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RESEARCH ARTICLE

Synergistic Combination of Chitosan and Cinnamid Acid as Edible Coating to Extend Self-life of Acelora Cherry (*Malpighia emarginata*) Fruit

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Abstract

Chitosan is edible coating which is a safe, biodegradable and non-toxic substance derived from chitin shells of crustaceans. It's considered as an antifungal agent that can control postharvest diseases. Cinnamic acid is a plant-derived soluble compound that impedes microbial growth. Acerola cherry **(***Malpighia emarginata***)** fruit is well known as one of the richest natural sources of ascorbic acid as well as its high concentration of health-relevant phytochemicals. It has a red color and highly perishable at complete maturity, resulting in serious quality losses during post-harvest. Purpose of this research demonstrated the efficacy of chitosan combined with cinnamid acid as edible coating to extend self-life of acelora cherry fruit. Finding results revealed that chitosan 1.5% + 2 mM cinnamid acid was appropriate to limit weight loss; maintain firmness, chlorophyll, and vitamin C content effectively. Microbial safety was also monitored on treated samples during 8 days of storage at ambient temperature. The combination of chitosan and cinnamic acid provides synergistic effect as a semi-permeable barrier to delay the respiration rate.

Keywords: *Acelora cherry, Chitosan, Cinnamid acid, Coating, Weight loss, Firmness, Chlorophyll, vitamin C.*

Introduction

Acerola cherry (*Malpighia emarginata)* is a tropical fruit-bearing shrub or small tree in the family Malpighiaceae. It's highlighted as one of the best natural sources of amino acids, phenolics, anthocyanins, carotenoids and bioflavonoids, vitamin C, surpassing fruits like guava, cashew, orange and lemon [1, 4]. Mallic acid is also found in high amount in Acerola fruits, with trace amounts of citric and tartaric acids as well.

The vitamin C content is high in immature fruits and decreases during ripening [5] its extracts also showed potent antioxidative, anti-inflammatory, antihyperglycemic, antitumor, antigenotoxic and hepatoprotective activity [6]. There's a rising global demand for natural bioactive-rich fruits and derivatives, such as acerola [7, 8].Chitosan is a biodegradable film derived from chitin [9]. Chitosan is more durable than chitin to depolymerization due to stabilizing effect of the free amino group. Chitosan has an antifungal characteristic that affects internal metabolic reaction of pathogen [10].Chitosan coating is useful to maintaining the storage quality and prolonging the shelf life of postharvest fruits and vegetables, which is always used as the carrier film for the antimicrobial agents [11]. Cinnamic acid is well known as an antimicrobial agent [12].

Cinnamic acid was found to be potent inhibitors of both the subsequent end products and the glycation reaction [13]. The reaction of chitosan with cinnamic acid gave the corresponding N-cinnamoyl chitosan (NCC) polymer [14]. The utilization of chitosan and cinnamic acid aims to replace the application of synthetic fungicides to control spoilage by the pathogen [15]. Chitosan and cinnamic acid are believed as safer alternatives to the potentially toxic agents to cure early deterioration and prolong the stability of foodstuff [16, 17]. The coatings also minimize the effects of unfavourable conditions and accidental contamination during postharvest handling [18]. Edible coating is an eco-friendly technique which slows deterioration of vegetables by controlling gas exchange, moisture transfer, and oxidation [19].

The effect of combined coatings of chitosan (Ch) and cinnamic acid (CA) in extending the tomato shelf life was evaluated [20]. Gelatin-based coating was applied for acerola preservation [21]. Coating of acerola fruit with xanthan gum has been found to delay the ripening process [22]. However there was not any research mentioned to the application of chitosan and cinnamid acid to create a synergistic effect during preservation of acelora cherry fruit. Objective of our study focused on the edible coating efficiency prepared by different formulas of chitosan and cinnamid acid to prolong stability of acelora cherry fruit.

Material and Method

Material

Acelora cherry fruits were harvested from rural gardens in Can Tho city, Vietnam. After collecting, they must be conveyed to laboratory for experiments. They were subjected to washing and treatment. Chitosan, cinnamic acid and acetic acid were purchased from Sigma-Aldrich. Chitosan was dissolved in 1.0% (v/v) acetic acid. Cinnamic acid was dissolved in distilled water.

