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Trial Application of Bacterial Cellulose Membrane in Minimally Pre-Prepared Fresh Coconut

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Abstract: *In this publication, we have conducted experiments in using bacterial cellulose to create preservation coating for minimally pre-prepared fresh coconut with different experimental formulas, including the use of only bacterial cellulose membrane and combined with bacterial cellulose membranes which have absorbed Benzoate Sodium (preservation agent) at different concentrations. The results obtained are as follow:*

- *First step in using thin bacterial cellulose membrane to preserve minimally pre-prepared fresh coconut: bacterial cellulose membrane can be used as a preservation method for minimally pre-prepared fresh coconut for at least up to 14 days. Bacterial cellulose membrane with Benzoate Sodium 0.2% will deliver the best preservation capability, assure the quality of fresh coconut even after 28 days of preservation, all at normal temperature condition.*
- *The result also contributes to the survey of new application of bacterial cellulose: using bacterial cellulose as food coatings*

Keywords: *Bacterial cellulose, fresh coconut*

I. Introduction

Thin bacterial cellulose membrane is a biological polymer membrane, which has similar chemical structures to plant cellulose membrane but with some other physiochemical characteristics such as: high mechanical durability and water absorption ability, small fiber diameter, high level of purity, great polymerization capability... At the moment, BC membrane is considered as the new material source with the potential to be applied for lots of different fields such as: food industry, medical industry, cosmetic,...[2, 6, 7] In food industry, BC membrane has been researched for their applications of preserving food as a kind of coating [3, 4].

Fresh coconut is a popular, nutritious product and widely loved by everyone. Coconut fresh juice is not only refreshing but also contains many nutrients which benefit health [5]. However, using fresh coconut is still inconvenient due to its hard and thick shells. Therefore, the peeling process is really necessary for easier usages, as the product has been minimally pre-prepared. Short shelf-life would be the main disadvantage of those kind of products, only lasting for around 1 to 2 days. For this reason, an efficient and safety way should be developed to preserve those minimally pre-prepared coconuts, yet a money-saving one. In this research, a preservation method of minimally pre-prepared fresh coconut using BC and using BC with Benzoate Sodium at different concentrations to evaluate and determine which formula is the best to extend the shelf-life of the minimally pre-prepared fresh coconut.

II. Materials And Methodology

2.1. Materials

BC membrane is formed by *Gluconacetobacter intermedius* through thin-layer static fermentation process [4].
Xiem Coconut: using the species of Xiem Dua Coconut (a local Vietnamese species of coconut) aged around 8 months.

2.2. Research method

Xiem Dua Coconuts are peeled off into shape, then treat the surface with Acetic Acid 5%, dry the surface of the fruit and store it at room temperature ($30 – 32^0$C) with 6 experimental formulas (Table 1).

Table 1. Preservation experimental formulas of minimally pre-prepared fresh coconut

Experimental formulas	Symbol
Initial sample	IS_1
Initial sample after 3 days preservation	IS_2
Coconut sample preserved by BC	BC
Coconut sample preserved by BC + Benzoate Sodium 0,1%	BC + 0,1% B.Sodium
Coconut sample preserved by BC + Benzoate Sodium 0,2%	BC + 0,2% B.Sodium
Coconut sample preserved by BC + Benzoate Sodium 0,3%	BC + 0,3% B.Sodium

The coconut sample preserved by BC membrane combined with Benzoate Sodium: thin BC membrane will be cultured and collected, after soaking it for 30 minutes in Benzoate Sodium solution at those concentrations: 0.1, 0.2 and 0.3% respectively. Thin BC membrane absorbed B.Sodium will be used to make coating for minimally pre-prepared fresh coconut in the preservation experiment.

Analyze and evaluate the sample quality after each 7-day preservation period

Quality monitoring criterion in preserving fresh coconut:

- Total aerobic bacteria number: counted by pour plate method and incubated in aerobic condition at 30^0C for 72 hours. All steps are perform in sterile condition, equipments and environment are all sterilized and aseptic. [1].
- Total glucose level: Determine total glucose level based on standard glucose measured at wavelength 490nm [8].
- Total acid level: Determine total acid level neutral method, direct titration of acids in the sample by sodium hydroxide with phenolphtalein indicator [8].
- pH sample: pH of coconut juice is determined by portable pH meter HI 8424 with glass electrode.
- Vitamin C level: determined by redox method, Vitamin C reduces iodine solution, based on the ammount of reduced iodine by Vitamin C in the sample, indicate the vitamin C level [8].

