Effect of antifungal hydroxypropyl methylcellulose-beeswax edible coatings on gray mold development and quality attributes of cold-stored cherry tomato fruit

Cristiane Fagundes[a], Lluís Palou[b], Alcilene R. Monteiro[a], María B. Pérez-Gago[bc]*

[a] *Universidade Federal de Santa Catarina, Departamento de Engenharia Química e Engenharia de Alimentos, Campus Universitário-Trindade, 88040-900 Florianópolis, SC, Brazil*

[b] *Centre de Tecnologia Postcollita (CTP), Institut Valencià d'Investigacions Agràries (IVIA), Apartat Oficial, 46113 Montcada, València, Spain.*

[c] *Fundación AGROALIMED*

**** Corresponding author: M.B. Pérez-Gago, E-mail: perez_mbe@gva.es; Tel +34 963424000***

Abstract

Edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW), and food preservatives with antifungal properties were evaluated on cherry tomatoes during cold storage. Food preservatives selected from previous research work included sodium propionate (SP), potassium carbonate (PC), ammonium phosphate (APh) and ammonium carbonate (AC). Cherry tomatoes artificially inoculated with *Botrytis cinerea* were coated and stored up to 15 d at 5 °C followed by 7 d of shelf-life at 20 °C. All antifungal HPMC-BW coatings significantly reduced gray mold development on inoculated and cold-stored cherry tomatoes, being the SP-based coating the most effective. Analytical and sensory fruit quality was also evaluated after cold storage and shelf-life. The AC-based coating was the most effective to control weight loss and maintain the firmness of coated cherry tomatoes. Respiration rate, firmness, color, sensory flavor, off-flavor, and fruit appearance were not adversely affected by the application of the antifungal coatings. Overall, the application of HPMC-BW edible composite coatings containing AC could be a promising treatment to extend the postharvest life of cherry tomatoes. Further studies should focus on the modification of some physical characteristics of the coatings in order to enhance the general performance and provide higher peel gloss.

Keywords: cherry tomato; *Solanum lycopersicum;* postharvest quality; food additives; *Botrytis cinerea;* gray mold.

1. Introduction

During the last decades, there has been an increased interest by consumers in natural healthy fresh fruits and vegetables. Tomato (*Solanum lycopersicum* L.), being a climacteric fruit, has a relatively short postharvest life, generally limited by transpiration, postharvest diseases, increased ripening and senescence (Zapata et al., 2008). Although storage under optimum cold storage conditions have been effective in extending shelf-life as it reduces the rate of respiration of the fruit, the benefits from refrigeration are not important enough to preserve produce quality. Tomato fruit is susceptible to postharvest diseases caused by various pathogenic fungi that cause important economical losses. *Botrytis cinerea* Pers.: Fr. and *Alternaria alternata* (Fr.) Keissl., causing gray mold and black spot, respectively, are among the most common fungal pathogens responsible for postharvest decay on cherry tomato fruit (Wang et al., 2010).

Several technologies have been developed to extend the shelf-life of fruits and vegetables, which include the control of diseases caused by fungi. One of these techniques is the release of antimicrobial agents incorporated into biodegradable edible films and coatings (Valencia-Chamorro et al., 2011). Edible coatings are considered an environmentally-friendly technology able to extend the shelf-life of fruits and vegetables by reducing moisture loss and respiration rate, preventing physical damage, and enhancing product appearance. These coatings are commonly based on polysaccharides, proteins, and lipids, alone or in combination. In fruits and vegetables, composite coatings based on polysaccharides or proteins and lipids are usually used to achieve good moisture and gas barriers provided by the lipid and polymer components, respectively. Among the hydrophobic materials, waxes such as BW have been the most widely used for protective moisture barrier of fresh commodities. Furthermore, the addition of food preservatives can improve the functional properties of the coatings by retarding the growth of bacteria, yeasts, and molds during storage and distribution of fresh fruits and vegetables (Valencia-Chamorro et al., 2011; Lucera et al., 2012). In

tomato, the development of antifungal edible coatings has been mainly focused on chitosan-based formulations. These coatings have been effective controlling black spot caused by *A. alternata* (Reddy et al., 2000), gray and blue molds caused by *B. cinerea* and *Penicillium expansum*, respectively (Liu et al., 2007; Badawy and Rabea, 2009), anthracnose caused by *Colletotrichum* spp. (Muñoz et al., 2009), and rhizopus rot caused by *Rhizopus stolonifer*, when combined with essential oils (Ramos-García et al., 2012). The addition of food additives or 'generally recognized as safe' (GRAS) compounds with antifungal properties to other hydrocolloids has been less studied in tomato. Pea starch coatings amended with potassium sorbate showed some antifungal activity against *P. expansum* and *Cladosporium fluvum*; however, decay was only significantly controlled for 5 days at 5 ºC (Mehyar et al., 2011). In a very recent work, we studied the *in vitro* activity of a wide variety of food additives (mineral salts, organic acid salts, paraben salts, and other GRAS compounds) with antifungal properties against *B. cinerea*, formulate stable hydroxypropyl methylcellulose (HPMC)-beeswax (BW) edible composite coatings containing selected antifungal food preservatives, and determined the curative activity of these coatings against gray mold on cherry tomatoes artificially inoculated with *B. cinerea* (Fagundes et al., 2013). Overall, the best results for reduction of gray mold on cherry tomato fruit incubated at 20 ºC were obtained with coatings containing 2.0% of sodium propionate (SP), potassium carbonate (PC), ammonium phosphate (APh), or ammonium carbonate (AC). The next research step for potential commercial development of these antifungal coatings is the evaluation of their performance on cold-stored cherry tomatoes. Therefore, the objective of this work was to determine the effect of selected HPMC-lipid edible composite coatings containing food additives with antifungal properties on the development of gray mold and the physico-chemical and sensory quality of cherry tomatoes during cold storage.

