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Application of Transglutaminase Crosslinked Whey Protein/Pectin as Edible Film Coating for Preservation of Mango Fresh-Cut

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Abstract.
Mango is a climacteric tropical fruit with a high nutritional value by its wealth of antioxidants (total carotenoids and vitamin C). Fresh-cut mango is a very interesting product to attend the increasing demand for fresh-cut fruits. Fresh cut mango pulp is very perishable and so has a short shelf life due to cut-damaged surface, exposed tissues and availability of cell nutrients to pathogenic organisms. Objective of this present research focused on the application of transglutaminase crosslinked whey protein/pectin in different ratios as edible coating film for preservation of mango fresh-cut. Weight loss, total phenolic, carotenoid, ascorbic acid, total plate count accumulation, sensory score were major evaluated parameters to decide the optimal whey protein:pectin in film forming and coating to preserve mango fresh cut. Results revealed that 75%: 25% (whey protein:pectin) was adequate for application. This approach opens an alternative strategy to prolong fresh cut product shelf life in commercial distribution.

Keywords: *Mango fresh cut, whey protein, pectin, shelf life, coating*

I. INTRODUCTION

Recognized for its attractive color, delicious taste, rich aroma, exotic flavor, and high nutritional value, mango is a rich source of carotenoids and provides high contents of ascorbic acid and phenolic compounds (Shahnawaz et al., 2012; Liu et al., 2013). Mango being a highly perishable fruit possesses a very short shelf life and reach to respiration peak of ripening process on 3rd or 4th day after harvesting at ambient temperature (Narayana et al., 1996). The shelf life of mango varies among its varieties depending on storage conditions. It ranges from 4 to 8 days at room temperature and 2-3 weeks in cold storage at 13ºC (Carrillo et al., 2000). After harvesting, the ripening process in mature green mango takes 9-12 days (Herianus et al., 2003). The ripening process of mango fruit involves a series of biochemical reactions, resulting into increased respiration, ethylene production, change in structural polysaccharides causing softening, degradation of chlorophyll, developing pigments by carotenoids biosynthesis, change in carbohydrates or starch conversion into sugars, organic acids, lipids, phenolics and volatile compounds, thus leading to ripening of fruit with softening of texture to acceptable quality (Herianus et al., 2003).

Deterioration of fresh produce occurs gradually during storage and its cumulative effect renders food unacceptable to consumers. Fresh-cut processing induces chemical and biochemical changes besides increasing product respiration rate leading to a reduction of storage time (Azarakhsh et al., 2014). Packing, and packaging methods, help maintain, and extend, shelf-life of fresh produce. Impact of temperature and relative humidity management on produce influences quality during transit and storage. Use of edible coating is a novel approach to improve the quality of food for consumer acceptance. Edible coatings are an eco-friendly technique, which slows deterioration of vegetables by controlling gas exchange, moisture transfer, and oxidation. Major advantage of these coatings is to improve nutritional and sensory quality of food by incorporating active ingredients into the polymer matrix that are consumed with food products (Poorva Sharma et al., 2018).

Whey protein is a thin left-over liquid obtained during cheese formation after precipitation of casein protein. It is dried to form whey protein concentrate (WPC), protein content ranging from 25 to 80%, or whey protein isolate having protein content > 90%. Edible films formed by whey proteins are transparent, flexible, colorless and flavorless, with a poor moisture barrier. Protein-based films have a good aroma barrier (Miller and Krochta, 1997), and low permeability to oxygen (Hong and Krochta, 2006).

