Chapter 5: Results and Discussion

I. Edible Coating Development

Edible coatings in this study were created from lipid-hydrocolloid emulsions. The goal of coating development was to produce a durable coating that would maintain its structure during storage and provide protective moisture and gas barrier properties. Such coatings would not flake or become brittle upon refrigeration. The coatings should also be easy to apply and generate no undesirable textural or taste characteristics. Final coating formulations are presented in Appendices I-L.

A. Lipid Selection

The lipid portion of the edible coating is the main barrier for prevention of moisture loss. In order for the coating to be effective, the lipid must adhere to the fruit's surface, form a continuous barrier, and remain intact over a wide temperature range. Previous coating trials were conducted using liquid fats (Ball, 1997) including Apex B (AC Humko, Memphis, TN) and Durafresh (PacRite, Ecoscience Produce Systems, Orlando, FL). These fluid lipids did not adhere well to the fruit's surface at warmer temperatures, which resulted in incomplete coverage. Attempts to modify the adhesion properties of the liquid fats previously used included incorporation of hydrocolloids. However, results soon showed that the nature of the fat was the determining factor in coating adherence. Liquid fats simply bead up on the pepper's surface and do not afford maximum even coverage.

Fats that were solid at room temperature, including shortening-type plastic fats: Humkote (AC Humko, Memphis, TN), Astral (AC Humko, Memphis, TN), Vegetable Shortening (Food Lion, Salisbury, NC), as well as paraffin wax (Gulf Wax, Gulf Lite and Wizard, Inc., Memphis, TN) were tested next. Such fats were too thick to apply directly, and required melting prior to application. Melting allowed the lipid to be spread evenly on the fruit's surface. Upon cooling, the fats provided an even barrier. However, depending upon the nature of the fat, some fats became too brittle as they cooled. For example, paraffin wax began cooling during coating application, and resulted in very thick uneven wax distribution, and upon refrigeration the wax became very brittle. In order to determine which of the three plastic fats was best, pepper slices were coated evenly with each molten fat, and then refrigerated for 48 hours. All three fats remained intact on the pepper surface, however, they differed in their level of opacity. The fats with a higher solid fat content at lower temperatures (Astral and Humkote) appeared slightly milky upon cooling. Vegetable shortening however, did not appear to provide a durable barrier. Therefore, only Astral and Humkote were used as the lipids to be combined with polysaccharides.

Humkote (Product number 54358) is composed of partially hydrogenated soybean and cottonseed oil. Typical uses include confectionery coatings as well as use in dressings and dips. The melting point for Humkote is 105±1.5°F making it solid at room temperature, but easily melted with low heat. Astral R (Product number 52604) is also made from partially hydrogenated cottonseed and soybean oils. The melting point of Astral (99±2°F) is slightly lower than Humkote. Comparison of the solid fat indices at various temperatures for both fats are listed in Table 5.1.

B. Polysaccharide Components

Initial testing of polysaccharide components was conducted using gum products. Polysaccharide gums were selected based on their ability to promote coating viscosity, coating adhesion to the fruit, as well as their ability to reduce the thickness of the lipid layer, and thereby prevent anaerobic metabolism.

C. Emulsifier Selection

Emulsifiers were selected for the ability to stabilize the coating emulsions, as well as their ability to be easily incorporated into the coating formulations. Two plastic emulsifiers (Super G #1, Product number 86095, and Super G #10, Product number 84178, AC Humko, Memphis TN) were tested. Both emulsifiers were composed of

Table 5.1: Solid fat indices at various temperatures for Astral and Humkote lipid coating components.

Temperature	**Solid Fat Index:**	
	Astral	**Humkote**
50°F	69	62.5
70°F	59	53
80°F	52	46
92°F	22	26
100°F	5	10.5

mono- and di-glycerides, however, Super G 10 was ultimately selected due to the added antioxidants tert-butyl hydroquinnone (TBHQ) and citric acid which could help prevent any rancid flavor changes that may develop. A second emulsifier composed of xanthan gum (TIC Gums, Belcamp, MD) and propylene glycol alginate (TIC Gums, Belcamp, MD) was used in a ratio of 1:2 at a total percentage of no more than 1.8%.

D. Gum Coatings

Lipid and gum coatings were constructed as emulsions in the following manner. First, the lipid portion was melted and then transferred to a blender. The dried gums are then added to the agitated molten lipid. Water is then slowly added to the lipid base, so as to hydrate the gum. In most cases, use of an emulsifier was needed to combine the hydrophobic and hydrophilic phases. Emulsifier selection will be discussed in a later section.

