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Process for selective extraction of pectins from plant material by differential pH

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Abstract

Extraction of natural hydrocolloid carbohydrate polymers, such as pectin, from plant matter is accomplished at somewhat elevated temperature and controlled conditions of acidity/alkalinity. In many cases the plant material contains a variety of different extractables, including non-polymeric carbohydrates (sugars) in addition to the pectins. Very recently two different kinds of pectin, a calcium-sensitive pectin (CSP) and a non-calcium-sensitive pectin (NCSP), have become interesting commercially. What is described in this work is a process to selectively extract NCSP and CSP by varying the pH of the extracting solution. In a first extraction with acidic aqueous solution, a pH between 3.0 and 3.3 without addition of polyvalent salt is sufficient to extract NCSP pectin. A second extraction under stronger acid conditions (pH of about 2.0) is sufficient to extract the remaining pectin, which is primarily CSP.

Keywords: Selective extraction; Differential pH; Calcium-sensitive pectin extraction

1. Introduction

Hydrocolloids extractable from plant materials include pectin, carrageenan, guar and other less well known and less commercially important polysaccharides. These materials are generally considered foodstuffs and are controlled in the USA by Food and Drug Administration (FDA) regulations (Food Chemicals Codex, 1981; Code of Federal Regulations, 1983). The focus of this paper is on pectin, but the process described herein is not limited to pectin and might be applicable to other hydrocolloid extraction processes.

Pectin, which is chemically similar to cellulose, may be thought of as the “glue” that holds the cellulose together in the cell walls of plants. Pectin and cellulose are both polysaccharides, but pectin is primarily α-linked polygalacturonic acid (partly esterified with methyl ester) in which rhamnose may be found, and cellulose is essentially a polymer chain made up of β-1,4 linked sucrose units with no esterification. Not all plant materials are rich in pectin. The oldest pectin source is apple pomace left over from juicing. The most common source of pectin nowadays is from citrus peel, primarily lemon—but lime, orange and grapefruit may also be used. Novel sources of pectin include sugar beets and sunflower heads, but these are not, at the moment, commercially significant. In order to qualify as pectin, the anhydro-galacturonic acid must make up at least 65% of the ash-free dry matter in pectin sold as a commercial product.

There are many processes for extraction of pectin, and there are many uses for these products (Aspinall, 1982; Whistler & BeMiller, 1973; Yalpani, 1988). Typically, a pectin process comprises

1. aqueous extraction from plant starting material;
2. purification of the liquid extract; and
3. isolation of the extracted pectin from the liquid.

Extraction technology is being studied continually (Hwang, Kim & Kim, 1998; Minkov, Minchev & Paev, 1996; Panchev, Kirtchev & Kratchanov, 1989), because pectin is a commercially important product.

Extraction of pectin may be by aqueous acid or base. The basic extraction process yields a pectin of low degree of esterification (low DE pectin) as a result of saponification of the ester groups, whereas the acid extraction process generally yields a pectin of high degree of esterification (high DE pectin), approximately equal to the naturally occurring DE. High DE pectin has a degree of esterification of 50% or greater. Low DE and high DE pectin generally have different uses in foodstuffs, because they gel by different mechanisms. Both are sold commercially. In addition,

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certain other modifications of pectin have found commercial utility, for example, amidated pectin in which the methoxyl groups are partly replaced by amine groups.

In the acid extraction process, plant material is treated with acid at temperatures between 70 and 90°C for a time sufficient to remove desired amounts and quality of pectin from the cellulose plant material. Extract juice from the extraction step is separated from the reaction mixture by filtration. Rotary drum vacuum filtration is common in the industry because the cake is very mushy and difficult to handle. The cake is neutralized and sold as cattle feed or put through a re-extraction step to extract more pectin before being filtered and disposed of. All kinds of equipment common to the extraction arts can be used for pectin extraction—from stirred tanks or pots to continuous counter-current extractors, for example, those described by Leach, Schols, Pyle and Niranjan (1993).

Pectin is precipitated from the extract juice by specified means—either alcohol precipitation (ethyl or isopropyl can be used) or by salting out with aluminum chloride. The precipitated pectin is separated from the precipitating solution by filtration or other means; it is then washed, dried and milled to the desired particle size. During processing the pectin may undergo an ion exchange step to put it in the sodium form for ease of use in foodstuffs applications.