There were several researchs mentioned to edible coating for fresh cut mango. A study was aimed at evaluating the effect of coatings with alginate (AL), pectin (PE), carboxymethyl cellulose (CMC) or chitosan (CH) on microbial stability, physicochemical attributes, total phenolics and carotenoids content, antioxidant capacity and sensory properties of fresh-cut mango during 14 days at 4 ± 1 °C (Blanca Salinas-Roca et al., 2018). Fresh-cut mango was wrapped with mango film and packed in modified atmosphere packaging (MAP) (Rungsinee Sothornvit et al., 2010). The effect of various chitosan coatings on fruit ripening behaviour, biochemical and organoleptic characteristics were evaluated during storage (Nadeem Akhtar Abbasi et al., 2009). Enhancing safety and shelf life of fresh-cut mango by application of edible coatings and microencapsulation technique was examined (Majid Alikhani et al., 2014). The influence of heat treatment combined with coating on sensory quality, physico-chemicals characteristics (firmness, colour, pH, titratable acidity, total soluble solids and total carotenoids content) and microbiological quality of fresh-cut mangoes were studied (Tassadit Djioua et al., 2010). A work aimed at evaluating the effect of an alginatechitosan nanomultilayer coating, obtained by electrostatic layerby-layer self-assembling, in the quality and shelf life of fresh-cut mangoes (Marthyna P. Souza et al., 2015). Application of MAP to extend freshcut mango shelf-life was examined (Nguyen Phuoc Minh, 2014).

Fresh-cut mango fruits are highly perishable due to cut-damaged surface, exposed tissues and availability of cell nutrients to pathogenic organisms (Soliva-Fortunay & Martı´n-Belloso, 2003; Charles et al., 2008). The typical response of mango fruit to the process is softening and decrease in overall appearance by browning of the cut surface (Plotto et al., 2004). Therefore the present study focused on using whey protein/pectin containing transglutaminase as edible coating to improve the storability of this fruit.

II. Material And Method

2.1 Material

Mango fruits were harvested from Vinh Long province, Vietnam. They must be cultivated following VietGAP without pesticide and fertilizer residue to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. Apart from collecting mango, we also used other materials such as whey protein isolate, transglutaminase, pectin, sorbitol, folin-Ciocalteu's phenol reagent, 2,6-dichlorophenolindophenol, sodium bicarbonate, gallic acid, peracetic acid. Lab utensils and equipments included knife, weight balance, refractometer, pH meter, buret.

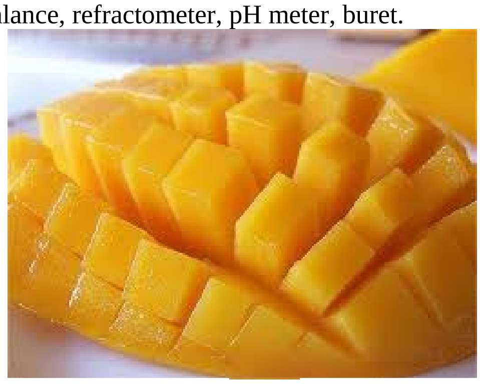

Figure 1. Fresh cut mango *(Mangifera indica)*

2.2 Researching method

2.2.1 Film coating preparation

Whey protein/pectin in different ratio (25%: 75%, 50%: 50%, 75%: 25%) film-forming solution containing transglutaminase was prepared by solubilizing whey protein isolate: pectin (2.5 g: 7.5g, 5.0g: 5.0g, 7.5g: 2.5) with 0.5 g of sorbitol in 100 mL of water. The pH of the solution was adjusted to 5.0 and then transglutaminase was added by stirring overnight at ambient temperature. Control whey protein film forming solution was also prepared as mentioned above without pectin and transglutaminase. All film forming solutions were cast by pipetting 50 mL of each film forming solution into Petri dishes (150 mm 15 mm) and drying at 50^oC and 25% relative humidity.

2.2.2 Sample preparation

Mango fruits were peeled and cut into 2 x 2 x 2 cm pieces by knife then washed. Control and coated samples were arranged randomly. All samples were soaked in a peracetic acid solution (0.05 g/L) for 2 min, then in a 5 g/L $CaCl_2$ solution for 5 min and left to dry for 5 min. Coated samples were dipped into film coating solution for 2 min and then let drained for 5 min before storage at 4^oC for either 2, 6, 8, 10, 12, 14 days.

