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INFLUENCE OF PREPARATION AND PROCESSING CONDITIONS ON CRANBERRY GEL PROPERTIES

A Thesis Presented

by

MAUREEN ALANNA PEASE

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

September 2007

Department of Food Science

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DEDICATION

For John and Ruth Pearse. You are a continual source of inspiration.

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ABSTRACT

INFLUENCE OF PREPARATION AND PROCESSING CONDITIONS ON CRANBERRY GEL PROPERTIES

SEPTEMBER 2007

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ABSTRACT

Four formulations of cranberry gels using raw materials manufactured by a variety of different processes were examined for their rheological and textural properties. Generally, with higher treatment temperature and holding times, the gels' rheological and textural properties improved. Gels were examined 24 hours, 48 hours, and 4 days after being prepared. Gels generally showed some further improvement of gel strength during the storage, particularly for gels that were initially processed for the shortest time at the lowest temperature.

The pectin molecular properties of five different types of raw cranberry purees were examined for the effect of processing conditions. Pectin was extracted from each type of puree, yield determined and FT-IR analysis was performed in order to determine degree of esterification of each type of extracted pectin. Degree of esterification ranged from ~60-90%. Puree from fresh fruit and cranberry puree concentrate had the highest yield and degree of esterification, whereas purees from the byproducts of puree processing had lower yields and degree of esterification. Purees exposed to elevated temperatures and prolonged heating times showed signs of hydrolysis.

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CHAPTER 1

INTRODUCTION

The purpose of this study was two-fold. Firstly, the effects of product formulation (raw materials and pre-processing) on the rheological and textural properties of cranberry gels were examined. Secondly, the effect of gelation conditions (time, temperature) on the texture and rheology of cranberry gels were investigated. The conditions mimicked during the study were meant to imitate processing conditions of cranberry gels closely related to those of interest to or currently in practice at Ocean Spray Inc. In addition, changes in the rheological properties upon storage (up to 4 days) were evaluated. Determination of the pectin characteristics of the raw material was intended to determine the role that the properties of the pectin in the cranberry raw materials play in terms of the overall gelation behavior. Gelation of cranberry gels is complex due to the compositional complexity of the raw materials. For example, cell fragments are present in raw materials with sizes varying depending on the pre-processing conditions.

Ocean Spray Cranberries, Inc. (Lakeville-Middleboro, MA) is in the process of changing their production method of cranberry gel from a batch open kettle cook to a continuous process. A major concern is the ability to produce a consistent product with a continuous process. The study encompassed several formulations, (pre-set by Ocean Spray) and conditions in order to determine the formulation and processing methods most conducive to the result desired by Ocean Spray. The formulations provided by Ocean Spray included several different types of raw material, each of which had been processed

in a different manner. This study also allowed Ocean Spray to determine the most effective processing method for the raw material used for producing cranberry gels.

Gels were manufactured under controlled conditions in an oil bath and stored in sealed cans. Rheological and textural properties of cranberry gels were examined after gelation to determine the mechanical properties of the formed gel. The ability of the materials to withstand a prolonged holding period prior to filling was determined by evaluating whether pectin hydrolysis may take place in the event a holding or recirculation step may be required prior to filling.

The pectic substances in cranberries have long been of interest due to the unique properties of cranberry gels. Pectin content and quality is known to have a direct effect on gel strength. To gain some insights into differences between the different formulations, pectin was extracted from each of the raw materials and yield was determined. Raw materials were briefly heat-treated for these experiments in the absence of gel-inducing sugars to prevent microbial growth and to denature any enzymes that may lead to the degradation of the pectic substances in the raw materials. Degree of esterification was then determined. Differences in gel quality could be attributed to variations in pectin content in the raw materials and processing conditions.

The overall objective of this study was to determine which formulation is the most effective in withstanding the rigors and potential variations encountered in the processing plant. Due to the complexity of the raw materials, thorough investigation of the properties of raw materials and the resultant gels is of the upmost importance. The outcome of this study was to recommend a particular formulation as well as processing

conditions to form a high-quality gels (as defined by their textural and rheological properties).

CHAPTER 2

LITERATURE REVIEW

2.1. Fundamentals of Cranberry Gel Processing

Cranberry gel is a traditional product that is commonly consumed by many consumers as an essential part of many holiday meals. In addition, consumption of pectin has attracted increased interest due to the health-promoting effects of polyphenolic compounds present in cranberry. Recent research has shown that cranberry polyphenolics may prevent urinary tract infections by preventing adhesion of pathogenic microorganisms to the intestinal tract wall. (Leahy, M., et al., 2002).

2.1.1 Influence of Processing Conditions on Cranberry Gels

Cranberry pectin has long been of interest for its ability to produce a strong gel at significantly lower soluble solids levels than other commercially produced jellies and jams (Baker and Kneeland, 1936). The gelling ability of cranberries has been attributed to the concentration of pectic substances, soluble solids, and acids in the fruit (Weckel and Swanson, 1972). Cranberry gels are commonly sold as either "whole berry" or "strained" formulations. In the case of the former, gels are either composed of strained cranberry puree mixed with whole fruits. In the case of the latter, gels are composed of strained puree. Cranberry pectin content from raw fruit has been reported to vary greatly with values ranging from 0.4% to as high as 1.2% (Flynn, 1950). This is significantly lower than the pectin content of commercially viable citrus peel (20-30%) and apple pomace (10-15%) (Pedersen, 1978). Cranberry gels are known as slow-setting gels. The processed raw materials after addition of sugar and exposure to high temperatures will

form a gel network within 24 hours after processing, but the gel will continue to gain strength for up to a week after processing (Baker and Goodwin, 1941a).

Due to the low pH of the macerated cranberry fruits (pH < 2.0-3.0), the raw materials may form a gel with less sugar than is typically required in citrus or apple pectin-based jams and jellies. For example, 38-41% soluble solids are sufficient for cranberry gels while 65% soluble solids are used in most commercial jellies (Baker and Kneeland, 1936, Pintauro, 1965). Pintauro (1965) confirmed that cranberry pectin formed a gel at lower than expected pH (≤3.6). It has been found that the addition of citric acid decreases set time and increases final gel strength of cranberry gels at 40% soluble solids (Pintauro, 1970). Pintauro also reported that cranberry pectin has a high degree of methylation, the average being between 79-90%. Process control of cranberry gels is most often accomplished by refractometry or verification of the degree of soluble solids (Weckel and Swanson, 1972). However, this method of controlling gel quality does not always produce a consistent product. A more consistent product may be obtained by either monitoring the cooked product to a standard viscosity, or monitoring pectin content of the raw material and adjusting the gel formulation accordingly (Baker and Goodwin, 1941, b, Weckel and Swanson, 1972). Newer inline sensors capable of conducting these measurements may thus be able to provide a better control over the process outcomes.

The acid content of the raw material can have a significant effect on the degree of methylation, so pH of the raw material should be carefully monitored. The sensitivity of the cranberry gel to the naturally-present acid is due to the high methoxyl content of cranberry pectin. The optimum pH of the gels falls within the range of 2.7-2.9. At lower

pH or at increased exposure to temperatures, possibility for alterations in the molecular properties of cranberry pectin may occur (Pintauro, 1970). The high viscosity of cranberry gels can be attributed to the high molecular weight of cranberry pectin (Pintauro, 1967). Viscosity of extracted cranberry juice has also been shown to be inversely proportional to the concentrations of the characteristic red pigment of cranberry juice in some cranberry varieties (Zuckerman et al., 1966).

When cranberry pectin solutions are exposed to temperatures in excess of 165°F, the solution can undergo as much as a 50% decrease in viscosity in the first 15 minutes of heating (Flynn, 1950, Pintauro, 1967). These studies showed that significant depolymerization of pectin occurs during the first 15 minutes of heating at 165°F or greater (Pintauro, 1967). The hydrolysis of pectin is a major concern during gel processing since it may decrease the strength of the resulting gel, and for that reason temperature exceeding 180°F are usually not recommended. During cranberry gel manufacture, the mixing of the raw materials has been shown to be of great importance. Improper mixing can lead to incomplete incorporation of sugar, which may lead to loss of gel strength (Baker and Goodwin, 1941b). To help insure gel stability manufacturers incorporate pressed cranberries into the formulation. Also, holding cranberry gels for extended periods of time in either refrigerated or elevated temperatures may prove detrimental to gel quality (Pintauro, 1970). Freezing of cranberry gel can weaken the gel network and cause increased syneresis (Boggs and Johnson, 1947).

2.1.2 Influence of Quality of Raw Materials

There is evidence that the maturity of cranberry fruits can play an important role in the strength of gels obtained from these fruits (Pintauro, 1970). As the amount of

insoluble pectin increases in the fruit, the texture becomes firmer (VanBuren, 1974). As the fruit matures pectic substances ("proto-pectin") begin to be converted into pectin (Thakur et al., 1997). Thus immature fruits typically have a lower pectin yield and may produce a weaker gel. Between different varieties of cranberries, there are significant differences in terms of their gelling properties (Flynn, 1950, Pintauro, 1967). It has also been shown that significant differences in pectin concentration and gelation behavior existed in cranberry varieties grown at separate bogs (Flynn, 1950). Weckel and Swanson (1972) reported that degree of maturity, season, horticultural methods, and storage conditions affected the pectin content of cranberries.

All raw materials used in this study were processed from materials previously harvested from Ocean Spray cooperative bogs in Massachusetts, New Jersey and Wisconsin. Purees used for formulations were processed by Ocean Spray (Markham, WA and Kenosha, WI) and then transported to the Lakeville, MA facility. From the storage at the Lakeville facility purees were shipped overnight to the University of Massachusetts, where they were kept frozen at -40ºC until use.

The manufacture of stable cranberry gels is of great concern to industries selling these types of products. Consumption of cranberry gels generally occurs only on occasion, but with regularity, thus a high quality of the product is essential to satisfy consumers. To help ensure gel stability, manufacturers incorporate the hulls of previously processed fruit into the formulation mix. Pectin and pectic substances are contained within the cell wall, so the incorporation of hulls (known as press cake) into the formulation increases the amount of pectin in the formulation, and thus the gel strength.

2.1.3 Gel Processing Method

The processing method for cranberry gels at Ocean Spray has remained the same for over 50 years. The method involves an open kettle cook. The cook is separated into two parts. During the first kettle cook cranberries, water, and press cake are mixed. The mixture is agitated under elevated temperature (heating is accomplished by steam) until a puree is formed. The puree from the first kettle cook is then sent to a finisher to produce a more homogeneous product. The puree is pumped through a finisher screen to remove skins and seeds from the cranberry puree. After finishing, the weighed puree is transferred into a second kettle, where a blend of corn syrups is added. The mixture is agitated through boiling at elevated temperature (heating is accomplished by steam). Upon completion of this step, the sauce is pumped to a can-filler where lids are applied and seamed. The cans are then conveyed into a can cooler and submerged in cooling water. Upon cooling the cans are labeled, packed into cases, and then transferred into the warehouse. The cans of sauce will be stored for several days in order to assure then gels have sufficiently formed before distribution to customers.

This method is known within the plant to be highly variable and it is hard to obtain a consistent product using the open kettle cook. The time of cook can vary, as well as the evaporation of moisture from batch to batch, which can affect the appearance and quality of the gel. Maintaining a consistent temperature throughout the process is also a concern. Too low or too high a processing temperature can adversely affect gel strength. A continuous processing method would be more conducive to a more consistent, higher quality product.

2.1.4 Improved Processing Method

In order to reduce product variability and improve consistency, Ocean Spray is evaluating continuous methods of sauce manufacturing which would eliminate variation associated with the batch process. The continuous process will still employ a two step method of puree extraction followed by blending with sweeteners. However the continuous process utilizes tubular heat exchangers for temperature control, and mass flow meters for blending accuracy. Extracted purees were generated from a continuous pilot plant process using cranberries and two sources of pressed cranberries (West Coast and Mid West varieties) in order to evaluate pectin content and hydrolysis under processing conditions. One stream of cranberry puree was concentrated in an evaporator at low temperature for evaluation. The goal of this study was to compare whether raw materials produced using this method yield gels with comparable gel strength and whether these raw materials may be more robust to variations in subsequent processing conditions.

2.2 Fundamentals of Pectin

2.2.1 Physical and Chemical Properties of Pectin

Pectin is the key component in cranberries responsible for the formation of a gel after heating and addition of sugars. Pectin is generally defined as water-soluble pectinic acids with varying methyl ester contents which are capable of forming gels in addition with sugar and acid when exposed to the correct conditions. Pectin is found in the cell wall between cell wall tissues. Pectin is a component of the middle lamella, or "cement" between cell walls. Softening of fruit during ripening is partially due to the breakdown of pectin and pectic substances in the middle lamella (Bennion, 1980). Pectin can be

extracted from a range of botanically different tissue, and each type of tissue has different pectin properties. Pectin is also stable to acidic conditions (which is a necessity considering the low pH of many fruits). However, covalent bonds are known to be broken in neutral or alkaline pH (May, 1990). Due to its outstanding gelling ability, pectin is a common food ingredient. Pectin is made up of α-(1, 4) linked D-galacturonic acid units linked in a linear fashion. Pectin molecules also contain rhamnogalacturonan, a neutral sugar, which is responsible for splitting and causing kinks in the galacturonic acid chain (Thakur et al., 1997). Pectin is a subgroup of the group of substances classified as pectic substances (Pedersen, 1978).

Pectic substances are "complex colloidal carbohydrate derivatives that occur in, or are prepared from, plants and contain a large proportion of anhydro-galacturonic acid units." Several other substances besides pectin fall under this classification. Protopectin is a substance found in plant cell walls from which pectin is created. Unlike pectin, protopectin is insoluble in water due to the fact that all of its carboxyl groups are esterified with methanol. Enzyme hydrolysis of protopectin within the plant will yield pectinic acids. This leads to the softening associated with the ripening of fruit (Bennion, 1980). During the ripening process, protopectin is converted to water soluble pectin (Yamaki et al., 1979). Storage and heat treatment of fruit may also cause protopectin to be converted to pectin (Luh and Dastur, 1966).

Pectinic acids are polygalacturnonic acid units that contain more than a minimal number of methoxyl groups. Pectinic acids are capable of forming gels when combined with sugar, acid, or with divalent cations, (if the methoxyl content is sufficiently small) (Bennion, 1980). Pectinic acids also contain several neutral sugars such as arabinose,

galactose, rhamnose, and xylose. (McCready and Gee, 1959; Barrett and Northcote, 1965). Pectic acids are made up of polygalacturonic acid units, but contain no attached methoxyl groups (Bennion, 1980). Ripeness and storage temperature of fruit should be carefully monitored, since over-ripe fruit will yield more pectic acid and less pectin, and cold storage may be detrimental to pectin quality (May, 1990).