III. Results And Discussion

3.1. Total aerobic bacteria

Microorganism is one of the reasons that lead to spoiled food. As preservation time flow, microorganism will develop in quantity, in that process they will produce metabolic wastes, which alter the color, states, flavour and nutritious components of the coconut, leads to spoiling and degraded quality. Therefore, total aerobic bacteria is one of the most important microbiological criteria in food and beverages. Generally, anaerobic bacteria number still increased throughout the whole preservation period. However, the number is lower when compared with IS_2 sample. In which, batch which is preserved by BC has the fastest rate of bacteria reproduction, next is the formula BC + 0.1% B.Sodium. with the preservation sample at day 28 indicating the same result with control sample IS_2. BC + 0.2% B.Sodium and BC + 0.3% B.Sodium have the same aerobic bacteria reproduction and considerably low, after 28 days of preservation, the result is significantly lower than the IS_2 sample (Chart 1)

With this criteria, BC + 0,2% B.Sodium and BC + 0,3% B.Sodium are the 2 most experimental formulas and could be used to assure the quality of fresh coconut after 28 days preservation, BC và BC + 0,1% B.Sodium had the capability of preservation for around 21 days.

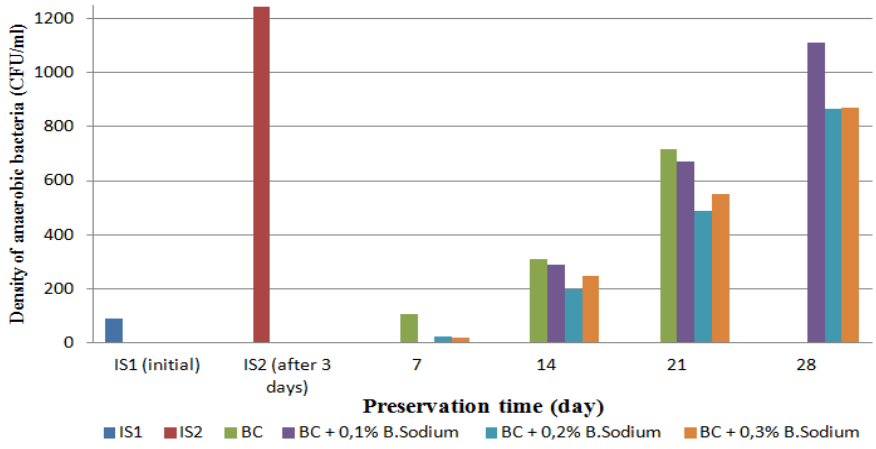

Chart 1. Chart for total aerobic number after preservation period

3.2. Total glucose

Glucose is one of the main substances which provide carbon source for bacteria development. Therefore, the total glucose level in coconut juice will decrease when total aerobic bacteria increase. Total glucose concentration tend to decrease gradually during preservation process (chart 2). In which, fresh coconut preserved by BC membrane started decreasing at day 14 with 16.8% and then dropped to day 21 with over 65% compared to the control sample IS_2. Next formula is the BC + 0.1% B.Sodium, total glucose level dropped 1.4% compared to initial control sample after 28 days of preservation. Only in this particular formula BC + 0,3% B.Sodium from 7^{th} day to 28^{th} day during preservation, total glucose level slightly drop but still maintain higher level than the control sample IS_1, this could be explained by the insignificant differences among those coconuts in the same source.

So, at total glucose level criteria, the 3 formulas BC + 0,1% B.Sodium, BC + 0,2% B.Sodium and BC + 0,3% B.Sodium had performed really well, be able to assure the quality of fresh coconut for 28 days preservation, in which BC + 0.2% B.Sodium and BC + 0.3% B.Sodium are the most optimal choices. Other than that, the fresh coconut sample preserved by only BC membrane lasted for 14 days

