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**Interaction of Cloud Proteins, Pectins and
Pectinesterases in Flocculation of Citrus Cloud**

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Project Title: Interactions of Cloud Proteins, Pectins and Pectinesterases in Flocculation
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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order:

AISS - alcohol insoluble solids of the serum; HMP - high-methoxyl-pectin;

LMP - low-methoxyl-pectin; TL - PE - thermolabile PME; TS-PE - thermostable PME;

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Principal Investigator

Signature
Authorizing Official, Principal Institution

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Cooperation Summary (numbers)

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Description Cooperation:

Mutual efforts of both teams, U.S.A. and Israel, were invested to understand the affinity and availability of PME and non-PME proteins to sensitized pectins. The optimal pH activity of PME is 7 whereas pectin is sensitized and cloud is flocculated at the juice pH, 3-4. Thus, considering the natural juice pH (3-4), the roles of proteins, pectins and PME activity in the cloud instability, were studied in pH ranges of 2-7. The researchers of both teams performed and/or demonstrated together experiments in both countries Israel and USA. Firstly, controlled and repeatable conditions and factors responsible to cloud instability were identified and verified in juice-cloud of two orange varieties and from fruits of different areas or countries. Together, both research groups propose that the affinity and availability of PME/non-PME cloud-proteins to sensitized pectins and neutral sugars play role in juice clarification.

Interactions of Cloud Proteins, Pectins, and Cloud Pectinesterases in Flocculation of Citrus

Abstract: The overall objective was to understand the cloud flocculation of citrus juice by characterization of the interactions between proteins and pectins, and to determine the role of PE isozymes in catalyzing this phenomenon. Specific objectives were to: 1. identify/characterize cloud-proteins in relation to their coagulable properties and affinity to pectins; 2. to determine structural changes of PME and other proteins induced by cation/pectin interactions; 3. localize cloud proteins, PME and bound protein/pectates in unheated and pasteurized juices; 4. to create "sensitized" pectins and determine their effect on clarification. The original objectives were not changed but the methods and approach were modified due to specific research requirements. Two postulates were: 1. there is a specific interaction of cloud proteins with de-esterified regions of pectin and this contributes to cloud loss; 2. isozymes of pectin-methyl-esterase (PME) vary in efficiency to create sensitized pectins.

The appearance of citrus fruit juice is an important quality factor and is determined by the color and turbidity that are conferred by the suspended particles, i.e., by the cloud and its homogeneity. Under some circumstances the cloud tend to flocculate and the juice clarifies. The accepted approach to explain the clarification is based on pectin demethoxylation by PME that promotes formation of Ca-pectate. Therefore, the juice includes immediate heat-inactivation upon squeezing. Protein coagulation also promotes cloud instability of citrus fruit extracts. However, the clarification mechanism is not fully understood. Information accumulated from several laboratories indicates that clarification is a more complex process than can be explained by a single mechanism. The increasing trend to consume natural-fresh juice emphasizing the importance of the knowledge to assure homogeneity of fresh juice.

The research included complementary directions: Conditions that induce cloud-instability of natural- juice [IL]. Evaluate purification schemes of protein [USA]. Identifications of proteins, pectin and neutral sugars ([IL]; Structure of the cloud components using light and electron microscopy and immuno-labeling of PME, high-methoxyl-pectin (HMP) and low-methoxyl-pectin (LMP); Molecular weight of calcium sensitized pectins [US]; Evaluation of the products of PME activity [US]. Fractions and size distribution and cloud components [IL-US].

The optimal pH activity of PME is 7 and the flocculation pH of the cloud is 3-4. Thus, the roles of PME, proteins and pectins in the cloud instability, were studied in pH ranges of 2-7. The experiments led to establish firstly repeatable simulate conditions for cloud instability [IL].