Commercial pectins are produced mainly by acid extraction of apple pomace and citrus peel (BeMiller and Whistler, 1996). The type of acid used for extraction may have an effect on the quality of the extracted pectin (Pruthi, 1965). Commercial pectin is a by-product of the fruit juice industry. Viscosity of fruit juices has been used as a gauge of pectin content (Bennion, 1980). Pectin is known to be a heat sensitive material, so temperature is monitored closely during extraction in order to obtain the highest quality product possible (May, 1990).

The raw material (pomace or peel) is dried after juice is extracted in order to prevent bacterial or mold growth. Enzymes produced by bacteria and mold are known to produce the enzymes pectin methylesterase and polygalacturonase. Fungal poly-galacturonase cleaves the α-1, 4 bonds between D-galacturonic acid units, which degrades pectin quality and can have an adverse effect on the gelling ability of pectin. These enzymes may work within the pectin chain (endo-enzymes) or may remove poly-galacturonic acid units from the ends of pectin chains (exo-enzymes). Fungal pectin methylesterase attacks the bond between methanol and carboxyl groups in pectin. The enzyme de-esterifies the polygalacturonic acid units in groups, which makes the resultant pectin much more sensitive to calcium despite the degree of esterification. Both types of enzyme degradation can take place over the course of a few hours. These enzymes are

also extracted and produced commercially in order to obtain pectins with a specific molecular weight or degree of esterification (Pedersen, 1978, May, 1990). These enzymes also participate in the normal ripening process of plants containing pectin (Bennion, 1980). Pectin methylesterase and polygalacturonase are also susceptible to heat denaturation (VanBuren, 1974).

2.2.2 Degree of Esterification of Pectin

Some of the carboxyl groups of pectin chains may be esterified with methanol. If the percentage of carboxyl groups esterified with methanol is over 50%, the pectin is referred to as pectin with a high degree of esterification. When the percentage of carboxyl groups esterified with methanol is less than 50%, the resultant pectin is said to have a low degree of esterification (Thrakur et al., 1997).

As the number of carboxyl groups esterified with methanol approaches 100% (protopectin), pectin begins to lose its solubility in water and its ability to form a gel is lost (Bennion, 1980). Most pectins extracted from natural materials classified as having a high degree of esterification usually have less than 75% of their carboxyl groups esterified (Pedersen, 1978). Pectins with a degree of esterification between 60 and 80% are highly soluble in water (Gee et al., 1958).

When pectin is extracted from raw materials, the type of fruit, the degree of ripeness and extraction conditions all play a role in the degree of esterification that the extracted pectin will have. As fruit ripens and is converted from protopectin to pectin, the degree of esterification decreases (Gee et al., 1959). As pectin is extracted from raw material, an alkaline pH can de-esterify methyl groups and thus decrease the degree of esterification of the extracted pectin (Koch and Nevins, 1989). Extended heat processing

seems to have a minimal effect on the degree of esterification (Postlmayr et al., 1956, Yapo et al., 2007).

2.2.3 Molecular Weight of Pectin

Molecular weight of pectin is a key parameter in its ability to form a gel due to the fact that the linear polymer chain must be long enough to accumulate a sufficient number of weak interactions to form stable cross-links (Rees, 1972). The molecular weight of the pectin molecule corresponds to the chain length. Neutral sugars, such as α-L-rhamnopyranosyl, are thought to be responsible for the chain irregularities that limit the size of the chains (BeMiller and Whistler, 1996). Chain length may also be affected by the presence of covalently linked side chains. The linked side chains give the molecule a "hairy" appearance.

Pectic substances have a wide range of molecular weights from 10,000 Da to 400,000 Da (Bennion, 1980). Pectin is known to have molecular weights in the range of 50,000 Da to 150,000 Da. Pectin chains can be composed of a few hundred D-galacturonic acid units or contain as much as 1,000 units. This variability in molecular weight can result in large variations of pectin gels (Pedersen, 1978). A decrease in molecular weight can increase pectin solubility (Thakur et al., 1997). Alkali treatments can decrease pectin chain length, thus decreasing molecular weight (Koch and Nevins, 1989).

Molecular weight of a pectin chain is known to affect its ability to form a gel (BeMiller and Whistler, 1996). Pectin with a higher molecular weight is typically considered to be of a better grade than pectin with a lower molecular weight. Processing of pectin limits the molecular weight of the finished product due to the depolymerization

of pectin chains (Smit and Bryant, 1969). Harsh processing conditions (high shear, temperature abuse etc.) can greatly influence molecular weight. Extreme acid or alkaline conditions can further reduce molecular weight of extracted pectin (Yapo et al., 2007). Alkaline conditions degrade pectin through a β-elimination reaction (May, 1990). In general, a higher molecular weight will yield stronger gel networks (Kertzes, 1951).

2.3 Pectin Gelation

2.3.1 Introduction

One important characteristic of pectin is its ability to form gels. Gelation is the association or cross-linking of long polymer chains to form a three dimensional continuous network, which traps and immobilizes liquid within to form a firm and rigid structure (Glicksman, 1982). The sol to gel transition occurs because randomly dispersed polymer segments associate in such a way that solvent is contained in its interstices.

On a molecular basis, a gel consists of three major elements (Thakur, 1997):

- Associated or cross-linked regions known as junction zones, which may involve a single covalent bond between two chains or a combination of hydrogen bonds and hydrophobic interactions between two polymer chains.
- Interjunction segments of polymers that are relatively mobile cause the gel to be highly flexible.
- Water or solvent that is entrapped in the polymer network.

The characteristic differences between gels arise because of variations in network flexibility, the number and nature of cross-links, attraction and repulsions between network elements, and polymer-solvent interactions. The degree of esterification (DE) in

pectin determines its solubility and gelling behavior. The ester group is less hydrophilic than the carboxyl group; so high ester pectin will gel at a faster rate and at a higher temperature than low methoxyl pectin. High methoxyl and low methoxyl pectins gel under completely different conditions. High methoxyl pectin requires a minimum amount of sugar (usually more than 55%) and a low pH (below 3) to form a gel whereas low methoxyl pectin requires bivalent cations like calcium in order to a form a gel (BeMiller and Whistler, 1996). It should be noted that in high methoxyl pectin gels, an excess of 70% soluble solids will cause pectin to fall out of solution and thus decrease gel strength (Molyneux et al., 1971). In low methoxyl pectin gels, gelation may also be affected by the hardness of the water used during processing, due to the presence of calcium in hard water. (Wilkes, 1992)

The gelation process can be affected by many parameters. These include the degree of methylation, distribution of charge along the backbone, average molecular weight, ionic strength, pH, temperature, and presence of soluble solids. The neutral sugar side chains, (such as α-L-rhamnopyranosyl), can hinder gel formation. These side chains may be able to form weak bonds on their own, thus interfering with the pectin's ability to form a gel network (Thakur et al., 1997). Extracted pectins with the same degree of esterification may show different sensitivities to calcium or different set rates. This difference may be attributed to the differences in fruit the pectin was extracted from (May, 1990).

Pectins are typically negatively charged molecules. The charge can interfere with gelation if the charge density is high enough so that pectin molecules begin to repel one another. As pH increases, the charge density increases, which can prevent pectin chains

from forming junction zones (Thakur et al., 1997). An increase in pH can also cause splitting of glycosidic bonds within the pectin molecule, lowering the molecular weight of the molecule and decreasing its gelling ability (Albersheim et al., 1960). If the pH is well above the p*Ka* value for the acid groups of the pectin chain, then the negative charge of the chain will likely prevent gelation (May, 1990)

2.3.2 Gels from Low Methoxyl Pectins

The strength of low methoxyl gels is dependent on the pectin molecular weight, degree of polymerization, and calcium binding power (Thakur et al., 1997). Amidation, (via reaction with ammonia) of low methoxyl pectin also increases the strength of the resultant gel. An increase in ionic strength and a neutral pH both decrease the amount of calcium needed to form a gel (Thakur et al., 1997). Low methoxyl gels may also be formed over a wider pH range than their high methoxyl counterparts (pH 3-6), but as pH decreases the rate of gelation generally increases (May, 1990). Thus low acid pectin gels may require significantly longer setting time. The addition of sequestrants such as citrate or other fruit anions has a noticeable effect on low methoxyl gels and can be beneficial to gel strength. Care should be taken, however, when adding citric acid to a low methoxyl gel, since it may interfere with the complexing ability of divalent ions such as calcium (May, 1990).

Low methoxyl pectin forms gels through divalent ion, such as Ca^{++} or Mg^{++} (Bennion, 1980). Low methoxyl pectins do not require sugar in order to form a gel network, and thus are used to produce and formulate reduced and no-sugar gels (BeMiller and Whistler, 1996). The relative abundance of free carboxyl groups found in low methoxyl pectin allows positively charged divalent cations to form “bridges” with the

negatively charged free carboxyl groups. Calcium "bridges" are formed between two separate chains that are in thus eventually forming a three-dimensional network (Thakur et al., 1997). A section of backbone of the pectin molecule must be free of ester groups for calcium ions to be able to form this "bridge". The structure of the gel formed with divalent ions and pectin is known as 'egg box' model (May, 1990). These "bridges" are formed by unbranched, non-esterified galacturonan blocks bound by calcium ions. By increasing the amount of calcium in a gel, the gel strength and gel temperature increase as well (BeMiller and Whistler, 1996).

The addition of excess amounts of calcium can have adverse effects on the pectin gel, however. At pH 3-5, excess calcium can cause extensive cross-linking within the gel and syneresis may occur (Thakur et al., 1997). If the concentration of pectin is high in a low methoxyl gel, an addition of a small amount of calcium will yield an elastic gel. However, increasing the level of calcium when the pectin concentration is low will cause a brittle gel to be formed and in many cases syneresis will result (May, 1990). Low methoxyl pectin generally produces more brittle, inelastic gels than high methoxyl pectins and thus mouth feel of the two gels are completely different (Speiser et al., 1946). Due to the versatile properties of low methoxyl pectin, it can be used as not only as a gelling agent but also as a thickener (May, 1990).

The gel temperature of low methoxyl pectins is dependent on several factors: percentage of soluble solids, calcium concentration, pH, and pectin structure (Pedersen, 1978). Low methoxyl gels are also highly dependent on the degree of esterification, (between 30-50%) and are not less affected by changes in molecular weight (Speiser and Eddy, 1946).

2.3.3 Gels from High Methoxyl Pectins

The formation of high methoxyl gels requires the presence of sugar and acid (Bennion, 1980). As acid is added, the highly charged and hydrated carboxyl groups are converted into uncharged, only slightly hydrated carboxylic acid groups. The loss of charge and hydration allows for the pectin molecule to associate with other chains and for junction zones to form. The formation of junction zones is aided by the addition of sugar, which competes for the water and thus lowers the solvation of the polymer chain, which promotes chain-chain interactions (BeMiller and Whistler, 1996). For this reason, properties of high methoxyl gels depend strongly on molecular weight of pectin due to the increase in hydrogen bonding as a result of increased chain length (Speiser and Eddy, 1946).

Junction zones in high methoxyl pectin gels are stabilized by the presence of hydrogen bonds and hydrophobic interactions between pectin chains (Thakur et al., 1997). Hydrophobic interactions within the gel are promoted by the unfavorable interactions between water and the methoxyl groups of the pectin chain. Consequently, the methoxyl groups have a tendency to associate with each other in order to reduce the contact area with water. By reducing the number of non-polar methoxyl groups that are in direct contact with the water, the conformational stability of the network is increased (Thakur et al., 1997). It is thought that pectin molecules form a 'random coil' promoted by these interactions are controlled by the degree of hydrogen bonding and hydrophobic interactions (Chou et al., 1991).

The amount and type of soluble solids used in the gel also contributes to the overall gel strength. The strength of hydrophobic interactions are affected by both

amount and type of solute (soluble solids), and thus the temperature of gelation, gel strength and kinetics of the development of the gel structure are all affected by the type of soluble solid (sugar) used to produce a high methoxyl pectin gel (Thakur et al., 1997). High proportions of glucose or fructose in a gel are known to be detrimental to forming a high methoxyl pectin gel network. Large concentrations of glucose may lead to pH increases, thereby increasing gel setting temperature. Conversely, a large proportion of fructose in a gel can lower the gel setting temperature. A common solution in industry is using a mixture of glucose and fructose syrups to obtain desired the gel quality (May, 1990).

As mentioned, hydrogen bonding in high methoxyl gels contributes gel stability. Hydrogen bonding may occur between the oxygen atoms of carboxyl groups. Hydrogen bonds are very weak and are easily broken, but due to the sheer number of bonds that may occur in a gel network, they make contribute substantially to the thermodynamic stability of the gel (Thakur et al., 1997). With an increase in pectin concentration, the number of hydrogen bonds in a gel network will increase (Kjøniksen et al., 1995).

The firmness of high methoxyl pectin gels is dependent on temperature of storage, pH, pectin concentration, and the type of sugar used in the gel (Thakur et al., 1997). At pH values well above the *pKa* value for carboxyl groups, the pectin molecule has a sufficiently negative charge to prevent gelation. By reducing the pH, gelation may occur but require initially a very high (~80% soluble solids) sugar content. The required soluble solids level may be reduced by continuing to decrease the pH. However, a minimum 55% soluble solids is needed to prevent that high methoxyl pectin gels from beginning to lose gel strength. (May, 1990)

Setting temperature in conjunction with pH may also affect the quality of a high methoxyl pectin gel. When soluble solids content is constant in a gel, an increase in pH can cause a decrease in strength above a certain critical limit. As the pH increases, the setting temperature decreases. As pH decreases, the setting temperature of the gel increases. If the setting temperature increases to the boiling point, this can have an adverse effect on the gel structure. The action of the boil can cause shearing and disruption of the gel network and syneresis and an incomplete gel network are the common result (May, 1990). Temperature during storage of the gels is also very important, and can have a major impact on pectin molecule interactions and the time needed to form a gel (daSilva and Gonçalves, 1994).

2.4 Summary

Gelation of pectin is a complex process that is influenced by both the raw materials properties as well as processing conditions. As a result, production of consistent gels with pectin raw materials is difficult at best and variations may readily occur. Strength may be build into the product by ensuring that sufficiently high levels of pectin are present and by carefully controlling the environmental conditions, namely pH, mineral levels and soluble solids content.

In this study, a variety of pectin gels will be manufactured from various raw materials and their properties is investigated. Differences between these gels in terms of their behavior and strength of formulation will be discussed.

CHAPTER 3

METHODS AND MATERIALS

3.1 Materials

Raw materials were provided by Ocean Spray Cranberries, Inc., (Lakeville-Middleboro, MA) (Table3.1). All raw materials were pasteurized prior to shipment.

Table 3.1. List of ingredients supplied by Ocean Spray Inc.

