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Extrusion/Spheronization of Pectin-Based Formulations. II. Effect of Additive Concentration in the Granulation Liquid

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ABSTRACT Purpose. The aim of this study was to improve the formation of spherical pectin pellets by investigating the effect of additive concentration in the granulation liquid on the shape and size of the products as well as by identifying an optimal additive concentration. **Methods.** High-methoxylated, low-methoxylated, and amidated low-methoxylated pectin types were evaluated in combination with different concentrations of methanol, ethanol, citric acid, lactic acid, and calcium chloride. Pellets were prepared in a power-consumption-controlled twin-screw extruder, then spheronized and dried. The moisture content of the extrudate was determined, and the final products were characterized by image analysis and sieving analysis. A cloud point test was employed for the identification of an optimal additive concentration. **Results.** The concentration of additive in the granulation liquid affected the moisture content of the extrudate and the shape, size, and mechanical stability of the pectin pellets. Improvements in the pellet characteristics are dependent on the pectin type employed. The 2 low-methoxylated pectins were more sensitive to concentration changes than was the high-methoxylated type. Above a certain threshold concentration, the quality of the pellets are improved. This additive concentration differs according to type of pectin and type of additive. **Conclusion.** It was demonstrated that there is a concentration-dependent interaction between pectin and substances added to the granulation liquid that can be utilized to improve the formation of spherical pectin pellets.

Keywords: Extrusion, Spheronization, Pectin, Granulation Liquid, Concentration Effects.

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INTRODUCTION

Part 1 of this work presents a broad processability assessment of pectin-based formulations in an extrusion/spheronization process [1] . It was shown that the swelling properties of pectin could be modified and the shape and size of the pellets improved by adding substances to the granulation liquid.

Pectin, being a swelling hydrocolloid, must be expected to swell to a larger degree than the most commonly used excipient for pellet production by extrusion/spheronization, microcrystalline cellulose (MCC) [2] . It must therefore be expected that pectin-based formulations are more susceptible to alterations in the amount and constituencies of the granulation liquid than are MCC-based formulations.

The importance of extrudate moisture content on the pellet shape and size parameters in the extrusion/spheronization process has often been reported [3,4] . Water content is claimed to be one of the most important variables in the extrusion process [5] , and Kleinebudde recommends either strictly controlling the moisture content or including it as a formulation variable [3] . Strict control of the moisture content can be achieved by performing the extrusion experiment at a constant power consumption-for example, by using a power-consumption-controlled twin-screw extruder [6] .

Based on previous experience, our working hypothesis was that the quality of the pectin pellets is affected by the concentration of additive in the granulation liquid. By performing the experiments with a power-consumption-controlled twin-screw extruder, it would be possible to ensure extrusion at optimal moisture content. Any changes occurring in the moisture content of the extrudate as a result of the increasing additive concentration would therefore be interpreted as a change in the properties of pectin. Changes causing reduced solubility of pectin can be detected with the cloud point test [7] . Consequently, the optimally shaped pellets should be produced at the additive concentration corresponding to the cloud point.

The objective of this study was to improve the formation of spherical pectin pellets produced by extrusion/spheronization; this required, primarily, evaluation of a concentration-dependent effect on the quality of the products with respect to size and shape and, secondly, identification of the optimal additive concentration.

MATERIALS AND METHODS

Materials

A high-methoxylated (HM) pectin (Pectin Classic CU202, batch no. 0810679), a low-methoxylated (LM) pectin (Pectin Classic CU701, batch no. 0903185)-both from Herbstreith & Fox GmbH, Neuenburg, Germany-and an amidated LM pectin (Pectin type 920, batch no. 6753/01 from Citrus Colloids) were tested. Characteristics are listed in **Table 1** . For pellet preparation, acetylsalicylic acid (NMD, Oslo, Norway) was added as a model drug to 20% of the total powder mixture. As references, pellets without pectin were made. These consisted of MCC and model drug (80:20).

Table 1. Characteristics of Pectin1

Pectin Type	Degree of Methoxylation (%)	Degree of Amidation (%)	Degree of Free Acid Groups (%)	pH[2]
amidated LM	25	23	52	4.7
LM	35	--	65	2.9
HM	72	--	28	3.4

Note: -- indicates data not available, LM = low methoxylated, HM = high methoxylated.
[1]Analysis provided by the producer
[2]Measured in a 1% solution of pectin in water at 20°C

As granulation liquid, aqueous solutions of 5 additives were tested at different concentrations, referred to as % wt/wt. The additives were calcium chloride, citric acid, lactic acid, methanol, and ethanol. Hydrochloric acid was used for the cloud point test. All chemicals were of analytical grade (Merck, Darmstadt, Germany).