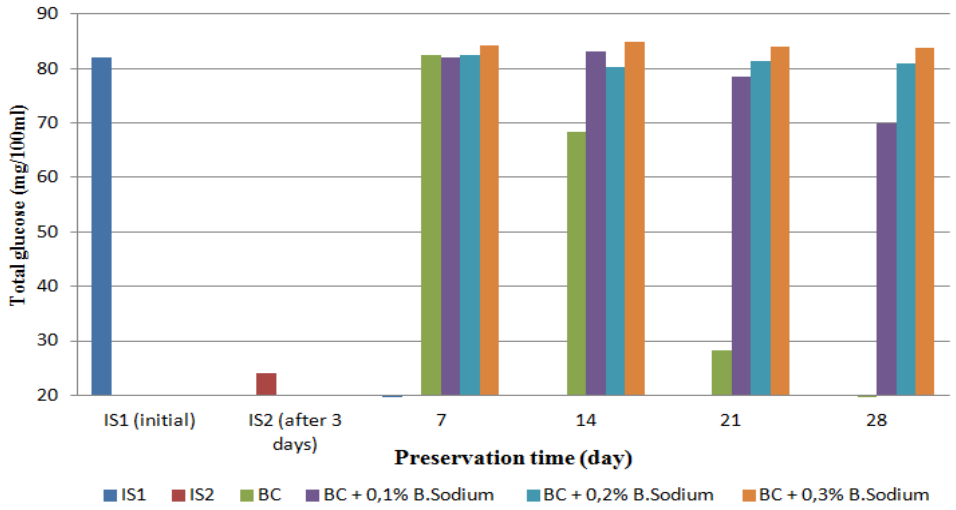

Chart 2. Chart for total glucose level of coconut after preservation time

3.3. pH level

During preservation period, when the sample has the spoiling tendency, pH level will tend to decrease. Specifically, formula BC's pH level strongly drops from 5.09 (control sample IS_1) to 4.15 after 21 days preservation, lower than IS_2 sample with pH = 4.75. The preservation capability of the two formulas are the same BC + 0.2% B.Sodium and BC + 0.3% B.Sodium when pH = 5,04 after 28 days. In which, BC + 0.1% B.Sodium got the best preservation result and relatively stable from day 21 to day 28 maintaining the pH level = 5.05, equivalent to preservation sample of formula BC at day 14 (Chart 3).

So with this criteria, minimally pre-prepared fresh coconut will be well preserved within 14 days. Other formulas, BC + 0.1% B.Sodium, BC + 0.2% B.Sodium và BC + 0,3% B.Na gave the best preservation capability within 28 days.

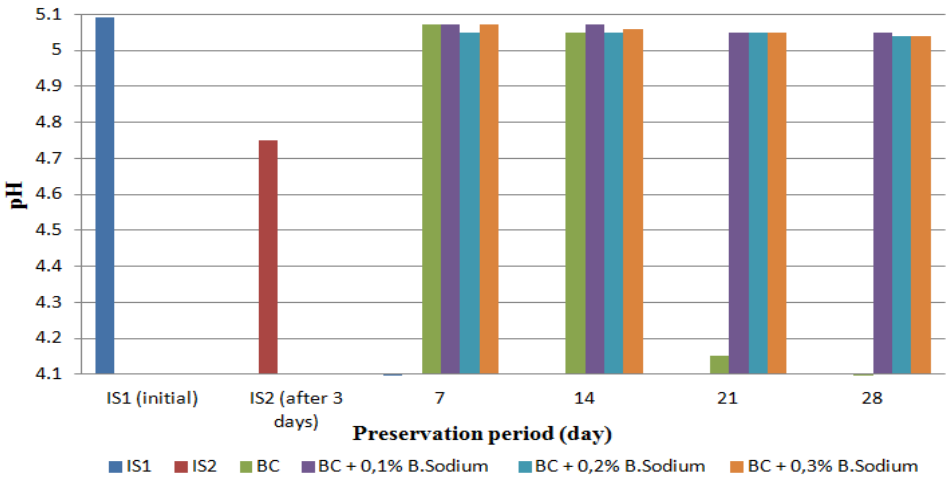

Chart 3. Chart for pH of coconut after preservation period

3.4. Total acid level

During preservation period, the minimally pre-prepared fresh coconut sample could more or less, be contaminated with deteriorating microorganism include: bacteria, yeast and mold... Therefore, together with the increasing in total aerobic bacteria number throughout preservation period as analyzed in section 3.1, the organic acid also increases significantly. This could be inferred that during the proliferation of bacteria, they will discharge metabolic products which contain many kinds of organic acid, lead to the increasing of total acid level in the products, pH tend to decrease (this totally matches with the result at section 3.3) and the product will have sour taste. Those are the main reasons that cause spoiling of the products.

During preservation period, total acid level rise strongly and fast at formula BC, increase by 12.5% after 14 preservation days and gain up to 140% at day 21th compared to control sample IS_1, while even being higher than control sample IS_2. For preservation batch with formula BC + 0,1% B.Sodium, total acid level increases more than 14% after 21 days of preservation and rise insignificantly at 28th day. Other 2 remaining formulas BC + 0,2% B.Sodium and BC + 0,3% B.Sodium gave the equivalent results, only increase around 9% after 28th day of preservation (Chart 4).