Ingredients	Weight of sample, grams
Hot extracted cranberry puree	3250
Hot extracted puree from pressed cranberries (west coast)	3250
Hot extracted puree from pressed cranberries (mid-west)	3250
60/40 sweetener blend	2750
Standard jellied sauce cranberry puree	2740
24 Brix cranberry puree concentrate	~600

3.1.1 Standard jellied sauce puree

This puree was formulated from a mixture of whole cranberries, water and pressed cranberries. Press cake puree is a mixture of rice hulls and the cranberry hull waste produced during production of the cranberry fruit juice. Cranberry fruit puree is produced from thawed frozen cranberry fruit. The mixture is heated in a kettle, milled, and then run though a finisher screen to remove skins and seeds. The puree was immediately cooled and frozen prior to shipment.

3.1.2 Hot extracted puree

This puree was produced from whole cranberries. The fruit was milled and heated in a heat exchanger prior to finishing. The puree was immediately cooled and frozen prior to shipment.

3.1.3 Hot extracted purees from pressed cranberries (west coast and mid-west)

These purees were produced from two sources of cranberries. The pressed cranberries are frozen after pressing and have a moisture content of 70-75%. The frozen pressed cranberries are milled, rehydrated with water at approximately 4:1 ratio (water/fruit). The mixture was heated in a heat exchanger prior to finishing. The purees were immediately cooled and frozen prior to shipment.

3.1.4 Cranberry puree concentrate

This puree was produced from hot extracted cranberry puree. Puree was concentrated at ~145°F (63°C) by evaporation. Ideal concentration ratio was 3:1.

Gels were formulated with these raw materials according to pre-set formulations provided by Ocean Spray (Table 3.2). Raw materials were frozen until use and then stored at refrigeration temperatures during use.

3.2 Methods

3.2.1 Preparation of Samples

3.2.1.1 Gel Preparation

The first study involved mixing and heating pre-mixed formulation to form a gel. Raw materials were weighed and thoroughly mixed according to the formulation chart provided by Ocean Spray using a blender at low speeds (Table 2). Mixed formulations were first heated to 70-80°C in a microwave. The mixed formulations were then poured into lined tin cans and immediately heated to one of the pre-determined temperatures, (88, 93, or 99°C) and held there for one of three pre-determined time spans (2, 5, or 15 minutes). Cans were sealed and quickly set in an ice bath to rapidly cool them. Once

cooled, cans were stored at room temperature for 1, 2, or 4 days. After storage, cans were opened and gels were subjected to texture analysis and small strain rheological tests.

In the second part of this thesis, tests were conducted to monitor the gelation process itself. In this case, the raw materials were prepared as mentioned before by mixing of raw ingredients. According to formulations shown in Table 3.2 formulations were then loaded in a rheometer and subjected to a simulated heating cooling cycle (see later).

Table 3.2 Composition of four cranberry formulations used to produce gels after heating and cooling processes. Formulations were specified by Ocean Spray Inc. and represent typical formulations used in conventional batch cooks and in newer continuous cooks.

Formula A	**Weight %**
Hot extracted Cranberry Puree	35.2
Hot extracted puree from pressed cranberries (west coast)	18.1
60/40 sweetener blend	46.7
Formula B	**Weight %**
Hot extracted Cranberry Puree	35.2
Hot extracted puree from pressed cranberries (mid-west)	18.1
60/40 sweetener blend	46.7
Formula C	**Weight %**
60/40 sweetener blend	46.7
Standard Jellied Sauce Cranberry Puree	53.3
Formula D	**Weight %**
Water	24.2
24 Brix cranberry puree concentrate	11
Hot extracted puree from pressed cranberries (mid-west)	18.1
60/40 sweetener blend	46.7

3.2.1.1 Pectin Extraction

In the second part of the study, pectin was also subjected to degree of esterification analysis. To this purpose, pectin was extracted from thawed raw material using a modified method described by Pagán et al. (2001). Raw materials were freeze dried before extraction began. Freeze dried material was mixed with distilled water acidified to pH 2 at 150 rpm at 60°C for 1 hour. Solids were then separated from the extract using a Buchner funnel with Whatman No. 1, (Sigma, St. Louis, MO) filter paper. Pectin was extracted from the solution by using an equal volume of 95% w/w ethanol to induce phase separation and flocculation. The pectin solution was stirred at 150 rpm for 15 minutes and then refrigerated for 1 hour. The pectin was extracted again using a Buchner funnel and Whatman No. 1 filter paper. The resultant mass was freeze dried and then weighed to calculate yield.

3.2.2 Rheology Experiments

3.2.2.1 Introduction

All rheological investigations have been carried out using a coaxial cylinder rheometer (Sherman, 1970; Pal and Rhodes, 1989). The MCR 300 from Paar Physica rheometer with built in coaxial cylinder setup was used for the measurements. The principal design of a coaxial cylinder rheometer is shown in figure 3.1.

Rotating the inner cylinder of the system creates a flow profile within the gap between inner and outer cylinder. The Bohlin CS-10 is a Searle type system in which the inner cylinder is driven as opposed to a Couette type rheometer where the outer cylinder rotates. A Searle type system is easier to adjust in temperature however at high shear rates; the development of Taylor instabilities can cause turbulent flow thereby rendering the measurements invalid.

The basic fluid mechanical equations that govern the flow behavior of the sample in the gap stem from a force balance done in the three principal directions of the cylindrical coordinates r, θ and z:

$$r: -\rho \frac{v_\theta^2}{r} = \frac{1}{r}\frac{\partial\left(r\tau_{rr}\right)}{\partial r} - \frac{\tau_{\theta\theta}}{r} \tag{3.1}$$

$$\theta: 0 = \frac{\partial\left(r^2\tau_{\theta r}\right)}{\partial r} \tag{3.2}$$

$$z: z: 0 = -\frac{\partial p}{\partial z} + \rho g z \tag{3.3}$$

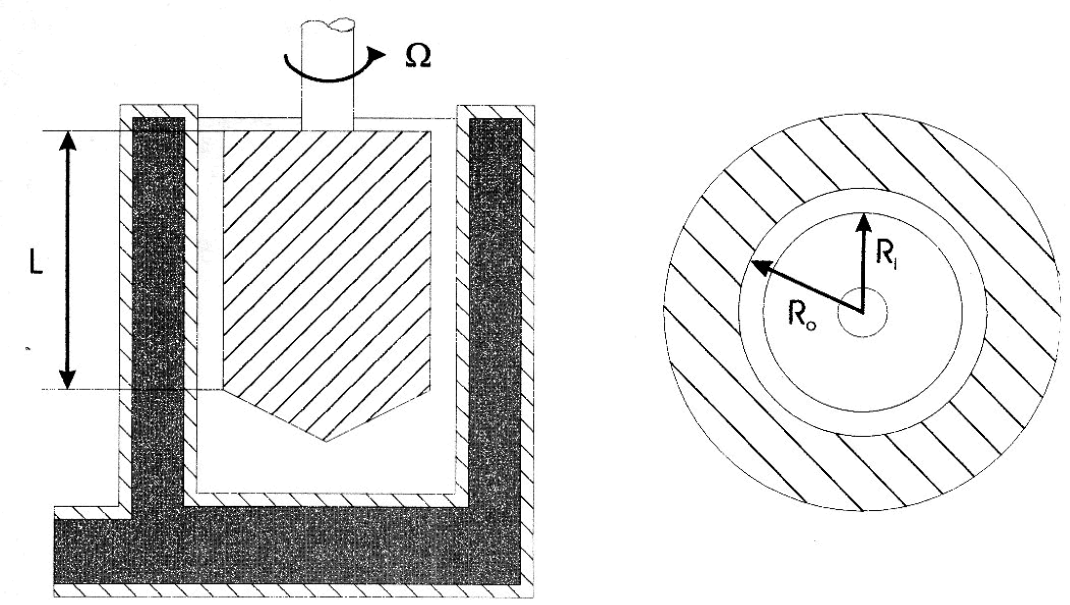


Figure 3.1. Side view and birds eye of the experimental setup for a coaxial cylinder rheometer.

By inserting the boundary conditions we get the following expression for the stress at the inner cylinder:

$$\tau_i = -\frac{M}{2\pi R_i^2 L} \tag{3.4}$$

Where M is the momentum, L the length of the inner cylinder and R_i the radius of the inner cylinder. Correspondingly the stress on the outer cylinder is given by the same

equation using R_o for the radius. The ratio between inner and outer stress is therefore defined as $\beta = (R_i/R_o)^2$ .The shear rate is given by:

$$\gamma(r) = -r\frac{d\omega}{dr} = \frac{2\Omega_i}{1-\beta} \tag{3.5}$$

Here ω is the angular velocity and can generally be a function of time. Therefore all the basic rheometrical experiments can be conducted depending on the time dependent function of the angular velocity. Figure 3.2 illustrates the actual experimental setup of the Paar Physica - MCR 300.

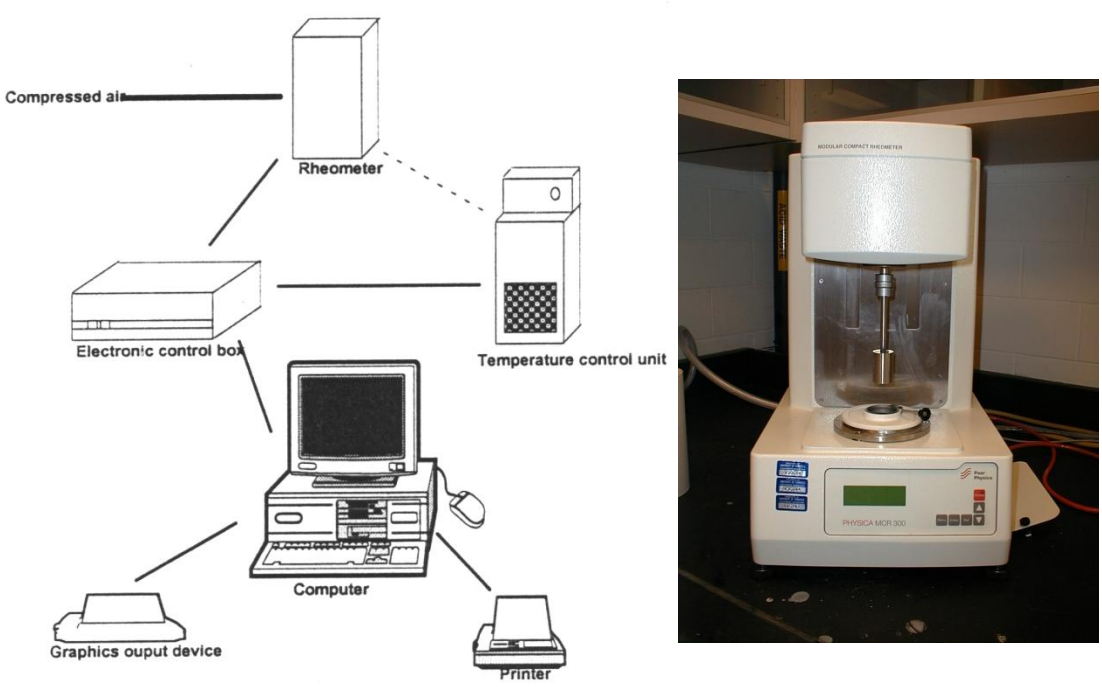


Figure 3.2. Setup of the Paar Physica MCR 300 rotational rheometer.

3.2.2.2 Flow Curves

For hydrolysis experiments conducted in the second part of the study, flow curves of pectin raw materials in the absence of soluble solids (to prevent gelation) were conducted. Raw materials were exposed to the same processing temperatures used in the gelation experiment (88, 93, 99°C) for 0, 15, 30, 45, 60, and 90 minutes. After heat treatment, raw materials were allowed to come to room temperature (25°C ±1). Once heat treated, shear tests were performed on all raw materials using the rheometer. Viscosity of solutions was measured as a function of strain rate using coaxial cylinder geometry. Strain rates varied from 0.01 to 500. The shear tests were used in order to

determine changes in viscosity with heat treatment due to hydrolysis of pectic material in the raw cranberry material. Approximately 19ml of sample was loaded into the cylinder. The single gap cylinder (CC27) was used with an outer radius of 14.46mm (Anton Paar, Graz, Austria).

3.2.2.3 Dynamic Strain Sweep and Frequency Sweep Test

Two types of tests were performed to determine properties of gels after gelation had occurred and samples had been stored for various times (Figure 3.3). For these tests, plate-plate geometry was used for the analyses. Strain and frequency sweep data was obtained using the PP25 plate, 24.94 mm diameter (Anton Paar, Graz, Austria) and a gap size of 1mm. The bottom plate used during the experimentation was a TEK150P/MC1 (Anton Paar, Graz, Austria) equipped with a Peltier-type temperature controller in combination with a water bath. The maximum shear rate used during testing was 2,618 1/s and the maximum shear stress obtained was 6,111 Pa. Samples were cored using a stainless steel corer provided by Ocean Spray, Inc. The cored gel was sliced into samples approximately 1mm in height. The strain sweep was conducted using small amplitude oscillatory strain of 0.001-1 at 1Hz frequency at 25°C.

Frequency sweeps were used to determine the dynamic rheological properties of the gels (G', G"). Frequency sweeps were conducted from 0.1-100Hz at 25, 50, and 70°C at a strain of 0.1. All data was collected using a computer program (US 200 version 2.21, Stuttgart, Germany).

3.2.2.4 Gelation Kinetics Tests. To determine kinetics of gelation, dynamic rheological properties were determined during heating and cooling. Formulations were mixed as previously described and loaded into the cup and bob system. A second series of tests were conducted with the addition of 50mM $CaCl_2 \cdot H_2O$. Tests were conducted over 30-95°C, held at 95°C, and 95-30°C over 2 hours at 1 Hz.

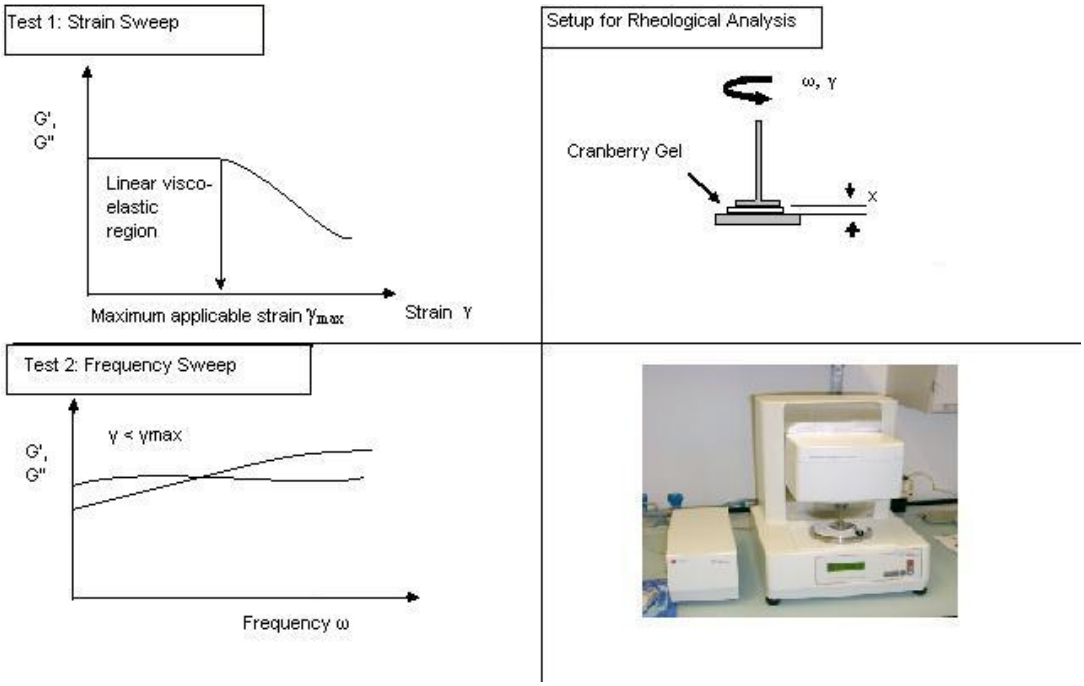


Figure 3.3. Dynamic Strain Sweep and Frequency Sweep Test.