Thermostable PME (TS-PE) known to induce cloud instability, but also thermolabile forms of PME (TL-PE) caused clarification, most likely due to the formation and dissolution of inactive PE-pectin complexes and displacement of a protective colloid from the cloud surface [US]. Furthermore, elimination of non-PME protein increases TS-PE activity, indicating that non-PME proteins moderate PME activity [US]. Other experiments Concomitantly with the study of the PME activity but promotes the association of cloud-proteins to pectin. Adjusting of the juice pH to 7 retains the cloud stability and re-adjusting of the pH to <5.5, accelerate cloud instability clarification at pH 3-4. Heat inactivated juice remained relatively stable in the course of the pH changes. Only PME of >40% DE reacts to immuno-labeling in the cloud fragments, whereas <25% DE induce clarification. These findings concomitantly with the structural study and the chemical analyses showed that the cloud flocculation is a result of the association of cloud-protein, pectin and neutral sugars [IL].

Together, the research groups propose that the affinity and association availability of PME and non-PME cloud-proteins to sensitized pectins and neutral sugars influence juice clarification.

Major conclusions, solutions achievements

The pH is one of the important factors in relation to cloud instability. It is well known that optimal PME activity is at pH 7 and 30-60°C, whereas the natural pH of the juice is 3-4, in which the cloud-proteins tend to flocculate. Thus, in order to understand the roles of PME, proteins and pectins in the clarification, the juice was studied within the ranges of pH 2-7, incubation temperature 0-60°C and heat-inactivation at 95°C with the required controls. This experimental system led to establish firstly accurate repeatable conditions for cloud instability, identified according to clarification measurements [IL]. Scattered light transmittance measurements showed significant alterations of juice clarification as a result of pH, considering protein-pectin interactions. The most stable cloud was obtained by altering the pH from 3.5 to 7. Dramatic acceleration of the cloud instability was appeared by reverse decreasing of the pH from 7 to <5.5, whereas total clarification was obtained at pH range 3-4. Heat inactivated juice was remained relatively stable in the course of the pH changes. The structural study of the cloud and analyses were performed for three types of juices: Natural fresh juice (natural-juice), juice that was adjusted to pH 7 (turbid-juice) and juice that was adjusted to pH 7 and readjusted to pH 3.5 (clear-juice).

Major findings from the US group were that not only TS-PE forms but also thermolabile of PE TL-PE forms consistently caused clarification and this was most likely due to the formation and dissolution of inactive PE-pectin complexes and displacement of a protective colloid from the cloud surface. Major findings from the IL group were that cloud stabilization and de-stabilization were due to changes in electrostatic interactions and rapid clarification was observed only after adjustment of juice pH to 7.5 and readjustment to pH 3.5. Together, the research groups propose that PE sensitized pectins competitively bind several cations, including organic and inorganic cations, PE, and non-PE protein and these binding interactions influence clarification of juices.

Cloud fractionation by both continuous and stepped sucrose gradient led to identification of 3 prominent colloidal fractions. In juice of Shamouti orange, the densities the cloud fractions were equivalent to those cushioned upon sucrose densities of 1.8, 1.6 and 1.51 g ml⁻¹ and of Valencia juice 1.8, 1.55 and 1.36 g ml⁻¹. Protein characterization showed differences in SDS-PAGE profiles of both cloud and AISS between natural-juice, stable juice at pH 7 and clarified juice after reverse

change of the pH from 7 to 3.5. Protein profiles of Shamouti and Valencia orange juices in all the treatments were similar. Differences were found between the SDS-PAGE profiles of the whole juice and those of the separated fractions. No significant differences in the protein profiles were observed between the cloud fractions. However, marked differences in SDS-PAGE profiles of the fractionated cloud were found between Shamouti and Valencia juice cloud. Four isoforms of 24.5, 26, 37.5 and 53 kDa of PME were identified in both cloud and AISS proteins by the Western blot procedure. Differences in the band intensities were seen between the treatments. Immunogold labeling localized PME in cytoplasmatic fragment and as expected in the pectin components in cell wall fragments. Only high-methoxyl pectin was detected in cloud cell wall fragments in all of the juice treatments, i.e. in the natural- turbid- and clarified-juices.