The extract obtained by typical commercial acid extraction is composed of those molecules that are soluble under the conditions of pH, time and temperature used during the extraction. The extract is composed of a mixture of pectins of different molecular weight and degree of esterification. Molecular weights can range from 100,000 to 200,000, but weight-average molecular weights are more typically about 140,000. Control of specific pectin properties for desired end use is achieved by selection of raw material, blending, standardizing by adding sugar or dextrose, etc.

One of the main functional variations between high DE and low DE pectins is their sensitivity to the presence of polyvalent cations such as calcium. This property of the pectin is defined as calcium sensitivity. Calcium sensitive pectins can be gelled in the presence of calcium ions without sugar and are therefore useful for low-fat or sugarless, acidic foodstuff formulations—for which there is a large market, or fat hold-out applications—for which a strong market is developing. Low DE pectins are highly calcium sensitive and are used in low sugar applications where the mix must be gelled (diet jams and jellies, for example). These pectins tend to form very firm, hard gels and are highly pH sensitive. In most commercial applications, a blend of low DE pectins is formed in order to obtain a more broadly adaptable product. High DE pectins that are calcium sensitive tend to retain more water in the gel than low DE pectins (Glahn, 1995a,b) and therefore tend to be softer and more desirable for many end use applications. Thus, the development of efficient ways of producing high DE, calcium sensitive pectins is commercially desirable.

Pectins extracted by the acid process are normally high DE pectins and contain a mixture of both calcium sensitive (CSP) and non-calcium sensitive pectins (NCSP). Analytical and chemical methods have yet to distinguish structurally between the two types, and many theories exist; one of which is that "hairy" regions (Ros, Schols & Voragen, 1998) formed by neutral sugars along the backbone of the chain may "trap" calcium ions or hold them in position, thereby contributing to the gel structure. Another theory is that the distribution of esterified groups along the chain backbone is the critical feature. A random distribution is thought to give relatively low calcium sensitivity, but a block distribution gives much higher calcium sensitivity. The block distribution gives a charge concentration, which may hold calcium ions in place in a gel structure.

Separation of NCSP and CSP from pectin extract juice by selective precipitation is described by Glahn (1995a,b). Selective precipitation is achieved by a precipitating solution of water and alcohol that contains, additionally, a dissolved polyvalent cation, such as calcium. The nitrate salt is generally used for minimization of corrosion in the process equipment and is compatible with the extracting acid (nitric). This solution causes the calcium sensitive pectin to gel, whereas the non-calcium sensitive pectin remains dissolved. By manipulating the concentrations of alcohol and calcium salt and temperature, gel particles can be formed and then separated by screen filtration methods. This approach for producing purified CSP involves the substantial additional use of a calcium salt (for the partial gelation step) and additional separation equipment not normally used for pectin production. The ion exchange step, which is used in typical pectin production processes, is significantly enlarged by this method, because a substantial amount of calcium needs to be added; the calcium concentration in the pectin is therefore high and must be reduced in the product, which is usually the acid or the sodium form of pectin (sometimes referred to as "sodium pectate"). This process also adds significantly to the solvent (alcohol) recovery system, since it uses additional alcohol.

A very recent process disclosed in International Publication WO 9703574, now issued as a European patent (Buchholt et al., 1998) uses a recombinant enzyme to block de-esterify pectin, thereby making it calcium sensitive and useful for the aforementioned commercial applications. The pectin is used to stabilize protein suspensions, e.g. acidified yogurt drinks, whey protein drinks, etc.—a typical application for CSP pectin. This is a sophisticated biotech process, and rather than separating CSP and NCSP, it creates CSP pectin. Because of the advanced technology of this process, it may not be commercially viable at the present time.