3.2.3 Texture Analysis

Large strain rheological tests or texture analysis were performed using an Instron Texture Analyzer 5542, (Norwood, MA).

A series of three tests were performed in order to determine the gels' final texture properties. Gels were cored and sliced into samples approximately 1 inch in height. Tests performed included compression, compression-relaxation, and compression cycle (Figure 3.4). Data was collected using a computer program (Instron Merlin version 3.30, Norwood, MA). For all tests, a 10kN compression load cell with a diameter of 25 mm

was used. The load cell compression speed was set at 10 mm/min for all tests. The surface of the compression plates was stainless steel. Plates were lubricated with mineral oil before testing.

3.2.3.1 Compression Test

Compression tests were used to determine the breaking point of the gel. The gels were compressed to 25% of their initial height. Data was analyzed to determine force deformation and break load of the gels.

3.2.3.2 Relaxation Test

Compression-relaxation tests were performed in order to determine characteristic relaxation times. Samples were compressed to 75% of their original height for a span of 200s. A mathematical model was used in order to calculate the relaxation time from the raw data.

$$\sigma = f(t) = \sigma_e + (\sigma_o - \sigma_e)\exp(-t/\lambda_{rel}) \quad (3.1)$$

A simple version of the Maxwell model was used to analyze data. It included a coefficient to represent the equilibrium stress (σ_e). The mechanical model represents the energy storage portion of the material behavior with a characteristic elasticity of σ_e while the viscous part if represented by a characteristic relaxation time constant λ_{rel} (Steffe, 1996).

3.2.3.3 Recoverable Work.

Finally, compression cycling was performed in order to determine recoverable work of the sample. Samples were compressed to 65% of their original height and the load released. This stress test was then repeated a second time.

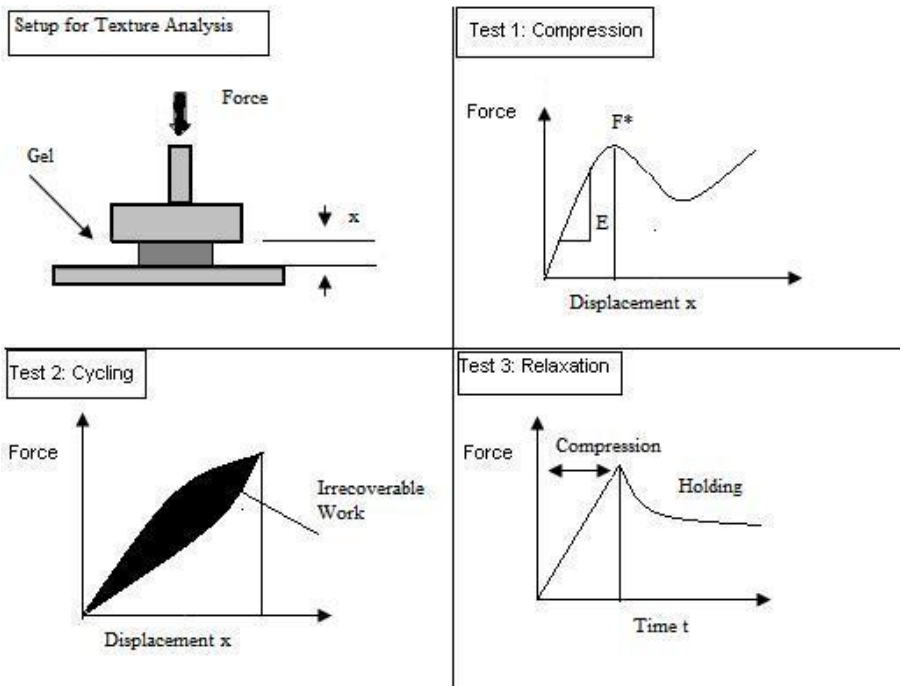


Figure 3.4. Schematics of the three different texture analysis protocols.

3.2.4 Infrared Spectroscopy to Determine Degree of Esterification

The degree of esterification of the extracted pectin was analyzed by FTIR, (Fourier transform infrared spectrophotometer) analysis. A standard curve was prepared using pectin with known degrees of esterification, (30, 60, and 90%), (Sigma, St. Louis, MO). FTIR spectra were collected using an IR Prestige-21 FTIR outfitted with a DLATGS detector. Spectra were collected using an Attenuated Total Reflectance, (ATR) accessory against a KBr background, with 4cm^{-1} resolution, 64 scans, and range of 600-4,000 cm^{-1}. All spectra were analyzed using the computer program IR Solution version 1.20, (Shimadzu, Kyoto, Japan) Peakfit software for line curve fitting. Spectra were analyzed using the method described by Chatjigakis et al., (1998). Degree of esterification is calculated as the number of esterified carboxylic groups / number of total carboxylic groups) x 100. Two specific frequencies, namely the bands at 1749 and 1630 cm-1 were used in order to calculate degree of esterification. The carboxyl ester groups

absorb at about 1740 cm-1 whereas the corresponding carboxylate groups absorb at about 1600 cm-1. The ratio of the areas of the bands at 1749 cm-1 over the sum of the areas of the bands at 1749 and 1630 cm-1 should be proportional to the degree of esterification. When an appropriate calibration curve relating to the ratio of areas $A_{1749}/ (A_{1749}+A_{1630})$ to the degree of esterification is established, the degree of esterification of the pectic molecules can be determined from the FT-IR spectroscopic data.

CHAPTER 4

TEXTURE AND RHEOLOGY OF CRANBERRY GELS FROM CRANBERRY PUREE OR SAUCE AS A FUNCTION OF HOLDING TIME AND TEMPERATURE

4.1 Abstract

Four different formulations of cranberry gels using raw materials manufactured by a variety of different processes were examined for their rheological and textural properties. Generally, with higher treatment temperature and holding times, the gels' rheological and textural properties improved. Gels were examined 24 hours, 48 hours, and 4 days after being prepared. No discernible pattern was observed with increased storage time during these experiments. The lack of improvement over time could be due to the non-permanence of hydrophobic interactions between pectin chains. The lack of any increase in elastic modulus or gel strength could also be due to variations in the mobility of pectin chains and the duration of junction zones between pectin chains.

4.2 Introduction

The rheological properties of pectin gels containing apple and citrus pectin have been extensively studied (da Silva and Rao, 1995; da Silva and Gonçalves, 1994; Chou et al., 1991). However, there is a lack of studies into the rheological and textural properties of cranberry gels. The use of small amplitude oscillatory rheology can provided insight into the conformation of pectin gelation networks (Chou et al., 1991).

For example, measurement of storage (G') and loss (G") can be used to obtain information about changes in the material properties during formation of networks and during storage. The storage modulus represents the elastic properties while the loss

modulus represents the energy that is dissipated and is lost (the viscous contribution to the overall material function). Analysis of G' and G" over a range of frequencies allows for the analysis of the time-dependent behavior of the gels, i.e. the response to the superimposition of a short-term or long-term stress situation (Whittingstall, 2000). Testing textural properties of cranberry gels are important for determining its strength and deformation characteristics after setting and are a good tool to evaluate the overall quality of the gel after production (Whittingstall, 2000).

Time and temperature considerations can be critical to network formation in biopolymer systems. Both time and temperature can have a major impact on the number and type of bonds formed between the polymer chains. Rao et al., (1993) reported that the value of G' for pectin gels did not significantly increase during the first 24 hours of storage, but improvement was seen after 14 days of storage. Temperature of treatment for gels also must be favorable to the development of hydrophobic interactions and hydrogen bonding (da Silva and Rao, 1995; da Silva and Gonçalves, 1994).

In this study, four different cranberry formulations were subjected to a series of time – temperature profiles in order to observe the effect of varied time and temperature treatments on the gel properties with the goal of determining optimal conditions to produce stable and sufficiently elastic cranberry gels. After treatment, gels were held at ambient temperature for up to 4 days in order to observe whether changes in material properties took place after the initial gelation.

4.3 Materials and Methods

4.3.1 Rheometry

A rotational rheometer with a plate-plate geometry and cup and bob geometry (MCR 300, Paar Physica, NJ) and the accompanying US 200 software were used to measure the rheological properties of pectin gels. Dynamic strain sweep and frequency sweep tests were performed using plate-plate geometry and gelation kinetics tests were performed using cup and bob geometry. The temperature of the measuring system was controlled by a Peltier unit. Samples were filled in the temperature controlled cylindrical measuring cup and quickly equilibrated to the required temperature (30^{o}C) prior to each experiment.

Strain and frequency sweep data was obtained using the PP25 plate, 24.94 mm diameter (Anton Paar, Graz, Austria) and a gap size of 1mm. The strain sweep was conducted using small amplitude oscillatory strain of 0.001-1 at 1Hz frequency at 25°C. Frequency sweeps were used to determine the dynamic rheological properties of the gels (G', G"). Frequency sweeps were conducted from 0.1-100Hz at 25, 50, and 70°C at a strain of 0.1.

To determine kinetics of gelation, dynamic rheological properties were determined during heating and cooling. Formulations were mixed as previously described and loaded into the cup and bob system. A second series of tests were conducted with the addition of 50mM $CaCl_2 \cdot H_2O$. Tests were conducted over 30-95°C, held at 95°C, and 95-30°C over 2 hours at 1 Hz.

4.3.2 Texture Analysis.

A series of three tests were performed in order to determine the gels' final texture properties. Compression tests were used to determine the breaking point of the gel. The gels were compressed to 25% of their initial height. Data was analyzed to determine force deformation and break load of the gels. Compression-relaxation tests were performed in order to determine characteristic relaxation times. Samples were compressed to 75% of their original height for a span of 200s. A mathematical model was used in order to calculate the relaxation time from the raw data. Finally, compression cycling was performed in order to determine recoverable work of the sample. Samples were compressed to 65% of their original height and the load released. This stress test was then repeated a second time.

4.3.3 Statistics

All measurements were conducted in triplicate using duplicate samples. Values shown in figures correspond to mean averages.

4.4 Results

4.4.1 Dynamic Amplitude Sweeps of Cranberry Gels

4.4.1.1 Influence of Holding Time on Strength of Gel as Measured by Amplitude Sweeps

Amplitude sweep measurements were conducted for all gels. In these tests, G' and G" of gels was measured on the plate-plate system at 25C as a function of applied strain at an oscillation frequency of 1 Hz. Gels generally increased in gel strength with increasing holding time. Figure 1 shows as an example of the results obtained for formulation A formulated with 35.2% hot extracted cranberry puree, 18.1% hot extracted puree from

pressed cranberries (west coast) and 46.7% of 60/40 high fructose corn syrup sweetener blend (see materials).

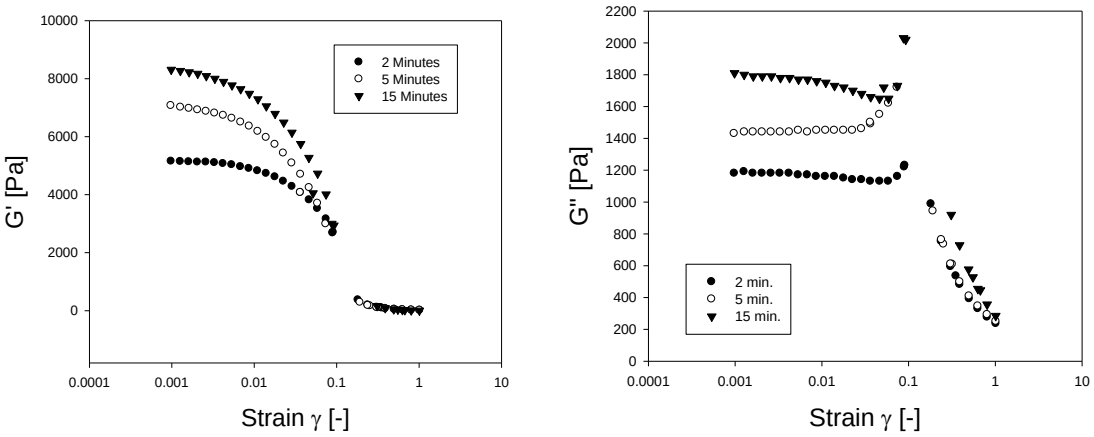


Figure 4.1 G' (left) and G" (right) measured at 1 Hz as a function of strain for formulation A at a holding temperature of 99C.

G' and G" decreased with increasing strain indicating a breakdown of the gel structure of strains above 0.1 regardless of holding time. G' and G" at strains below 0.1 increased with increasing holding time (2 to 15 minutes) suggesting the formation of a stronger gel network. Similar results were found for all other gels, that is, the magnitude of the storage and loss modulus generally increased at lower strains with increasing holding times. The results are in agreement with literature results, that is, gels typically are weaker if insufficient time is given prior to cooling to allow for optimal configuration biopolymers and maximum number of bonds and junction zones to be formed.

4.4.1.2 Influence of Holding Temperature on Strength of Gel as Measured by Amplitude Sweeps

Amplitude sweeps of gels formed at different hold temperatures revealed that the influence of the holding temperature on the overall gel strength was much less pronounced

than the influence of the holding time on the gel strength. Figure 4.2 illustrates this fact for formulation D. As previously reported, gel strength increased significantly with increasing holding time, e.g. G' at low strains increased from less than 3000 to approximately 6000 Pa when the holding time was increased from 2 to 15 minutes. However, gel strength as a function of holding temperature changed by less 500 Pa when the temperatures was increased from 88 to 99C. Similar tendencies were observed for all other gels, although absolute differences varied from gel to gel, indicating that different formulations were more or less sensitive to time and temperature. It should be noted though that formulation A did not form a gel at 88C at a holding time of 2 minutes, indicating that conditions were not sufficient to induce network formation. Note: This type of test only demonstrates the strain-dependent behavior of the gel and is comparably crude. The frequency sweeps may be more revealing as they demonstrate changes in the time-dependent (memory) behavior of the gel. While the strain dependence may not be influences as strongly by the holding temperature, the time dependent behavior may reveal more differences between formulations as influence by hold temperatures and times.

