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Determination of particle size distribution and analysis of a natural food supplement on pectin base

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Abstract : The pectin is among the most studied soluble dietary fiber with cholesterol lowering properties. The major difficulty in its use is the slow dissolution and the lumps formation when placed in water. The main objective of this research is through technological methods to achieve a more rapid and complete dissolution of high-esterified citrus pectin in water. The method used is wet granulation with different granulation liquids (sucrose solution, distilled water and water-ethanol mixtures) and the resulting variant granules are tested for particle size distribution, solubility and hydration rate.
Granulation of high-methoxyl citrus pectin improves its dissolution in water in comparison with the powder pectin. Also, the hydration rate at the 300th s is greater than 90% with the granulated pectin and only 54% with non-granulated. Among the obtained five variants of granules, the best results are observed in the variant, granulated with 40% (v/v) ethanol. The analysis of the size and distribution of the particles reports the least scattering of the results during the variants granulated with 25% and 40% ethanol, which corresponds to the highest degree of uniformity of the granules.

Keywords - *dissolution, granulation, particle size distribution, pectin*

I. INTRODUCTION

Reduction of serum levels of LDL cholesterol significantly lowered the risk of developing cardiovascular disease (CVD). The main dietary approach is reducing the consumption of saturated fats. However, the importance of diet and other approaches, such as increasing the daily intake of water soluble dietary fiber is increasingly recognized. Viscous dietary fiber generally lead to mild or moderate reduction of total cholesterol (TC) in humans. It is estimated that with every additional gram of water soluble fiber in your diet, the total serum cholesterol and LDL decreases by 0.028-mmol/l and-0.029 mmol/l, respectively [1].
The pectin is among the most studied soluble dietary fiber with cholesterol lowering properties. Several studies with animals [2] and clinical studies of hypercholesterolemic persons confirmed a significant reduction in the total serum cholesterol and LDL cholesterol with regular consumption of apple or citrus pectin [1];[3]. There is a need for a daily intake of at least 6 g pectin in order to achieve a significant effect of reduction of cholesterol. Quantities of less than 6 g/day are not effective [4].

With the term pectin are referenced a group of complex polysaccharides, which constitute approximately one third of the dry weight of the cell walls in higher plants [5]. The pectin substances are particularly common in fruits, mostly in citrus and apples that are basic raw materials for its production, but pectin can be obtained from many other sources [6].

The main chain in the pectin molecule consists of α (1-4)-linked residues of D-galacturonic acid. Many of the carboxylic groups are esterified with methanol. Theoretically, the degree of esterification (DE) can vary from 0 to 100%. The pectins with a degree of esterification over 50% are known as high-methoxyl (HM) pectins and therefore low-methoxyl (LM) pectins have DE below 50% [5]. In the main chain are included and a certain amount of rhamnose residues. The side chains, containing arabinose and galactose, are randomly scattered or grouped in separate regions. The degree of esterification of the pectins has an important functional matter and affects their industrial use as jellifying and thickening agent. HM pectins form gels in low pH (4.0), presence of soluble solids (usually sucrose above 55%) and stabilize through hydrophobic interactions [7]. Conversely, LM pectins form electrostatically stabilized gel networks in the presence of divalent metal cations, usually calcium. Study of Brouns et al., shows that the degree of esterification of pectin affects the cholesterol lowering effect [8]. According to the authors, the best results are obtained with apple and citrus pectin with a high degree of esterification (DE > 70 %).

Pectin has always been a natural component of the human diet and according to the records of the WHO and the Food and Agriculture Organization is considered a safe supplement without restrictions in your daily intake [9]. The pectin substances have numerous applications in the food and pharmaceutical industries [7].

Due to adsorption and complex forming properties, pectin is also a suitable carrier for a variety of biologically active substances, including of plant origin [10];[11];[12].

There are certain difficulties in ensuring a daily intake of pectin in necessary quantities (more than 6 g/day). Such doses is hard to be accepted in the form of tablets or capsules, and the pectin powder must dissolves in water, which creates a practical problem related with his dissolution mechanism – the formation of aggregates, slow and difficult dissolving and getting a viscous solution or gel, which is not pleasant to consume. These difficulties may refuse user from regular consumption and thus compromise the lowering cholesterol effect.