2.3 Physico-chemical and biological analysis

Untreated and treated mango fruits were weighed regularly to determine weight loss which was calculated cumulatively by comparing the weights of the sample with the electronic balance. Total phenolic content was determined using the FolinCiocalteu method described by Rocha and Morais (2002). Carotenoid content was calculated by measuring the absorbance at 450 nm using a spectrophotometer (Jagannath, Napjappa, Das Gupta, & Bawa, 2006). **Ascorbic acid content** was measured by 2,6-dichlorophenolindophenol titration. The total colony forming units (CFU) was enumerated during the storage period by Petrifilm - 3M. The sensory attributes such as visual appearance, color, taste, flavor and acceptability was carried out by selected panel of judges (9 members) rated on a five point hedonic scale. All measurements were sampled at an interval of 2 days for the total 14 days storage period and the results were expressed as percentages.

2.4 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVI.

III. Result & Discussion

3.1 Weight loss (%) of coated fresh-cut mango by transglutaminase crosslinked protein/pectin coating

Mango fruits are highly susceptible to weight loss. The main reason behind weight loss is vapor pressure gradient and respiration, which causes wilting and shrivelling resulting in low- market value and acceptability by consumers (Ali et al., 2010). Edible coatings act as a barrier to water loss to the atmosphere by maintaining high relative humidity in the tissue atmosphere and reducing moisture loss (Olivas et al., 2003). Fresh fruit and vegetable moisture loss after harvest represents a serious problem causing shrinkage and weight decrease of the products, and this phenomenon is more prominent in processed and ready-to-eat samples (Giovanna Rossi Marquez et al., 2017).

The good whey protein/pectin film performance could be explained by the formation of transglutaminase-crosslinked whey protein/pectin complexes that significantly reduced the interstitial space within the film network and consequently increased film water vapor and gas barrier properties (Di Pierro et al., 2013). A factor contributing to postharvest loss is respiration rate. Edible coatings have a potential to decrease respiration rate by creating an internal modified atmosphere providing a barrier to oxygen and carbon dioxide (González Aguilar et al., 2009). The lipophilic nature of essential oils increases resistance of coatings to gas diffusion. Addition of lipophilic additives improves barrier properties of coatings and decreases respiration rate.

3.2 Total phenolic content (GAE/kg) of coated fresh-cut mango by transglutaminase crosslinked protein/pectin coating

Mango fruits are a good source of phenolics and flavonoids and contribute to the diet as antioxidants, reducing the risk of several diseases. During maturity, these compounds decrease due to metabolic rate of fruits. These compounds are secondary metabolites in plants with the ability to protect human body tissues against oxidative attacks (Romanazzi et al., 2002). Processed fruit and vegetables have a reduced shelf-life also because of their susceptibility to the oxidative degradation induced by their cutting, peeling and washing that may increase oxidative stress through introduction of oxygen, loss of water, removal or inactivation of endogenous antioxidants (Giovanna Rossi Marquez et al., 2017). Edible coatings produce an abiotic challenge to fresh produce, modifying the metabolism and affecting production of secondary metabolites.

Total phenolics were expressed as g of gallic acid equivalents (g GAE)/kg of each sample (fresh weight). Loss of phenolics in uncoated tissues was due to senescence and breakdown of cell structure. Edible coating effectively delayed senescence by controlling the metabolic rate, retaining phenolics for a longer storage period.

3.3 Carotenoid content (mg/ 100g) of coated fresh-cut mango by transglutaminase crosslinked protein/pectin coating

As ripening occurs, carotenoid is degraded, and the antioxidant activity decreases. With application of coatings, respiration rate can be slowed, and antioxidant activity can be maintained for a longer storage period.