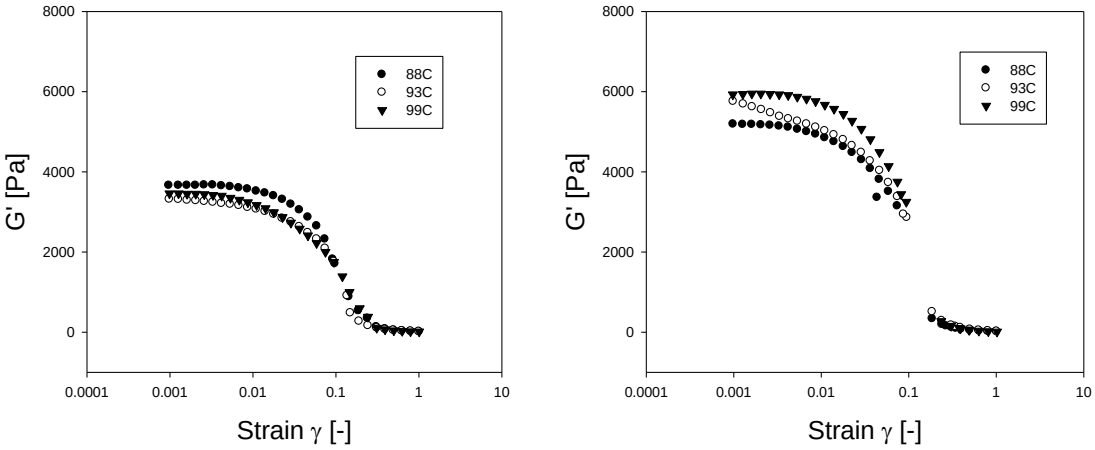


Figure 4.2 G’ measured at 1 Hz as a function of strain for formulation D for different holding temperatures (88, 93, 99C) at a holding time of 2 minutes (left) and 15 minutes (right).

4.4.1.3 Influence Gel Composition on Strength of Gel as Measured by Amplitude Sweeps

Significant differences between different formulations were observed in terms of final gel strength and their dependency on time-temperature profiles. While formulation A did not form a gel at all at 2 minutes holding time, under all other tested time-temperature combinations, formulation D was the weakest gel overall. Its strength as measured by G’ was 30% that of the strongest gel. Formulation D is the only gel that was composed of concentrate and was reconstituted with water. We hypothesize that the concentration and reconstitution process may have altered ability of cranberry plant fragments to strengthen and support the pectin gel structures.

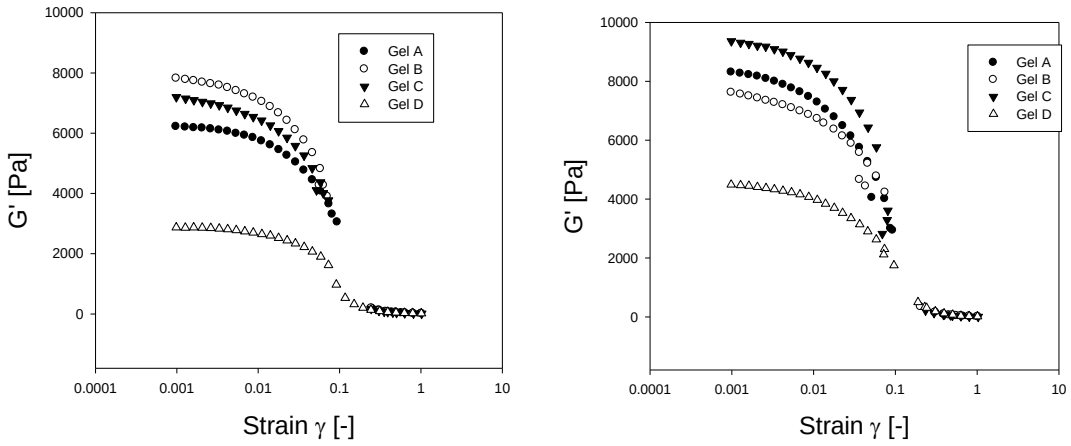


Figure 4.3 G' measured at 1 Hz as a function of strain for different formulations, holding time 5 Minutes at 88C, (left) holding time 15 Minutes, 99C, (right).

4.4.1.4 Influence of Storage Time on Gel Strength of Cranberry Gels as Measured by Amplitude Sweeps

The loss and storage moduli of all gels were measured after storage for 1, 2 and 4 days as a function of applied strain. For example, G' and G" as a function of strain of formulation A formed with a 15 minute holding time at 99C (Figure 4.4) or 5 minutes at 88C (Figure 4.5) stored for 1, 2 and 4 days indicated that both the elastic and loss moduli increased with increasing storage time. Similar effects were observed with all other gels (data not shown) indicating that the gels continued to gain strength during storage at room temperatures. When the gel strength for formulation D manufactured at 99C/15 minutes was compared with the strength of the gel manufactured at 88C/5 minutes, it can be seen though, that the relative increase for the gel that was formed at the higher temperature was larger (Figure 4.6).

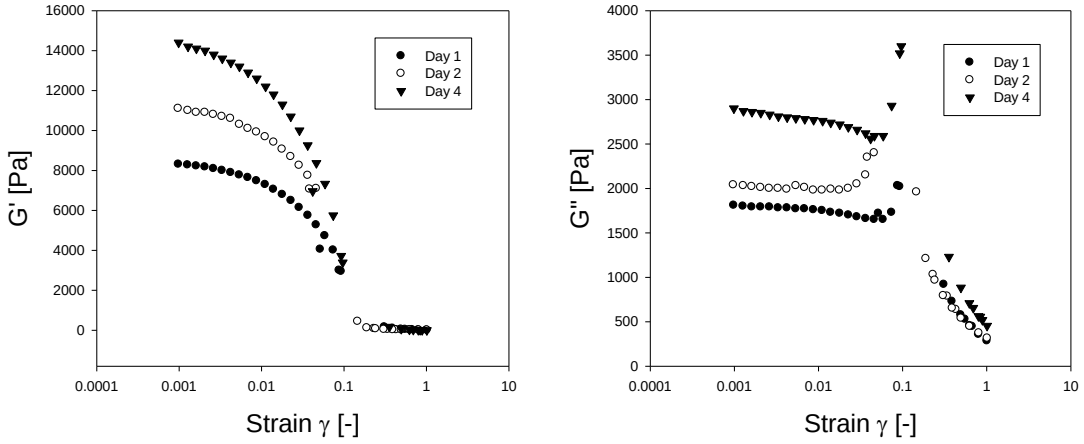


Figure 4.4 G’ (left) and G” (right) measured at 1 Hz as a function of strain for formulation A stored for 1, 2, and 4 days at a holding time of 15 minutes at 99C.

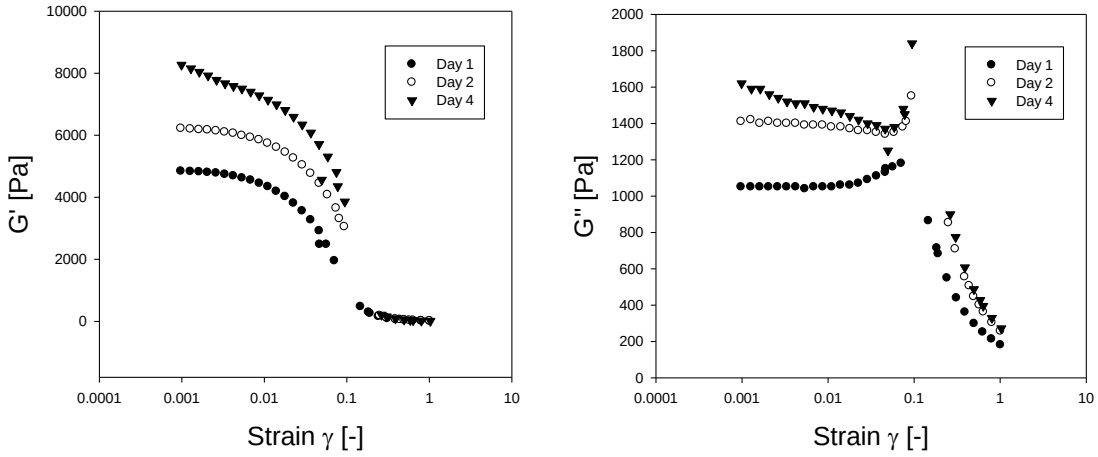


Figure 4.5 G’ (left) and G” (right) measured at 1 Hz as a function of strain for formulation A stored for 1, 2, and 4 days at a holding time of 5 minutes at 88C.

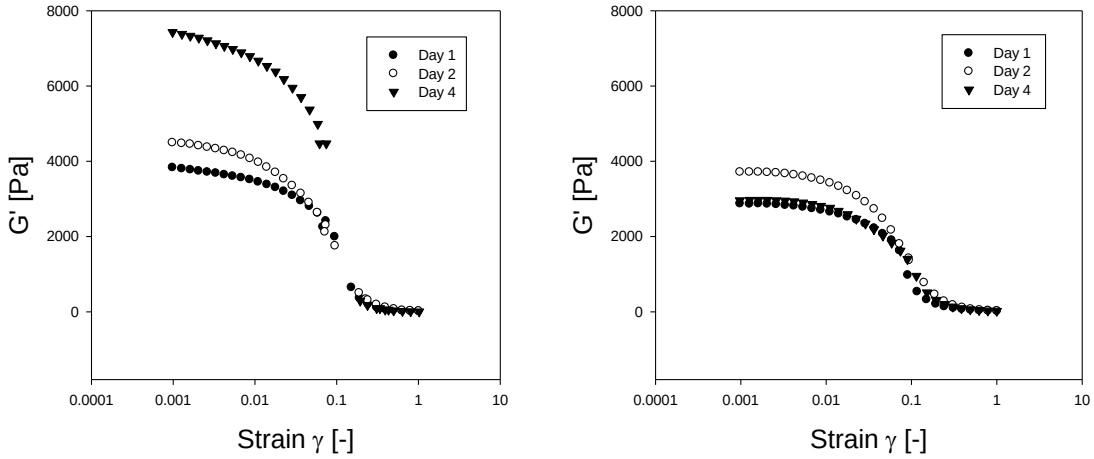


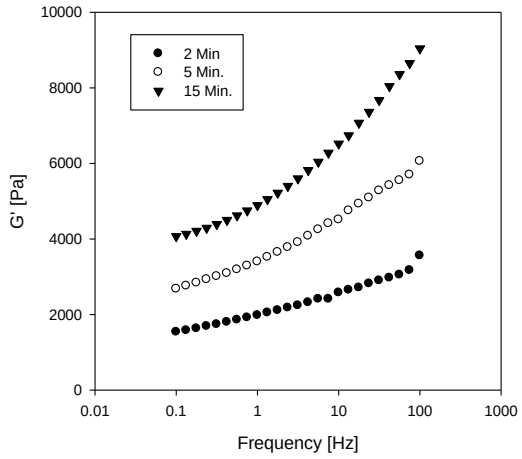
Figure 4.6 G’ measured at 1 Hz as a function of strain for formulation D stored for 1, 2, and 4 days at a holding time of 15 minutes at 99C (right) and 5 minutes at 88C (left)

4.4.2 Dynamic Frequency Sweeps of Cranberry Gels

4.4.2.1 Influence of Holding Time on Strength of Gel as Measured by Frequency Sweep

Frequency sweeps were conducted to obtain information about the time-dependent behavior of cranberry gels as a function of gel holding time. Figure 4.7 shows results of the measurement of the storage and loss moduli as a function of oscillation frequencies (0.1 to 100 Hz) at a strain of 0.01 for formulation A as a function of different holding times. Similar to results seen in the amplitude sweeps, the gel strength generally increases with increasing holing time. In addition, the increased frequency dependence at the longer holding times indicates a transition in the material behavior towards a more rubbery behavior. This would suggest increased elasticity in the gel which would correspond to an increased number of junctions and bonds in the gel. Similar tendencies were seen in all other gels, albeit the degree of changes in the gel structure depended on formulation (see

below).



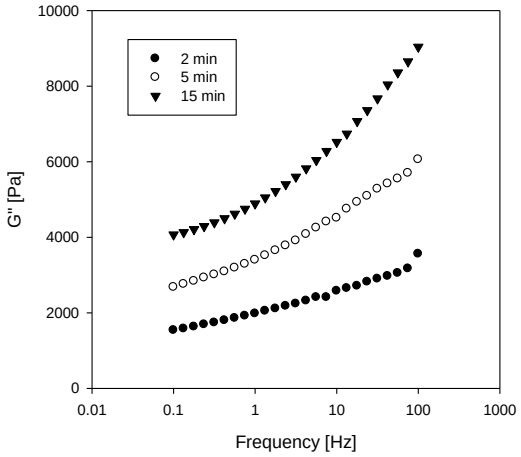


Figure 4.7 G' (left) and G" (right) measured as a function of frequency at a strain of 0.01 for formulation A on day one, measured at 99C, at different holding times.

4.4.2.2 Influence of Holding Temperature on Strength of Gel as Measured by Amplitude Sweeps

With increased holding temperatures, the gel strength of the material further increased, although the improvement in the gel strength was less pronounced than the improvement due to increased holding times. Figure 4.8 shows the elastic and loss moduli of cranberry formulation A as a function of oscillation frequency (0.1 to 100 Hz) measured

for a holding time of 15 minutes. Both G' and G" increased with increasing temperatures but the frequency dependency of the curves remains essentially unchanged.

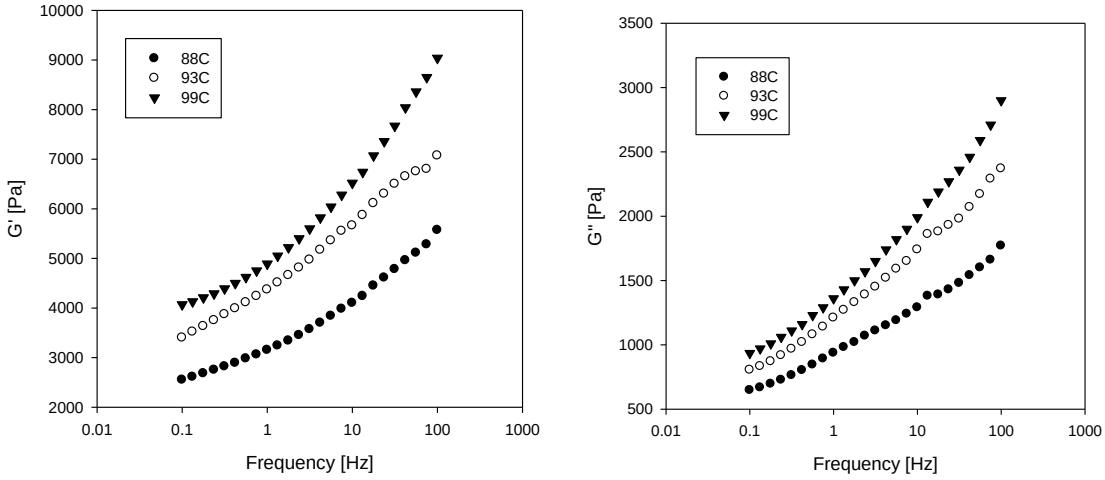


Figure 4.8 G' (left) and G" (right) measured as a function of frequency at a strain of 0.01 for formulation A on day one, at a holding time of 15 minutes.