The objective of this work is to improve dissolution in water of high-methoxyl citrus pectin. The method used is wet granulation with different granulation liquids and the resulting variant granules are tested for particle size distribution, solubility and hydration rate.

II. EXPERIMENTAL

Materials

Citrus pectin HM (GRINDSTED PECTIN MRS 351, Danisco), water-ethanol mixtures with different concentration, sucrose solution 45% (w/v), sucrose, ethanol, purified water, hydrochloric acid pa (Merck), sodium hydroxide pa (Merck), phenolphthalein.

Granulation

The applied method is wet granulation. In advance, a dry mixture of pectin and sugar in the ratio of 3:2 is prepared. Variants of granulating liquid: sucrose solution 45% w/v, distilled water, ethanol 25, 40 and 70% v/v. Granulation is performed on ERWEKA AR 400 apparatus, through a sieve with a mesh size of 1.00 mm. The resulting granules are dried in an oven with air circulation at a temperature of 50 ºC for 1 to 3h and re-granulated of the sieve sizes 0.80 mm.

Analysis of the pectin powder

Analysis of the pectin powder is carried out with the method described in the monograph for pectin in USP 23 [13]. Samples of 5.000 g pectin are shaken for 10 min with mixture of 100.0 ml 60% v/v ethanol and 5 ml hydrochloric acid. The resulting slurry is transferred on a weighed sintered-glass filter and washed with six 15 ml portions of the same mixture followed by 60% v/v ethanol to the negative reaction of the filtrate for chlorides. The obtained sludge is washed with 20 ml ethanol and dried at 105ºC to constant weight.

Samples of exactly 1/10 of the dry residue are moistened with 2 ml ethanol and dissolved in 100.0 ml carbon dioxide-free water. The pectin solution is titrated with a standard solution of sodium hydroxide (0.5 mol/l) with phenolphthalein indicator and the actual volume is referred to as initial titer. 20 ml of sodium hydroxide solution (0.5 mol/l) are added and 15 min later - 20 ml of hydrochloric acid solution (0.5 mol/l) are added as well and titrate with 0.5 mol/l sodium hydroxide solution to light pink. This result is reported as the saponification titer.

1 ml 0.5 mol/l sodium hydroxide solution of the saponification titer is equivalent to 15.52 mg methoxy groups in pectin.

1 ml sodium hydroxide solution (0.5 mol/l) of the amount of the initial titer and saponification titer and the equivalent of 97.07 mg D-galacturonic acid.

From the content of D-galacturonic acid and methoxy groups is calculated the degree of esterification of pectin.

Particle Size Distribution (PSD)

The size and distribution of particles for each variant of the granules are laser measured with ANALYSETTE 22 NanoTecplus (FRITSCH). The ANALYSETTE 22 NanoTec plus works on the basis of laser diffraction and therefore determines volume i.e. how many percent of the complete sample volume is filled with particles smaller than x µm. The obtained results for the three samples are grouped into 102 channels from 0 to 2000 micrometers.

Measurement of dissolution

The solubility and the hydration rate of granules are determined by the method of Kravtchenko et al. [14]. 330 ml distilled water is placed in a glass container equipped with a magnetic stirrer (700 rpm). Accurately weighed quantity of each sample is added to the water at final concentration of pectin 2.5% (w/v). Measurement of viscosity of the resulting solutions is conducted on a rotational viscometer Rheomat 120, at the speed 1280 s^{-1} and a temperature of 20±1°C. Once the viscosity reached a stable value, that value is considered the final viscosity corresponding to complete hydration. The hydration rate (%) for each time is obtained by dividing the viscosity read at time by the final viscosity, and multiplying by 100.

Statistical analysis

Data represent mean ± standard deviation (SD) of three independent experiments. The data are analyzed by one-way analysis of variance. Differences are considered statistically significant when the p level was less than 0.01.