Table 1. Weight loss (%) of coated fresh-cut mango by whey protein/pectin in different ratio

Day of preservation	Control	25%: 75%	50%: 50%	75%: 25%
0	0	0	0	0
2	1.75±0.02a	1.02±0.01ab	0.94±0.02ab	0.22±0.03b
4	2.35±0.01a	1.14±0.03ab	1.05±0.03ab	0.31±0.01b
6	2.78±0.02a	1.35±0.02ab	1.19±0.00ab	0.39±0.03b
8	2.95±0.01a	1.47±0.01ab	1.26±0.02ab	0.44±0.00b
10	3.19±0.02a	1.51±0.02ab	1.33±0.01ab	0.49±0.02b
12	3.55±0.01a	1.60±0.02ab	1.37±0.02ab	0.53±0.01b
14	3.63±0.01a	1.74±0.01ab	1.44±0.02ab	0.62±0.01b

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 2. Total phenolic content (g GAE/kg) of coated fresh-cut mango by whey protein/pectin in different ratio

Day of preservation	Control	25%: 75%	50%: 50%	75%: 25%
0	63.19±0.02a	63.19±0.02a	63.19±0.02a	63.19±0.02a
2	62.90±0.01b	62.98±0.01ab	63.02±0.01ab	63.17±0.01a
4	62.77±0.03b	62.90±0.02ab	62.95±0.02ab	63.05±0.02a
6	62.70±0.01b	62.79±0.01ab	62.87±0.01ab	62.99±0.03a
8	62.42±0.03b	62.58±0.02ab	62.69±0.01ab	62.91±0.00a
10	62.37±0.02b	62.43±0.02ab	62.57±0.02ab	62.86±0.01a
12	62.25±0.03b	62.31±0.01ab	62.46±0.03ab	62.79±0.02a
14	62.03±0.02b	62.21±0.01ab	62.34±0.01ab	62.70±0.00a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3. Carotenoid content (mg/ 100g) of coated fresh-cut mango by whey protein/pectin in different ratio

Day of preservation	Control	25%: 75%	50%: 50%	75%: 25%
0	5.85±0.02a	5.85±0.02a	5.85±0.02a	5.85±0.02a
2	5.68±0.01b	5.80±0.02ab	5.82±0.01ab	5.84±0.01a
4	5.65±0.02b	5.78±0.01ab	5.82±0.01ab	5.84±0.01a
6	5.60±0.03b	5.76±0.01ab	5.81±0.02ab	5.83±0.02a
8	5.54±0.01b	5.75±0.02ab	5.79±0.01ab	5.82±0.01a
10	5.49±0.01b	5.72±0.01ab	5.75±0.02ab	5.79±0.02a
12	5.41±0.01b	5.68±0.01ab	5.70±0.01ab	5.74±0.01a
14	5.34±0.00b	5.62±0.01ab	5.67±0.02ab	5.72±0.00a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 4. Ascorbic acid content (mg/ 100g) of coated fresh-cut mango by whey protein/pectin in different ratio

Day of preservation	Control	25%: 75%	50%: 50%	75%: 25%
0	58.45±0.01a	58.45±0.01a	58.45±0.01a	58.45±0.01a
2	58.30±0.02b	58.37±0.02ab	58.40±0.01ab	58.42±0.02a
4	58.21±0.03b	58.33±0.01ab	58.37±0.02ab	58.40±0.01a
6	58.17±0.01b	58.28±0.02ab	58.32±0.03ab	58.37±0.03a
8	58.12±0.01b	58.19±0.02ab	58.26±0.01ab	58.35±0.02a
10	58.04±0.02b	58.12±0.01ab	58.21±0.03ab	58.32±0.01a
12	57.93±0.01b	58.03±0.02ab	58.17±0.02ab	58.29±0.02a
14	57.82±0.02b	57.94±0.01ab	58.10±0.02ab	58.25±0.01

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 5. Microbial accumulation (cfu/g) of coated fresh-cut mango by whey protein/pectin in different ratio