Experimental design

Pellets were prepared from the powder mixtures, which were extruded with granulation liquids containing the selected additives in different concentrations. The additives were the same as reported in part 1 of the study [1] . Aqueous solutions of the additives were tested in concentrations ranging from pure water (0% additive) up to the maximum concentration for each additive that allowed formation of a product with the extrusion/spheronization technique. Concentrations producing nothing but powder or a gelled extrudate were regarded as not suitable for product formation.

The 3 pectin types were tested with granulation liquids containing the same types of additives, but the concentration of additives had to be adjusted for each pectin type as the different types responded differently to the additive type and concentration. In total, 77 experiments were performed using the 3 pectin types and the 5 different additive types.

Preparation of pellets

A power-consumption-controlled twin-screw extruder (type ZE25x18D, Berstorff AG, Hannover, Germany) with 48 dies 1 mm in diameter and 2.5 mm long was used for the preparation of pellets. All experiments were run at a power consumption of 180 W [6] and a powder feed rate of 25 g to 26 g. The extruded mass was rounded in a spheronizer (Type S-320, Nica, Molndal, Sweden) with a cross-hatched plate with a diameter of 320 mm at 800 rpm for 5 minutes. The pellets were dried in a fluid-bed dryer at 50°C for 30 minutes (Glatt, Binzen, Germany).

Moisture content of the extrudate

The moisture content of the extrudate was determined by collecting samples during the extrusion process. Percentage moisture content was determined gravimetrically after drying at 105°C for 24 hours.

Size and shape of the pellets

All products were characterized according to shape, size, and size distribution using an image analysis system (Leica Q500MC, Qwin, Cambridge, UK). Prior to processing of the images, care was taken to ensure that all pellets were detected as single entities. One pixel corresponds to 54 mm. Six feret diameters were measured around each individual particle for 300 ± 100 items (median, D10, D25, D75, and D90 were calculated). The length was defined as the longest feret diameter and the breadth as the shortest. The area (total number of detected pixels) and roundness (= perimeter^2 /{4*p*area*1.064}) were calculated for each particle.

Mechanical stability of the pellets

The mechanical strength of the pellets was determined by sieving the product after drying, as described in part 1 [1] . Particles that could pass through 0.3 mm openings were regarded as fines. A high amount of fines was regarded as an indication of a less mechanically stable product.

Cloud point

Preparations of pectin (3% wt/wt) in aqueous solutions of selected additives at different concentrations were prepared. Precipitation of pectin, indicating phase separation, was detected as the cloud point by visual inspection of the preparations [8] . The concentration of each additive was incremented at intervals ranging from 0.1% to 5% wt/wt starting with pure water (no additive) until the cloud point was reached.

Scanning electron microscopy (SEM) photomicrographs

Samples of pectin pellets were mounted on aluminum stubs using double-sided sticky tape sputter-coated with Au/Pd in a 60/40 combination (Polaron E500 Sputter-coater, Cambridge, UK) and examined using a scanning electron microscope (JMS-6400 SEM, JEOL, Tokyo, Japan).

RESULTS

Effect of additive concentration on pellet size

None of the pectin pellets obtained was perfectly spherical. The shape ranged from short, rod-shaped particles with a median length of about 1.5 mm to longer rods up to 10 mm. The length could be employed as a single parameter to describe size and shape of the pellets [1] . For a perfect sphere, the length is equal to the diameter, which in this case should be close to 1 mm.

Figure 1 shows the size distribution of a typical formulation. This batch has a median length of 3.5 mm; the longest particle is measured to be 6.8 mm and the shortest to be 1.3 mm. An important observation is that the size distribution (interquartile range) becomes broader (**Table 2**) as the median pellet length increases. It is typical for batches of poor quality to have some really long particles (8-10 mm) and smaller fragments of particles (<1 mm), while the average pellets have a length of 5 to 6 mm. The batches of better quality have a narrow size distribution and a length between 1 and 2 mm. This can be seen in **Figure 2** , which shows the median length of pellets of amidated LM pectin and MCC granulated with different amounts of calcium chloride. Compared with pellets of MCC, the pellets of amidated LM pectin were 2 to 3 times the length and the size distribution was broader. The error bars, which indicate the upper and lower quartile (D75 and D25) for 300 ± 100 measured particles, are small for the MCC pellets. The bars are also somewhat smaller for the pellets of amidated LM pectin obtained with a calcium chloride concentration of 10% compared with those obtained with pure water or low additive concentrations (not shown). In spite of the large variability of the poor batches, a significant reduction in pellet length for amidated LM pectin with increasing concentration of calcium chloride in the granulation liquid can be seen (**Figure 2**).

