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Preservation of Jackfruit Bulb By Transglutaminase Crosslinked Whey Protein/Pectin As Edible Film Coating

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Abstract.

Jackfruit is rich in nutrients including carbohydrates, proteins, vitamins, minerals, and phytochemicals. This fruit is underutilized in commercial scale processing in regions where it is grown, mainly due to higher percentage of inedible portion which leads to more waste generation, difculty in peeling and separation of edible bulbs from the rind. Fresh-cut jackfruit is a very interesting product to attend the increasing demand for fresh-cut fruits. Jackfruit bulb 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 jackfruit bulb. 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 jackfruit bulb. Results revealed that 80%: 20% (whey protein: pectin) was adequate for application. This approach opens an alternative strategy to prolong jackfruit bulb product shelf life in commercial distribution.

Keywords: *Jackfruit bulb, whey protein, pectin, shelf life, coating*

I. INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* L.) is native to parts of South and Southeast Asia. It is the rich source of carbohydrates, minerals, carboxylic acids, dietary fibre, and vitamins such as ascorbic acid (AA), and thiamine (Rahman et al., 1999). The edible fleshy pericarp amounts to only 35 per cent of the whole fruit, which is often prone to flavor loss, tissue softening, cut-surface browning and postharvest decay owing to the highly perishable nature (Saxena et al., 2009). It is a rich source of several high-value compounds with potential benefcial physiological activities (U. B. Jagtap et al., 2010). Jackfruit contains functional compounds that have capability to reduce various diseases such as high blood pressure, heart diseases, strokes, and bone loss. It is also capable of improving muscle and nerve function, reducing homocysteine levels in the blood (S. B. Swami et al., 2012). Phytonutrients such as lignans, isofavones, and saponins in jackfruit contribute to its anticancer, antihypertensive, antiulcer, and antiaging properties (M. S. Baliga et al., 2011).

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 jackfruit bulb. An experiment was carried out to investigate the effect of pretreatments on quality and shelf life of fresh cut jackfruit bulbs (SC Prathibha et al., 2019). Effect of controlled atmosphere storage and chitosan coating on quality of fresh-cut jackfruit bulbs was examined (Alok Saxena et al., 2013). Optimization of chemical treatment on fresh cut tender jackfruit slices for prevention of browning by using response surface methodology was carried out (Rana, S. S. et al., 2018).

Jackfruit bulbs 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 jackfruit 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. MATERIALS AND METHOD

2.1 Material

Jackfruit 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 jackfruit, 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. Jackfruit (*Artocarpus heterophyllus* L.) bulbs

2.2 Researching method

2.2.1 Film coating preparation

Whey protein/pectin in different ratio (20%: 80%, 40%: 60%, 60%: 40%, 80%: 20%) film-forming solution containing transglutaminase was prepared by solubilizing whey protein isolate: pectin (2.0 g: 8.0g, 4.0g: 6.0g, 6.0g: 4.0g, 8.0g: 2.0g) 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 40^oC and 35% relative humidity.

2.2.2 Sample preparation

Jackfruit fruits were peeled 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 8 g/L $CaCl_2$ solution for 2 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 jackfruit 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 jackfruit bulb by transglutaminase crosslinked protein/pectin coating

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 jackfruit bulb by transglutaminase crosslinked protein/pectin coating

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.

Table 1. Weight loss (%) of coated jackfruit bulb by whey protein/pectin in different ratio