In this work we present a process by which naturally occurring CSP and NCSP can be separated directly from the peel, in-situ, by fractional extraction with differential pH and without the addition of polyvalent cation salt. This is done by a sequential, two-step process using different pH conditions in each extraction step. In the first step, mild pH

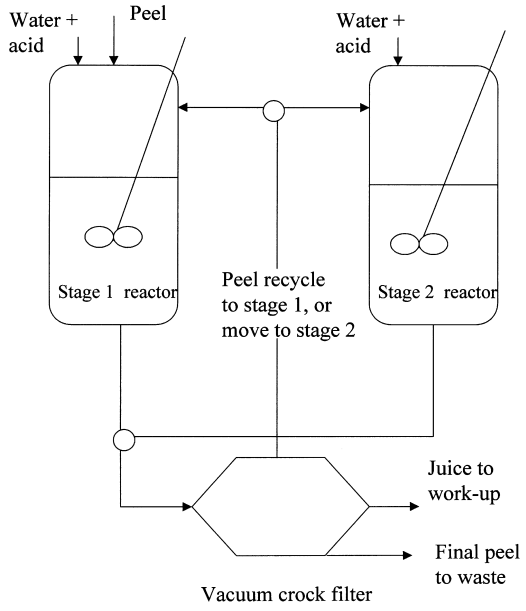

Fig. 1. Experimental apparatus.

conditions (pH of about 3.0 without addition of polyvalent salt) are used to extract pectin that turns out to be NCSP, as determined by analytical tests. In the second step, stronger acid conditions (pH of about 2.0, which is typical of normal pectin extractions) are used to extract pectin which turns out to be CSP, as determined by analytical tests. The general principal of fractional extraction is not new. Various solvents have been used in sequence to extract pectin fractions in the past (Fuchigami, 1990; Raynal, Mourges & Conte, 1991), but the novel feature of the present work is to use the same solvent at different concentration of acid to extract two kinds of pectin, NCSP and CSP.

2. Experimental

Experimental extractions were done by typical pot extraction methods using various sized glass vessels up to 12 l. Fig. 1 is a schematic of the experimental apparatus. Typical peel charges were in the range 100–400 g of dried peel (about 12% moisture) from various sources—mostly lemon peel from Argentina. This is the most popular source for peel, commercially. The extraction vessels were jacket-heated, and slow agitation was used. Temperature was measured by a temperature probe and was 70°C for all experiments. Extraction times lasted from 1 to 3 h, but after about 2 h not much additional extraction takes place. Water-to-peel ratios ranged from 20 to 30, and nitric acid was used for lowering the pH to desired extraction conditions. In some experiments calcium salt was added to inhibit extraction of CSP.

At the conclusion of the reaction step, the mixture was dumped into a large Buechner funnel or vacuum crock into which nylon filter cloth was placed. A rubber diaphragm was used with the vacuum to press the cake. Generally, this simulated the action of a rotary drum vacuum filter typically used in pectin extractions. The filter cake typically contained about 20% solids after this treatment (4/1 liquid-to-solid ratio). In the cases where yield study was important, the cake was re-extracted in the same vessel with an appropriate amount of water and acid. In most cases, the liquid/solid ratio was between 20/1 and 30/1, as this is a typical optimal range in commercial practice. For NCSP extractions (in stage 1 of Fig. 1), the pH was in the range 3.0–3.3. The yields fell off significantly at higher pHs, and at lower pHs CSP started to be extracted. As an alternative, the pH could be lowered to about 2.5 for NCSP extraction, but addition of calcium salt in the rage 1–50 mM was necessary under these conditions to prevent bleed through of CSP. In stage 2 of Fig. 1 CSP is extracted owing to a higher concentration of acid.

The extract juice was then evaporated to increase concentration to about 2% by weight. Then the liquid was ion exchanged with sodium ion exchange resin by stirring in a beaker for about 20 min. The liquid was then precipitated with isopropyl alcohol to about 50% by liquid weight. The resulting gummy mass was then dried overnight at about 50°C in a vacuum oven with nitrogen sweep. The product was then ground to size, typically 100% through an 80 US mesh screen. Samples were then analyzed for molecular weight, degree of esterification, galacturonic acid content, calcium sensitivity, and other chemical features. These tests are generally standard analytical procedures. Some further detail can be found in Joye, Luzio, Soederberg and Taytelbaum (1998).