III. RESULTS

Chemical analyses of pectin

The analysis of the samples from the source citrus pectin (GRINDSTED PECTIN MRS 351), showed the pectin substances content – 0.850g/g product. The commercial pectin products contain also neutral sugars and other dietary fiber, and to some brands of pectin extra added dextrose or sugar for standardizing to „150 jelly grade". The contents of D-galacturonic acid is 701.36 ± 6.05 mg/g pectin, and the methoxy groups – 78.38 ± 1.71 mg/g pectin. From the results obtained has been calculated the degree of esterification – 69.93%. With these parameters, the pectin is defined as high-metoxyl (HM). The measured pH value of 1.0 % aqueous solution is 3.35, which is explained by the presence of free carboxyl groups in the molecule of the pectin and checked by the data referred to in the literature [7].

Granulation of pectin

Five variants pectin granules are developed (Table 1).

Table 1. Variants of pectin granules

Variants	Composition of 100g powder		Granulation liquid
	Pectin(g)	Sugar (g)	
Variant 1	60	40	Sucrose solution 45%
Variant 2	60	40	Ethanol 25%
Variant 3	60	40	Ethanol 40%
Variant 4	60	40	Ethanol 70 %
Variant 5	60	40	Distilled water

Obtained granules are with a light beige color and irregular in shape. There are differences in the appearance of particles depending on the granulation liquid (Fig. 1).

Fig. 1. Appearance of pectin granules

Granulometric analysis of pectin granules

The results of the granulometric analysis of pectin granules are presented in Figures 2 to 6.