In both the natural- and the turbid-juice, different relative portions of protein were found in the cloud matter ($\sim 850/\sim 560$ and $\sim 1375/\sim 617 \mu\text{g ml}^{-1}$, in natural-/turbid- Shamouti and Valencia orange juice, respectively) and in the AISS ($\sim 158/\sim 436$ and $252/1328 \mu\text{g ml}^{-1}$, in natural-/turbid- Shamouti and Valencia orange juice, respectively). In the clear juice, most of the protein is found in the cloud (~ 1005 and $\sim 1466 \mu\text{g ml}^{-1}$, in Shamouti and Valencia orange juice, respectively) and a relative small portion in the AISS (~ 110 and $\sim 120 \mu\text{g ml}^{-1}$, in Shamouti and Valencia orange juice, respectively). Similar trends were found also for the relative amounts of both pectins and neutral sugars. These results show that pectin is associated with the cloud-protein in relation to the clarification phenomenon. In cloud of both Shamouti and Valencia orange, the relative lowest content of pectin was found in the relative higher-density cloud fraction and vice versa. In cloud of Shamouti orange juice, no correlation was found between the fraction density and the relative total amount of neutral sugars ($36\text{-}61 \mu\text{g ml}^{-1}$). A Relative high content of total neutral sugars was found in cloud of Valencia orange juice, with a distinct decreasing trend with the fraction density (142 , 132 and $16.9 \mu\text{g ml}^{-1}$ in the upper, medium and lower cloud fractions, respectively). In general, higher relative amounts of neutral sugars were found than of pectin.

Light and electron microscopy study showed cloud constituents of cytoplasmatic matrix, membrane-like fragments, pigment bodies, cell wall residues and unidentified matter. Relative high structural density of cloud particles and their clusters were found in the precipitate of clear juice (particularly after increase of the pH to 7 and reversing it to 3.5) and dispersed

structural patterns in the turbid-juice. The dense precipitate included osmophilic bodies of essential oil and pigments. The cloud is composed of a wide spectrum of dimensions and shapes. Marked differences in the relative portions of osmophilic structures were observed between the cloud fractions and between clouds of both Shamouti and Valencia juices. It appears that the differences are a result of variability in the relative content of oily components and their connection to other cloud constituents.

The findings of the present study showed the sensitive responses of the cloud constituents to flocculate and in the juice clarification, under conditions that induce protein flocculation. These results indicate the role of cloud protein-pectin binding in the cloud instability.

According to other publications and due to the present results, binding of colloidal proteins and pectins causes cloud instability. The cloud is sensitive to flocculation at the juice pH, indicating that this is the isoelectric range of the cloud-proteins. According to other studies, to our previous work and the present results, it can be suggested that the cloud stability is dependent on both the electrostatic equilibrium between the cloud proteins to the pectins and the tendency of the hydrophobic $-OCH_3$ sphere to be attracted to the hydrophobic proteins zones.

PE activity generates blocks of negatively charged pectin. The de-esterified pectin binds calcium to form large, unstable aggregates. Depending on the conditions of de-esterification and sensitization (pH, PE source, PE purity, PE concentration, presence of other juice components), some juices will clarify more rapidly than others. Proteins, PE, and other large MW cations that bind pectins form complexes that are stabilized by electrostatic repulsion. Cloud loss may be initiated by loss of electrostatic repulsion and/or by formation of large, unstable aggregates. A second mechanism of clarification may be explained by displacement of protein or PE from the surface of pectin and aggregation of sensitized pectin.