Xanthan gum (0.6%) (TIC Gums, Belcamp, MD) and propylene glycol alginate (1.2%) (TIC Gums, Belcamp, MD) were initially used because of their ability to stabilize emulsions when used together. Use of these two polysaccharides resulted in a smooth creamy mixture that did not separate upon standing. Therefore, this coating was used as a model to test other ingredients.

In order to determine the optimum amount of lipid to use, various levels of molten fat were incorporated into this emulsion. The total level of gums remained constant (1.8%), while the amount of fat (38%, 48%, 58%) and water (60.2%, 50.2%, and 40.2%, respectively) was varied. Results showed that emulsions made with 38% fat and 60.2% water were thick and showed no separation. However, as the fat content increased and the water content decreased the emulsions became thinner. The reduced water content was most likely the reason for the thinning of the emulsions. Sufficient water was needed for the gums to hydrate. If there is not enough water the gums do not swell, and therefore, do not increase the viscosity and stability of the emulsion. Finally, coatings were made with a maximum of 38% lipid, and a minimum of 60% water for gum hydration.

The amount of time needed for emulsion mixing was also a factor that needed to be determined. Increased blending of emulsions resulted in increased shear stress, and resulted in coating thinning and fat flocculation. Therefore, coatings were blended for the minimum time necessary for all of the molten fat to be incorporated. Timing ranged from approximately 5 to 10 seconds depending on the quantity of lipid used.

After determining the correct amount of fat, and the appropriate mixing time, different polysaccharide gum combinations were tested. Gum combinations tested included the following:

1% carboxy-methyl-cellulose (CMC)
3% gum arabic
1% CMC and 3% gum arabic
1% locust bean gum
1% locust bean gum and 1% xanthan gum
0.6% xanthan gum and 1.2% propylene glycol alginate

The aforementioned hydrocolloids were selected for their chemical properties including their ability to stabilize emulsions, enhance viscosity of solutions, and their overall ability to act as a barrier film (BeMiller and Whistler, 1996). Carboxy-methyl-cellulose (CMC) is a linear polysaccharide that is primarily used as a thickener in sauces and dressings. CMC was examined for its ability to thicken coatings and promote adhesion. Locust bean gum was used for its ability to react with xanthan gum and form gels as well as promote smooth texture. Xanthan gum was selected for it's ability to promote "cling" as it functions in salad dressings, as well as for its emulsification properties when it is combined with propylene glycol alginate. Gum arabic was also selected for both its ability to stabilize emulsions and its potential coating properties.

Acceptable coatings, which were well emulsified, smooth, and viscous enough to be applied to the fruit, included both the combination of xanthan and propylene glycol alginate, and the xanthan and locust bean gum coating. Both of these coatings were refined to improve emulsion stability by adding mono- and di- glycerides (Super G 10 (AC Humko, Memphis, TN)). The final formulations for xanthan gum coating and locust bean gum coating can be seen in Appendices I and J, respectively.

E. Acid Modified Starch Coating

Acid modified starch is known for its film forming ability. Initially, a mixture of acid modified starch (30%) (PCote, Grain Processing Corporation, Muscatine, IA) and water (70%) were blended and then heated to dissolve the starch. The mixture was spread onto the fruit and allowed to cool at room temperature. In addition to room temperature cooling, the coating was also oven-dried by placing the pepper pieces on a baking sheet in a 250°F oven for approximately 10 minutes. Both drying methods produced a coating that formed a film, however, the film failed to adhere to the fruit's surface, and pulled off in one solid piece. To determine if altering the fruits surface would improve adhesion, the starch and water mixture (30:70) was applied over a base coating of solidified lipid. This bi-layer coating also peeled after refrigeration.

Next, separate trial coatings were made with the addition of Astral and Humkote lipids. Lipids were incorporated into the starch mixture during the heating step for the purpose of improving adhesion, as well as providing moisture barrier properties. However, the coating still failed to adhere to the fruit's surface. The level of starch was suspected to be too high, so the amount was reduced to 18.9%, while the water content was increased to 75% and the fat level was changed to 2.8% lipid and 2.8% emulsifier. This modified coating adhered to the fruit well after drying, however, after refrigeration the coating became brittle and flaky. Next, a formulation of 10% emulsifier, 15% fat, 15% starch and 60% water was made. This formulation was thick and smooth and turned clear and shiny when dried. Glycerol (5%) (Vitusa, Berkley Heights, NJ) was added to aid in the plasticity of the coating. The other ingredients were used as follows: 3.5% fat, 3.5%

emulsifiers, 15% starch, and 73% water. This coating was heated until it began to thicken. The coating was then applied to the peppers and resulted in excellent coating and drying properties. After four days in refrigerated storage the coating still remained intact and did not peel. Upon trying to reproduce this coating, it became apparent that the cooking time was important. The duration of the heating step was directly related to the solids content of the coating. A solids content of 53% ± 2% was determined to be optimum. Both Astral and Humkote performed well in this formula, however Astral was eventually determined to be too cloudy and thick after refrigeration, and therefore, Humkote was used in the final formulation. The final formulation for the acid modified starch coating can be seen in Appendix K.