For optimal yield, the peel could be extracted four times, two for NCSP and two for CSP. This usually results in over 90% theoretical pectin extracted. Following Fig. 1, the peel would be extracted for NCSP in the first stage, then separated in the vacuum crock filter. The juice from the filter would be worked up to recover the pectin: the peel would be returned to stage 1 for another extraction. The first procedure would be repeated, the juice going to a second work-up and the peel going to stage 2 for CSP extraction. The CSP extraction procedure would be done twice, each time the juice going to work-up. The stage-2 peel would be recycled the first time and sent to waste the second.

2.1. Calcium sensitivity test

The calcium sensitivity test has many variants. It is simply the measure of viscosity increase in a pectin solution when calcium ions are added. The specific test used here is as follows:

1. A pectin solution of about 0.6 wt% is prepared using distilled water. The pH is adjusted to 1.5 by using 1 M hydrochloric acid. The pectin must be in the acid or monovalent salt form.
2. 145 g aliquots of #1 are added to viscosity measurement vials.

Table 1
Results of fractional extraction experiments

Conditions and results	4a	5a	8a	11a	4b	4c
STEP 1						
Dilution/pH/time (h)	20/3.3/3	17/2.9/2	20/2.5/3	14/2.5/1	20/3.3/3	20/3.3/3
Calcium added (mM)	0	22.5	45	0	0	0
NCSP yield (g)	23.18	16.31	30.00	32.58	21.92	36.28
Molecular weight	122,000	124,000	158,000	184,000		
Calcium sensitivity (mPa s or cP)	3	5.5	2.5	200	6.5	0.5
GA	76.6	75.4	78.9	77.6	74.5	77.5
DE	74.6	76.9	76.2	74.5	77.9	78.5
Calcium content (ppm)	586	1500	4650	730		
STEP 2						
Dilution/pH/time (h)	17.5/2.0/0.75	17.5/1.75/1.5	17.5/1.5/0.75	17.5/1.5/0.75	17.5/2.0/0.75	17.5/2.0/0.75
CSP yield (g)	47.85	56.93	49.68	43.44	56.07	56.63
Molecular weight	149,000	174,000	177,000	195,000		
Calcium sensitivity (mPa s or cP)	871	634	542	456	556	355
GA	70.7	80.7	76.9	72.7	72.0	68.9
DE	64.8	67.4	66.3	65.9	69.7	68.1
Calcium content (ppm)	195	229	401	222		
YOG	215	202	172	169		
NCSP/CSP split (%)	33/67	22/78	38/62	43/57	22/78	39/61

3. 5 ml of a solution containing 250 mM calcium chloride is added to the 145 g pectin solution to give a final concentration of 8.3 mM calcium.
4. With stirring by a magnetic stirrer, 25 ml of an acetate buffer containing 1 M acetate ions and a pH of 4.75 is added to the pectin solution to bring the pH to 4.2. This also brings the final concentration of pectin to about 0.5 wt%.
5. The magnet is removed, and the glass is left at room temperature (18–25°C) until the next day, when the viscosity is measured at 25°C with a Brookfield viscometer.

Viscosities up to about 800 cP (or mPa s) can be measured in this way. Higher viscosities usually indicate gelification of the solution, and the results are less reliable. If the application demands this kind of performance, the concentration of pectin in the solutions is reduced. The method can be adapted to give a good relative indication of the calcium sensitivity of the samples.

When the viscosity of the same pectin samples is measured without the addition of calcium chloride—diluting with distilled water instead, a base line is established. This is termed CS − , and the former test is CS + , and the difference between the two is the viscosity increase due to addition of calcium ions and is termed “delta CS.” For pectin samples of low calcium sensitivity, this difference is typically less than 20 cP. Viscosities of calcium sensitive pectins, on the other hand, are typically greater than 100 cP.

2.2. YOG test

This test measures the stabilizing power of pectin in acid protein drinks. Solutions of pectin with different concentrations are mixed with yogurt, homogenized and heat treated at 70°C for 10 min. The amount of sediment and the viscosity are measured after cooling to 5°C. The minimum amount of pectin producing the lowest sediment and viscosity is desired. Why stabilization is at a lower viscosity and not a higher is a complex matter of colloid chemistry. Basically pectin will coat the particles up to a point, thereby stabilizing them and preventing coalescence and precipitation; any additional pectin will go to viscosify the solution and will not contribute to stabilization. Therefore, the minimum amount of pectin to stabilize is the desired end. The performance of an unknown batch is compared to that of a standard batch of pectin. This test is very complex and somewhat operator-sensitive, so the exact and lengthy details are not reported here. About 10 different concentrations of pectin are used. The milk solids content of the yogurt can vary, but 8.5% non-fat milk solids concentration was used in these tests. The sedimentation and viscosity of the solution as a function of pectin concentration are plotted for the standard and the unknown. A shift factor between the sedimentation curves and the viscosity curves is determined by a complex procedure, and the YOG grade calculated.