2. Materials and methods

2.1. Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). BW (grade 1) was supplied by Fomesa Fruitech, S.L. (Beniparrell, València, Spain). Oleic acid and glycerol were from Panreac Química, S.A (Barcelona, Spain). Laboratory reagent grade preservatives (99% minimum purity) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany) and included SP (CH_3CH_2COONa; E-number E-281), PC (K_2CO_3; E-501 (i)), Aph ($NH_4H_2PO_4$; E-342 (i)), and AC [$(NH_4)_2CO_3$; E-503 (i)]. All these chemicals are classified as food additives (with their correspondent E-number) or GRAS compounds by the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (US FDA).

2.2. Emulsions preparation

HPMC-lipid edible composite emulsions were prepared combining the hydrophilic phase (HPMC) and the hydrophobic phase (BW) suspended in water. Glycerol and oleic acid were used as plasticizer and emulsifier, respectively. All the emulsions contained 30% BW (w/w, db). Ratios of HPMC-glycerol (3:1) (dry basis, db) and BW-oleic acid (5:1) (db) were kept constant throughout the study. Tween 80 was also added to the formulations at a concentration of 1.5% (w/w, wet basis; Panreac-Química S.A., Barcelona, Spain) to improve wetting of the coating and adherence to tomato surface. All formulations contained 2% (w/w, wet basis) of food preservative. Emulsions were prepared as described by Valencia-Chamorro et al. (2008). Briefly, an aqueous solution of HPMC (5% w/w) was prepared by dispersing the HPMC in hot water at 90 ºC and later hydration at 20 ºC. The corresponding food preservative, BW, glycerol, oleic acid, Tween 80, and water were added to the HPMC solution and heated at 98 °C to melt the lipid. Samples were homogenized with a high-shear probe mixer (Ultra-Turrax model T25, IKA-Werke, Steufen, Germany) for 1 min at 12,000 and 3 min at 22,000 rpm. Emulsions were cooled under agitation to a temperature lower than 25 ºC by placing them in a water bath and agitation was continued during 25 min to ensure complete hydration of the HPMC. The final solid concentration of the emulsions were

optimized to obtain formulations with a viscosity range of 100-150 cp. Table 1 shows the solid concentration, viscosity and pH of the emulsions containing selected food preservatives. Emulsions were kept 1 day at 5 °C before use. These formulations were stable and no phase separation was observed.

2.3. Effect of coatings on disease development

2.3.1. Fungal inoculum

The strain TAA-1 of *B. cinerea*, obtained from decayed tomatoes in Valencia packinghouses, was isolated, identified, tested for pathogenicity, and maintained in the IVIA culture collection of postharvest pathogens. Prior to each experiment, the isolate was grown on potato dextrose agar (PDA; Sigma-Aldrich Chemie, Steinheim, Germany) in petri dishes at 25 °C for 7-14 days. A high-density conidial suspension was prepared in Tween 80 (0.05%, w/v) in sterile water, passed through two layers of cheesecloth, measured with a haemacytometer, and diluted with sterile water to achieve an inoculum density of 1 x 10^6 spores/mL of *B. cinerea*.

2.3.2. Fruit inoculation and coating application

Cherry tomatoes (*Solanum lycopersicum* L. var. *cerasiforme* cv. Josefina*;* syn.: *Lycopersicon esculentum* Mill.) used in the experiments were commercially grown and collected in the Valencia area (Spain). Fruit were free from previous postharvest treatments or coatings. Before each experiment, fruit were selected, randomized, washed with fruit biodegradable detergent at 6% (v/v) (Essasol V., Dydsa, Potries, Valencia), rinsed with tap water, and allowed to air-dry at room temperature. Cherry tomatoes were superficially wounded once in the equator with a stainless steel rod with a probe tip 1 mm wide and 2 mm in length. This wound was inoculated with the pathogen by placing 10 µl of a spore suspension containing 1 x 10^6 spores/ml of *B. cinerea*. After incubation at 20 °C for 24 h to resemble common fungal infections, inoculated fruit were coated by immersion for 30 s in the selected HPMC-lipid edible

composite emulsions, drained, and allowed to air-dry at 20 °C. Inoculated but uncoated fruit were used as control. Coated fruit were placed on plastic trays on corrugated cartons that avoided fruit contact and stored for 14 days at 5 °C, followed by 7 d at 20 °C and 85-90% RH. These conditions simulated typical commercial cold storage and shelf-life for Spanish cherry tomatoes. In every experiment, each treatment was applied to 3 replicates of 10 fruit each. The experiments were repeated twice.

2.3.3. Determination of disease incidence and severity

Gray mold incidence was calculated as the percentage of decayed fruit. Disease severity was determined as the diameter of the lesion (mm). Both incidence and severity were assessed after 7 and 14 d of storage at 5 ºC, and also after a shelf-life period of 7 d at 20 ºC following cold storage.

2.4. Effect of coatings on fruit quality

2.4.1. Fruit coating and storage

For the quality study, fruit were selected, randomized, washed with biodegradable detergent, rinsed with tap water, and allowed to air-dry at room temperature. Fruit were then divided into 5 groups of 120 fruit each, which corresponded to the four coating treatments described in Table 1 and one control (uncoated fruit). Cherry tomatoes were coated as described above, drained of excess coating, dried and stored for up to 15 d at 5 ºC and 90-95 % RH. Physico-chemical and sensory fruit quality was assessed after 10 and 15 d of storage at 5 ºC plus a shelf-life period of 5 d at 20 ºC.

2.4.2. Assessment of fruit quality

2.4.2.1. Weight loss

Lots of 30 non-inoculated fruit per treatment were used to measure weight loss. The same marked cherry tomato were weighted at the beginning and at the end of each storage period. The results were expressed as the percentage of initial weight lost.

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181*2.4.2.2. Fruit firmness*

182Firmness of 20 fruit per treatment was determined at the end of each storage period 183using an Instron Universal testing machine (Model 4301, Instron Corp., Canton, MA, 184USA). Each fruit was compressed between two flat surfaces closing together at a rate 185of 5 mm/min. The machine gave the deformation (mm) after application of a load of 9.8 186N to the equatorial region of the fruit. Results were expressed as percentage of 187deformation, related to initial diameter.