F. Maltodextrin Coating

Maltodextrin solutions can form gels after heating and refrigeration. Such gels have been used to mimic fat in baked goods. Maltodextrin gels are also capable of forming films when applied in thin layers and allowed to dry. The dextrose equivalent (DE) value for a starch is inversely related to molecular weight of the dextrin fragments. Lower molecular weight fragments are more hygroscopic and tend to form more uniform films. For example, a 30% maltodextrin gel (DE 11, 62.1% solids) creates a thick syrup-like coating over the fruit's surface that eventually dries to a brittle film. Several other maltodextrin gel concentrations were also examined for film forming ability, including two levels of Maltodextrin (DE 18) solution, 30% and 50%. However, the film was still flaky upon drying. Therefore, as with the other trial, both Astral and Humkote lipids were used in separate trials to plasticize the coating. First, maltodextrin (27%) (Star-Dri 18, Staley, Decatur, IL, DE 18), water (63.6%), lipid (4.5%) and emulsifier (4.5%) was added to a saucepan and brought to a boil. In this formulation the lipid separated, indicating that the mono- and di-glyceride emulsifier was not sufficient. This heated mixture was then added to a blender and emulsified by first adding xanthan and algin gums (0.6g and 1.2g respectively) and then adding 60 ml water. The resulting product provided excellent coverage and dried smoothly without flaking, however, if the pepper fruit was bent or distorted the coating would wrinkle and develop cracks. To prevent cracking the fat content was increased to 9.1% lipid, 9.1% emulsifier, and the maltodextrin level was decreased to 18.2%, and 38.6%. After heating, 40 ml of this mixture was then added to a blender. Next, 1.2 g of algin and 0.6 g of xanthan gum were added to the maltodextrin mixture. Sixty grams of distilled water were then added to the blender with constant agitation. The resulting emulsion was then refrigerated. After 24 hours of refrigeration the coating was thick and viscous. After application the coating dried shiny and clear. After refrigeration the coating still remained intact and did not peel or flake. As with previous coatings Humkote lipid was selected for its clarity upon refrigeration. The final formulation of the maltodextrin coating can be seen in Appendix L.

G. Sensory Results

Preference testing of fresh and stored coatings was conducted using a ranking test. Testing involved panelists ranking the appearance and taste of freshly coated peppers or coated peppers stored for ten days.

Sensory results (Figure 5.1) indicated significant ($p<0.05$) differences in both appearance and taste of freshly coated peppers. Peppers coated with the locust bean gum emulsion were significantly ($p<0.05$) preferred over peppers coated with the maltodextrin coating (Figure 5.1). However, no preference differences between maltodextrin coatings and other coated groups were observed.

The appearance and taste of stored peppers differed significantly ($p<0.05$). Results indicated that peppers coated with the acid-modified starch were unacceptable, as determined by significantly lower preference ratings (Figure 5.2).

Sensory testing indicated that only one of the four coatings prototypes, acid-modified starch, was unacceptable for use due to its low preference rankings after storage. Therefore, xanthan gum, locust bean gum, and maltodextrin coatings were selected to undergo objective testing during a 5 week storage study.