Implications – scientific and agricultural

There are multiple mechanisms of cloud stabilization/destabilization, both involve changes in electrostatic repulsion between cloud constituents. The present study showed that reversible alterations of the juice pH cause cloud instability and irreversible clarification. This finding shows that usually, cloud proteins react to flocculation due to both structural and charge modifications, thus leading to interactions of soluble and insoluble pectin and cloud-proteins. It is assumed that

the cloud proteins subjected to structural modifications as a result of pH increase. Re-decreasing of the pH to that of the natural-juice is accompanied by reconstruction changes of the cloud-proteins that accompanied by chemical and structural interactions with pectin polymers. It also can be assumed that heat-inactivation (not only inactivate the PE, but) also stabilizes the cloud-proteins and prevent their structural modifications due to changes of both pH and charge. The results of the present study and the fact that the optimal PE activity is at pH 7 (whereas the natural-juice pH is 3-4) indicate that cloud instability is a consequence of the availability of the cloud-proteins (including the PE protein itself) to interact and interweave with the pectin. Thus, the cloud instability is influenced by both structure of the cloud-proteins and changes in the charge/charge-distribution on the pectin surface due to PE activity. .

After PME sensitization of juice pectins, other components of the juice (pH, cations, minerals, non-PME protein, blocks of de-esterified zones etc.) will influence stability of juices. Most likely, the mineral composition of water to reconstitute juices will influence stability of some juices and propensity of under-processed juices to clarify. Further, production areas, harvest season, and other production practices may influence the natural mineral composition of juices and susceptibility to PE activity.

Significance of main scientific achievements or innovations

Cloud is a prominent quality factor and its instability causes serious marketing damage to the citrus industry whereas juice is the highest bulky product. The accepted theory of cloud instability is based on the formation of Ca-pectate gel, occluding the cloud elements that tend to flocculate and precipitate.

Juices from various batches of citrus fruits are variable in their cloud instability and clarification. Therefore, study of the cloud-instability mechanism requires repeatable and controlled conditions under which stable cloud and/or clarification occurs. The present study has established, firstly, an experimental system for controlled and reliable colloidal flocculation in natural fresh juice. These findings enabled simulation of juice clarification and showed that the cloud instability is due to changes in the properties of the cloud constituents as evidenced by dependence on pH. Considering the approach that attributes PME to cloud instability, findings of

the present study present first indications that deserve consideration of the following aspects: (1) at pH 7, which is the optimal for the PME activity, the juice is quite stable. The juice is totally clarified when adjusted and readjust to pH >5.5-7 and <4.5, respectively. Heat-inactivated juice doesn't clarified following pH modifications; (2) Relatively higher amount of proteins, pectin and neutral sugars are found in the cloud of clear juice than the of the natural- and turbid-juice, and vice versa in the AISS, indicating that polysaccharides are associated with the cloud-protein in relation to the clarification phenomenon. (3) Only HMP was identified by immunogold-labeling in cell wall fragments of the cloud in both stable and instable juice. In this context, it should be mentioned that monoclonal antibodies for HMP (Jim7) react with pectins with DE of about >40%, whereas the classic definition of HMP is DE >50%. However, recent results of the US team (unpublished data) showed that the total DE that causes clarification is relatively low (<25%). (4) PME was identified by Western blot procedure in proteins of both the cloud and the AISS. Immunogold-labeling detected PME in cytoplasmatic fragments of the cloud. (5) No significant differences were found between juices of the various treatments in the content of calcium and other elements of both cloud and serum.

These findings reinforce evidence about a mechanism that leading to formation of protein-pectin-colloid complex responsible for cloud instability syndrome. The cloud proteins play major role in the colloidal instability syndrome. PME isozymes are integral cloud constituents that promote the binding of protein-pectin-colloids. Evaluation of several protein purification schemes showed that thermostable PE was not selectively extracted from Valencia as previously reported for Marsh grapefruit PE. Because insufficient quantities of TS-PE could be obtained to do extensive structural or binding studies either from Valencia or Marsh grapefruit, (CD, DSC of pectin after PE sensitization, gelation studies of pectin after sensitization, etc.), the focus was directed towards evaluation of the products of PME activity. The US team developed the methodology to estimate molecular weight of calcium sensitized pectins (#1, Wicker, Corredig, Kerr, 1999, #2 Corredig, Kerr, Wicker, 2000) but the MW of calcium added PME sensitized pectins were too large to be filtered, a necessary step, before MW analysis. By modifying the extraction and concentration methods, the US team obtained PME fractions with the highest specific activity from Marsh grapefruit (# 3. Corredig, Kerr, Wicker, 2000) and Valencia orange