Day of preservation	Control	25%: 75%	50%: 50%	75%: 25%
0	6.1 x 101 ±0.02a	6.1 x 101 ±0.02a	6.1 x 101 ±0.02a	6.1 x 101 ±0.02a
2	7.3 x 101 ±0.00a	6.4 x 101 ±0.03ab	6.2 x 101 ±0.03ab	6.2 x 101 ±0.03b
4	8.4 x 101 ±0.01a	6.9 x 101 ±0.00ab	6.6 x 101 ±0.02ab	6.3 x 101 ±0.00b
6	9.5 x 101 ±0.00a	7. 4 x 101 ±0.00ab	6.9 x 101 ±0.00ab	6.5 x 101 ±0.01b
8	1.2 x 102 ±0.00a	8.4 x 101 ±0.01ab	8.0 x 101 ±0.03ab	7.3 x 101 ±0.03b
10	2.4 x 102 ±0.01a	9.3 x 101 ±0.02ab	8.7 x 101 ±0.01ab	7.5 x 101 ±0.00b
12	4.7 x 102 ±0.01a	1.1 x 102 ±0.01ab	9.3 x 101 ±0.01ab	7.7 x 101 ±0.02b
14	6.8 x 102 ±0.02a	2.3 x 102 ±0.03ab	1.1 x 102 ±0.02ab	7.9 x 101 ±0.01b

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 6. Sensory score of coated fresh-cut mango by whey protein/pectin in different ratio

Day of preservation	Control	25%: 75%	50%: 50%	75%: 25%
0	8.21±0.02a	8.21±0.02a	8.21±0.02a	8.21±0.02a
2	8.07±0.01b	8.11±0.01ab	8.15±0.02ab	8.18±0.01a
4	7.94±0.02b	8.03±0.02ab	8.11±0.03ab	8.15±0.03a
6	7.87±0.00b	7.98±0.01ab	8.07±0.01ab	8.12±0.02a
8	7.80±0.00b	7.93±0.03ab	8.02±0.02ab	8.10±0.00a
10	7.71±0.03b	7.89±0.02ab	7.97±0.03ab	8.07±0.00a
12	7.67±0.02b	7.82±0.00ab	7.90±0.01ab	8.04±0.02a
14	7.60±0.01b	7.76±0.00ab	7.84±0.01ab	8.01±0.02a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

A study was aimed at evaluating the effect of coatings with alginate (AL), pectin (PE), carboxymethyl cellulose (CMC) or chitosan (CH) on microbial stability, physicochemical attributes, total phenolics and carotenoids content, antioxidant capacity and sensory properties of fresh-cut mango during 14 days at 4 ± 1 °C. Coated fresh-cut mango kept microbial counts below 6 logs CFU g^{-1}, being CH-coated fresh-cut mango those that exhibited the lowest microbial counts (1 log CFU g^{-1}) along entire storage. AL, PE and CMC coatings maintained yellow colour of fresh-cut mango throughout storage. AL and CH coatings, which have different monomers in their chain, improved the content of antioxidant compounds in fresh-cut mango as related to uncoated. AL-coated fresh-cut mangoes were the toughest, among those coated, during 14 days. The highest consumer acceptance was achieved in AL (90.2%) coated fresh-cut mango (Blanca Salinas-Roca et al., 2018).

3.4 Ascorbic acid content (mg/ 100g) of coated fresh-cut mango by transglutaminase crosslinked protein/pectin coating

Ascorbic acid decreases during ripening or maturity as this organic acid act as a substrate for respiration. Edible coatings effectively delay decrement of ascorbic acid by slowing the respiration rate.