Day of preservation	Control	20%: 80%	40%: 60%	60%: 40%	80%: 20%
0	0	0	0	0	0
2	1.73±0.01[a]	1.00±0.02[ab]	0.91±0.02[b]	0.20±0.01[bc]	0.13±0.01[c]
4	2.31±0.02[a]	1.11±0.01[ab]	1.02±0.01[1b]	0.29±0.02[bc]	0.24±0.03[c]
6	2.75±0.03[a]	1.31±0.01[ab]	1.16±0.01[b]	0.36±0.01[bc]	0.27±0.04[c]
8	2.93±0.01[a]	1.44±0.02[ab]	1.23±0.01[b]	0.42±0.03[bc]	0.38±0.02[c]
10	3.17±0.01[a]	1.48±0.03[ab]	1.30±0.00[b]	0.47±0.01[bc]	0.40±0.01[c]
12	3.53±0.03[a]	1.57±0.00[ab]	1.33±0.01[b]	0.51±0.00[bc]	0.43±0.01[c]
14	3.61±0.02[a]	1.72±0.00[ab]	1.42±0.03[b]	0.60±0.02[bc]	0.48±0.02[c]

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 jackfruit bulb by whey protein/pectin in different ratio

Day of preservation	Control	20%: 80%	40%: 60%	60%: 40%	80%: 20%
0	63.22±0.02[a]	63.22±0.02[a]	63.22±0.02[a]	63.22±0.02[a]	63.22±0.02[a]
2	62.87±0.00[c]	62.94±0.00[bc]	63.00±0.02[b]	63.16±0.02[ab]	63.18±0.01[a]
4	62.70±0.01[c]	62.84±0.01[bc]	62.91±0.01[b]	63.03±0.01[ab]	63.11±0.02[a]
6	62.59±0.03[c]	62.72±0.02[bc]	62.80±0.02[b]	62.97±0.01[ab]	63.05±0.03[a]
8	62.35±0.02[c]	62.51±0.01[bc]	62.62±0.02[b]	62.88±0.02[ab]	63.02±0.00[a]
10	62.26±0.01[c]	62.39±0.01[bc]	62.51±0.01[b]	62.85±0.02[ab]	62.94±0.01[a]
12	62.11±0.01[c]	62.27±0.02[bc]	62.41±0.01[b]	62.73±0.01[ab]	62.89±0.02[a]
14	62.00±0.01[c]	62.15±0.00[bc]	62.30±0.00[b]	62.66±0.03[ab]	62.84±0.00[a]

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 jackfruit bulb by whey protein/pectin in different ratio

Day of preservation	Control	20%: 80%	40%: 60%	60%: 40%	80%: 20%
0	6.45±0.02[a]	6.45±0.02[a]	6.45±0.02	6.45±0.02[a]	6.45±0.02[a]
2	6.27±0.03[c]	6.32±0.01[bc]	6.36±0.02[b]	6.38±0.02[ab]	6.40±0.03[a]
4	6.20±0.03[c]	6.25±0.03[bc]	6.29±0.01[b]	6.32±0.01[ab]	6.36±0.02[a]
6	6.14±0.00[c]	6.19±0.02[bc]	6.23±0.01[b]	6.27±0.01[ab]	6.33±0.01[a]
8	6.06±0.02[c]	6.14±0.01[bc]	6.19±0.03[b]	6.23±0.03[ab]	6.31±0.03[a]
10	6.01±0.00[c]	6.06±0.02[bc]	6.14±0.01[b]	6.17±0.01[ab]	6.25±0.01[a]
12	5.94±0.02[c]	6.01±0.03[bc]	6.07±0.03[b]	6.11±0.02[ab]	6.21±0.00[a]
14	5.88±0.01[c]	5.96±0.00[bc]	6.01±0.01[b]	6.07±0.03[ab]	6.16±0.01[a]

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%).

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. An experiment was carried out to investigate the effect of pretreatments on quality and shelf life of fresh cut jackfruit bulbs. The jackfruit bulbs were pretreated with calcium chloride 0.50 and 1.0% alone or in combination with 0.25% ascorbic acid, 10% aloe vera gel, 0.10% potassium metabisulphite. Untreated samples were used as experimental control Jack fruit bulbs pretreated with 1% $CaCl_2$ along with 0.25% ascorbic acid registered better in terms of maintaining quality with extended shelf life. The treatment recorded lower PLW, better retention of ascorbic acid, total carotenoids, total antioxidants and lower microbial load at the end of the experiment up to 3 weeks (SC Prathibha et al., 2019).