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189*2.4.2.3. Color*

190Skin color of cherry tomatoes was measured with a Minolta (Model CR-400, Minolta, 191Tokyo, Japan) on 20 fruit per treatment, using the CIELAB color parameters lightness 192(L*), a*, b*, chroma (C*) and hue angle (hº). Each measurement was taken at three 193locations for each cherry tomato. A standard white calibration plate was employed to 194calibrate the colorimeter.

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196*2.4.2.4. Internal quality*

197The assessed internal quality attributes were soluble solids content (SSC), titratable 198acidity (TA), and pH of tomato juice. SSC of the juice was measured using a digital 199refractometer (model PR1; Atago Co. Ltd., Japan) and values were expressed as g 200sucrose per 100 g of juice. TA of tomato juice was determined by titrating 5 mL of juice 201sample with 0.1 mol/L sodium hydroxide to an end point of pH 8.1 and expressed as g 202of citric acid per 1 L. pH of the juice was determined using a pH-meter (model C830, 203Consort bvba, Turnhout, Belgium). For each treatment, 3 juice samples from 7 fruit 204each were prepared and three different readings were performed.

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206*2.4.2.5. Respiration rate*

Respiration of coated and uncoated cherry tomatoes was measured by the closed system. Three replicates of 5 fruit each were used to determine the CO_2 production at the end of the storage. Samples were weighed and placed in sealed containers of known volume. The accumulation of CO_2 in the headspace atmosphere was measured at 20 °C over a period of 3 h. The gas sample (1 mL) was injected into a gas chromatograph (GC) (Thermo Trace, Thermo Fisher Scientific, Inc. Waltham, MA, USA) equipped with a thermal conductivity detector (TCD) and fitted with a Poropack QS 80/100 column (1.2 m x 0.32 cm i.d.). Temperatures were 35, 115, and 150 ºC, respectively for the oven, injector, and thermal conductivity detector. Helium was used, as carrier gas at a flow rate of 22 mL/min. The CO_2 concentration was calculated using the peak area obtained from a standard gas mixture of 15.0:2.5% O_2:CO_2. Results were expressed as mg CO_2 kg^{-1} h^{-1}.

2.4.2.6. Ethanol and acetaldehyde contents

Ethanol and acetaldehyde were analyzed from the head-space of juice samples using a GC (Thermo Trace, Thermo Fisher Scientific) equipped with an auto-sampler (Model HS 2000), flame ionization detector (FID), and 1.2 m x 0.32 cm (i.d.) Poropack QS 80/100 column. The injector was set at 175 ºC, the column at 150 ºC, the detector at 200 ºC, and the carrier gas at 28 mL min^{-1}. A composite juice of three replicates of 7 fruit per treatment was analyzed. Five mL of juice were transferred to 10-mL vials with crimptop caps and TFE/silicone septum seals. Samples were frozen and stored at –18 ºC until analyses. A 1-mL sample of the headspace was withdrawn from vials previously equilibrated in a water bath at 20 ºC for 1 h, followed by 15 min at 40 ºC, to reach equilibrium in the headspace, and then injected into the GC. Ethanol and acetaldehyde was identified by comparison of retention times with standards. Results were expressed as mg of volatile component per 1 L of juice.

2.4.2.7. Sensory evaluation

Sensory quality of treated samples was evaluated by 10 trained judges at the end of each storage period (ISO 8586-1:1993). Each judge was given samples from each batch and requested to evaluate flavor on a 9-point scale where 1 = very poor and 9 = optimum and off-flavor on a 5-point scale where 0 = absence of off-flavor and 5 = high presence of off-flavor. Ten fruit per treatment were halved cut and separated into individual segments. Two segments from two different fruit were presented to judges in trays labeled with 3-digit random codes and served to them at room temperature. The judges had to taste the segments of each sample in order to compensate, as far as possible, for biological variation of the material. Spring water was provided for palate rinsing between samples. External aspect of the fruit (coating cracks, spots, etc.) was also evaluated by the panelists. A 3-point scale was used in which the aspect was classified as 1 = bad, 2 = acceptable, and 3 = good. Panelists were also asked to rank visually the coated fruit from highest to lowest gloss.

2.5. Statistical analysis

Statistical analysis was performed using Statgraphics 5.1. (Manugistics Inc., Rockville, MD, USA). Specific differences between means were determined by Fisher's protected least significant difference test (LSD, $P < 0.05$) applied after an analysis of variance (ANOVA). For disease incidence data, the ANOVA was applied to the arcsine of the square root of the percentage of infected fruit in order to assure the homogeneity of variances. Non-transformed means are shown. For sensory gloss, specific differences were determined by Friedman test, which is recommended for ranking by the UNE 87023 (AENOR, 1997). Means from two equivalent experiments are presented.

3. Results and discussion

3.1. Effect of coatings on disease development

The antifungal performance of the coatings was evaluated according to the reduction of disease incidence and severity on coated tomatoes previously inoculated with *B.*

cinerea. This methodology allowed the assessment of the curative activity of the antifungal coatings. This was necessary because gray mold is a postharvest disease caused to a great extent by latent field infections (Barkai-Golan, 2001). The effect of the different HPMC-BW edible coatings containing food preservatives on gray mold development on cherry tomato artificially inoculated with *B. cinerea* and stored for 14 d at 5 °C followed by 7d at 20 °C is shown in Fig. 1. During cold storage at 5 °C, all the coatings significantly reduced gray mold incidence and severity compared with uncoated control samples. In general, the reduction of disease severity by coating application was considerably higher than the reduction of disease incidence. The coating containing SP was the most effective to reduce the incidence of gray mold in cherry tomatoes during cold storage (reduction of 100 and 30% after 7 and 14 d at 5 ºC, respectively). When tomatoes were transferred to 20 °C to simulate shelf-life, the coatings did not prevent fungal decay and disease incidence reached 100% for all treatments. This result might have been influenced by the high concentration of fungal inoculum that was used in these trials (10^6 spores/mL) and the prolonged period of shelf-life simulation (7 d at 20 ºC). This high inoculum density of *B. cinerea* was used to obtain high percentages of decay on control fruit and to conservatively select only those coatings with the highest potential for effective commercial usage. After 1 week at 5 °C, the severity of gray mold was low (less than 5 mm) and the SP, AC and APh-based coatings were the most effective to reduce it. After 2 weeks, disease severity increased to about 15 mm on control fruit and the SP-based coating was the most effective, with lesion diameters surrounding 5 mm. When cherry tomatoes were transferred to 20 °C for shelf-life, disease severity notably increased, but it was significantly lower on coated samples than on uncoated controls (about 40 mm). The SP-based coating was the most effective to reduce fungal growth, with a severity reduction of 44% compared to control fruit. From these results regarding disease incidence and severity, it is confirmed that the mode of action of the coatings was fungistatic rather than fungicidal, because fungal growth was only retarded, but not

completely prevented. In general, important differences in performance depending on fruit species and cultivars and fruit physical and physiological condition have been observed after application of most of the alternative antifungal treatments which mode of action is rather fungistatic than fungicidal (Palou et al., 2008).