Researching Procedure

The coating formulation was arranged for 8 different sets of coating treatment with formulas as follows: Control: 1.0% acetic acid; (A): Chitosan 0.5%; (B): Chitosan 0.5% + 2 mM cinnamid acid; (C): Chitosan 1.0%; (D): Chitosan 1.0% + 2mM cinnamid acid; (E): Chitosan 1.5%; (F): Chitosan 1.5% + 2 mM cinnamid acid; (G): Chitosan 2.0%; (H): Chitosan 2.0% + 2mM cinnamid acid. Fruits were dipped prior into chitosan solution for 15 s and then permitted to dry for one hour at room temperature, followed by layer by layer encapsulating of 1 min in cinnamic acid (as 8 different above formulas which were prepared), and then dried for one hour at ambient temperature on a tray. The 0th, 2nd, 4th, 6th and 8th observational days were monitored by measurements of weight loss (%), firmness (N), chlorophyll (mg/g), vitamin C (mg/100g), total plate count (cfu/g).

Physico-chemical, Microbial and Statistical Analysis

Weight losses (%) were determined by comparing weight of samples before and after the storage period. Firmness (N) was measured by penetrometer. Chlorophyll contents (mg/g) were determined following to the method proposed by Zhang et al [23].Vitamin C (g/100g) was evaluated by 2, 6-dichlorophenolindophenol titration. Total plate count (TPC, cfu/g) was analyzed by Petrifilm-3M. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Stat graphics Centurion XVI.

Result & Discussion

Weight Loss (%) of Coated acelora Cherry by Chitosan and Cinnamid Acid During Preservation

Respiration rate is one of the major factors contributing to postharvest losses of fruit [24]. Weight losses (%) of treated and non-treated samples were carefully monitored within 8 days of storage. Our results revealed that acelora cherry fruits coated by chitosan 1.5% + 2 mM cinnamid acid showed the lowest weight loss (%).

Table 1: Weight loss (%) of coated acelora cherry by chitosan and cinnamid acid

Storage days	0	2	4	6	8
Control	0	3.28±0.05^{a}	6.18±0.00^{a}	10.12±0.03^{a}	14.27±0.01^{a}
Formula A	0	2.10±0.02^{b}	4.05±0.01^{b}	7.54±0.01^{b}	9.43±0.00^{b}
Formula B	0	1.94±0.01^{bc}	3.84±0.02^{bc}	6.89±0.00^{bc}	8.21±0.00^{c}
Formula C	0	1.85±0.00^{bc}	3.43±0.04^{c}	6.21±0.02^{c}	7.19±0.04^{d}
Formula D	0	1.46±0.00^{c}	3.07±0.02^{cd}	5.93±0.05^{cd}	6.45±0.02^{de}
Formula E	0	1.23±0.02^{cd}	2.75±0.01^{cd}	5.12±0.01^{d}	5.88±0.03^{e}
Formula F	0	1.04±0.01^{cd}	2.31±0.00^{d}	4.67±0.02^{de}	5.14±0.01^{ef}

Formula G	0	0.99±0.03d	2.11±0.04de	4.22±0.00e	4.76±0.02f
Formula H	0	0.95±0.02d	2.06±0.02e	4.15±0.00e	4.63±0.00f

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%).

Combined coating of chitosan and cinnamic acid were demonstrated to influence firmness and weight loss [20].

Firmness of Coated acelora Cherry by Chitosan and Cinnamid Acid during Preservation

Firmness is another important factor for quality of fruit [25]. The firmness of fruits is closely associated with acceptability levels of the fruits. Firmness (N) of treated and non-treated samples was carefully monitored within 8 days of storage. Our results revealed that acelora cherry fruits coated by chitosan 1.5% + 2 mM cinnamid acid still maintained their firmness until the 6th day of storage.

Table 2: Firmness (N) of coated acelora cherry by chitosan and cinnamid acid

Storage days	0	2	4	6	8
Control	4.35±0.02a	2.03±0.01d	1.51±0.04d	1.24±0.04e	1.06±0.03f
Formula A	4.35±0.02a	2.84±0.03c	2.77±0.02c	2.61±0.02d	1.73±0.04e
Formula B	4.35±0.02a	3.01±0.00bc	2.95±0.01bc	2.84±0.01cd	2.05±0.00d
Formula C	4.35±0.02a	3.22±0.02bc	3.14±0.03bc	3.02±0.00cd	2.49±0.02cd
Formula D	4.35±0.02a	3.57±0.01b	3.41±0.00b	3.16±0.04c	2.83±0.02c
Formula E	4.35±0.02a	3.92±0.03ab	3.85±0.02ab	3.23±0.03bc	3.01±0.01bc
Formula F	4.35±0.02a	4.04±0.00ab	3.96±0.01ab	3.55±0.01b	3.14±0.04b
Formula G	4.35±0.02a	4.19±0.01a	4.13±0.03a	3.89±0.02ab	3.52±0.00ab
Formula H	4.35±0.02a	4.22±0.00a	4.17±0.01a	4.04±0.02a	3.79±0.03a