3.3 Carotenoid content (mg/ 100g) of coated jackfruit bulb 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.

In one study, fresh-cut jackfruit bulbs were evaluated for quality changes as effect of an additive pretreatment with $CaCl_2$, ascorbic acid, citric acid, and sodium benzoate followed by chitosan coating. Different types of samples such as pretreated and coated, only pretreated, only coated, and untreated were subjected to controlled atmosphere (CA) storage (3 kPa O2+6 or 3 kPa CO_2; N_2 balance) or normal air at 6 °C. CA conditions, pretreatment, as well as chitosan coating in synergy with each other, could significantly minimize the loss in total phenolics and ascorbic acid content of the samples to the levels of around 5% and 17%, respectively, during extended storage up to 50 days. Chitosan coating could also restrict the changes in microbial load. The CA condition of 3 kPa O_2+ 6 kPa CO_2 was found to render higher efficacy in retaining quality attributes of the samples (Alok Saxena et al., 2013).

3.4 Ascorbic acid content (mg/ 100g) of coated jackfruit bulb 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.

Vargas-Torres et al. (2017) have discovered the ability of pretreatment with 1-methylcyclopropene and the application of edible coatings (xanthan, sodium alginate, or gellan) to extend the shelf life of precut jackfruit up to 12 days, while preserving the original quality attributes when stored at 5∘ C. Te fndings of the study revealed that the pretreatment and application of edible coating were able to reduce the weight loss, respiration, and ripening rates while maintaining the desired sensory and nutritional attributes such as colour, frmness, pH, total soluble solids, and titratable acidity of the products.

3.5 Microbial accumulation (cfu/g) of coated jackfruit bulb 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).

3.6 Sensory score of coated jackfruit bulb 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.

Results from table 1, 2, 3, 4, 5, 6 revealed that the optimal ratio of whey protein:pectin should be 80%:20% for film coating of jackfruit bulb.

Table 4. Ascorbic acid content (mg/ 100g) of coated jackfruit bulb by whey protein/pectin in different ratio

Day of preservation	Control	20%: 80%	40%: 60%	60%: 40%	80%: 20%
0	54.23±0.01^{a}	54.23±0.01^{a}	54.23±0.01^{a}	54.23±0.01^{a}	54.23±0.01^{a}
2	54.02±0.02^{c}	54.08±0.01^{bcc}	54.13±0.02^{b}	54.17±0.02^{ab}	54.20±0.02^{a}
4	53.97±0.01^{c}	54.00±0.02^{bc}	54.05±0.01^{b}	54.11±0.02^{ab}	54.16±0.01^{a}
6	53.85±0.00^{c}	53.92±0.01^{bc}	53.97±0.01^{b}	54.05±0.01^{ab}	54.11±0.03^{a}
8	53.77±0.03^{c}	53.88±0.03^{bc}	53.91±0.00^{b}	53.98±0.00^{ab}	54.05±0.00^{a}
10	53.61±0.01^{c}	53.78±0.00^{bc}	53.83±0.01^{b}	53.90±0.00^{ab}	54.00±0.02^{a}
12	53.48±0.00^{c}	53.71±0.01^{bc}	53.77±0.01^{b}	53.82±0.01^{ab}	53.93±0.01^{a}
14	53.40±0.01^{c}	53.62±0.00^{bc}	53.70±0.00^{b}	53.77±0.02^{ab}	53.88±0.00^{a}

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 jackfruit bulb by whey protein/pectin in different ratio

