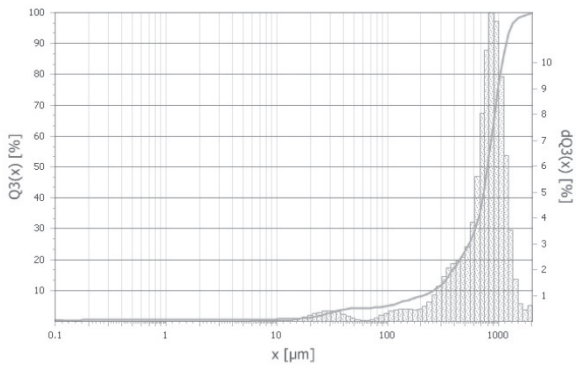

Figure 5. Histogram and cumulative frequency in Variant 4 of granular LIO - Milk strawberry

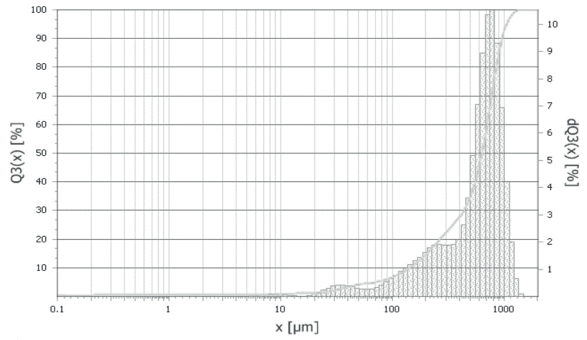

Figure 6. Histogram and cumulative frequency in Variant 1 of granular LIO - Milk chokeberry

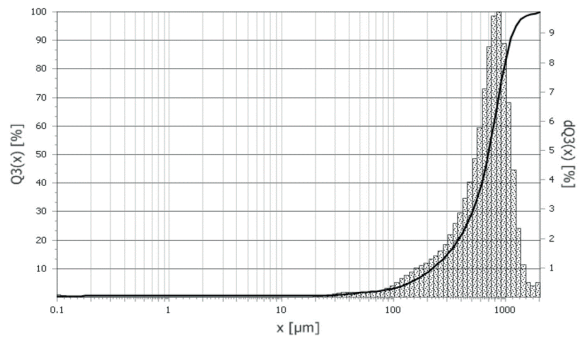

Figure 7. Histogram and cumulative frequency in Variant 2 of granular LIO - Milk chokeberry

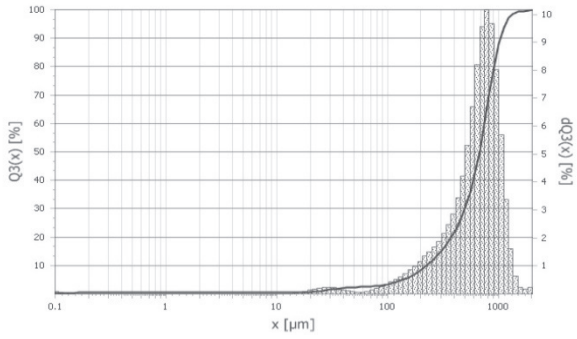

Figure 8. Histogram and cumulative frequency in Variant 3 of granular LIO - Milk chokeberry

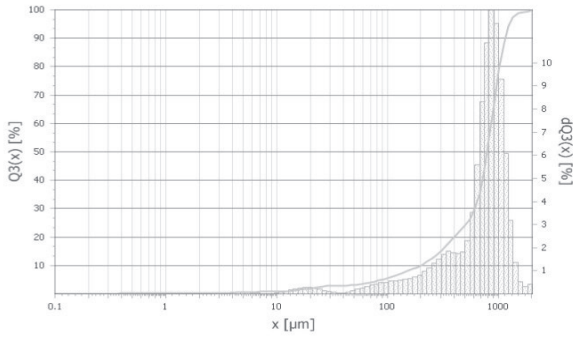

Figure 9. Histogram and cumulative frequency in Variant 4 of granular LIO - Milk chokeberry

In Variant 2, a granular product with FOS and sucrose powder, the frequency of distribution is asymmetric (Figures 3 and 7). Particle volumes with size between 0 and 20 µm and size larger than 1500 µm are very small (closer to zero), which means that they are practically not represented. The largest number of particles is concentrated in the range between 340 and 1200 µm.

In Variant 3 - granular product with locust bean gum and sucrose powder, the frequency distribution is again not symmetrical (Figures 4 and 8). Volumes of particles with size between 0 and 20 µm and size larger than 1500 µm are very small (closer to zero), which means that in practice they do not exist. The largest number of particles is concentrated in the range between 300 and 1100 µm.

In Variant 4, a combination of FOS and locust bean gum was used in the granulation solution. The particle volumes are concentrated in the range between 300 and 1200 µm (Figures 5 and 9).

The differences in the particle distribution at the different granule variants are significant. As can be seen from the histograms at variants 2, 3 and 4, there is less scattering of the sample data. These results are confirmed by statistical processing of the experimental data (Tables 1 and 2).

Table 1. Statistical parameters of the variant samples granulated product LIO - Milk strawberry

Product LIO - Milk strawberry	D [43] (µm)	Mode (µm)	Span
Variant 1	600.5	750.17	1.28
Variant 2	628.2	800.52	1.26
Variant 3	657.2	800.52	1.23
Variant 4	762.0	882.46	1.13

Table 2. Statistical parameters of the variant samples granulated product LIO - Milk chokeberry

Product LIO - Milk chokeberry	D [43] (µm)	Mode (µm)	Span
Variant 1	591.65	750.17	1.29
Variant 2	686.3	826.95	1.26
Variant 3	658.3	800.52	1.21
Variant 4	741.6	802.46	1.19

In variants 2, 3 and 4, the results obtained for the mean volume diameter (D [43]) and Mode are higher than the controls. An important

indicator is and the width of the distribution (Span), as it gives grounds to assess the homogeneity of the granules and their distribution by fraction of different sizes. Lower values correspond to less scattering of particles by size and volume. Lower results were calculated for Variants 2, 3 and 4. According to this indicator at Variant 4 of the two products the highest degree of homogeneity of the granules is reported.

The obtained experimental data show a deviation in the particle distribution of the studied products. As expected, the change in the composition of the product leads to a change in the distribution caused by the different physically characteristics of the ingredients of the product. The inclusion of locust bean gum and FOS to the granules leads to stabilization of the structure and increase of the number of particles concentrated in the range between 300 and 1200 μm.

Granules with only added sucrose can be more easily crushed and dusted during transport or due to the weight of the product itself. This adversely affects the dissolution rate, dosing accuracy and commercial appearance of the product.

As a result of the experiments performed and after analysis of the obtained data, we found that for the preparation of granules with a low degree of dusting it is optimal to use 10% sucrose, 1% locust bean gum and 1% FOS for processing the lyophilized mixture. This will achieve optimization of the technological process for the production of synbiotic products "LIO - Milk strawberry" and "LIO - Milk chokeberry".

CONCLUSIONS

The granulation technology allows to obtain homogeneous in size particles, which improves the quality of solubility and increases the accuracy in dosing the amount of product. Determining the distribution of granules is directly dependent on their composition and purpose.

The natural composition of the granulated "LIO - Milk strawberry" and "LIO - Milk chokeberry" guarantees exceptional uniformity in use, as well as prevention of possible oxidation. The developed synbiotic concentrates are characterized by pronounced probiotic activity and high biological value, which defines them as products for prophylactic and therapeutic nutrition.

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