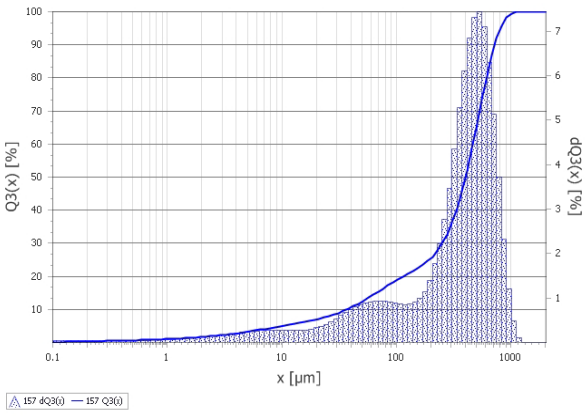

Fig. 2. Granulometric analysis of variant 1

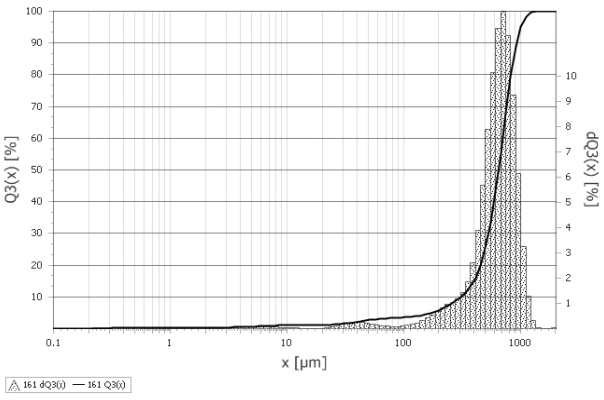

Fig. 3. Granulometric analysis of variant 2

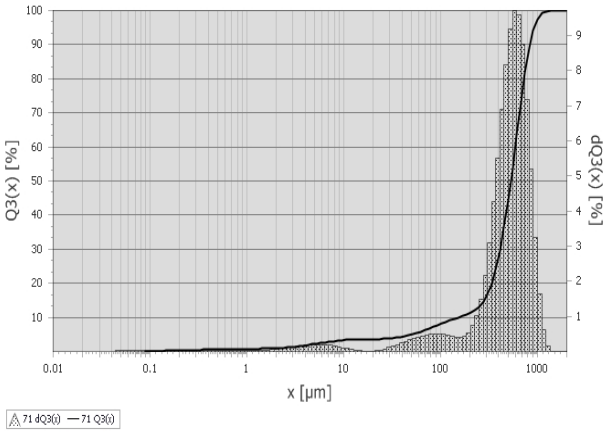

Fig. 4. Granulometric analysis of variant 3

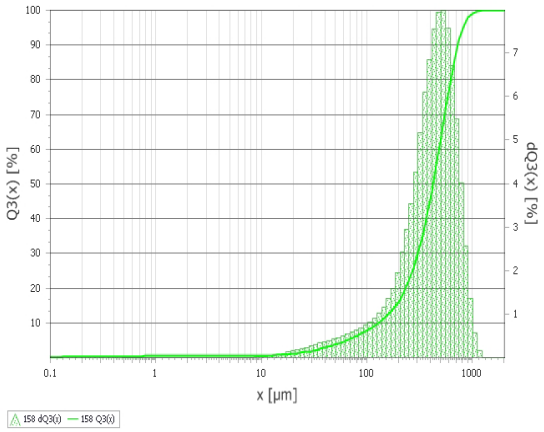

Fig. 5. Granulometric analysis of variant 4

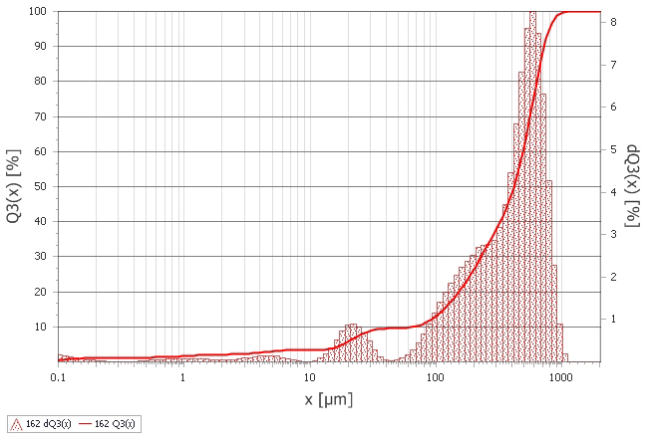

Fig. 6. Granulometric analysis of variant 5

The size of the particles has a major influence on a number of properties of materials and is a valuable indicator for quality and performance. There are various methods for the determination of the particle size, as they are most commonly associated with the target device. In this study the real tests are made with the help of laser particle meter ANALYSETTE 22 NanoTec plus. The ANALYSETTE 22 NanoTec plus works on the basis of laser diffraction and therefore determines volume i.e. how many percent of the complete sample volume is filled with particles smaller than x µm. The obtained results for the three samples are grouped into 102 channels from 0 to 2000 micrometers.

For the Particle Size Distribution (PSD) the central values are mean, median and mode. For symmetric distributions all these values are equivalent: mean = median = mode. Respectively, for non-symmetric distribution mean, median and mode will be three different values. Since laser diffraction results are reported on a volume basis, so the volume mean can be used to define the central point although the median is more frequently used than the mean when using this technique.

The best way to determine the average volume is by using the histogram (graphical method for presentation of frequency distribution), showing a lower and an upper limit of the chanel and what percentage of the total volume of the sample fall within it.

The equation for defining the mean volume is:

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

where Di value for each channel is the geometric mean, the square root of upper x lower diameters. The geometric mean is a type of mean or average, which indicates the central tendency or typical value of a set of numbers by using the product of their values (as opposed to the arithmetic mean which uses their sum). The

geometric mean is defined as the nth root of the product of n numbers $\{x_i\}_{i=1}^{N}$, i.e., for a set of numbers , the geometric mean is defined as follow:

$$\left(\prod_{i=1}^{N} x_i\right)^{1/N} \qquad (2)$$

Thus, for the numerator in equation (1) take the geometric Di to the fourth power x the percent in that channel, summed over all channels. For the denominator take the geometric Di to the third power x the percent in that channel, summed over all channels.

Median values (Md) are defined as the value where half of the population resides above this point, and half resides below this point. For particle size distribution the median is called the D50. The D50 is the size in microns that splits the distribution with half above and half below this diameter. The Dv50 (or Dv0.5) is the median for a volume distribution, Dn50 is used for number distributions, and Ds50 is used for surface distributions. Since the primary result from laser diffraction is a volume distribution, the default D50 cited is the volume median and D50 typically refers to the Dv50 without including the v. This value is one of the easier statistics to understand and also one of the most meaningful for particle size distributions.