In this work, HPMC-BW edible coatings containing SP were the most effective against *B. cinerea*, although those formulated with AC, and also with APh or PC also reduced disease severity during cold storage. Propionates are classical preservation agents. Droby et al. (2003) showed that calcium propionate completely inhibited the mycelial growth of *B. cinerea* at a level of 5% (w/v). Similarly, the activity of carbonates in preventing decay by modes of action such as inhibition of spore germination or germ tube elongation, or production of pectinolytic enzymes is well recognized (Palou et al., 2001; Mills et al., 2004; Smilanick et al., 2005). These salts strongly inhibited mycelial growth and spore germination of *B. cinerea* as well as polygalacturonase activity (Palmer et al., 1997). Considering that the proportion of CO_3^{2-} ions is elevated at high pH (>11), the CO_3^{2-} form has been suggested to be responsible in aqueous solutions of the inhibitory activity that leads to reductions of mycelial growth and spore germination. In our work, the HPMC-BW formulations containing CO_3^{2-} ions had a pH close to 11 (Table 1), which could explain their effect in controlling mold growth.

3.2. Effect of coatings on fruit quality

3.2.1. Weight loss

Fig. 2 shows the weight loss of coated and uncoated samples. After storage for 10 and 15 d at 5 ºC, followed by 5 d at 20 ºC, weight losses were in the ranges of 1.54-2.98% and 1.95-3.25%, respectively. The coating containing AC significantly reduced weight loss of coated cherry tomatoes during storage compared to uncoated samples, which indicates the effectiveness of this coating as a moisture barrier. However, the coating containing APh did not improve the moisture barrier in cherry tomatoes and those

amended with SP and PC induced higher weight loss than that observed in uncoated controls.

Tomatoes are naturally covered by a continuous wax layer that provides high resistance to water movement across the cuticle. Coatings containing hydrophobic compounds, deposited as an additional layer over the natural waxes, should improve the moisture resistance of the fruit. In our work, the barrier properties of the coatings were greatly influenced by the different food additives incorporated to the HPMC-BW matrix. Whereas AC significantly reduced weight loss of cherry tomatoes, SP and PC increased it compared to uncoated samples. This might indicate a partial removal of the natural waxes present on the peel of cherry tomatoes. Other research works have also reported that the addition of lipids to polysaccharides not always results in a reduction on weight loss of coated commodities, such as cherries or cucumbers (Baldwin et al., 1997), apples (Bai et al., 2002), or plums (Navarro-Tarazaga et al., 2008). In addition, Valencia-Chamorro et al. (2008) reported that the water vapor permeability (WVP) of HPMC-lipid films is greatly affected by the addition of food preservatives. These films, although with the same lipid content, presented significant different WVPs depending on the food additive, which was attributed to changes in the network structure of the polymer matrix. Among the different salts of organic acids and parabens tested, those films that contained potassium sorbate or SP presented the highest WVP. The application of similar coating formulations did not reduce weight loss of 'Valencia' oranges after 60 d at 5 °C plus 7 d at 20 °C (Valencia-Chamorro et al., 2009). In fact, the application of potassium sorbate and SP-based coatings even resulted in higher weight loss on coated oranges than on uncoated controls. These results were correlated with the WVPs values of the stand-alone films and also with the mechanical properties of the films that showed them as very brittle and stiff, which could be responsible for the formation of pits or cracks of the coatings on the fruit surface, leading to an increase in weight loss.

3.2.2. Fruit firmness

Firmness, expressed as percentage deformation, of cherry tomatoes was around 17% after 15 d of storage at 5 °C plus 5 d at 20 °C (Fig. 3). In general no differences were observed between coated and uncoated samples, although at the end of storage samples treated with the PC-based coating showed slightly higher deformation value than the uncoated control. When comparing among coating treatments, cherry tomatoes coated with APh and AC-based coatings presented lower percentage deformation than SP and PC-coated samples.

Fruit softening is triggered by biochemical processes involving the hydrolysis of pectin and starch at the cell wall by enzymes, such as pectinesterase and polygalacturonase (Seymour et al., 1993). Low respiration rate can limit the activities of these enzymes. Therefore, firmness retention in coated tomato has been repeatedly related to a reduction in enzymatic activities caused by a modification of the internal atmosphere of the fruit (Park et al., 1994; Tasdelen and Bayindirli, 1998; Zhuang and Huang, 2003; Zapata et al., 2008; Ali et al., 2010; Ahmed et al., 2013). In this work, cherry tomatoes coated with AC and APh-based coatings showed lower respiration rates than uncoated control fruit after 10 and 15 d of storage at 5 ºC, respectively (Fig. 4), but they did not show significant differences in firmness with the uncoated control. On the other hand, the effect of coatings on the maintenance of fruit firmness has also been related to their ability to control weight loss (Baldwin et al., 1997). This fact was confirmed in this work since cherry tomatoes coated with SP and PC-based formulations presented significantly higher weight loss than uncoated samples (Fig. 2), and these samples were also the ones with the highest deformation values.