4.4.2.3 Influence Gel Composition on Strength of Gel as Measured by Amplitude Sweep

Again, significant changes in the material behavior as a function of formulation were found. Figure 4.9 shows the G' and G" as a function of formulation measured at 99C at a holding time of 15 minutes. As previous amplitude measurements revealed, formulation D has the lowest G' and G" compared to all other gels, suggesting that this gel was not as strong and as elastic as all other gels – possibly due to the dehydration – rehydration effect. In addition, formulation D shows less frequency dependence in G' and G" as compared to all other gels, suggesting that the gels is less elastic possibly due to a reduced number of bonds that may have been formed.

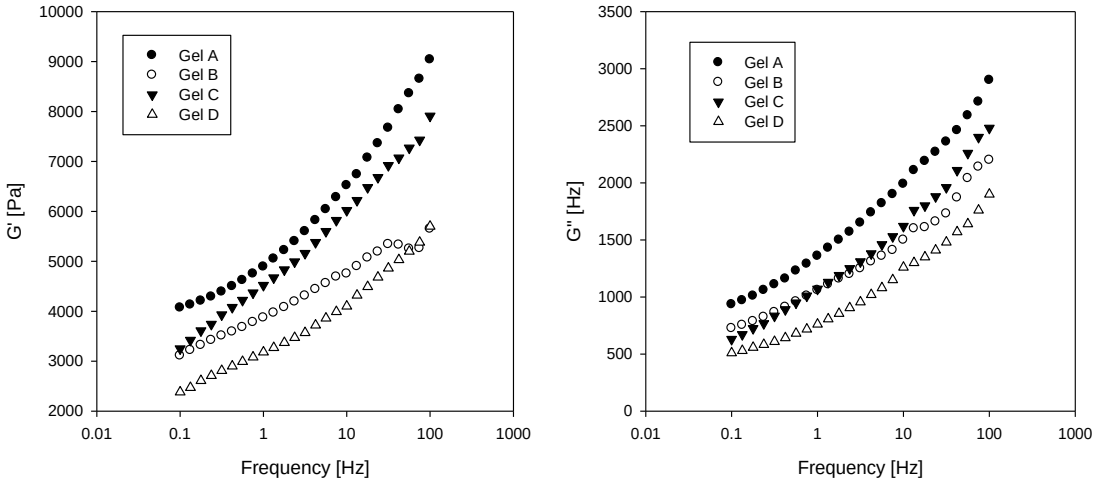


Figure 4.9 G’ (left) and G” (right) measured as a function of frequency at a strain of 0.01 for the different gels measured at 99C at a holding time of 15 minutes.

4.4.2.4 Influence of Storage Time on Gel Strength of Cranberry Gels as Measured by Frequency Sweeps

The storage and loss moduli of gels stored for 1, 2 and 4 days were measured as a function of frequency. Some small increases in both G’ and G” were observed as gels were sampled after increasing storage times. Gels stored for 4 days had higher G’ and G” as a function of frequency indicating a shift of the general material function towards a more rubbery behavior.

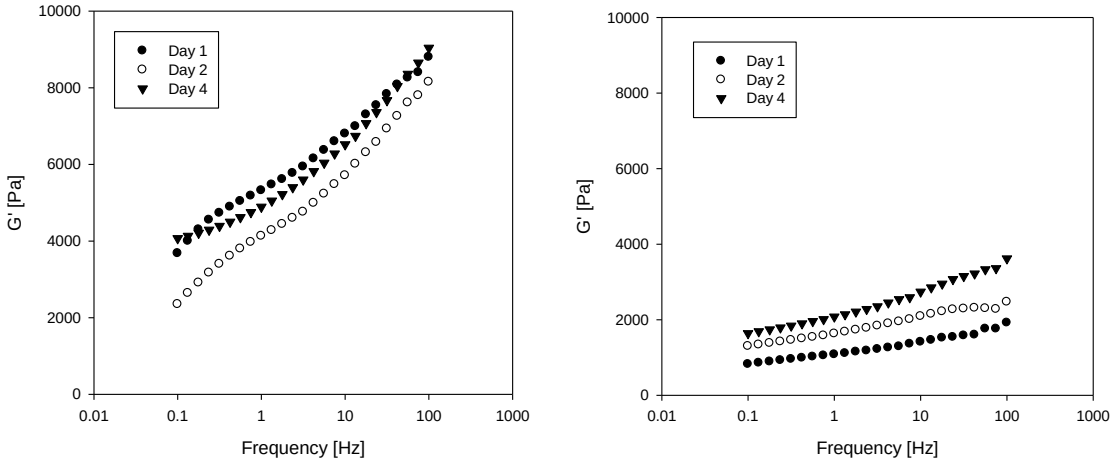


Figure 4.10 G' of formulation A (left) and formulation D (right) measured as a function of frequency at a strain of 0.01 at 99C at a holding time of 15 minutes.

4.4.3 Texture Analysis of Cranberry Gels

Gels were subjected to a variety of texture analysis tests using an Instron Texture Analyzer. Tests included force deformation measurements to determine the maximum load and elastic modulus prior to break as well as compression relaxation measurements to obtain irrecoverable work, recoverable work, and total work data.

4.4.3.1 Maximum Load and Elastic Modulus of Cranberry Gels as a Function of Holding Time and Temperature

Gels formed at the various conditions and stored for 1, 2, and 4 days were subjected to a simple compression test. Samples were compressed between two parallel stainless steel plates and force versus compression was recorded. Extension (or strain) was calculated as $L\text{-}L_0/L_0$ where L is the instrumental coordinate at an applied compression force and L0 is the initial instrumental coordinate. Figure 4.11 shows the effect of holding time and temperature for formulation A after one day of storage on the force-deformation curve. With increasing holding time and temperature increased load is required to achieve

a given deformation indicating that the gel is becoming stronger. From this set of data, the maximum load prior to breakage was calculated. The maximum load is an indication of the force required to cause irreversible damage to the gel. If higher forces are applied, the structure of the gel is damaged leading to a weakening of the structure and thus a drop in the load required to cause deformation. Gels increased in strength with both holding time and holding temperature, but holding time led to greater increases in gel strength than hold time. An overview of the maximum load under the different conditions for formulation A is shown in Figure 4.13.

Significant differences in gel behavior under compression were found for different gel formation. Figure 4.12 shows the compression curve for the 4 gels formed at 99C and 15 minute holding time. Formulation D was the weakest of the 4 gels with the lowest maximum load. An overview of the maximum load for all gels after day 1 is shown in Fig. 4.14.

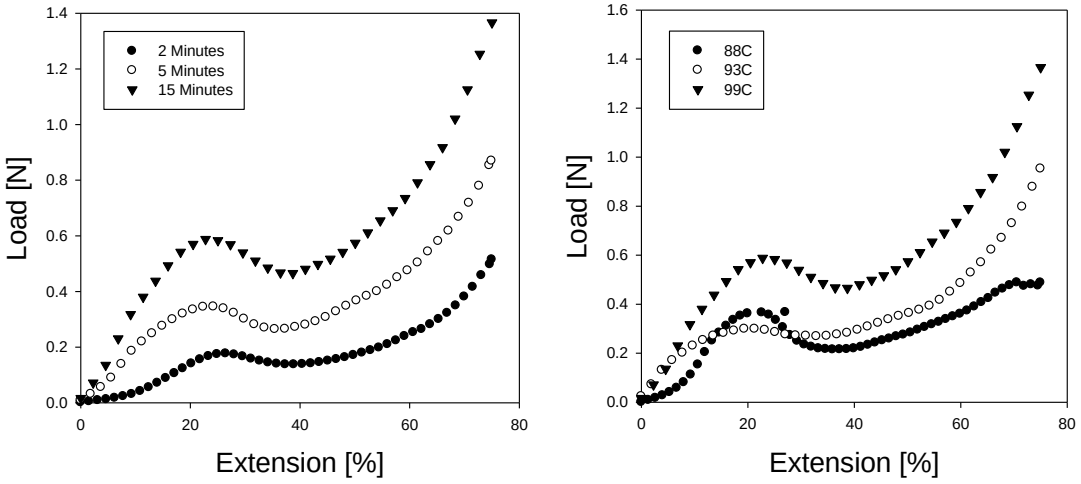


Figure 4.11 Load of formulation A as a function of extension at (left) 99C at three different holding times (2, 5, 15 minutes) and (right) at a holding time of 15 minutes at three different holding temperatures (88, 93 and 99C).

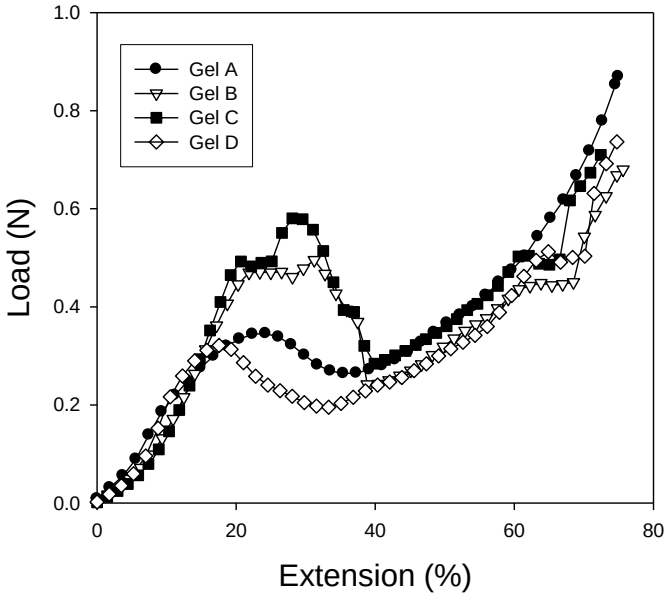


Figure 4.12 Load of different gels measured as a function of extension at 99C at a holding time of 15 minutes.

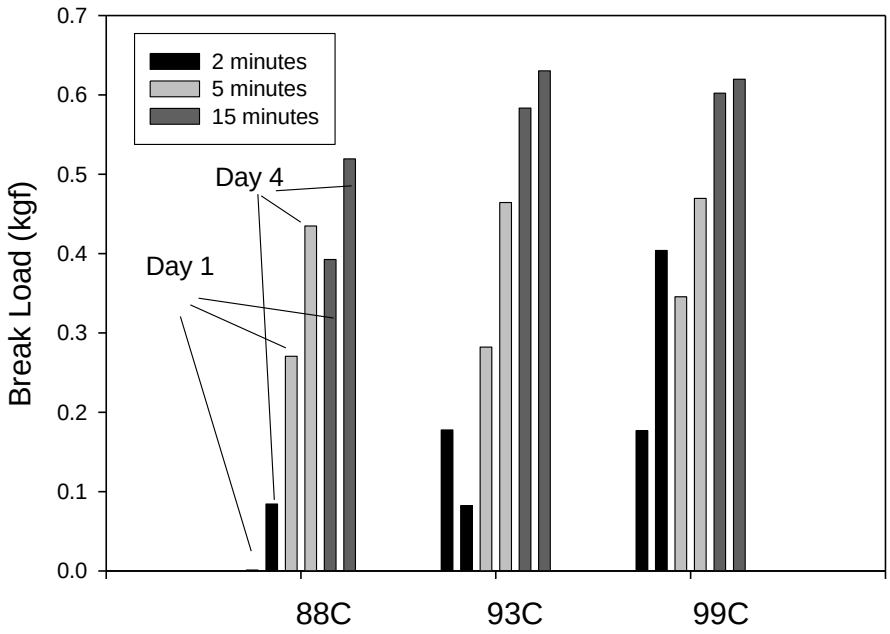


Figure 4.13 Critical load of Gel A as a function of hold time and temperature as well as storage time.

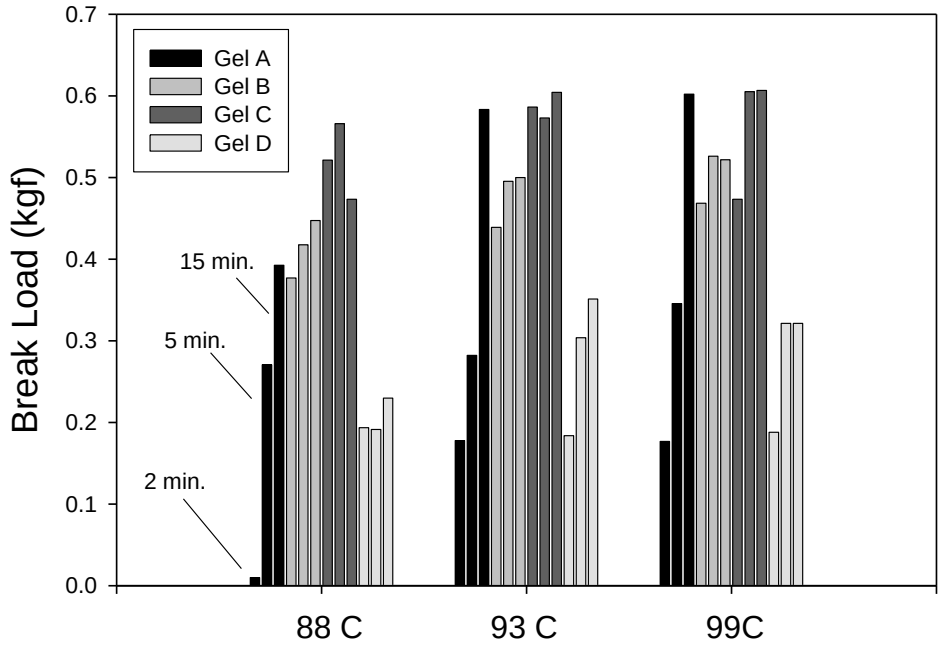


Figure 4.14 Load of different gel formulations measured as a function of extension at 99C at different holding times on day one.

4.4.3.2 Recoverable, irrecoverable and total work calculated from compression cycle test for cranberry gels formed at various times holding times and temperature stored for 1 and 4 days

Gels were subjected to a maximum strain of 25% and load during deformation and recovery was recorded. Recoverable, irrecoverable and total work was calculated from the compression cycle (Table 4.1 – 4.3). Gels had high irrecoverable work und low recoverable work. Total work generally increased with increasing holding time and temperature and storage time. Results are in agreement with previously presented texture analysis and rheology data.

Table 4.1 Irrecoverable work of gels formed at various hold times and temperatures.