The standard YOG grade is 100. If all the stabilizing power of pectin comes from CSP, and if CSP is 50% of the total pectin, a pure CSP sample should give a YOG grade of 200. If the sample was not pure CSP, the YOG grade would be lower than 200. The parent pectin from which the CSP was separated can also be subjected to this test to give a measure of selectivity.

3. Results and discussion

Of the many results obtained, those given in Table 1 summarize our findings. In the original experiment there were 11 different conditions with one repeat run to make a total of 12 runs. This was done by a fractional statistical design, so that a response surface could be generated to find the significant variables for the extraction method. Time of extraction, peel source, dilution ratio in each step, and calcium level in the first extraction were thought to be significant variables as well as the pH in each step. Three different lemon peel sources from different growing sites in Argentina, designated a, b and c, were used for the data in Table 1. All runs included a short (15–20 min) re-extraction/wash step in each stage. This was done with hot water and additional acid just to maintain pH conditions. Subsequent experiments were then carried out with other peel sources, e.g. grapefruit, for comparison. We report here only the runs from the original experiments that gave significantly positive results for defining the best process conditions for the extraction. The number preceding the letter is a run number indicating type of conditions. All run 4s, for example 4a, 4b and 4c, have the same conditions. In runs 4b and 4c however, the molecular weight information was not available and the calcium content was not measured. In runs 4 and 11 no additional calcium was added to the reactor, so the calcium content of all run 4 materials should be about the same. Some calcium enters the reactor with the peel. This gives about 11 mM concentration at 25/1 dilution ratio. Increasing levels of calcium, added as calcium nitrate, are shown in runs 5 and 8. This is known to inhibit CSP extraction and may thereby increase selectivity as in Glahn (1995a,b) and Joye et al. (1998). We have only reported total weights in the table, not weight of pectin from each individual extraction step.

Runs 4 (without calcium, but at higher pH) and 5 (with calcium, but at lower pH) show similar results. Both give low calcium sensitivity numbers for the NCSP fraction, indicating pure NCSP, but give high calcium sensitivity numbers for the CSP, indicating good purity CSP and a clean split between the two. The overall yields and molecular weights of the two runs are similar, but the split is somewhat different. Run 4 gave a 1:2 NCSP/CSP split, whereas run 5 gave a 1:3 NCSP/CSP split. The CSP in run 5 is less pure than in run 4 as indicated by a lower calcium sensitivity number (871 vs. 634), thus it is likely that more NCSP is in this sample, and adding calcium may not be preferred. As a general guideline, calcium sensitivity numbers less than about 600 are less desirable as product.

Run 8a was done under more aggressive acid conditions (pH of 2.5), but increased calcium addition (45 mM added), and showed similar results to runs 4 and 5 with the “a” peel. However, the calcium sensitivity of the CSP fraction was significantly less, below 600, and the split was somewhat more evenly divided. The YOG grade was much lower than 200, indicating some impurity in the CSP. Therefore, these would not be the desired extraction conditions.

Run 11a also shows loss of YOG, though the split is good. There does not appear to be any CSP in the NCSP fraction, so the NCSP seems to be pure, but there could have been some bleed-through of the NCSP into the CSP fraction which would have lowered the YOG and the calcium sensitivity. The amount of NCSP in the extracted pectin varies with peel source (location) and other variables including time of harvest, but the expected maximum may be as high as 45% of the total pectin. Table 1 shows about 40% for the peels tested here.