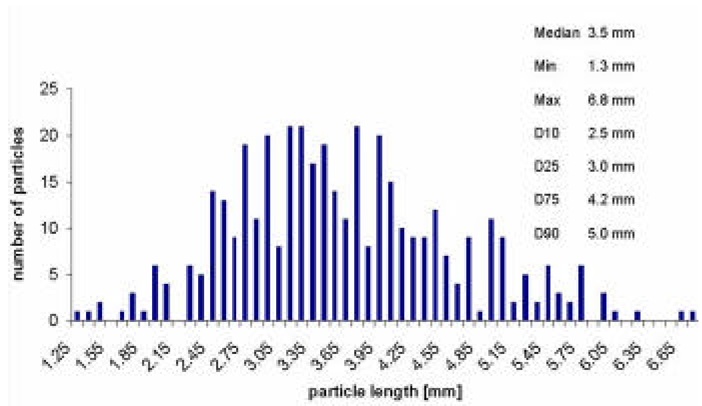

Figure 1. Example of the distribution of particle length (amidated low-methoxylated pectin extruded with water, 405 particles measured).

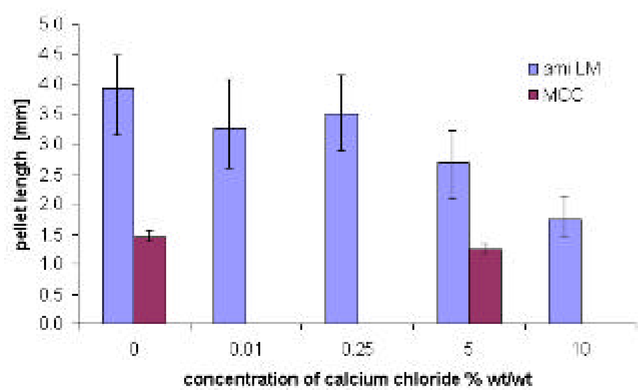

Figure 2. Reduction in the pellet length as a function of increasing concentration of calcium chloride. The pellet length is given as the median (D50), and the error bars represent the upper and lower quartile (D75 and D25) calculated for 300 ± 100 particles. Note the discontinuous scaling of the x-axis.

The concentration necessary to obtain a size reduction as well as the degree of reduction in pellet length differed for each type of additive. A summary for all pectin types and additives can be found in **Table 2**. Calcium chloride had a size-reducing effect for the 2 LM types (amidated LM and LM). Citric acid and lactic acid showed a reducing effect only for the amidated LM pectin type. The reduction in average pellet length was less pronounced for the 2 acids, from 3.9 mm to 3.7 mm and 3.4 mm, respectively (**Figure 3A**).

Increasing the concentrations of methanol and ethanol resulted in a reduction in the pellet length for all pectin types. **Figure 3B** shows the results for amidated LM pectin. Methanol seems to be capable of reducing the pellet length to a higher degree tha? ethanol. However, the response of the individual pectin types to increasing concentrations of methanol and ethanol showed large variations.

Figure 4 shows the pellet length of all pectin types as a function of increased ethanol concentration. The highest concentrations tested (25% and 30% ethanol) did not result in formation of a product, only powder, for amidated LM and LM pectin. LM pectin produced the shortest pellets, while HM pectin showed the largest size reduction in length when produced with ethanol compared with pellets extruded with no additive.