Therefore, with this criteria, minimally pre-prepared fresh coconut preserved by BC membrane could be assured of quality for up to 14 days. BC + 0.1% B.Sodium is capable of preserving the product well for 21 days. BC + 0.2% B.Sodium and BC + 0,3% B.Sodium had more advantages, assure the quality for up to 28 days

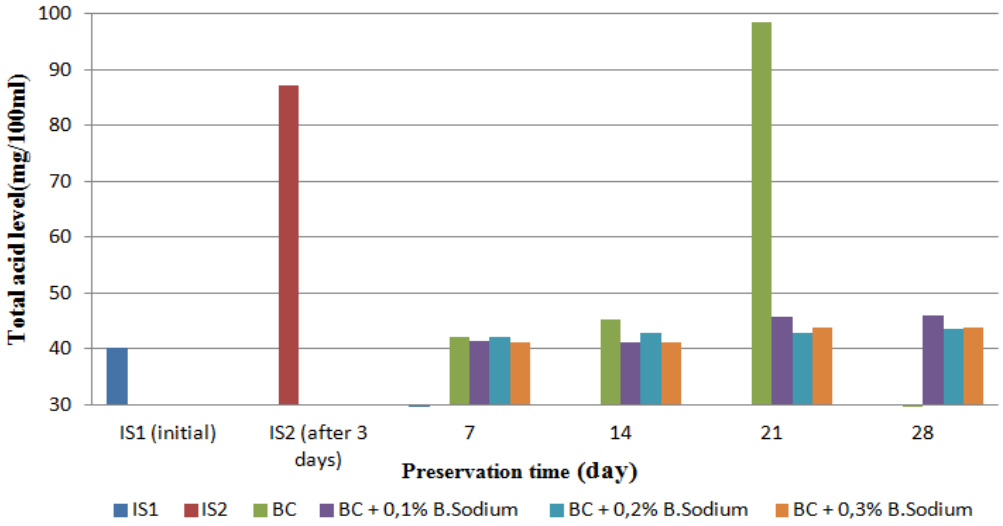

Chart4. Chart for total acid level of coconut after preservation period

As the results had shown, there are really tight connections between criterion, total aerobic number, total glucose level, pH and total acid of the coconut water during preservation. Once the total aerobic bacteria number increase will lead to the decline in total glucose level in the coconut water as they used glucose as a substrate to grow, simultaneously discharge lots of byproducts of metabolism process, causing the increasing of total acid level and follow the set, pH level will also decrease.

3.5. Vitamin C

Vitamin C level also contribute a small amount in the nutrition contents of coconut water, at the same time partly affect the sweet taste of the coconut. High vitamin C level means that the coconut water will be sour. Therefore, a suitable method should be taken to keep balancing the vitamin C level in the coconut. Preserved by BC membrane for the coconut will result in a great decline in vitamin C level, more than 41% compared to the initial control sample (IS_1) and are the same to control sample IS_2. Other sample at remaining formulas generally shown that there is no difference between 7th day and 28th day. However after 28 days of preservation, vitamin C level are a little higher than control sample IS_1, but insignificant. This could be explained by the differences between coconuts in the same group (Chart 5).

So with this criteria, the minimally pre-prepared fresh coconut preserved by BC membrane could assure the quality for up to 14 days, other formulas gave the best results after 28 days of preservations.