3.2.3. Color

Table 2 shows the CIELAB color parameters of coated and uncoated cherry tomatoes after 15 d of storage at 5 ºC plus 5 d at 20 ºC. There was a decrease in L* and hº with storage time, but these values were not affected by coating application. The rest of the

color parameters (a*, b* and C*) showed significant differences among treatments at the end of storage. The a* value (red color) for PC-based coated tomatoes was significantly higher than for uncoated fruit, whereas no differences were observed between the uncoated control and the fruit treated with the other coatings. While C* values were maintained during storage on coated tomatoes, they significantly decrease on uncoated samples after cold storage plus 5 d at 20 ºC. Since no differences were found in hº among treatments, higher C* values on coated samples indicated same colors but with higher purity or saturation.

Different colors are present simultaneously during tomato ripening, since chlorophyll is degraded from green to colorless compounds and, at the same time, carotenoids are synthesized from colorless precursor (phytoene) to carotene (pale yellow), lycopene (red), β-carotene (orange), xanthophylls, and hydroxylated carotenoids (yellow) (Giuliano et al., 1993). In the presence of high CO_2 levels, color changes are delayed due to a decrease in the synthesis of ethylene (Buescher, 1979). In this sense, it has been described that the application of gum arabic, zein, alginate or HPMC coatings was able to delay color changes in tomatoes during storage at 20 ºC by creating a modified atmosphere in the fruit (Zhuang and Huang, 2003; Zapata et al., 2008; Ali et al., 2010). In our case, tomatoes were stored under cold storage followed by a shelf-life storage period of 5 days at 20 ºC. Although coating could influence the tomato respiration rate and volatile levels under these conditions, such influence might have been insufficient to raise a significant effect on peel color parameters of coated cherry tomatoes. Furthermore, cherry tomatoes were selected and processed with a full-developed red color, which could also explain the small changes observed in color during cold storage time.

3.2.4. Fruit internal quality

Coating application did not significantly affect TA, SSC, and pH of cherry tomatoes (Table 2). The effect of coating application on internal quality parameters is typically

dependent on coating type, fruit cultivar, and storage conditions. In tomato, Das et al. (2013) found greater values for TA in uncoated fruit than in fruit coated with rice starch-based coatings, which was attributed to higher ethylene production and respiration rate in uncoated fruit during ripening. In the same work, higher values of pH and SSC were also reported in uncoated than in coated tomatoes. The pH increase was attributed to the loss of citric acid in tomatoes as fruit ripened. Similarly, the application to tomato of gum arabic and sucrose polyester-based coatings (Semperfresh®) slowed the reduction of SSC during storage at 20 ºC (Tasdelen and Bayindirli, 1998; Ali et al., 2010). Nevertheless, tomatoes coated with alginate and zein presented higher SSC than uncoated samples after 9 d of storage at 20 ºC (Zapata et al., 2008).

3.2.5. Respiration rate

The effect of the coatings on respiration rate of cherry tomatoes during cold storage plus 5 d at 20 ºC is shown in Fig. 4. Tomatoes are generally classified as having a moderate respiration rate, in the range of 10-20 mg/Kg h at 5 ºC (Kader, 2002). In this work, all the samples showed an increase in the respiration rate during storage, which indicates an increase in the fruit metabolic activity. After 10 d at 5 ºC plus 5 d at 20 ºC, the samples coated with the AC-based coating presented the lowest CO_2 production, whereas after 15 d of cold storage, tomatoes coated with the APh and AC-based coatings had lower respiration rates than uncoated samples, indicating that these coatings might have modified the internal atmosphere of cherry tomatoes. The effect of coatings on respiration of horticultural products is related to their ability to create a barrier to oxygen diffusion through the coating. In general, polysaccharide based coatings, such as HPMC, present a good oxygen barrier at low or intermediate relative humidity (Valencia-Chamorro et al., 2011). In tomatoes, the application of coatings based on gum arabic (Ali et al., 2010), alginate or zein (Zapata et al., 2008) reduced the respiration rate of the fruit during storage, showing that these edible coatings were effective as gas barriers. In other fruits such as plums and grapes, HPMC-based

coatings also reduced the fruit respiration rate (Navarro-Tarazaga et al., 2008; Sánchez-González et al., 2011). However, the oxygen barrier of coatings greatly depends on the presence of minor ingredients, such as antimicrobial food additives, that might modify their effectiveness. Valencia-Chamorro et al. (2008) showed significant differences in oxygen permeability (OP) values of HPMC-lipid films depending on the food additive and lipid type. For similar coating formulations, films containing sodium benzoate presented lower OP than films containing potassium sorbate, and the combination of these additives with other organic salts like SP increased the OP about 2-fold. According to our results, the addition of ammonium salts (AC and APh) could be more appropriate than that of PC and SP to obtain HPMC-BW coatings able to reduce the respiration rate of cherry tomatoes.

3.2.6. Ethanol and acetaldehyde content

The application of HPMC-BW coatings to cherry tomatoes created a modified atmosphere within the fruit, which translated in a significant increase in the contents of ethanol and acetaldehyde in the juice ($P < 0.05$; Fig. 5). The concentration of ethanol and acetaldehyde in the juice of coated cherry tomatoes after storage periods of 10 and 15 d at 5 ºC plus 5 d at 20 ºC was in the range of 1.24-2.95 mg/L and 0.33-0.82 mg/L, respectively, while they were in the range of 0.24-0.81 mg/L and 0.20-0.36 mg/L, respectively, on uncoated samples. Although with some variability during both storage periods, the highest levels of ethanol and acetaldehyde were found in cherry tomatoes coated with the PC-based coating ($P < 0.05$). Dávila-Aviña et al. (2011) also found an accumulation of acetaldehyde in tomatoes treated with an edible mineral oil wax-based coating and stored at 10 °C, whereas a carnauba-wax coating had no effect on this off-flavor aroma volatile.

3.2.7. Sensory evaluation

HPMC-BW coatings containing food preservatives did not significantly modify the flavor of cherry tomatoes compared to uncoated samples during storage, as determined by the judges ($P > 0.05$). The overall flavor of coated and uncoated tomatoes at the end of both storage periods was evaluated with scores in the range 6.6-7.0 (considered as acceptable) and in any case the judges found off-flavor development (scores in the range 0.1-0.4) (data not shown). These results indicate that the ethanol and acetaldehyde levels reached after 10 and 15 d of storage at 5 ºC plus 5 d at 20 ºC were below the threshold of off-flavor detection for cherry tomatoes.