The influence of heat treatment combined with coating on sensory quality, physico-chemicals characteristics (firmness, colour, pH, titratable acidity, total soluble solids and total carotenoids content) and microbiological quality of fresh-cut mangoes were studied. Hot water dipping 50 C for 30 min and chitosan coating, either alone or combined, did not affect the taste and the flavour of mangoes slices. The chitosan coating combined with HWD or not inhibited the microbial growth for 9 days at 6 C. Indeed chitosan coating was used for his antimicrobial proprieties. Hot water dipping 50 C for 30 min was the beneficial treatment to maintain firmness and colour during 9 days at 6 C (Tassadit Djioua et al., 2010).

3.5 Microbial accumulation (cfu/g) of coated fresh-cut mango by transglutaminase crosslinked protein/pectin coating

Microbial decay is a factor responsible for postharvest loss in vegetables due to direct exposure to contaminating microorganisms through soil, dust, water and postharvest processing equipment (Nigro and Ippolito, 2016). Spoilage microorganisms produce extracellular lytic enzymes that degrade the cell wall polymer leading to the release of intracellular constituents, which provide favorable conditions for the growth of other microorganisms. Minimally processed vegetables offer a favorable environment for microbial growth due to the amount of surface exposed to the environment. Moisture increase is generally known to foster the growth of mold and bacteria and be responsible for their development in packed food products. In particular, the loss of water by packed fruit and vegetables during storage significantly increases the relative humidity existing inside the packaging and generates a favorable microenvironment for microorganism development (Giovanna Rossi Marquez et al., 2017).

Coating of the cut fresh fruit and vegetables before packaging could be an effective process to avoid microbial growth (Jagannath et al., 2006; Lee, Park, Lee, & Choi, 2003; Rojas-Grau et al., 2008). A work aimed at evaluating the effect of an alginatechitosan nanomultilayer coating, obtained by electrostatic layerby-layer self-assembling, in the quality and shelf life of fresh-cut mangoes. Chitosan-alginate nanomultilayer edible coating extended the shelf life of fresh-cut mangoes up to 8 days (Marthyna P. Souza et al., 2015).

3.6 Sensory score of coated fresh-cut mango by transglutaminase crosslinked protein/pectin coating

Spoilage processes happening during fruit and vegetable storage damage qualitatively the products, generating changes of their color, flavor and texture.

Fresh-cut mango was wrapped with mango film and packed in modified atmosphere packaging (MAP). The shelf life of uncoated and coated fresh-cut mango pieces was 6 days for each at 5°C and was 3 and 4 days respectively, at 30°C. The sensory evaluation indicated that coated fresh-cut mango was slower to produce an off-flavour and maintained better visual quality than uncoated mango at 30°C (Rungsinee Sothornvit et al., 2010). Manually sliced mango was treated by coating opuntia mucilage-rosemary oil (Mu + RO), 2 g rosemary oil microencapsul (ROM), and 2 g (ROM) plus (Mu + RO); the treated mango pieces were placed in plastic trays, and overwrapped with PVDC film and then stored at 6°C. Changes in the quality parameters and activity of peroxidase (POD) enzyme were evaluated for 9 days of storage period. These treatments retarded loss of ascorbic acid and the drop in sensory acceptability, fewer changes in color, decreasing activity POD enzyme. These also inhibited the decay incidence and slowed microbial growth. The (Mu + RO) treatment was more effective in controlling postharvest quality as compared to the (ROM) treatment (Majid Alikhanial et al., 2014).

Results from table 1, 2, 3, 4, 5, 6 revealed that the optimal ratio of whey protein:pectin should be 75%:25% for film coating of mango fresh cut.

IV. CONCLUSION

Minimally processed fruits are one of the major growing sectors in food retail market. Although the freshly cut mango still has a very limited offer in the world market. Edible coatings are also promising to improve the quality and extend the shelf-life of fresh-cut products. Coatings can control the internal atmosphere of fruits and retard their senescence, helping in their preservation once they provide a partial barrier to moisture, O_2, and CO_2 while also avoiding volatiles loss. They act as barriers to water loss and gas exchange by creating a micro-modified atmosphere around the product. Therefore edible coatings can contribute to extend the shelf life of these products.

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