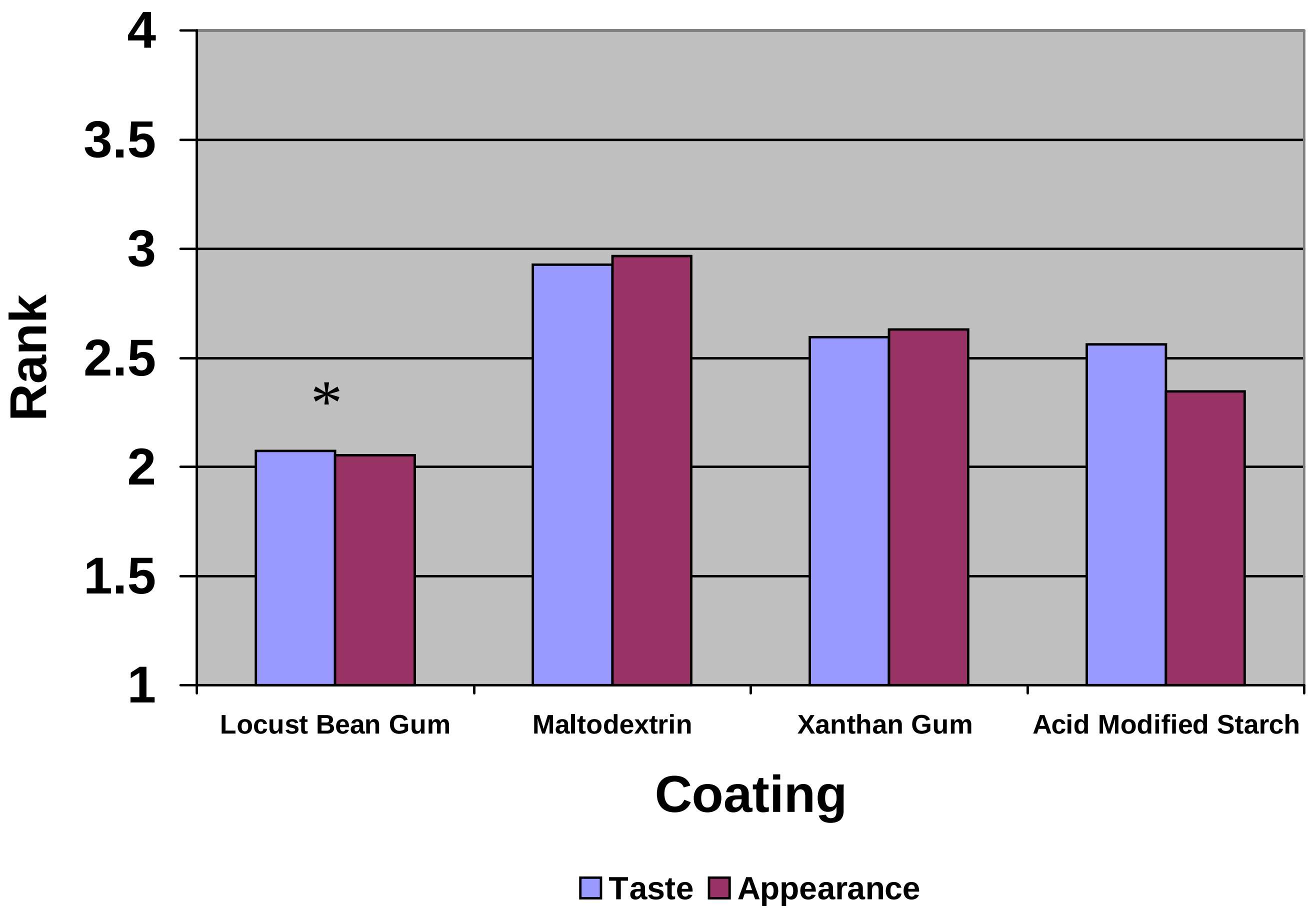

Figure 5.1: Ranking preference scores for freshly coated peppers (1 = preferred most, 4= preferred least).
*Locust bean gum coated peppers were preferred over maltodextrin coated peppers ($p<0.05$) while no differences ($p>0.05$) between other groups were seen.