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%)

Chlorophyll (5mg/g) of Coated acelora Cherry by Chitosan and Cinnamid Acid during Preservation

Chlorophyll is mainly responsible for green color of fruit and vegetable peel. It plays vital role in their appearance and acceptance [26]. When chlorophyll is decomposed, it leads to the development of yellowness that reduces their sensory appeal and market value [27]. Chlorophyll content (mg/g) of treated and non-treated samples was carefully monitored within 8 days of storage. Our results revealed that acelora cherry fruits coated by chitosan 1.5% + 2 mM cinnamid acid still maintained their chlorophyll content (mg/g) until the 8th day of storage.

Table 3: Chlorophyl (mg/g) of coated acelora cherry by chitosan and cinnamid acid

Storage days	0	2	4	6	8
Control	6.13±0.04a	4.07±0.02d	3.51±0.03f	2.38±0.03e	2.15±0.01e
Formula A	6.13±0.04a	4.95±0.04c	4.14±0.00e	3.03±0.01d	2.86±0.03de
Formula B	6.13±0.04a	5.12±0.01bc	4.52±0.02d	3.19±0.02cd	3.03±0.01d
Formula C	6.13±0.04a	5.46±0.03bc	4.89±0.01cd	3.85±0.04cd	3.72±0.04cd
Formula D	6.13±0.04a	5.73±0.04b	5.03±0.03c	4.17±0.01c	4.01±0.03c
Formula E	6.13±0.04a	5.87±0.02ab	5.17±0.04bc	4.52±0.02bc	4.14±0.02bc
Formula F	6.13±0.04a	5.95±0.01ab	5.52±0.02b	4.86±0.00b	4.63±0.00b
Formula G	6.13±0.04a	6.01±0.02a	5.73±0.00ab	5.15±0.00ab	4.92±0.05ab
Formula H	6.13±0.04a	6.06±0.03a	5.91±0.02a	5.42±0.05a	5.11±0.01a

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%)

Vitamin C of Coated acelora Cherry by Chitosan and Cinnamid Acid during Preservation

Vitamin C (g/100g) of treated and non-treated samples was carefully monitored within 8 days of storage. Our results revealed that acelora cherry fruits coated by chitosan 1.5% + 2 mM cinnamid acid still maintained their chlorophyll content (mg/g) until the 6th day of storage. This amount would be gradually decreased at the 8th day.

Table 4: Vitamin C (g/100g) of coated acelora cherry by chitosan and cinnamid acid

Storage days	0	2	4	6	8
Control	2.49 ± 0.02^{a}	1.85 ± 0.03^{e}	1.12 ± 0.01^{f}	1.04 ± 0.01^{e}	0.35 ± 0.03^{f}
Formula A	2.49 ± 0.02^{a}	2.09 ± 0.00^{d}	1.75 ± 0.01^{e}	1.39 ± 0.03^{de}	0.57 ± 0.00^{e}
Formula B	2.49 ± 0.02^{a}	2.13 ± 0.02^{cd}	1.88 ± 0.04^{d}	1.65 ± 0.01^{d}	1.14 ± 0.00^{d}
Formula C	2.49 ± 0.02^{a}	2.17 ± 0.01^{c}	1.95 ± 0.02^{cd}	1.74 ± 0.05^{cd}	1.35 ± 0.02^{cd}
Formula D	2.49 ± 0.02^{a}	2.21 ± 0.05^{bc}	2.04 ± 0.00^{c}	1.83 ± 0.02^{c}	1.66 ± 0.05^{c}
Formula E	2.49 ± 0.02^{a}	2.28 ± 0.02^{b}	2.11 ± 0.01^{bc}	1.94 ± 0.01^{bc}	1.78 ± 0.01^{bc}
Formula F	2.49 ± 0.02^{a}	2.35 ± 0.00^{ab}	2.19 ± 0.03^{b}	2.01 ± 0.03^{b}	1.92 ± 0.03^{b}
Formula G	2.49 ± 0.02^{a}	2.39 ± 0.03^{a}	2.28 ± 0.01^{ab}	2.13 ± 0.01^{ab}	2.05 ± 0.02^{ab}
Formula H	2.49 ± 0.02^{a}	2.42 ± 0.00^{a}	2.35 ± 0.00^{a}	2.28 ± 0.02^{a}	2.16 ± 0.04^{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 Plate Count (TPC) of Coated acelora Cherry by Chitosan and Cinnamid Acid during Preservation