(#7. Wicker, Ackerley, Corredig) that has been reported in the literature. Of the PME fractions tested, different isozymes had markedly different affinities for the pectin substrate and different clarification ability (#8, Wicker, Shomer, Corredig, Kerr, Merin, 2000), and different clarification abilities (#6, Corredig and Wicker, 2001; #7, Wicker, Ackerley, Corredig, 2001).

Based on communication with the Israeli team and early results, the US team focused on the effect of cations on PME activity, stability, clarification of juices with PME, and clarification of juices with sensitized pectins made from PME. They showed that even relatively high amounts of PME (1-2 U/ml juice) did not clarify juices. This was true for PME added to pasteurized juices as well as freshly squeezed juice. Furthermore, not all pectins that were sensitized by PME, clarified juices. Juices with added 42% DE pectin, sensitized by PME or by alkali, did not clarify. Only juices with 5% DE pectin (alkali sensitized) clarified juice. This suggests that juices are clarified by (either calcium- or protein-pectate) formation, only if highly de-esterified pectins, representing a small percentage of the population, are present. Pectins with moderate or high %DE in juice are stable, in spite of PME activity (#5. Corredig, Kerr, Wicker, 2001). The US team confirmed the role of cations on increasing PME activity, but also showed that cations could increase the propensity to clarification of juices at equal amounts of PME activity (#7 Wicker, Ackerley, Corredig 2001). Another major finding of the US group was the increase in thermostable PME activity by separation of a non-PME protein during ion exchange fractionation. This suggested that not only inorganic cations, but also a non-PME protein could moderate PME activity in juice (#3 Corredig, Kerr, Wicker, 2000, #4, Corredig and Wicker). Major conclusions from the US team is that (1) PME is actively involved in juice clarification due to association of cloud-protein and pectin, (2) other parameters influence PME activity and clarification of juices, such as cations, non-PME protein, (3) particle size of PME active juices or sensitized pectin added juices increases before onset of clarification. These results support the possibility of an additional mechanism of clarification, such as displacement of a protective colloid from the cloud particulates and aggregation.

List of Published papers –US as corresponding author

1. Wicker, L. Corredig, M., Kerr, W.L. 1999. Multi-Angle Light Scattering Estimation of Pectin Molecular Weight and the Effect of Homogenization. Proceedings: 10th Gums and Stabilizers for the Food Industry Conference, The Royal Society of Chemistry, Cambridge, UK. (Refereed)
2. Corredig, M., Kerr, W.L., and Wicker, L. 2000. Molecular Characterization of Commercial Pectins by Separation with Linear Mix Gel Permeation Columns In-line with Multi-angle Light Scattering Detection. Food Hydrocoll. 14: 41-47.
3. Corredig, M., Kerr, W. and Wicker, L. 2000. Separation of Thermostable Pectinmethylesterase from Marsh Grapefruit Pulp. J. Agric. Food Chem. 48: 4918-4923.
4. Corredig, M. and Wicker, L. 2000. Role of Cations in the Catalysis of Thermostable Pectinmethylesterase Extracted from Marsh Grapefruit Pulp. J. Agric. Food Chem. 48: 3238-3244.
5. Corredig, M., Kerr, W.L., and Wicker, L. 2001. Particle Size Distribution of Orange Juice Cloud after Addition of Sensitized Pectin. J. Agric. Food Chem. 49: 2523-2526

Publications in preparation process

1. Corredig, M. and Wicker, L. 2001. Juice Clarification by Thermostable Fractions of Marsh Grapefruit Pectinmethylesterase. Submitted to J. Food Sci.
2. Wicker, L., Ackerley, J.A., Corredig, M. 2001. Clarification of Juice by Thermolabile Pectinmethylesterase is accelerated by Cations. Submitted to J. Agric. Food Chem.