Table 2. Effect of Increasing the Additive Concentration in the Granulation Liquid on Properties of the Pectin Pellets

Pectin Type / Additive	Optimum Additive Concentration[1] (%)	Effects of Increased Additive Concentration			
		Pellet length median (mm)	Size variation (D75-D25)	Moisture content (%)	Fines (%)
amidated LM pectin					
No additive	--	3.9	1.32	51	1.1
Calcium chloride	10	1.7	0.65	64	0.3
Citric acid	1.5	3.7	1.44	52	2.8
Lactic acid	3	3.4	1.08	54	4.4
Methanol	19	1.8	0.70	60	>10
Ethanol	21	2.1	0.50	54	>10
LM pectin					
No additive	--	3.3	1.25	64	0.4
Calcium chloride	0.1	3.0	1.03	65	0.2
Citric acid	30	5.8	2.95	48	0.7
Lactic acid	15	5.6	2.15	59	3.0
Methanol	17	1.6	0.50	68	>10
Ethanol	20	1.4	0.37	71	>10
HM pectin					
No additive	--	6.3	1.97	53	2.1
Calcium chloride	30	9.0	2.40	44	0.5
Citric acid	30	8.0	2.74	42	1.5
Lactic acid	0–25[2]	6.3	3.09	53	5.3
Methanol	40	1.6	0.50	64	>10
Ethanol	30	1.7	0.63	60	>10

Notes: Data are given for the additive concentration producing maximum change in pellet length (median and interquartile range). LM = low methoxylated, HM = high methoxylated.
[1]Optimum additive concentration = concentration of additive showing maximum reduction in pellet size
[2]No differences in the concentration range 0%–25%