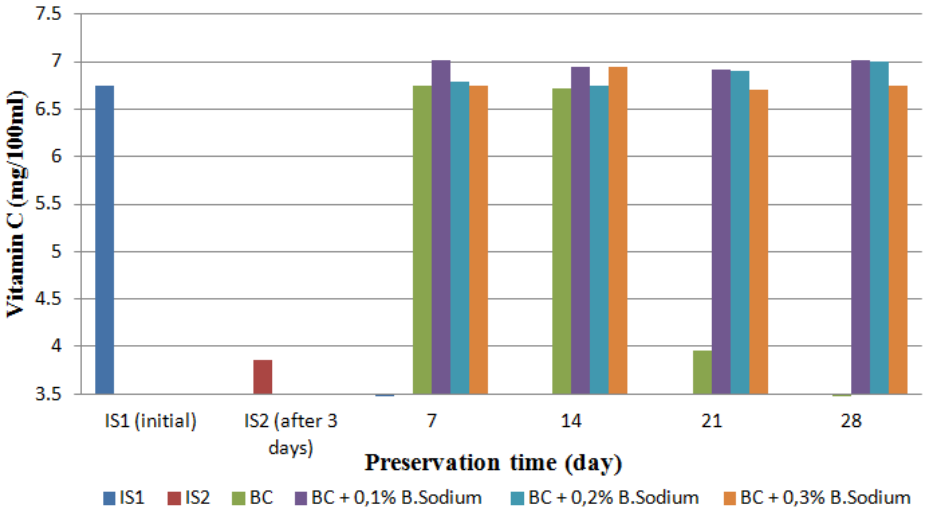

Chart 5. Chart for vitamin C level of the coconut after preservation period

3.6. Sensory evaluation and general argumentation

Minimally pre-prepared fresh coconut after preservation period will have signs of deterioration such as: dry sell, dark color, cloudy water, sour smell. Compared with the initial control sample (IS_1), the coconut had fresh shell, clear water, and sweet taste. But the preservation batch with BC membrane had the deterioration signs after 21 days preservation, continue monitoring to day 28^{th}, the coconut sample had some dented spots. Similar to that, at experimental batch BC + 0.1% B.Sodium, deterioration signs appeared after 28 days preservation. BC + 0.2% B.Sodium and BC + 0.3% B.Sodium are always considered the 2 most optimal formulas to preserve fresh coconut because of their preservation capability compared to other formulas. After their 28 days preservation period, there are only some changes of sensory quality such as dry shell, slightly dark color, while still maintaining the quality of coconut water: clear and sweet taste. However, after 35 days of preservation, these experimental samples had deterioration signs such as dry shell, bad looks, cloudy water, and sour smell.

So as an evaluation, this research has shown that the thin BC membrane played a role as moisture-keeping membrane, helped keeping the fresh level of the coconut, avoid drying and prevent loss of water, thanks to this, the sensory quality of the coconut are maintained and assured throughout the preservation period. At the same time, the BC membrane is a protective cover from microorganism factors. Benzoate Sodium is a preservative agent, it inhibits the growth of microorganism, especially mold. B.Sodium absorbed thin BC membrane has the ability to increase sensory quality and microbiological quality of the minimally pre-prepared coconut products.

Generalize the data and compare with fresh coconut from the tree (IS_1), we have some evaluations as below:

- Preserve the minimally pre-prepared fresh coconut by BC membrane can be assured of their quality for up to 14 days at normal temperature condition. Analyzing those monitoring criterion has shown that: total aerobic bacteria number has increased but not so significant, total acid level increased by 12.5%, total glucose level decreased by 16.8%, other criterion such as pH and vitamin C remained no change.
- Preserve the minimally pre-prepared fresh coconut by BC + 0.1% B.Sodium can be assured of their quality for up to 21 days at normal temperature condition. Analyzing those monitoring criterion has shown that: total aerobic bacteria number has increased but not so significant, total acid level increased by 14%, total glucose level decreased by 16.8%, pH remained the same, vitamin C level slightly increased compared with control sample IS_1. However, this could be explained because of different coconut among the whole group.
- Minimally pre-prepared fresh coconut preserved by BC membrane + 0,2% B.Sodium can be assured of their quality for up to 28 days at normal temperature condition. Analyzing those monitoring criterion has shown that: total aerobic bacteria number had increased but not so significant, total acid level increased by 8,7%, those pH criterion and total glucose level remained the same while vitamin C level slightly increased compared with control sample IS_1. However, this could be because of different coconut among the whole group.
- Preserve the minimally pre-prepared fresh coconut by BC + 0.1% B.Sodium can be assured of their quality for up to 28 days at normal temperature condition. Analyzing those monitoring criterion has shown that: total aerobic bacteria number and total acid level had increased but not so significant, those pH criterion and vitamin C level remained the same, total glucose level slightly increased. However, this could be explained because of different coconut among the whole group.

So, using the BC membrane + 0.2% B.Sodium as a preservation method would be the best and most optimal way to preserve minimally pre-prepared fresh coconut, the quality of the fresh coconut will be assured after 28 days of normal temperature condition.

Fig 6. Preservation sample: 1 (IS_1), 2 (IS_2), 3 (BC after 14 days), 4 (BC after 21 days)

IV. Conclusion

First step in using thin BC membrane to preserve minimally pre-prepared fresh coconut: this method could be used to preserve minimally pre-prepared coconut up to 14 days with BC membrane and up to 28 days with BC membrane and 0.2% B.Sodium at normal temperature condition, the quality of those formulas are still the same compared with the initial sample IS_1 and more stable than the control sample IS_2 after 3 days of preservation. The obtained results also contribute to the new application of bacterial cellulose (BC): using BC as food coatings.

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