The mode (Mo) is the peak of the frequency distribution, or it may be easier to visualize it as the highest peak seen in the distribution. The mode represents the particle size (or size range) most commonly found in the distribution. Less care is taken to denote whether the value is based on volume, surface or number, so either run the risk of assuming volume basis or check to assure the distribution basis. The mode is not as commonly used, but can be descriptive; in particular if there is more than one peak to the distribution, then the modes are helpful to describe the mid-point of the different peaks.

Most instruments are used to measure the particle size distribution, implying an interest in the width or breadth of the distribution (Span). The field of statistics provides several calculations 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 field of study.

Once "model independent" algorithms were introduced many scientists began using different calculations to describe distribution width. One of the common values used for laser diffraction results is the span, with the strict definition shown in the equation below:

$$Span = \frac{D_{v0.9} - D_{v0.1}}{D_{v0.5}} \qquad (3)$$

where Dv0.5 or D50 is the median, defined as the diameter where half of the population lies below this value. Similarly, 90 percent of the distribution lies below the D90, and 10 percent of the population lies below the D10. A summary of the statistical data described above for samples of pectin granules is made respectively in Table 2.

Table 2. A summary of statistical parameters for sample options of pectin granules

Sample	D[4,3] (μm)	Mo (μm)	Span
Variant 1	391.1	524.80	1.70
Variant 2	633.0	702.99	0.96
Variant 3	526.1	597.6	1.29
Variant 4	430.7	508.02	1.42
Variant 5	400.1	578.51	1.57

The differences in the size and distribution of particles in different variants granules are significant. As can be seen from the histograms (Fig. 2-6) in variants 2 and 3 there are less scattering from the sample data. These results are confirmed by the statistical treatment of experimental data (Table 2). Under variants 2 and 3, the results obtained for the mean volume diameter D (4.3) and Mode (Mo) are highest and therefore closest to the upper value of 800 μm particle size.

An important indicator also is the width of the distribution (Span), as it provides the basis to assess the homogeneity of the granules and their distribution in fractions of different sizes. Lower values correspond to less scattering particle size and volume. Lowest scores are calculated for variants 2 and 3. On this parameter in variants 2 and 3, taking into account the highest degree of uniformity of the granules.

Solubility of granules variants

The measured viscosity profiles of pectin granules variants are shown in Fig.7. After placing the samples in the water, the values increased until reaching the final viscosity, which is an indicator of the complete solution of pectin. The data for the final viscosity are presented in Table 3.

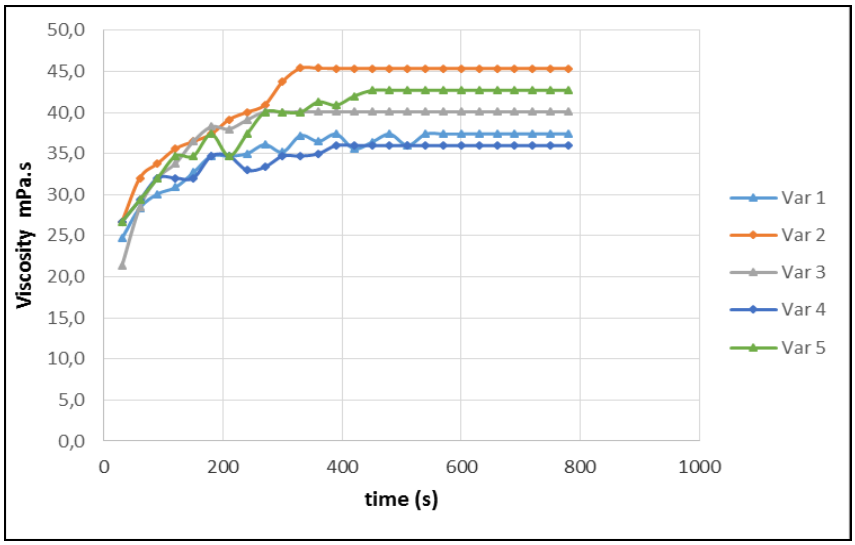

Fig. 7. Viscosity profiles of pectin granules. Citrus pectin granules were dispersed into water at final concentrations of 2.5% (w/v). The rheological analysis was conducted at 1280 s^{-1} and 20ºC. Data shown are from a typical experiment that was reproduced at least three times.