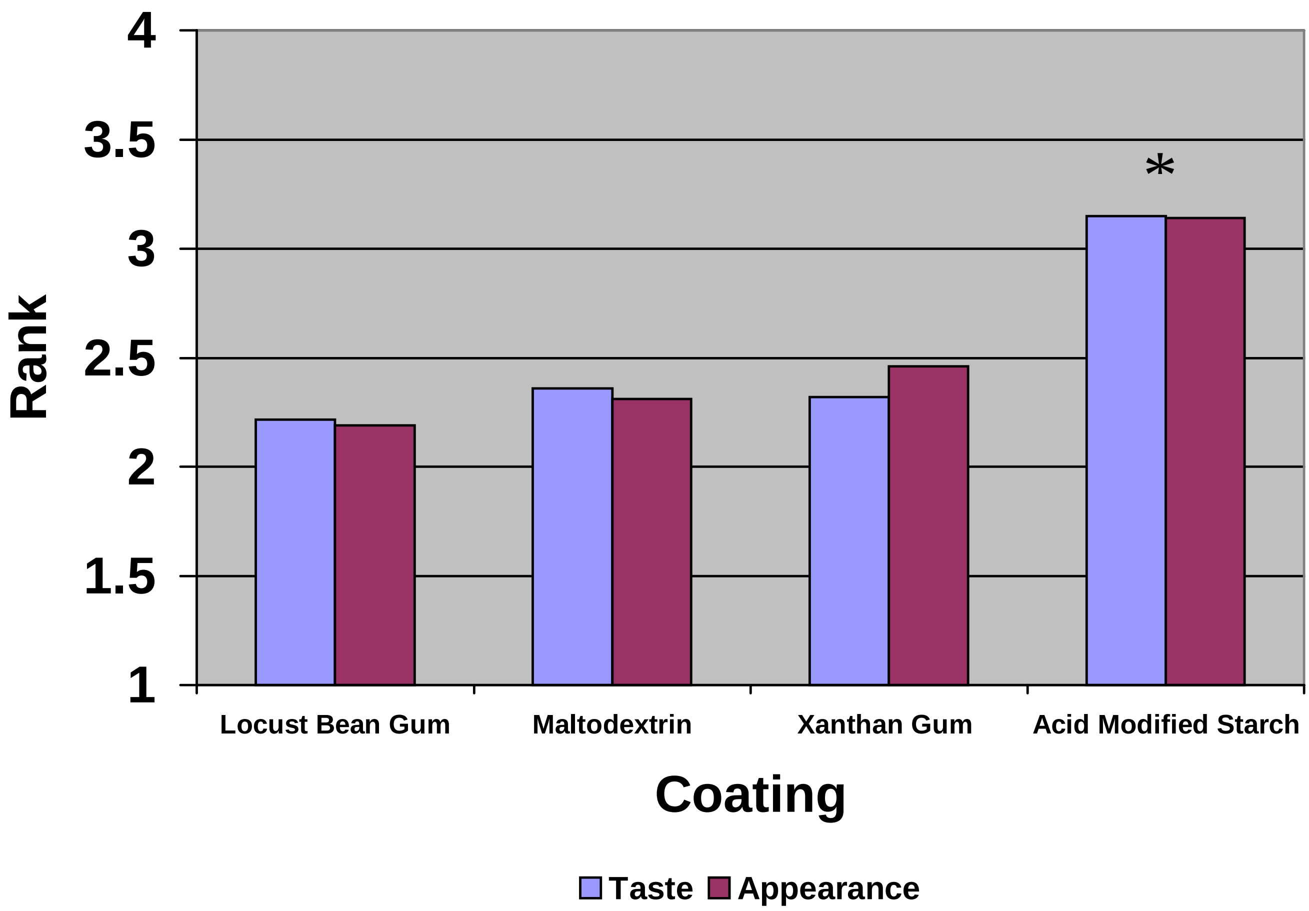

Figure 5.2: Ranking preference scores for coated peppers stored for 10 days (1=preferred most, 4= preferred least).

*Acid modified starch coated peppers were preferred significantly less ($p<0.05$) than all other coatings.

H. Coating Performance During Storage

During the course of the storage period several problems with the coatings became evident. First, when peppers were transferred from refrigerated storage into ambient temperatures moisture would condense on their surfaces. This condensation was not a problem in the higher lipid content coatings including xanthan gum and locust bean gum. However, when the fruits that were coated with the maltodextrin material were handled excessively the coating would become sticky and not maintain a uniform covering over the surface.

Another problem that was primarily associated with the xanthan gum and locust bean gum coated peppers was the hazy appearance of the coating after immediate removal from refrigerated storage. When the fruits from these coated groups stood alone the hazy or dull appearance was not pronounced. However, when they were compared to uncoated fruits, or fruits coated with the maltodextrin coating, they appeared less attractive. This dull appearance was due to the changes in the fat solidity between room temperatures and refrigeration temperature. At room temperature the fat is not as opaque as after refrigerated storage.

II. Quality Changes During Storage

A. Respiration

Respiration rates are directly related to fruit senescence. The goal of edible coating application was to provide a physical barrier to gasses that would mimic modified atmospheric storage. Under such conditions carbon dioxide could be trapped and inhibit metabolic processes leading to decay. Despite these goals, no significant differences ($p>0.05$) were observed between the log of respiration rates of uncoated and coated peppers (Table 5.2), and no significant interaction was observed. The lack of differences between coated and uncoated groups indicated that the coatings did not offer gas barrier

Table 5.2: Log of respiration rates (mg CO_2/kg/h) of coated and uncoated green peppers stored for five weeks.