3. Koltunov, V. and Merin, U. and Shomer, I. 2002. The role of pH and cloud-proteins in cloud instability of natural-fresh juice of Shamouti orange. (in preparation).
4. Koltunov, V., Merin, U. and Shomer, I., 2002. The role of pH and cloud-proteins in cloud instability of natural-fresh juice of Valencia orange. (in preparation).
5. Shomer, I., and Koltunov, V. 2002. Cloud structure in orange (*Citrus sinensis* L. osback) juice and localization of PME and pectins (in preparation).
6. Shomer, I., Wicker, Merin, U. L., Corredig, M., Kerr, W.L., and 2002. Structure and composition of proteins and polysaccharides in density-fractionated classes of Orange juice cloud. (in preparation).
7. Wicker, L., Shomer, I., Corredig, M., Kerr, W.L., and Merin, U. 2002. Cloud, clarification, pectin complexes and size distribution of colloidal systems of orange juice.

List of Abstracts – US as corresponding author

8. Wicker, L., Shomer, I., Corredig, M., Kerr, W.L., and Merin, U. 2000. Cloud, clarification, and pectin complexes in colloidal juice systems. Presented at the Food Hydrocolloids Conference, Sept. 10-15, 2000, Raleigh, N.C.
9. Corredig, M., Kerr, W.L., and Wicker, L. 1999. Isolation of three different forms of thermostable pectinesterase from Marsh grapefruit pulp. Institute of Food Technologists, 58th Annual Meeting, Chicago, IL, July 24-28, 1999
10. Wicker, L., Corredig, M., and Kerr, W.L. 1999. Separation and determination of molecular weight of pectins using linear mix gel permeation columns and multi-angle light scattering. 10th

Gums and Stabilizers for the Food Industry, The Past, Present, and Future of Food Hydrocolloids, The North East Wales Institute, Plas Coch, Mold Road, Wrexham, Wales, UK, July 5-9, 1999.

11. Corredig, M. and L. Wicker. 2000. Orange juice clarification by thermostable fractions of pectinmethylesterase. Institute of Food Technologists, 59th Annual Meeting, Atlanta, GA, June 10-14, 2000.
12. Corredig, M., W.L. Kerr, and L. Wicker. 2000. Particle size distribution of citrus cloud as affected by addition of pectin with different degrees of esterification. Institute of Food Technologists, 59th Annual Meeting, Atlanta, GA, June 10-14, 2000.
13. Ackerley, J.L., Corredig, M., Wicker, L. 2001. Clarification of citrus juice is influenced by specific activity of pectinmethylesterase (PME) and inactive PME-protein complexes. Institute of Food Technologists, 60th Annual Meeting, New Orleans, LA June 23-27, 2001.

Appendix

Activities of the US team towards objectives, but not published:

It was hypothesized that %DE would influence the properties of gels made from pectins, and that these would correlate with the propensity to clarify. We proposed to use dynamic mechanical analysis (DMA) to test gels formed from “sensitized” pectins reacted with different divalent cations. At this stage, sufficient quantities of “sensitized” pectins were not available to fully test this theory. However, we did develop the methodology for measuring complex gel properties using commercial LMP reacted with various divalent cations (Ca^{2+} , Mg^{2+} , Sr^{2+}). Gels were formed in DMA sample cups (20 mm diam x 5 mm high) from 0.5, 1 and 2% LMP reacted with 30 and 60 mM of cation (from CaCl_2 , MgCl_2 , or SrCl_2). Samples were analyzed under dynamic compression in the Perkin Elmer DMA7e as a function temperature and frequency, and using the storage modulus (E') and loss modulus (E'') as measures of solid-like and viscous-like behavior. In general, firm gels were formed from both Ca^{2+} and Sr^{2+} , while Mg^{2+} resulted in sol-like samples. No obvious transitions were observed when temperature was increased from 10°C to 80°C, although E' did decrease. Higher pectin or cation concentrations resulted in higher storage modulus (Table x). Gels formed from Sr^{2+} were much firmer (higher E') than those formed from Ca^{2+} . In addition, plots of E' versus frequency suggest that Sr^{2+} gels have higher cross-link density.