The addition of food preservatives to HPMC-BW formulations resulted in stable emulsions. Upon application, some coated tomatoes presented few small white spots on their surface that slightly reduced the general good appearance of the samples, but they were still classified as acceptable. Among all coated samples, tomatoes coated with APh-based coatings were evaluated with the highest external appearance value after 15 d at 5 ºC plus 5 d of shelf-life at 20 ºC (data not shown).

After both storage periods, none of the tested coatings provided higher gloss than the uncoated control. Cherry tomatoes coated with formulations containing PC and SP were significantly less glossy than the controls (Table 3). This behavior could be related to the macroemulsion character of coating formulations (Hagenmaier and Baker, 1994). Ali et al. (2010) reported that tomatoes coated with 10% gum arabic obtained the highest scores in flavor and overall acceptability after 20 d of storage at 20 ºC, while tomatoes coated with 15 and 20% gum developed off-flavor and were not acceptable to the panel of experts. Ahmed et al. (2013) evaluated the application of delactosed whey permeate coatings to tomatoes during 21 d of storage at 15 °C and they kept a good appearance and overall quality at the end of the storage period, while these parameters felt below the limit of marketability on control fruit.

4. Conclusions

All coatings significantly reduced the growth of *B. cinerea* on artificially inoculated cherry tomatoes during cold storage at 5 ºC, being the SP-based coating the most effective at inhibiting the pathogen. The moisture and gas barriers of the HPMC-BW coatings were affected by the food preservative incorporated into the formulation. The coating containing AC effectively reduced weight loss and those formulated with both ammonium salts (APh and AC) reduced the respiration rate of cherry tomatoes. None of the coatings affected negatively the physico-chemical and sensory quality of cherry tomatoes. Overall, HPMC-BW edible composite coatings containing AC as antifungal food additive could be a promising treatment for tomatoes that should be kept in cold storage. Further research should be conducted to improve the physical characteristics of these coatings in order to obtain better water loss control and enhance gloss and visual quality of coated fruit. Additional studies on the combination of these antifungal edible coatings with other control methods alternative to chemical synthetic fungicides could be also conducted to find synergistic and/or complementary activities for the control of gray mold caused by *B. cinerea* in cherry tomatoes.

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625**Table 1.** Characteristics of hydroxypropyl methylcellulose-beeswax edible composite coatings

626containing antifungal food preservatives

Food preservative	E-number	Molecular formula	Solid concentration (%)	Viscosity (cp)	pH
Sodium propionate	E-281	CH_3CH_2COONa	8.0	103.3	6.68
Potassium carbonate	E-501 (i)	K_2CO_3	10.0	123.8	10.98
Ammonium phosphate	E-342 (i)	$NH_4H_2PO_4$	6.5	123.1	7.87
Ammonium carbonate	E-503 (i)	$(NH_4)_2CO_3$	10.0	147.5	9.40

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629**Table 2.** Soluble solid content (SSC), titratable acidity (TA), pH and color parameters [lightness (L*), a*, b*, chroma (C*) and hue angle (hº)] of cherry

630tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing antifungal food preservatives

631and stored for 15 days at 5 ºC followed by 5 d of shelf-life at 20 ºC.

	TA (g citric acid L^{-1})	SSC (g sucrose 100 g^{-1})	pH	L*	a*	b*	C*	hº
Control	5.65 ab	8.77 a	4.54 ab	34.56 a	14.29 a	18.16 a	23.14 a	51.89 a
Sodium propionate	5.95 b	8.80 a	4.51 a	34.45 a	14.69 ab	19.45 b	24.42 b	52.99 a
Potassium carbonate	5.94 b	9.00 a	4.59 bc	34.41 a	15.55 b	19.81 b	25.21 b	51.95 a
Ammonium carbonate	5.83 b	8.70 a	4.51 a	34.37 a	15.11 ab	19.79 b	24.94 b	52.70 a
Ammonium phosphate	5.42 a	8.77 a	4.61 c	33.51 a	15.18 ab	19.24 b	24.53 b	51.77 a

632Values at harvest: TA = 6.58 g citric acid per L; SSC = 8.93 g sucrose per 100 g; pH = 4.21; L* = 37.31; a* = 14.73; b* = 19.94; C* = 21.84; hº =

63353.60

634Means in columns with different letters are significantly different according to Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

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644**Table 3.** Ranked gloss of cherry tomatoes uncoated (control) and coated with hydroxypropyl

645methylcellulose-beeswax edible composite coatings containing antifungal food preservatives

646and stored at 5 ºC followed by 5 d of shelf-life at 20 ºC.

Gloss rank	10 d 5 °C + 5 d 20 °C		15 d 5 °C + 5 d 20 °C	
More Glossy	Control	a	Control	a
	APh	ab	APh	ab
	AC	bc	AC	abc
	SP	bc	PC	bc
Less Glossy	PC	c	SP	c

647APh= ammonium phosphate; AC = ammonium carbonate; PC = potassium carbonate; SP = 648sodium propionate.

649Treatments in columns with different letters are significantly different according to Friedman test.

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Figure 1. Incidence and severity (±SD) of gray mold on cherry tomatoes artificially inoculated with *Botrytis cinerea*, uncoated (Control), or coated 24 h later with hydroxypropyl methylcellulose-beeswax edible composite coatings containing ammonium carbonate (AC), ammonium phosphate (APh), potassium carbonate (PC) or sodium propionate (SP), and stored up to 14 d at 5 ºC followed by 7 d at 20 °C. For each storage period, columns with different letters are significantly different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA. Values are means from two experiments. For disease incidence, the ANOVA was applied to arcsine-transformed values. Non-transformed means are shown.

Figure 2. Weight loss (±SD) of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing ammonium carbonate (AC), ammonium phosphate (APh), potassium carbonate (PC), or sodium propionate (SP), and stored up to 15 d at 5 ºC followed by 5 d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA.