The calcium sensitivity test can be used as a measure of selectivity. Run 11a done under aggressive acid conditions but no added calcium shows a split higher than 40%, but also shows a high calcium sensitivity for the NCSP fraction. This indicates bleed-through of CSP into the NCSP fraction and therefore a poor separation (low selectivity). Thus, runs 8a and 11a are not preferred conditions, whereas runs 4a and 5a are. If the extraction time for stage 1 is too long, or the pH is too low, CSP bleed-through will occur (see 11a in Table 1). Generally, stage 2 reaction times could be shorter than 2 h. We have no analytical measure to show that CSP produced in this way is “pure”. In this case, acceptable results are judged by performance in the calcium sensitivity test and by the YOG test described previously. Acceptable results are demonstrated by the data in Table 1, but the judicious use of additional calcium and its exact benefits, if any, await the results of future investigations.

Chemically, the galacturonic acid (GA) content of all extracted pectin samples in Table 1 are typical, between 70 and 80%. The degree of esterification (DE) is higher for the NCSP fraction (74–77%) than for the CSP fraction (64–68%). The reason for this is thought to be the higher acid concentration and the longer contact time of the CSP during the extraction process contributes to de-esterification. The dilution ratios are on a weight basis. The peel charge was 400 g in each experiment, thus the yields on a wt% basis can be computed, if desired. All experiments used a re-extract of about 10/1 liquid-to-wet peel ratio for each Step 1 and Step 2 reactions.

The calcium sensitivity numbers were obtained by the method discussed previously. In the test procedure the concentrations are such that the initial viscosity is very low for both NCSP and CSP samples. This can sometimes not be the case if the CSP sample already contains a significant amount of calcium. Therefore, the initial calcium level of CSP samples are rather important to the test. They should be less than 1500 ppm, preferably below about 500 ppm. This is accomplished by ion exchange before precipitation of the pectin. The calcium content of the NCSP is irrelevant, since this pectin is not calcium sensitive. The calcium levels for both are shown in the table.

The YOG test, as previously described, gives a quantitative measure of performance. A YOG grade of 100 is the standard. The parent pectin may or may not give this grade

(other YOG comparisons can be made with the parent pectin to get a measure of selectivity). If the CSP is pure, the YOG grade should be higher than 100. If the standard contained 50% CSP, the YOG grade of pure CSP should be 200. If the standard contained 60% CSP, then the YOG of pure CSP samples should be somewhat less than 200. If the standard contained 40% CSP, then the YOG grade of pure CSP should be greater than 200. The exact amount of CSP in the standard is not known precisely, but is thought to be about 55%. Then a YOG of 200 would indicate essentially pure CSP, and grades significantly lower than 200 would indicate some NCSP is mixed in with the CSP of the sample, and the extraction method needs improvement.

In the differential extraction process it is not essential to use the same type of equipment for each stage. For example, the NCSP extraction gives a firm peel, which can be separated from the mixture by screen filters, gravity belts, presses or the like. It does not require a rotary drum vacuum filter, as is otherwise common in the art. As a possible alternative, the whole NCSP extraction process could be done in a counter-current screw extractor, of the type common to sugar beet extraction, for example, or the one described in Leach et al. (1993). In this case, the exiting peel may be filtered only once, or not filtered at all—but the peel and juice can be gravity separated. In contrast, the CSP extraction is very messy; the peel is gummy and mushy, and continuous, countercurrent screw extractors do not work very well. Rotary vacuum filters work well separating the juice from this mushy peel, where other types of filters do not.

This work used batch or pot extractions, but the principle of differential pH could also be applied to continuous or other types of extraction technology. It is somewhat surprising that this process should work at all, but we theorize here that the NCSP pectin is the last laid down and has not had time to de-esterify (by enzymatic means, for example) to become CSP. Therefore, mildly acidic conditions are able to remove it from the biomass, whereas these are not strong enough to remove the CSP.

4. Conclusions

We have shown that a two-step process can be used to selectively extract a non-calcium sensitive pectin and a calcium sensitive pectin directly from the peel using a different pH in each step. The first step, extracting NCSP, should be done at a mild pH in the range 3.0–3.3. This can be done without additional calcium salt being added, but if it is desired, calcium salt can be added and the pH lowered somewhat to about the 2.7–3.0 range with similar results. However, a more aggressive acid extraction in stage 1 results in bleed-through of the CSP fraction and lowers the selectivity. The CSP extraction can be done conventionally.

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