Table 3. Final viscosity and degree of hydration at 300th s for granule variants.
The data are presented as means ± SD.

Variant	Time final viscosity (s)	Final viscosity (mPa.s)	Hydration rate at 300th s (%)
1	540±20	37±2.6	94.1
2	335±22	45±2.3	96.5
3	277±21	40±1.0	100.0
4	392±25	36±1.7	96.4
5	443±18	42±1.2	93.9

[1] Statistical significance was determined by One-Way ANOVA; significant differences between means ($p < 0.01$)

Time (s) for reaching the final viscosity is lowest in variants 2 and 3, respectively, 335±22 and 277±21. The solubility of all granules variants is improved compared to the control sample non granulated pectin (pectin and sugar mixture in the ratio 3:2), in which the complete solution is achieved in 32 min (32 ±2.6).
In addition the viscosity (mPa.s) after dissolution of granules of variants 1, 2, 3, 4 and 5 shall be respectively 37±2.6; 45±2.3; 40±1.0; 36±1.7 and 42±1.2, in the pectin concentration – 2.5% (w/v). The viscosity of the samples from the source non-granulated pectin is 38 ± 2.2 mPa.s.
The hydration rate at 300th s is over 90% for all variants reaching 100% for variant 3 and 96.5% for variant 2. In the non-granulated pectin the hydration rate at 300th s is only 54%.

IV. DISCUSSION

The main objective of this research is through technological methods to achieve a more rapid and complete dissolution of high-esterified citrus pectin in water. The mechanism of dissolving the pectin is different from that of the low molecular weight substances. In contact with water, the pectin particles absorb it very quickly and increase in size. If the individual particles are located in close proximity, they stick to one another, hold the air bubbles and form aggregates, which hinder the further penetration of the solvent and highly reduce the dissolution speed. To avoid this difficulty, it is necessary to carry out preliminary separation of the individual particles of the pectin. The methods to achieve this effect vary from high rotary speed homogenizing, dry mixing with sugars in the ratio 1:5 or using an inert medium for preliminary dispersion (sucrose solution,

alcohol, etc.) to the chemical modification of pectin [15];[16];[17]. From the dietary point of view, these methods are not appropriate for creating a natural food supplement with cholesterol-lowering action. Granulation with the addition of a minimum quantity of sugars is a promising way to improve solubility of the pectin. In this case, the granulating liquid is essential for the type, distribution and solubility of formed granules.
With our previous studies, we have defined that for pectin most appropriate is granulating with water, sucrose solution, and water-alcohol mixtures [18]. Different concentrations of ethanol in granulating fluid cause difference in shape, homogeneity and solubility of the granules. The samples prepared with sucrose solution or water (variants 1 and 5) have a large width distribution (Span of 1.70 and 1.57) which is an indicator of the non-homogeneity. Time to reach final viscosity is long. When granulation is carried out with hydro-alcoholic mixtures, the spread of distribution of the resulting granules ranges from 0.96 (variant 2) to 1.42 (variant 4). The following trend was observed - with the increasing concentration of ethanol over 40%, the fractions with particle sizes below 305 μm increase (on granulation with 70% ethanol they are 30% of the mass of the granules). Solubility tests have shown that up to a certain concentration, the presence of alcohol in granulating liquid leads to a faster dissolving of the resulting granules. The best results in this respect was reached when using 40% v/v ethanol (variant 3). After putting in water, this sample is dissolved in less than 5 minutes by forming a homogeneous solution.

V. CONCLUSION

Granulation of high-methoxyl citrus pectin with water, water-alcohol mixtures and sucrose solution, improves its dissolution in water. Compared with the starting pectin a significant reduction in time to reach the final viscosity after placement in water is reported. Also, the hydration rate at the 300^{th} s is greater than 90% with the granulated pectin and only 54% with non-granulated. Among the obtained five variants of granules, the best results are observed in the variant, granulated with 40% (v/v) ethanol. The analysis of the size and distribution of the particles reports the least scattering of the results during the variants granulated with 25% and 40% ethanol, which corresponds to the highest degree of uniformity of the granules.
The obtained pectin granules represent a good basis for obtaining ready for use natural pectin supplement with cholesterol-lowering action.

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