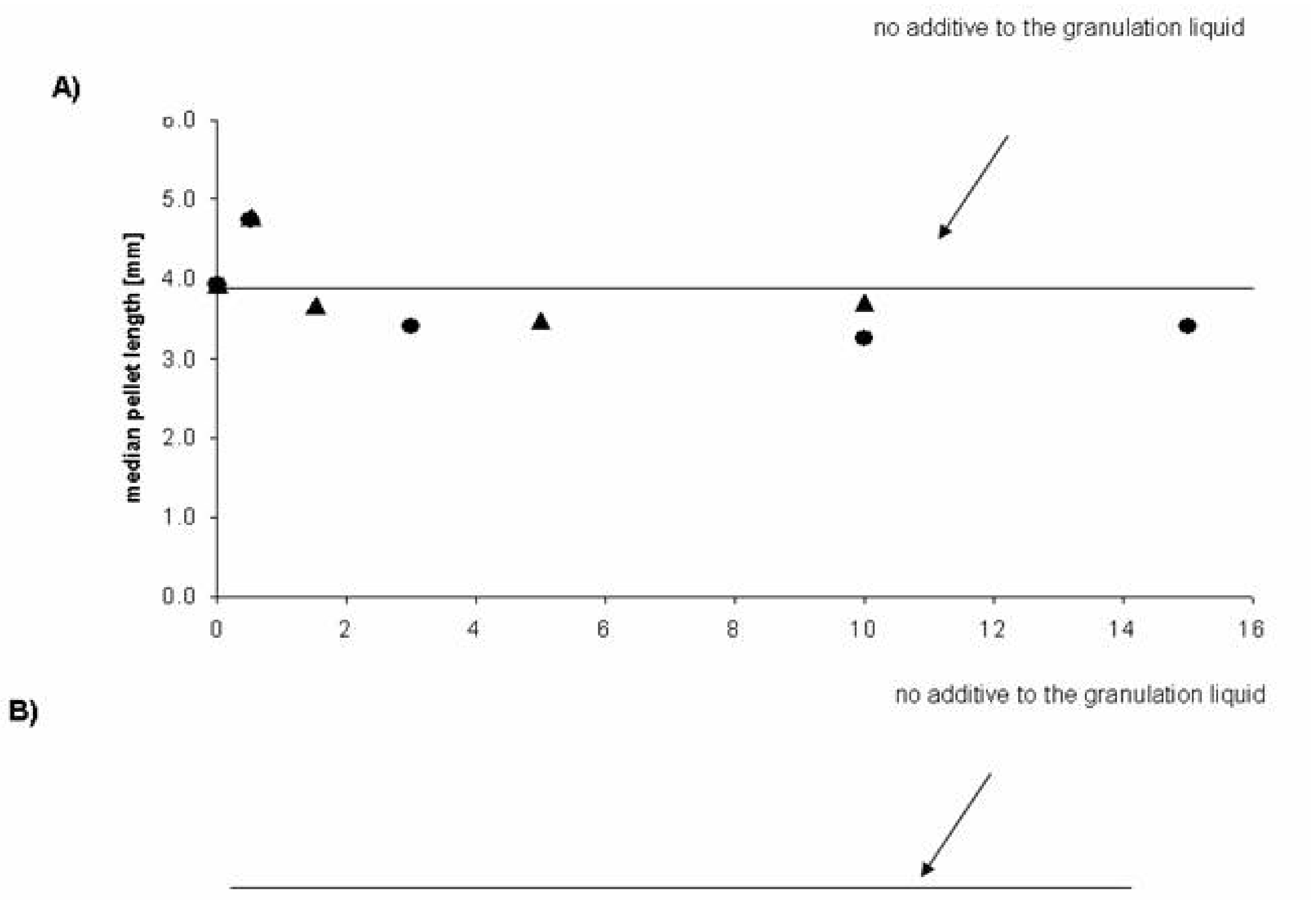

Figure 3. Examples of the concentration effect on the pellet length for amidated low-methoxylated pectin. A) Granulated with s citric acid and • lactic acid, B) Granulated with T methanol and ? ethanol.

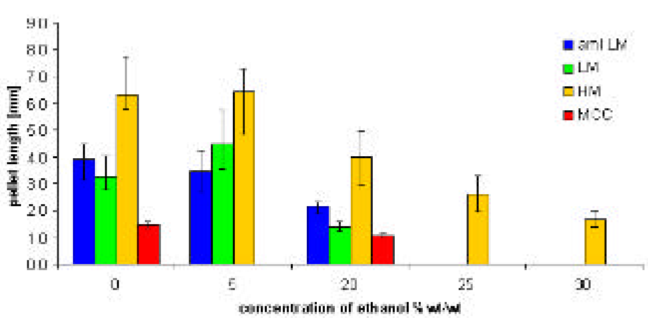

Figure 4. Example of reduction in pellet length as function of increasing concentration of ethanol for 3 pectin types (amidated low-methoxylated [LM], LM, and high-methoxylated [HM] pectin) and pellets of microcrystalline cellulose (MCC). The pellet length is given as the median (D50), and error bars represent the upper and lower quartile (D75 and D25) calculated for 300 ± 100 particles. Note the discontinuous scaling of the x-axis.

Effect of additive concentration on moisture content of the extrudate

The moisture content of the extrudate produced at a fixed level of power consumption is affected by the additive concentration. **Figure 5** shows an example of the concentration-dependent moisture level; data are from HM pectin extruded with methanol. The moisture content of the extrudate increases from 53% at an average pellet length of 6.8 mm to 64% at 1.7 mm. The moisture content as a function of increasing additive concentration is given in **Table 2** . In general, it may be stated that as the pellet length becomes shorter, the moisture content of the extrudate increases.

Effect of additive concentration on mechanical stability

The mechanical properties of the pectin pellets estimated as increased or reduced production of fines are given in **Table 2** . The most typical effect is an enormous increase in formation of fines with high concentrations of the freely evaporating additives. **Figure 5** shows how the amount of fines rises from 2.1% (no additive) to approximately 22% at a methanol concentration of 40% for the HM pectin. Further increase in the concentration resulted in an impossible manufacturing process because the extruded mass did not form pellets at all, as the fine particles lacked the necessary coherence.

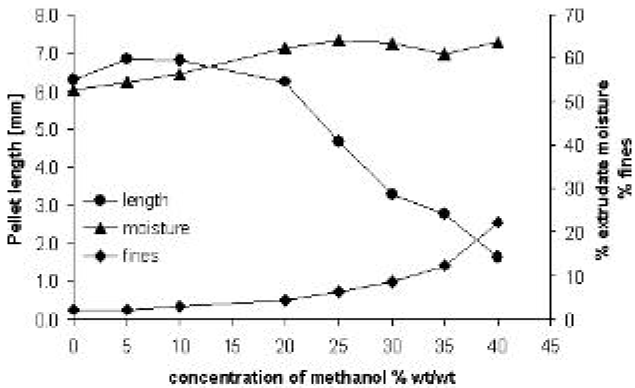

Figure 5. Effect of increasing methanol concentration for high-methoxylated (HM) pectin on median pellet length (•), moisture content of the extrudate (s), and amount of fines (?). Above 40% methanol, no pellets are formed, only powder.

Effect of additive concentration on the cloud point

The test must be regarded as a rough determination of cloud point because the appearance, color, and texture differ for solutions of different types of pectin. In cases where gels are formed, the cloud point is recorded as the concentration causing particle formation in the gel. **Table 3** shows the lowest additive concentration resulting in massive precipitation for the 3% pectin solutions. These results show a nearly perfect match, with the additive concentrations producing the maximum reduction in pellet length listed in **Table 2**. Thus, there seems to be a correlation between an additive concentration capable of causing phase separation and the improvement in pellet length.