Treatment [a]	Week				
	1	2	3	4	5
Control	1.30	1.40	1.40	1.40	1.80
Xanthan Gum	1.20	1.40	1.50	1.50	1.80
Maltodextrin	1.30	1.50	1.50	1.50	1.60
Locust Bean Gum	1.30	1.40	1.50	1.50	1.70
Coated Groups Average	1.27	1.43	1.50	1.50	1.70

[a] uncoated control peppers did not differ significantly ($p>0.05$) from coated peppers.

properties, that would have served to mimic modified atmospheric storage. Other studies have also failed to show reduced respiration rates in green peppers treated with a wide variety of coatings including lipid, carbohydrate and protein based types (Lerdthanangkul and Krochta, 1996). However, some types of coatings, including those made from chitosan (Ghaouth et al., 1991), have been shown to reduce respiration rates.

Respiration rates changed significantly ($p<0.01$) during the study. A significant linear relationship between weeks and log respiration rate was evident for each individual treatment group ($p<0.01$ for each treatment) (Figure 5.3). Typically, green peppers, which are non climateric fruits, do not show marked increases in respiration rates during storage. However, slight increases in respiration are expected when peppers are under physical stress, including physical handling, temperature extremes, and moisture deprivation (Saltveit, 1977). For these reasons, a slight increase in respiration rates with time was expected, however, the large differences between initial and final respiration rates were extreme. In addition, respiration rates of green peppers are typically much lower (5-10 ml/CO_2/kg/h) than values obtained in this study (Phan, 1987).

Increased respiration rates may be attributed to several factors including chilling injury, water stress, excessive handling and microorganism deterioration. When fruits become wounded or handled excessively respiration rates have been shown to permanently increase (Saltveit, 1977). Peppers during this study were handled on many occasions, and those fruit that remained in storage longer were handled more often over time.

Storing peppers in an environment that is below their lower temperature limits results in chilling injury, which will also increase respiration rates. In order to prevent chilling injury, peppers must be stored between 41 and 53.6°F at a relative humidity of 90-95% (Phan, 1987). Commercial refrigerators used for storage were placed at 40°F, which may have been too cold for pepper storage. Relative humidity levels were difficult to maintain under the refrigerated storage provided. The constant action of opening and