IRRECOVERABLE WORK [10^{-3}Nm]							
DAY 1				**DAY 4**			
Gel A							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	0	3.7	4.9	**2**	1.3	2.3	9.1
5	5.3	6.8	8.8	**5**	9.6	2.8	9.9
15	6.5	8.8	12.2	**15**	10.6	11.1	13.2
Gel B							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	6.5	6.3	8.9	**2**	7.7	9.8	9.2
5	8.4	9.5	11.2	**5**	9.4	10.17	11.4
15	9.9	12.6	14.5	**15**	10.6	7.22	13.7
Gel C							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	8.3	12.4	12.5	**2**	11.7	10.9	12.4
5	11.1	12.4	13.1	**5**	14	12.3	13
15	13.4	12.8	13.8	**15**	11.7	12.8	15.9
Gel D							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	3.1	6.4	4.56	**2**	5.8	8.5	5.5
5	4.2	7.55	6.7	**5**	5.8	8.6	6
15	6.9	7.11	7.9	**15**	6.2	4.3	7.5

Table 4.2. Recoverable work of gels formed at various times and temperatures.

RECOVERABLE WORK [10^{-3}Nm]							
Day 1				**Day 4**			
Gel A							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	0	0.3	0.29	**2**	0.5	1.2	0.9
5	0.7	0.41	0.79	**5**	0.7	1.3	1.2
15	0.75	0.97	0.73	**15**	0.9	1.8	1.5
Gel B							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	0.7	0.8	0.9	**2**	0.7	0.8	1.68
5	0.7	0.6	1.4	**5**	0.61	0.87	0.28
15	0.79	1	1.9	**15**	1.73	0.52	1.816
Gel C							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	0.9	1.2	2	**2**	2	1	2.7
5	2.1	1.92	1.3	**5**	1.2	2.3	2.4
15	0.9	1.7	1.3	**15**	1.7	1.9	2.3
Gel D [10^{-3}Nm]							
Time/Temp	**88C**	**93**	**99**	**Time/Temp**	**88C**	**93**	**99**
2	0.6	0.31	0.5	**2**	0.5	0.5	0.2
5	0.58	0.42	0.72	**5**	0.4	0.6	0.6
15	0.62	0.76	0.72	**15**	0.5	0.4	2.4

Table 4.3. Total work required for compression/relaxation of gels formed at various times and temperatures.

TOTAL WORK [10^{-3}Nm]							
DAY 1				DAY 4			
Gel A							
Time/Temp	**88C**	**93**	**99**		**88C**	**93**	**99**
2	0	4	5.19	**2**	1.8	3.5	10
5	6	7.21	9.59	**5**	10.3	4.1	11.1
15	7.25	9.77	12.93	**15**	11.5	12.9	14.7
Gel B							
Time/Temp	**88C**	**93**	**99**		**88C**	**93**	**99**
2	7.2	7.1	9.8	**2**	8.4	10.6	10.88
5	9.1	10.1	12.6	**5**	10.01	11.04	11.68
15	10.69	13.6	16.4	**15**	12.33	7.74	15.516
Gel C							
Time/Temp	**88C**	**93**	**99**		**88C**	**93**	**99**
2	9.2	13.6	14.5	**2**	13.7	11.9	15.1
5	13.2	14.32	14.4	**5**	15.2	14.6	15.4
15	14.3	14.5	15.1	**15**	13.4	14.7	18.2
Gel D							
	88C	**93**	**99**		**88C**	**93**	**99**
2	3.7	6.71	5.06	**2**	6.3	9	5.7
5	4.78	7.97	7.42	**5**	6.2	9.2	6.6
15	7.52	7.87	8.62	**15**	6.7	4.7	9.9

4.5 Discussion

Two types of small strain oscillatory tests were used to examine different cranberry gel formulations. Small strain rheological studies (<2%) are well-suited to examining the viscoelastic properties of gels. Amplitude (or strain) sweeps were performed in order to determine the linear viscoelastic region of the gel at a constant temperature. In the linear viscoelastic region G' and G" are constant when the amplitude of applied deformation (strain) is changed (Ideda, and Foegeding, 2000). G' can be defined as the "deformation energy stored in [a] sample during the shear process," (Tabilo-Munizaga, G. and Barbosa-Cánovas, G.V., 2005), or simply the elastic modulus. G" can be defined as "the

deformation energy used up in the sample during the shear and lost to [a] sample afterwards," (Tabilo-Munizaga, G. and Barbosa-Cánovas, G.V., 2005), or simply the loss modulus.

Frequency sweeps were used in order to elucidate the mechanical spectrum of the gels at a fixed temperature. G' and G" were determined as a function of oscillation frequency (ω). Examination of the rheological characteristics of the cranberry gels attempts to elucidate the behavior of the gel under stress and the resultant deformation that occurs (Tabilo-Munizaga, G. and Barbosa-Cánovas, G.V., 2005).

For amplitude sweeps, strain or deformation was varied and G' and G" were measured. Strain is a dimensionless quantity of relative deformation of a material (Tablio-Munizaga, G. and Barbosa-Cánovas, G.V., 2005). The results show that at 1 Hz, the critical limit of the linear viscoelastic region of all gel formulations was at a strain of .01.

During dynamic oscillatory testing, no sudden changes of moduli (indicative of a breakdown of structure) were observed (Aparicio-Saguilán et al., 2006). In most cases, formulations displayed a dependence on frequency of oscillation during frequency sweeps. As stated previously, G'>G" is indicative of a gel. As the distance between G' and G" curves increases, gel-like characteristics become more distinct. Gel-like characteristics also increase with a decreased dependency on frequency (Gilsenan, P.M. et al., 2000). As the frequency of oscillation increased, both G' and G" increased. The elastic character of the gels generally increased with an increase in frequency.

As storage time increased, formulation A showed a consistent dependency on frequency, but did not show an increase in G' from day 1 to day 4 (Fig. 4.9). Formulation D did not show a dependency on frequency. Unlike formulation A, formulation D showed

a distinct increase in G' from day 1 to day 4. The values of G' were much higher for formulation A, however. Pectin gels are known to be slow set gels and gel formation can improve over time (Rao et al., 1993). Cranberry gels have shown improvement in gel strength over a period of 7 days (Baker and Kneeland, 1936), and high methoxyl pectin/fructose gels have shown changes in G' and G" over a period of 22 days (Rao, M.A. and Cooley, H.J., 1993). Consequently, further testing with an increased storage time may be warranted.

Formulation also caused variation in G' and G". Formulation A exhibited the highest values of G' and G", while formulation D exhibited the lowest values of G' and G". This decrease in G' and G" may be due to changes in the amount of pectin in each formulation (see Table 2). Formulation A had the highest amount of pectin, whereas formulation D contained the lowest amount of pectin. Formulation B had a similar amount of pectin when compared to formulation A. The G' and G" values of formulation B were quite close to those of formulation A. Formulation C had a slightly smaller amount of pectin than formulation A or B. There was a corresponding drop in G' and G" values for formulation C. As pectin concentration of a gel increases, the G' and G" will increase (daSilva, J.A.L. and Rao, M.A., 1995).

Increasing the hold temperature caused an increase in G' and G". As temperatures increased from 88°C to 99°C, elasticity of the gels increased. This increase in G' could be due to the strengthening of hydrophobic interactions due to temperature increase (da Silva, J.A.L. and Gonçalves, M.P. 1994). This increase in G' may have a complex dependence on temperature, due to the fact that increased temperatures can have a softening effect on gels due to the weakening of hydrogen bonds within the gel.

Table 4.4 Calculation of pectin content per each formulation

Formula A	**Weight %**	**Pectin (g per 100g of formulation)**
Hot Extracted Cranberry Puree	35.2	0.22176
Hot Extracted Puree From Pressed Cranberries (West Coast)	18.1	0.007783
Sweetener Blend	46.7	0
	Total Pectin (g) per 100g of formulation	**0.229543**
Formula B	**Weight %**	**Pectin (g per 100g of formulation)**
Hot Extracted Cranberry Puree	35.2	0.22176
Hot Extracted Puree From Pressed Cranberries (Mid-West)	18.1	0.003801
Sweetener Blend	46.7	0
	Total Pectin (g) per 100g of formulation	**0.225561**
Formula C	**Weight %**	**Pectin (g per 100g of formulation)**
Standard Jellied Sauce Puree	53.3	0.2132
Sweetener Blend	46.7	0
	Total Pectin (g) per 100g of formulation	**0.2132**
Formula D	**Weight %**	**Pectin (g per 100g of formulation)**
Water	24.2	0
24 Brix Cranberry Puree Concentrate	11	0.0352
Hot Extracted Puree From Pressed Cranberries (West Coast)	18.1	0.003801
Sweetener Blend	46.7	0
	Total Pectin (g) per 100g of formulation	**0.039001**

Uniaxial compression of the gels provided information on the stress at failure of the gel formulations. Compression is also used as a measure of strength, which is defined as "the stress (force per unit area) that a specimen can sustain before failure," (Peleg, 2006). The critical load (kgf) of formulation A increased with temperature treatment, length of heat treatment, and length of ageing (Fig. 4.12). The critical or break load also varied with formulation. Formulation C showed the highest consistent break loads, and formulation B also had high consistent break loads. Formulation A showed marked improvement in break load with increased cook time. Formulation D had the lowest overall break loads of all formulations measured.

Pectin gels are disordered biopolymer networks and are generally weak gels (Tabilo-Munizaga, G., and Barbosa-Cánovas, G.V., 2005). When weak gels are exposed to a large stresses, the gel network is easily broken. The deformation caused by a given force is observable in Fig. 4.10. Differences in the load needed to achieve failure of the gels differed with both holding time and holding temperature. Holding time caused the most distinct differences in the force needed to cause permanent deformation of the gel. The load required increased with increased holding time. The differences between the curves of hold temperatures were not as dramatic as the differences in the hold time curves. However, holding formulation A at 99°C caused a distinct increased in the load required to cause permanent deformation of the gel.

Generally, gels B and C were found to be more brittle than gels A and D, a fact that can be seen from both the rheology and texture analysis studies. This brittleness is evidenced by the jaggedness of their break load curves in Fig 4.11. At the same time, formulations B and C had very high performances during break load analysis, and

produced gels that were quite strong and only broke at high applied loads. Formulations A only formed a strong gel after being held at elevated temperatures for an extended period of time. Gel strength increased in all gels as they were stored for a prolonged time. The formation of gels is a slow process and changes in the gel structure may continue past four days (Rao et al., 1993). The weakest gel was formed by formulation D. Increasing the time held from 2 minutes to 15 minutes increased the break load of formulation D. Increased hold times of 5 and 15 minutes also increased the break load in conjunction with increased hold temperature.

Recoverable work, irrecoverable work and total work are obtained by compression cycle tests. Recoverable work can be used as a measure of degree of elasticity. The high degree of irrecoverable work and low degree of recoverable work could be due to internal structural damage caused by the deformation caused by compression (Peleg, 2006). Pectin gels are classified as weak gels, thus their tenuous gel network can be easily broken under applied stress (Tabilo-Munizaga, G., and Barbosa-Cánovas, G.V., 2005). The increase in total work with increased holding time, temperature and holding temperature could be attributed to the weak interactions within the gel network.

Formulation D was observed as having a larger degree of hysteresis as compared to the other gel formulations. This large degree of hysteresis is indicative of unrecovered loss of structure within the sample (Rodd, A.B., et al., 2000). Formulation D had consistently weaker performances when compared to the other formulations, so loss of structure within the sample during testing could be partially responsible for the poor performance of this particular formulation.

There have been many studies as of late on the rheological properties of gels (Gallegos, C. and Franco, J.M., 1999); however, many issues exist with the reproducibility of gel systems (Tabilo-Munizaga, G., and Barbosa-Cánovas, G.V., 2005). As stated previously, the pectin content (and thus the gelation properties) of cranberries is affected by maturity, season, horticultural methods, storage conditions, variety, and location can all have an effect on gelation behavior in cranberry gels (Flynn, 1950; Weckel and Swanson, 1972). Another point to keep in mind is that cranberry gels have been demonstrated here to be non-linear viscoelastic materials. This being the case, when cranberry gels are subjected to large deformations they can undergo significant internal structural damage that can call into question the accuracy of results that measure properties such as the degree of elasticity (Peleg, 2006). Gel strength can be dependent on the deformation applied, speed of the cross-head, and if the test was conducted until gel rupture (Moresi, M. and Bruno, M., 2007). Relaxation time and recoverable work are also usually dependent on the level of strain the sample is exposed to and the rate at which loads are applied to the sample (Peleg, 2006).

4.6. Conclusions

Results show significant changes in gel properties as a function of holding time and temperature. Robustness of gel to form elastic gels depended on formulation and formulation D composed of rehydrated cranberry extract had the weakest performance as evidenced by all rheological measurements and by texture analysis. The results show that processing conditions as well as formulation are critical factors in ensuring consistency across production lines. Storage time of gels did not always result in increased elasticity, but literature suggests that increasing storage time past 4 days may confer increased

elasticity to the gels (Baker and Goodwin, 1941a; Rao et al., 1993). Increased holding time and temperature had a positive effect on formulation robustness. Formulations with an increased amount of pectin also increased the level of gel robustness.

CHAPTER 5

CHARACTERIZATION OF MOLECULAR PROPERTIES OF CRANBERRY PECTIN AS A FUNCTION OF PROCESSING CONDITIONS

5.1 Abstract

The pectin molecular properties of five different types of raw cranberry purees were examined for the effect of processing conditions. Pectin was extracted from each type of puree and FT-IR analysis was performed in order to determine degree of esterification of each type of extracted pectin. Puree from fresh fruit and cranberry puree concentrate had the highest degree of esterification, whereas purees from the byproducts of puree processing had lower degrees of esterification. Yield of pectin from each type of puree was also determined. Again, purees from fresh fruit and cranberry puree concentrate had higher yields than the purees produced from the byproducts of the puree production process. Purees were exposed to elevated temperatures to examine whether hydrolysis may occur at longer holding times. Degree of esterification ranged from 50 to 65% and yields varied between 0.4 and 1.2% (on dry weight basis). Each puree showed a pronounced drop in viscosity after 30-45 minutes of heat treatment at 99°C, indicative of pectin hydrolysis.

5.2 Introduction

There has been some work done on the properties of cranberry pectin (Flynn, 1950; Pintauro, 1965; Pintauro, 1970), but this work did not take into account current processing conditions and much of the work could be more accurately done using more

current research methods. Cranberry pectin is of interest because of its ability to form a gel at a low pH and low soluble solids content (Baker and Kneeland, 1936).

Temperature exposure tests were performed in order to determine the degree of pectin hydrolysis that may occur during processing. Prolonged heat can cause hydrolysis of pectin molecules. Hydrolysis of significant amounts of pectin could cause a decrease in final gel strength and is a concern for processors. Purees have already been heat treated during processing, so presumably some hydrolysis of pectic materials has already occurred.

Purees were treated in order to obtain a crude pectin extract in order to determine pectin yield and degree of esterification. Pectin extraction was carried out using an aqueous hot acid extraction and an ethanol flock. Wet pectin extract was freeze dried and analyzed for yield. Pectin yield may be affected by processing conditions of the purees and the extraction conditions (Yapo et al., 2007).