With few exceptions, such as amidated LM pectin precipitate with 0.5% calcium chloride, the shortest pellets are produced with a concentration of 10%. A low concentration of calcium ions also contributes to a size improvement (median length is reduced from 3.9 mm to 3.5 mm at 0.5%), although a concentration increase up to 10% further reduced the size (to 1.7 mm). A similar pattern was observed for LM pectin with ethanol and for HM pectin with both methanol and ethanol. A further increase in the concentration resulted in production of fine powder instead of pellets.

The addition of organic acids (citric and lactic acid), which creates an acidic environment, did not produce precipitation within the solubility range of the additive for the 2 nonamidated pectin types (LM and HM). Nor did they contribute to shortening of the pellet length. The same was seen for calcium in combination with HM pectin.

Table 3. Concentration of Additive at Cloud Point for Selected Additives to the Granulation Liquid

Pectin Type	Concentration of Additive at Cloud Point (% wt/wt)					
	Additives modifying polarity		Additives modifying acidity			Cross-linking additive
	methanol	ethanol	citric acid	lactic acid	HC1	Ca^{2+}
amidated LM pectin	20	21	1.5	3	0.5	0.5
LM pectin	15	7	none	none	1	0.1
HM pectin	25	21	none	none	1	none

Notes: Preparations of 3% wt/wt of pectin in aqueous solution. "None" indicates that no cloud point was reached within the solubility of the tested additive. LM = low methoxylated, HM = high methoxylated, HC1 = hydrochloric acid.

SEM photomicrographs

SEM photomicrographs of some selected batches are presented in **Figure 6**. **Figures 6A to 6C** show the shape and structure of 1 pellet (magnification x35) from 3 experiments with LM pectin extruded with water (A), 10% methanol (B), and 17% methanol (C), while **Figures 6D to 6F** show the surface structure of the corresponding pellets at a magnification of x200. It is obvious how the size and shape of the pectin pellets change with increasing concentration of methanol. A change in surface structure can be recognized for pellets made with high concentrations of methanol compared with those made with water (no additive). This might be related to changes in porosity, but as the differences in particle size and surface area were large between the products, porosity assessments were not permitted. Other studies have shown that the porosity of pellets increases with increasing concentrations of alcohol [9,10].

DISCUSSION

In most cases, an increased concentration of additive resulted in a reduction of the average pellet length. Increased concentration of additive is further correlated to increased moisture content in the extrudate. Hence, size of the pellets produced will be inversely correlated to the moisture content of the extrudate.

The mechanism for gel formation is different for HM and LM pectin. The structural formula of pectin is shown in **Figure 7**. In the case of HM pectin, gel formation is governed by both hydrogen bonds and hydrophobic interactions, whereas in LM pectin, gels are also formed in the presence of Ca^{2+}, which acts as a bridge between pairs of carboxyl groups of different pectin chains [7]. These interactions between Ca^{2+} ions and carboxyl groups are described by the "egg-box" model [11]. As the gel point and the cloud point is closely related [8], it can be assumed that the mechanisms important for gel formation are also important for phase separation.

The additives tested can be divided into 3 groups according to their expected mechanism of interaction with pectin: additives modifying the polarity/solubility, additives modifying the pH, and cross-linking additives.