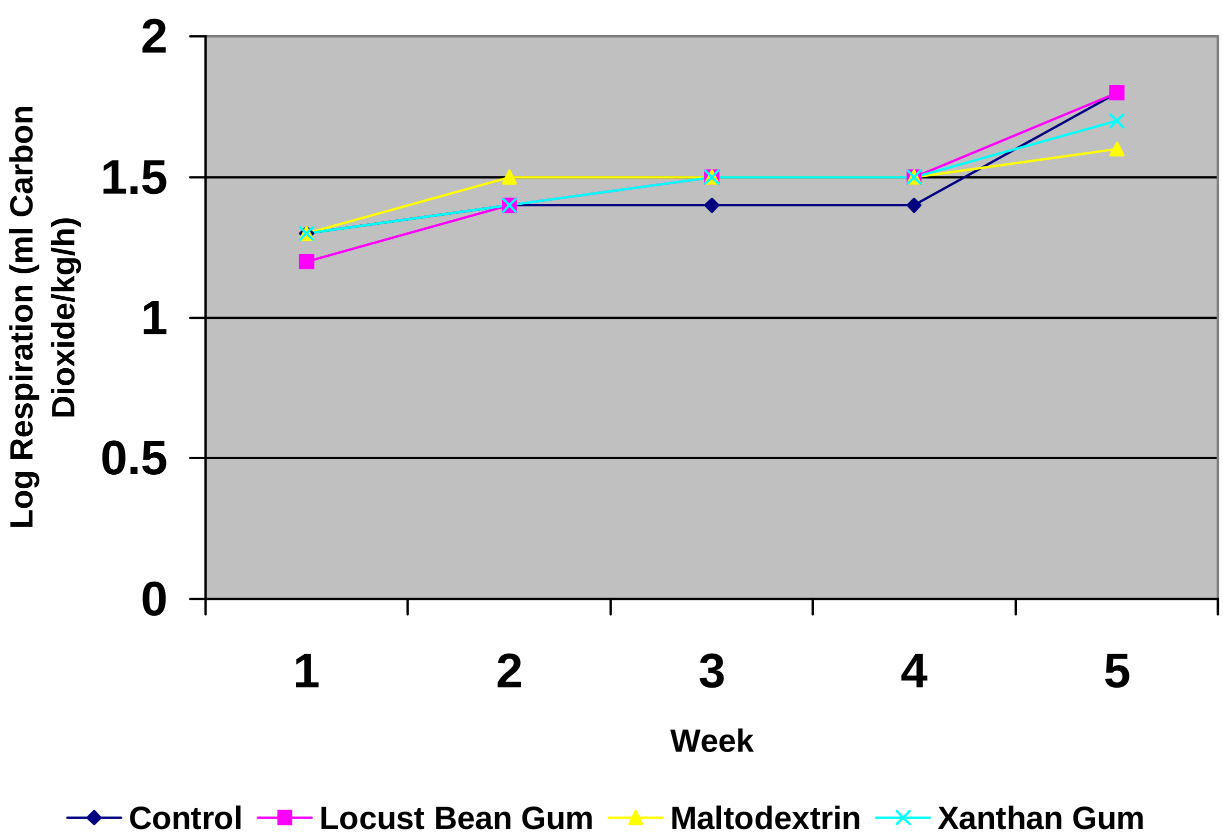

Figure 5.3: Linear relationship between log respiration rate of coated and uncoated green peppers and storage week ($p<0.01$)

closing of doors kept the humidity levels very low (31% ± 3%) which put added stress on the peppers during the five weeks of storage.

Increased rates have also been attributed to deterioration in general as well as microorganism infestation. Coatings may have allowed additional moisture to cling to the fruit's surface. This additional moisture would be an ideal environment for bacteria to thrive. Other types of moisture-trapping treatments, including packaging wraps, have been shown to foster high levels of yeast growth (Brackett, 1990) as well as increase the incidence of bacterial soft rot (Miller et al., 1986). Bacterial soft rot as well as high numbers of yeast both increase respiration rates.

Ripening in green peppers is characterized by a surface color change. Despite the lack of significant chlorophyll differences between treatments (Table 5.3), peppers began to change into the ripe fruit color. Color changes normally coincide with increases in respiration, however these increased rates are substantially less than those seen for climateric fruits (Saltveit, 1977).

B. Weight

The most obvious differences between treatments were observed in the evaluation of weight loss during storage. Weight loss, which is primarily a function of water loss, leads to textural changes as well as appearance changes, and is the primary contributor to spoilage of green bell peppers (Kader, 1983). Although textural differences were not detected by methods used in this study, significant differences in weight loss between treatments, and during the storage period, underscore the observed changes in texture that were not picked up by objective textural evaluation. All treatments exhibited significant moisture loss during storage ($p<0.01$) (Figure 5.4). Uncoated control peppers were found to lose significantly ($p<0.05$) greater amounts of weight during storage than any of the coated groups (Figure 5.4). Green peppers are not considered saleable after they lose 7% of their water weight (Burton, 1982a). Therefore, uncoated peppers were not saleable after 8 days, while coated groups were still saleable for an additional 6 to 8 days. As

Table 5.3: Chlorophyll content (mg/g) of green pepper fruits stored for five weeks.

Treatment [a]	Week				
	1	2	3	4	5
Control	0.145	0.106	0.133	0.122	0.126
Xanthan Gum	0.116	0.069	0.097	0.120	0.129
Maltodextrin	0.118	0.088	0.120	0.133	0.155
Locust Bean Gum	0.120	0.116	0.109	0.107	0.131
Coated Groups Average	0.118	0.091	0.108	0.120	0.138

[a] Coated treatments did not differ significantly ($p>0.05$) in chlorophyll content from uncoated control peppers.