IR-spectroscopy techniques can be used to characterize pectins according to their degree of esterification, by determining the differential localization of absorption bands originated by specific vibrational modes of atom groups in galacturonic acid and its methyl ester, respectively (Chatjigakis et al., 1998). FT-IR analysis was used in order to determine degree of esterification of extracted cranberry puree pectin. A high degree of esterification results in a faster, firmer set in less time compared to a low degree of esterification (Bennion, 1980).

In order to determine optimal processing conditions for raw materials, the molecular characteristics of the extracted pectin must be analyzed. The differences in

puree processing may also cause differences in the molecular characteristics of extracted pectin.

5.3 Materials and Methods

5.3.1 Rheometry

Flow curves of raw materials were performed in the absence of soluble solids (to prevent gelation). Raw materials were exposed to the same processing temperatures used in the gelation experiment (88, 93, 99°C) for 0, 15, 30, 45, 60, and 90 minutes. After heat treatment, raw materials were allowed to come to room temperature (25°C ±1). Once heat treated, shear tests were performed on all raw materials using the rheometer using cup and bob geometry. Viscosity of solutions was measured as a function of strain rate using coaxial cylinder geometry. Strain rates varied 0.01 to 500. The shear tests were used in order to determine changes in viscosity with heat treatment due to hydrolysis of pectic material in the raw cranberry material. Approximately 19ml of sample was loaded into the cylinder. The single gap cylinder (CC27) was used with an outer radius of 14.46mm (Anton Paar, Graz, Austria).

5.3.2 Extraction of Pectin

Pectin was extracted from thawed raw material using a modified method described by Pagán et al. (2001). Raw materials were freeze dried before extraction began. Freeze dried material was mixed with distilled water acidified to pH 2 at 150 rpm at 60°C for 1 hour. Solids were then separated from the extract using a Buchner funnel with Whatman No. 1, (Sigma, St. Louis, MO) filter paper. Pectin was extracted from the solution by using an equal volume of 95% w/w ethanol to induce phase separation and flocculation. The pectin solution was stirred at 150 rpm for 15 minutes and then

refrigerated for 1 hour. The pectin was extracted again using a Buchner funnel and Whatman No. 1 filter paper. The resultant mass was freeze dried and then weighed to calculate yield.

5.3.3 Determination of Degree of Esterification

The degree of esterification of the extracted pectin was analyzed by FTIR, (Fourier transform infrared spectrophotometer) analysis. A standard curve was prepared using pectin with known degrees of esterification, (30, 60, and 90%), (Sigma, St. Louis., MO). FTIR spectra were collected using an IR Prestige-21 FTIR outfitted with a DLATGS detector. Spectra were collected using an Attenuated Total Reflectance, (ATR) accessory against a KBr background, with $4cm^{-1}$ resolution, 64 scans, and range of 600-4,000 cm^{-1}. All spectra were analyzed using the computer program IR Solution version 1.20, (Shimadzu, Kyoto, Japan) Peakfit software for line curve fitting. Spectra were analyzed using the method described by Chatjigakis et al., (1998). Degree of esterification is calculated as the number of esterified carboxylic groups / number of total carboxylic groups) x 100. Two specific frequencies, namely the bands at 1749 and 1630 cm-1 were used in order to calculate degree of esterification. The carboxyl ester groups absorb at about 1740 cm-1 whereas the corresponding carboxylate groups absorb at about 1600 cm-1. The ratio of the areas of the bands at 1749 cm-1 over the sum of the areas of the bands at 1749 and 1630 cm-1 should be proportional to the degree of esterification. When an appropriate calibration curve relating to the ratio of areas $A_{1749}/ (A_{1749}+A_{1630})$ to the degree of esterification is established, the degree of esterification of the pectic molecules can be determined from the FT-IR spectroscopic data.

5.4 Results and Discussion

5.4.1 Yield Experiments

Pectin is a water soluble polysaccharide. The hot acid treatment dissolved the pectin and allowed for its extraction from the raw material. Subsequent filtration removed pulp and water-insoluble materials. The ethanol treatment extracted pectin and other alcohol-insoluble particles from the raw material (Sinclair and Jollifffe, 1960).

It was found the hot extracted cranberry puree contained the greatest amount of pectin (Table 5.1). The standard jellied sauce puree and the cranberry puree concentrate contained the next greatest amounts, respectively. The lowest amount of pectin was extracted from the hot extracted puree from pressed cranberries (west coast) and the hot extracted puree from pressed cranberries (mid-west).

5.4.2 Degree of Esterification

The cranberry puree concentrate exhibited the highest degree of esterification, (determined by area), but also had the highest standard deviation (Table 5.2). Standard jellied sauce puree and hot extracted cranberry puree were very close in degree of esterification. The degree of esterification of the hot extracted puree from pressed cranberries (west coast) dropped significantly compared to the first three materials, and the lowest degree of esterification value found was the hot extracted puree from pressed cranberries (mid-west).

Table 5.1. Pectin yield extracted from the various raw materials supplied by Ocean Spray Inc.

Puree	Dry Wt %	Wet Wt %
Hot extracted cranberry puree	4.97±1.92	1.26±0.24
Hot extracted puree from pressed cranberries (west coast)	1.65±0.84	0.043±0.02
Hot extracted puree from pressed cranberries (mid-west)	2.93±0.23	0.02±0.01
Standard jellied sauce puree	6.23±2.43	0.4±0.16
24 Brix cranberry puree concentrate	1.09±0.57	0.32±0.17

Table 5.2. Degree of esterification of pectin from various raw materials.

Sample		Degree of Esterification Determined by FT-IR
Hot Extracted Cranberry Puree		90.83 ± 8.18
Hot extracted puree from pressed cranberries (west coast)		82.28 ± 7.26
Hot extracted puree from pressed cranberries (mid-west)		64.53 ± 11.88
Standard Jellied Sauce Puree		87.86 ± 2.00
Cranberry Puree Concentrate		81.78 ± 9.70

5.4.3 Hydrolysis Experiments

Samples were subjected to prolonged temperature exposure at 88 to 99°C and flow curves were measured at 25°C after the heat treatment. All samples tested showed a decrease in viscosity after heat treatment indicative of pectin hydrolysis in samples. The cranberry puree concentrate became extremely difficult to work with after heating and was not included in this portion of the experiment. The decrease in viscosity depended on the treatment temperature. For example, at 88C, the decrease in viscosity in hot extracted puree from pressed cranberries (mid-west) was less than 20% when the sample was held for 90 minutes. On contrast, the viscosity decreased almost threefold after 90 minutes of heating at 99C.

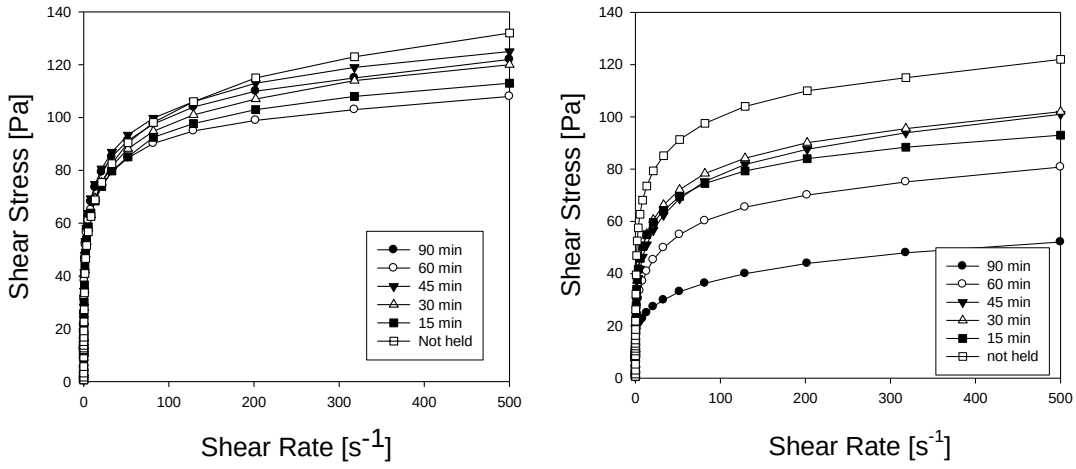


Figure 5.1. Hot extracted pressed cranberry puree (mid-west) held for up to 90 minutes at 88C (left) and 99C (right).

5.4.4 Gelation kinetics in the presence of calcium

The addition of calcium to the formulations did not have a strengthening effect on any of the gels. Figure 5.2 shows the results gelation kinetics experiment for Gel A in the absence and presence of 100mM calcium. Generally, differences were small compared with final gel strength that gels can attain after 24 hours with G' in excess of 10^3 Pa.

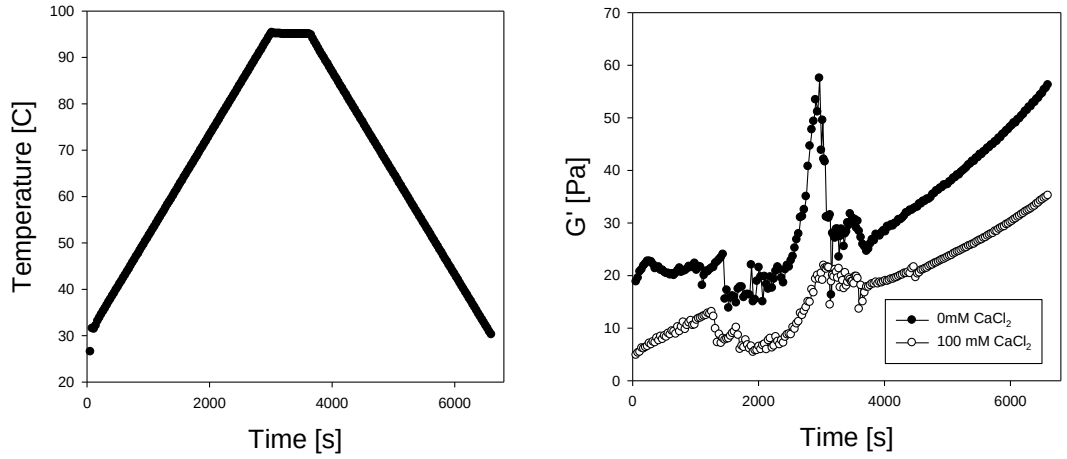


Figure 5.2. Temperature profile of gelation test (left). G' for Gel A with 0 or 100 mM $CaCl_2$ as a function of gelation time during the heating and cooling cycle, (right).

5.5 Discussion

Pectin extraction can damage the structural integrity of pectin, so care must be taken when extracting pectin from raw materials (Turquois, T. et al., 1999). Type of acid, length of acid treatment, and increased temperature can all have an effect on the extraction yield and quality of pectin (Sahari, M.A. et al., 2003). An increased temperature and harsh acid treatment can increase the yield of pectin from a raw material, but may also decrease the quality, (degree of esterification, molecular weight). The yield obtained had a very high standard deviation between samples. Sample size and the nature of the raw material may have played a role in the degree of variation between

samples (Canteri-Schemin, M.H. et al., 2005). In the case of apples, pectin extracted from pomace as opposed to flour had a lower yield. Because the nature of the extraction was crude, the substantial standard deviation may also be due to proteins or neutral sugars linked to the pectin that was not removed during the extraction process (Legentil et. al., 1995). Yield of cranberry pectin has been reported as between 0.4-1.2%, (Flynn, 1950). The yields (wet weight) found during this study were within the lower end of this range, with the exception of the purees made from already processed berries.

As degree of esterification decreases, the affinity of Ca^{2+} for the pectin chain increases. Increased length of un-substituted portions of the pectin chain is beneficial for calcium-induced gelation (Tibbits et al., 1998). The degree of esterification was so high for some of the raw materials; Ca^{2+} would not be able to form the 'bridges' between pectin molecules in order to help form a gel network. The degree of esterification of the pectin in the raw materials was high with the exception of the purees made from previously pressed cranberries. This concurs with previous work done by Pintauro (1967). The addition of Ca^{2+} also may have resulted in a more inhomogeneous gel structure, which could explain the decrease in gelling ability observed during the addition of Ca^{2+} (Löfgren et al., 2005). Pintauro (1967) also found that cranberry extracts did not complex with metal salts, (such as Ca^{2+}).

Pectin hydrolysis may occur for several reasons. Enzymatic hydrolysis by pectin methylesterase and polygalacturonase, acid hydrolysis, and heat can all cause the breakdown of pectin (Diaz et al., 2007). Hydrolysis by heat is dependent on the type of pectin present; for instance, pectate degrades due to heat much more quick than pectin. The rate of acid hydrolysis may also be reduced by a high degree of esterification. In the

case of cranberry pectin however, the prolonged heating of raw materials may have caused demethylation, β-elimination, and a decrease in molecular weight (Krall, S.M. and McFeeters, R.F., 1998). A high degree of esterification and a pH 3-4.5 make the pectin chain more resistant to hydrolysis. However, the cranberry fruit may have a lower pH than 3 and during prolonged heating hydrolysis of glycosidic bonds may occur, as well as chain cleavage by β-elimination (Axelos, M.A.V. and Branger, M., 1993).

Shear stress versus shear rate data was used in order to observe pectin hydrolysis in raw materials. Shear stress versus shear rate increased with increased time and temperature treatment. At 99°C, the shear stress raw materials could withstand decreased as holding time decreased. This is in comparison to treating raw materials at 88°C, where a much less significant change was observed between holding times. This increased holding temperature and increase in holding time could cause pectin hydrolysis, which would account for the decreased shear stress raw materials could withstand (Diaz et al., 2007).

5.6 Conclusions

The yield of pectin and degree of esterification from the raw materials fell within the expected range for cranberry puree with the exception of cranberry purees produced from previously processed berries. Raw materials were found to undergo hydrolysis when exposed to elevated temperatures for an extended period of time. Thus manufacturers have to be careful to control hold times or recirculation times that may be required during shutdown of the filling line. At long holding times at elevated temperatures, pectin may be irreversibly altered and thus no gel or very weak gels are formed after extremely long holding times at elevated temperatures.

CHAPTER 6

CONCLUSIONS

Results show significant changes in gel properties as a function of holding time and temperature. Ability of gels to form elastic gels depended on formulation and formulation D, composed of rehydrated cranberry extract, had the weakest performance as evidenced by all rheological measurements and by texture analysis. The results show that processing conditions as well as formulation are critical factors in ensuring consistency across production lines. Storage time of gels did not always result in increased elasticity, but literature suggests that increasing storage time past 4 days may confer increased elasticity to the gels (Baker and Goodwin, 1941a; Rao et al., 1993). Increased holding time and temperature had a positive effect on formulation robustness. Formulations with an increased amount of pectin had increased levels of gel robustness.

The yield of pectin and degree of esterification from the raw materials fell within the expected range for cranberry puree with the exception of cranberry purees produced from previously processed berries. Raw materials were found to undergo hydrolysis when exposed to elevated temperatures for an extended period of time.

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