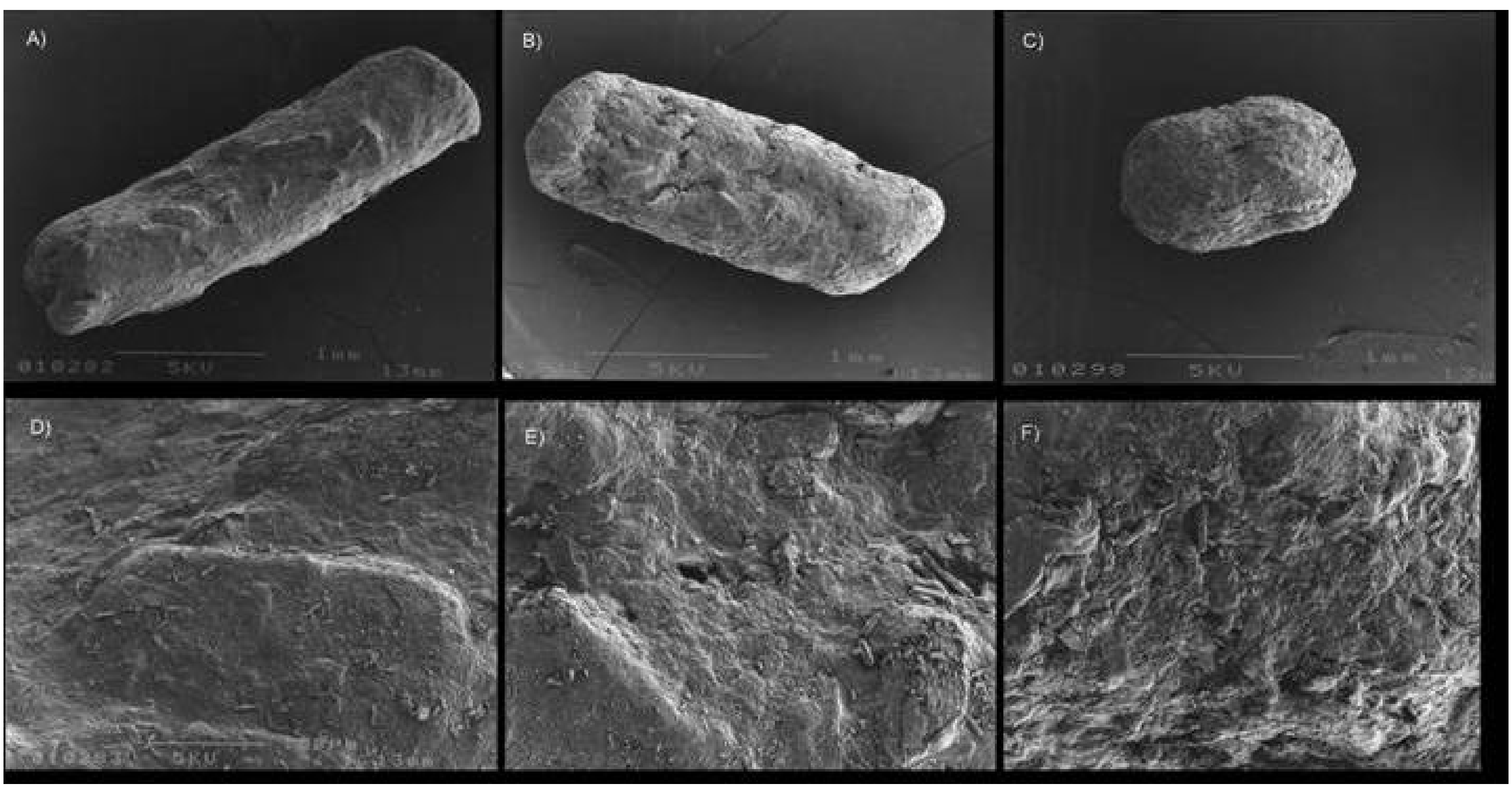

Figure 6. Scanning electron microscopy photomicrographs of pellets (magnification x35) of low-methoxylated pectin extruded with A) water (no additive), B) 10% methanol, and C) 17% methanol, and the corresponding pellet surface by another (magnification x200) in D-F.

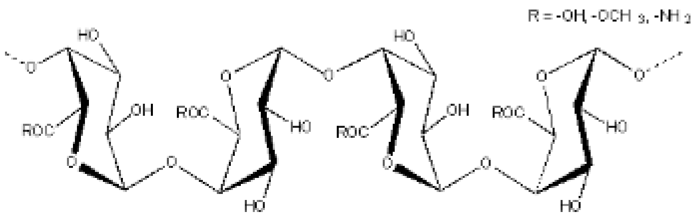

Figure 7. Structural formula of derivatives of polygalacturonic acid.

Additives modifying the polarity/solubility

The 2 additives that are capable of changing the polarity of the granulation liquids (methanol and ethanol) resulted in a reduction of the pellet size for all pectin types. By reducing the polarity of the solution, methanol and ethanol influence the solubility of pectin. Increased concentration of these additives result in "contraction" of the pectin molecules, thereby reducing the number of contact points between pectin molecules and ultimately reducing pectin precipitation. This is also seen in the cloud point test: Increased concentrations of the 2 alcohols result in phase separation.

The connectivity in a pectin gel is established mainly through entanglements. Close to phase separation, there is a fine balance between repulsive and attractive forces. Beyond the gel point, large polymer "lumps" are formed and the swelling is suppressed, leading to phase separation [12] . The conformation of pectin chains changes as a result of interaction between additive and the pectin molecules, resulting in phase separation. It is assumed that the hydrodynamic radius (R_h) is affected by the different additives as well as by the concentration of a certain additive, but we have not succeeded in showing this for the systems studied.