Figure 3. Firmness (±SD) of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing ammonium carbonate (AC), ammonium phosphate (APh), potassium carbonate (PC), or sodium propionate (SP), and stored up to 15 d at 5 ºC followed by 5 d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA. Values are means from two experiments.

Figure 4. Respiration rate (±SD) of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing ammonium carbonate (AC), ammonium phosphate (APh), potassium carbonate (PC) or sodium propionate (SP), and stored up to 15 d at 5 ºC followed by 5 d at 20 °C. For

each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA. Values are means from two experiments.

Figure 5. Ethanol and acetaldehyde content (±SD) in the juice of cherry tomatoes uncoated (Control) or coated with hydroxypropyl methylcellulose-beeswax edible composite coatings containing ammonium carbonate (AC), ammonium phosphate (APh), potassium carbonate (PC) or sodium propionate (SP), and stored up to 15 d at 5 ºC followed by 5 d at 20 °C. For each storage period, columns with different letters are different by Fisher's protected LSD test ($P < 0.05$) applied after an ANOVA. Values are means from two experiments.

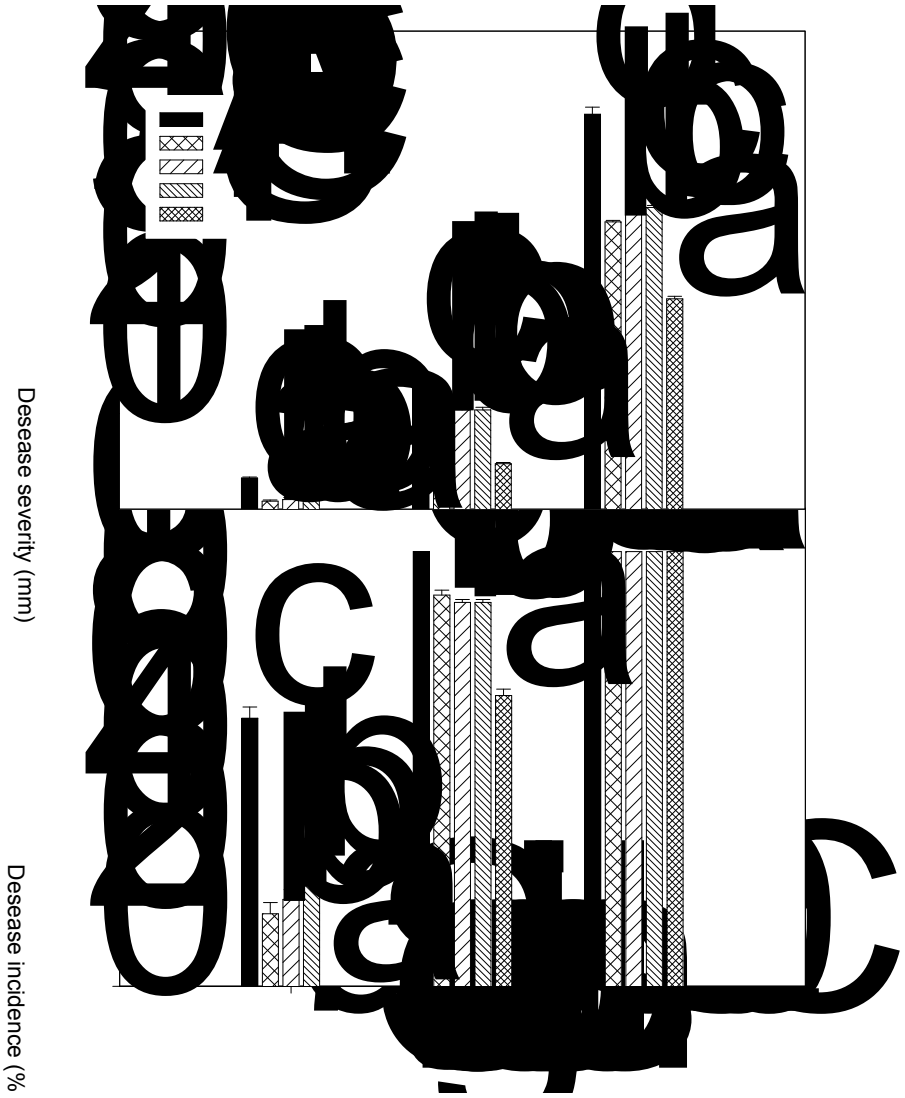
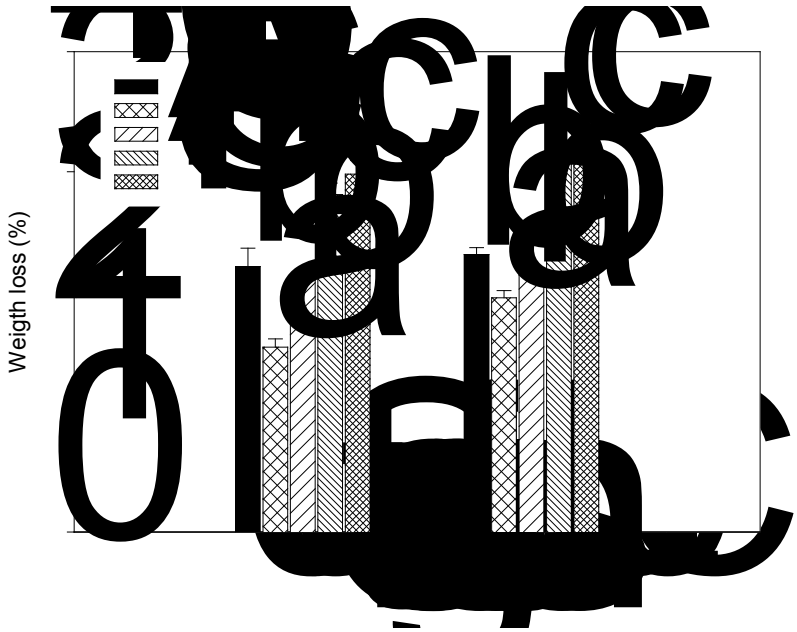


Figure 1.