Pectin gels were also studied by time-domain ^1H NMR to study water-binding and gel structure. Spin-spin relaxation (T_2) was measured using a Carr-Purcell-Meiboom-Gill pulse sequence on a 20 MHz NMR spectrometer. Relaxation curves were well fit by a single exponential model. Results indicate that T_2 values were lower for the gelled systems (Ca^{2+} and Sr^{2+}). Gels formed from Sr^{2+} had lower values of T_2 , suggesting a higher degree of water immobilization in these systems. Gels were also studied using a pulse-field gradient technique for studying water diffusion. Results showed little difference in moisture diffusion in gels formed from Ca or Sr. In addition, pectin concentration had little effect on diffusion coefficients at the gel strength studied. However, addition of sucrose to the mixture before gel formation did decrease water diffusion. In general, gels will reduce the apparent diffusion coefficient of water because of

effects of restricted diffusion. In turn, this has been used to measure distance between bounding surfaces. However, gels in which restricted diffusion has been observed are generally at higher gel concentration. Studies on more concentrated pectin gels will be continued if methods for making such gels can be developed.

Part of the proposed research was to evaluate the molecular weight, gelling and water relation profile of pectins extracted from PE sensitized juices. Obtaining sufficient quantity of PME was a persistent challenge. When enough PME was obtained to conduct clarification studies, not enough sensitized juice was available to reproducibly measure the gelation profile. In the case of estimation of molecular weight, the pectin from sensitized juice lost solubility, which precluded any type of chromatography. Differential scanning calorimetry (DSC) and fluorescence polarization were not attempted as described in the second year report.

Storage (G') and Loss Modulus for Low Methoxy Pectin Gels

	E' (Pa)	E'' (Pa)
0.5% LMP/25mM Ca^{2+}	2.37×10^4	5.25×10^3
1.0% LMP/25mM Ca^{2+}	4.78×10^4	6.12×10^3
2.0% LMP/25mM Ca^{2+}	9.23×10^4	8.01×10^3
0.5% LMP/25mM Sr^{2+}	5.52×10^4	7.97×10^3
1.0% LMP/25mM Sr^{2+}	8.89×10^4	8.03×10^3
2.0% LMP/25mM Sr^{2+}	1.02×10^5	9.88×10^3

Transverse Relaxation Times (T_2) for Low Methoxy Pectin Gels

	T_2 (msec)
Water	1889
2.0% LMP/25mM Ca^{2+}	901
3.0% LMP/25mM Ca^{2+}	687
2.0% LMP/25mM Sr^{2+}	695
3.0% LMP/25mM Sr^{2+}	494

Apparent Diffusion Coefficients for Water in LMP Gels

	D_w (m^2/s)
Water	1.89×10^{-9}
1.0% LMP/25mM Ca^{2+}	2.87×10^{-10}
2.0% LMP/25mM Ca^{2+}	2.92×10^{-10}
2.0% LMP/25mM Ca^{2+} plus 30% sucrose	3.81×10^{-10}
1.0% LMP/25mM Sr^{2+}	2.95×10^{-10}
2.0% LMP/25mM Sr^{2+}	3.02×10^{-10}
2.0% LMP/25mM Sr^{2+} plus 30% sucrose	3.92×10^{-10}