Day of preservation	Control	20%: 80%	40%: 60%	60%: 40%	80%: 20%
0	7.3 x 10^{1} ±0.02^{a}	7.3 x 10^{1} ±0.02^{a}	7.3 x 10^{1} ±0.02^{a}	7.3 x 10^{1} ±0.02^{a}	7.3 x 10^{1} ±0.02^{a}
2	7.9 x 10^{1} ±0.01^{a}	7.6 x 10^{1} ±0.01^{ab}	7.5 x 10^{1} ±0.02^{b}	7.4 x 10^{1} ±0.01^{bc}	7.4 x 10^{1} ±0.02^{c}
4	8.8 x 10^{1} ±0.02^{a}	7.9 x 10^{1} ±0.02^{ab}	7.8 x 10^{1} ±0.01^{b}	7.7 x 10^{1} ±0.02^{bc}	7.5 x 10^{1} ±0.03^{c}
6	9.9 x 10^{1} ±0.01^{a}	8.6 x 10^{1} ±0.01^{ab}	8.1 x 10^{1} ±0.03^{b}	7.9 x 10^{1} ±0.02^{bc}	7.6 x 10^{1} ±0.00^{c}
8	1.4 x 10^{2} ±0.02^{a}	8.9 x 10^{1} ±0.02^{ab}	8.4 x 10^{1} ±0.01^{b}	8.2 x 10^{1} ±0.00^{bc}	8.0 x 10^{1} ±0.01^{c}
10	2.7 x 10^{2} ±0.02^{a}	9.7 x 10^{1} ±0.01^{ab}	9.0 x 10^{1} ±0.02^{b}	8.6 x 10^{1} ±0.01^{bc}	8.2 x 10^{1} ±0.02^{c}
12	5.3 x 10^{2} ±0.00^{a}	1.3 x 10^{2} ±0.03^{ab}	9.9 x 10^{1} ±0.02^{b}	9.3 x 10^{1} ±0.03^{bc}	8.4 x 10^{1} ±0.00^{c}
14	7.9 x 10^{2} ±0.03^{a}	2.7 x 10^{2} ±0.01^{ab}	1.3 x 10^{2} ±0.01^{b}	9.8 x 10^{1} ±0.02^{bc}	8.6 x 10^{1} ±0.03^{c}

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 jackfruit bulb by whey protein/pectin in different ratio

Day of preservation	Control	20%: 80%	40%: 60%	60%: 40%	80%: 20%
0	8.35±0.01^{a}	8.35±0.01^{a}	8.35±0.01^{a}	8.35±0.01^{a}	8.35±0.01^{a}
2	8.17±0.01^{c}	8.21±0.01^{bc}	8.26±0.02^{b}	8.29±0.01^{ab}	8.31±0.02^{a}
4	8.13±0.02^{c}	8.17±0.02^{bc}	8.19±0.03^{b}	8.22±0.03^{ab}	8.29±0.03^{a}
6	8.03±0.00^{c}	8.09±0.01^{bc}	8.11±0.01^{b}	8.18±0.02^{ab}	8.27±0.00^{a}
8	7.93±0.00^{c}	8.04±0.03^{bc}	8.07±0.02^{b}	8.13±0.00^{ab}	8.24±0.00^{a}
10	7.82±0.03^{c}	7.98±0.02^{bc}	8.02±0.03^{b}	8.01±0.00^{ab}	8.20±0.02^{a}
12	7.77±0.02^{c}	7.91±0.00^{bc}	7.97±0.01^{b}	7.96±0.02^{ab}	8.13±0.02^{a}
14	7.71±0.01^{c}	7.88±0.00^{bc}	7.93±0.01^{b}	7.91±0.02^{ab}	8.11±0.01^{a}

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%).

IV. CONCLUSION

Minimally processed fruits and vegetables have gained rapid popularity among the consumer due their fresh like nature and convenience. Jackfruit bulb in a pre-cut form can provide convenience for shelf-life extension and facilitate easy transportation from production site to remote locations. It would also offer advantages such as ease in serving portions of large and difficult to peel fruits, reduced cost of packaging, extended shelf –life and quality products. The jackfruit bulb is highly perishable and ofen undergoes favour loss, tissue sofening, and cut surface browning. The sofening of the fruit makes it more susceptible for bruising and mechanical injury. The present study was undertaken to investigate the application of transglutaminase crosslinked whey protein/pectin in different ratios as edible coating film for preservation of jackfruit bulb.

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