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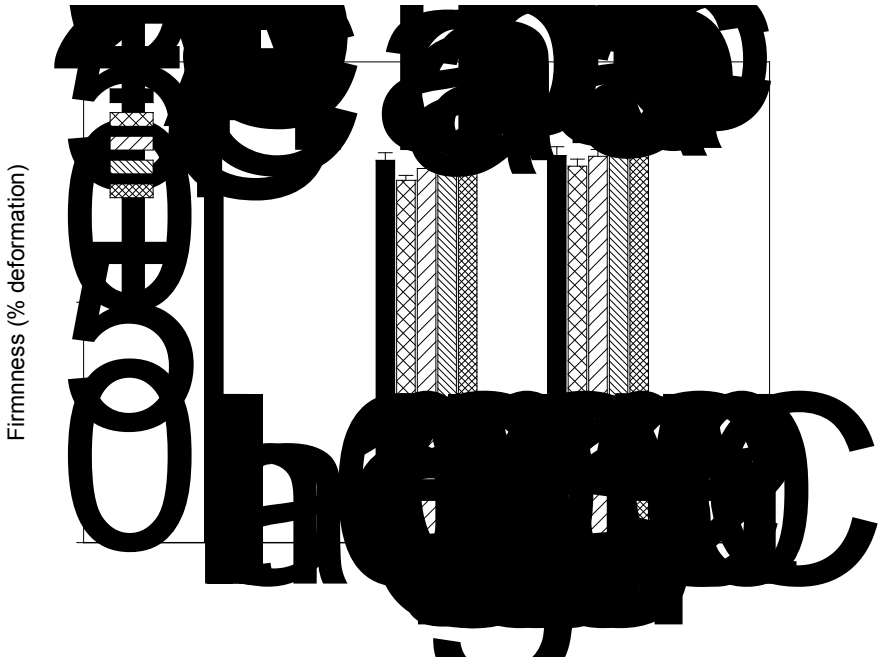

Figure 3.

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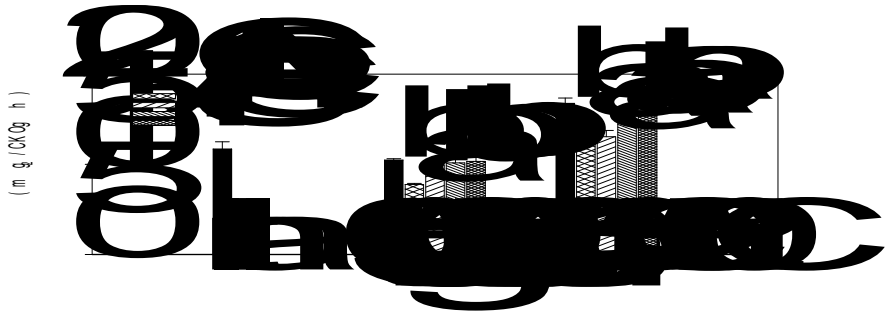

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744 **Figure 4**.

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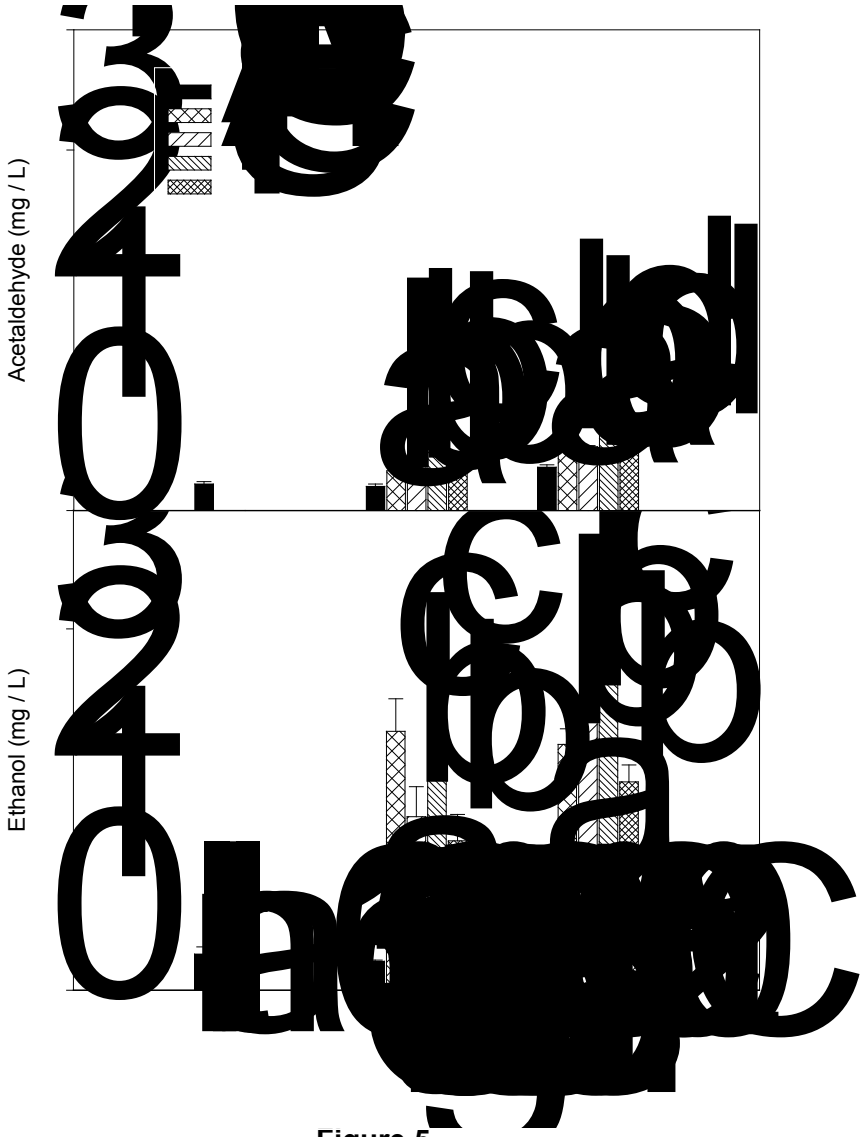

Figure 5.