Microbial safety is important in determining the quality and shelf life of fruit and vegetable [26]. Cinnamic acid helped chitosan in improving coating ability by serving better barrier from pathogen and oxidative gas penetration to prevent earlier spoilage problem [20]. Total plate count (TPC, cfu/g) was also monitored on treated samples during 8 days of storage at ambient temperature. Chitosan combined with cinnamid acid created an antimicrobial synergistic effect especially on acelora cherry.

Table 5: TPC (cfu/g) of coated acelora cherry by chitosan and cinnamid acid

Storage days	0	2	4	6	8
Control	$4.35x10^{4}\pm0.01^{a}$	$6.29x10^{4}\pm0.12^{a}$	$7.53x10^{4}\pm0.23^{a}$	$8.48x10^{4}\pm0.04^{a}$	$1.29x10^{5}\pm0.22^{a}$
Formula A	$4.35x10^{4}\pm0.01^{a}$	$3.24x10^{4}\pm0.27^{ab}$	$3.12x10^{4}\pm0.19^{ab}$	$2.81x10^{4}\pm0.17^{b}$	$7.59x10^{3}\pm0.17^{b}$
Formula B	$4.35x10^{4}\pm0.01^{a}$	$3.17x10^{4}\pm0.15^{b}$	$3.03x10^{4}\pm0.31^{b}$	$2.12x10^{4}\pm0.33^{bc}$	$4.23x10^{3}\pm0.06^{bc}$
Formula C	$4.35x10^{4}\pm0.01^{a}$	$2.42x10^{4}\pm0.08^{bc}$	$2.31x10^{4}\pm0.07^{bc}$	$1.75x10^{4}\pm0.26^{bc}$	$1.09x10^{3}\pm0.35^{c}$
Formula D	$4.35x10^{4}\pm0.01^{a}$	$1.28x10^{4}\pm0.16^{c}$	$1.19x10^{4}\pm0.22^{c}$	$1.43x10^{4}\pm0.14^{c}$	$7.64x10^{2}\pm0.12^{cd}$
Formula E	$4.35x10^{4}\pm0.01^{a}$	$8.53x10^{3}\pm0.09^{d}$	$7.95x10^{3}\pm0.29^{cd}$	$6.74x10^{3}\pm0.42^{d}$	$4.11x10^{2}\pm0.11^{d}$
Formula F	$4.35x10^{4}\pm0.01^{a}$	$6.39x10^{3}\pm0.15^{de}$	$5.21x10^{3}\pm0.11^{d}$	$3.16x10^{3}\pm0.24^{de}$	$1.25x10^{2}\pm0.05^{de}$
Formula G	$4.35x10^{4}\pm0.01^{a}$	$2.15x10^{3}\pm0.29^{de}$	$1.83x10^{3}\pm0.34^{de}$	$1.02x10^{3}\pm0.08^{de}$	$0.87x10^{2}\pm0.28^{e}$
Formula H	$4.35x10^{4}\pm0.01^{a}$	$2.04x10^{3}\pm0.06^{e}$	$1.01.x10^{3}\pm0.25^{e}$	$8.34x10^{2}\pm0.19^{e}$	$4.10x10^{1}\pm0.04^{f}$

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%)

Conclusion

The uniquely high concentration of ascorbic acid inside acelora cherry makes this fruit valuable for great demand on global market. However, this fruit is very perishable at ambient temperature during post-harvest. Deterioration leads short shelf life of fruit with unfavourable quality, resulting in potential rejection by customers. Chitosan filming is one of the potential approaches because of its excellent attributes to create the thin film on the fruit's surface, the property to prevent the loss of moisture and flavor, reduction of respiration rate, the inhibition of the oxygen penetration to the plant tissue or microbial growth. The chitosan-based coatings were developed by combining with antimicrobial such as cinnamid acid. The application of chitosan-cinnamid acid coating could prolong acelora fruit stability during preservation and consumption. Postharvest handlings are very important to obtain acelora cherry with acceptable quality.

Conflict of Interest

The authors declare that no conflict of interest exists in the course of conducting this research. First author had final decision regarding the manuscript and the decision to submit the findings for publication.

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