The massive production of fines at high additive concentrations for all pectin types extruded with methanol- and ethanol-containing granulation liquids supports the theory of reduced entanglements. As the contact points between pectin particles in the extrudate are reduced, it is not surprising that the mechanical stability of the pellets decreases. A high production of fines in the manufacturing process indicates low mechanical stability of the product. Figures 6D through 6F suggest weaker mechanical stability by indicating changes in the surface structure at high concentration. Low mechanical strength has also been reported for MCC pellets produced with high concentrations of ethanol [9] and 2-propanol [10] . This is explained by the use of volatile granulation liquids that evaporate and form highly porous pellets with low mechanical stability. In contrast to MCC, the solubility of pectin would be affected by the alcohol in the granulation liquid. The conformation of pectin chains is expected to change radically upon increase in concentration of the granulation liquid. The sum of these phenomena could explain the low mechanical strength of these products.

The increased moisture content of the extrudate as a result of increased alcohol concentration is another factor indicating reduced solubility of pectin. That a less-soluble substance needs a higher moisture level to form comparable pellets under equal conditions is known from the literature [13] .

Additives modifying the pH

Citric acid and lactic acid affected only the amidated LM pectin type. These granulation liquids will change the pH of the surroundings and thereby influence the number of dissociated carboxylic acid groups on the pectin chain. This would influence the hydrophilic-hydrophobic properties of pectin and thereby the solubility. A decrease in pH results in less dissociation, and the pectin could precipitate.

The pKa of lactic acid is 3.88, and the 3 pKa's of citric acid are 3.14, 4.77, and 6.39. Of the pH of 1% aqueous solutions of the 3 pectin types, only amidated LM (pH 4.7) has a potential of pH reduction with these weak acidic solutions. Neither LM (pH 2.9) nor HM (pH 3.4) pectin would be affected at these pH values. This is confirmed both with the cloud point test and the extrusion experiments. Stronger acids could possibly be used to achieve improvements for LM and HM pectins as well.

As a hypothetical granulation liquid, hydrochloric acid (HCl) was tested with the cloud point test. As shown in **Table 3** , HCl is capable of reducing the pH of all pectin types, thereby causing phase separation. As strong acids are not recommended for use in the extruder, the extrusion/spheronization experiments have not been performed with HCl, but the results of the cloud point test are a strong indication that the addition of strong acids would result in improvements in the average pellet length.

Cross-linking additives

As mentioned above, calcium ions are capable of cross-linking pectin chains. Addition of calcium chloride to the granulation liquid reduced the pellet size for the LM pectin types. It has been reported that only the LM pectin types form networks with calcium ions [7] ; thus, it seems likely that the cross-linking mechanism is important for the improved processability of these pectin types. The cloud point test indicates that the 2 LM pectins are very sensitive toward calcium ions. At concentrations of 0.1% and 0.5% calcium chloride, respectively, amidated LM and LM pectin form complexes that precipitate. A phase separation by HM pectin within the solubility range of calcium chloride was not observed. This might be explained by a too-low number of free carboxylic acid groups capable of forming the egg-box.

Increased concentration of an additive that is capable of cross-linking with the polymer should result in increased contact points in the network, thereby reducing the solubility of the complex and precipitation. As described earlier, the moisture content of the extrudate should increase, and this is exactly what was observed for the LM pectin types.

Increasing the concentration of calcium ions in the granulation liquid did not reduce the mechanical stability of the pectin pellets. As a result of the complex formation, the matrix has many contact points and, consequently, the products are mechanically strong.

CONCLUSION

It can be concluded that the optimal size and shape of pectin pellets is related to the concentration as well as type of additive in the granulation liquid. Moisture content of the extrudate, average pellet length, and the mechanical stability of the pellets were influenced by the concentration of additive in the granulation liquid, findings that suggest that a concentration-dependent modification of pectin occurs and that the mechanism and degree of modification are determined by both the type of pectin and the type of additive. An improvement in the size of pectin pellets was observed at concentrations above a threshold value of additives (the cloud point) capable of modifying the polarity of the granulation liquid (all pectin types), modifying the acidity of the granulation liquid (amidated LM), or cross-linking the pectin chains with calcium ions (amidated LM and LM).

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