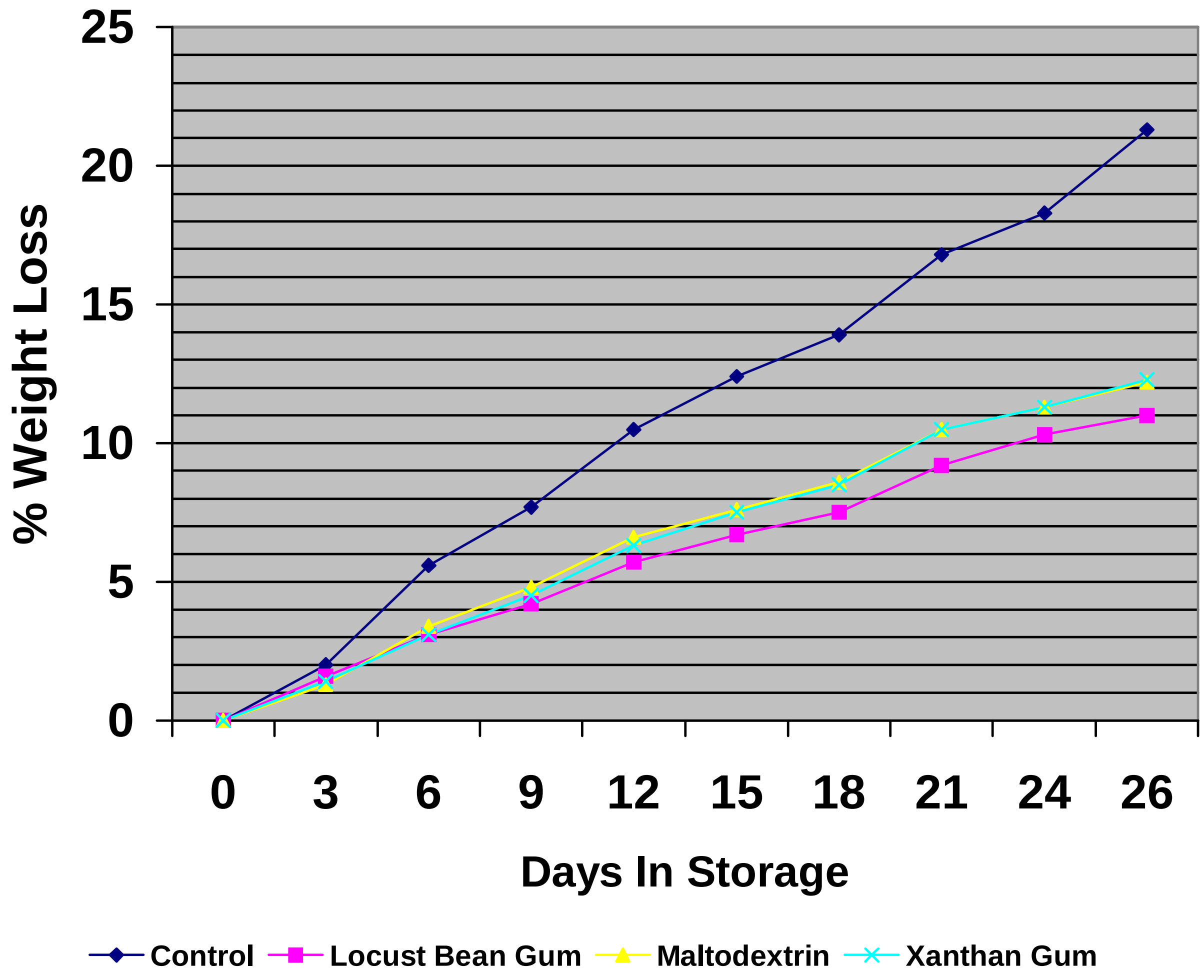

Figure 5.4: Cumulative percent weight loss of coated and uncoated green bell peppers stored for 26 days.

All treatments lost significant levels of moisture during storage ($p<0.01$), and all coated peppers retained significantly more moisture ($p<0.05$) than uncoated peppers.

mentioned previously, relative humidity levels were not optimum for storage, and may have increased the rate of water loss, and therefore accelerated deterioration. However, the increased loss observed in uncoated samples still emphasized the protective nature of the applied coatings.

The fact that none of the coated peppers differed significantly ($p>0.05$) in weight loss indicated that the use of a coating alone, and not the components of the coating, was protective against weight loss. Results concur with the fact that lipids are commonly thought to reduce moisture loss through transpiration from the plant's cuticle. Lipids act as a seal over the fruit's surface, and because water is not miscible with lipids the water is retained. However, results also show that the maltodextrin coating, which was much lower in lipid content than the xanthan gum and locust bean gum coatings, can still act as an effective moisture barrier. Other research has also confirmed the ability of coatings made from hydrocolloids and lipids to have good water vapor barrier properties (Greener and Fennema, 1989b). Other emulsion coatings, including those made from proteins and lipids, have also successfully reduced water loss (Avena-Bustillos and Krochta, 1993). The drying of the dextrin solution, with appropriate solids content, creates a polysaccharide matrix which water has difficulty penetrating. Dextrin-based coatings are known to provide better moisture resistance than starch coatings, as well as generate protective films when dried.

C. Texture

Textural changes in green peppers can be attributed to enzymatic breakdown of pectin and loss of cellular turgor pressure. Loss of turgor pressure within fruit cells results in shriveling and wilting. Edible coatings were applied to reduce moisture loss from the fruit and help retain turgor pressure. Fruits were tested for textural changes by analyzing the pectin content as well as analyzing the force required for a cylindrical probe to puncture a cut sample of the fruit.