

Original Article

The effect of edible film on properties and specific sensory attributes of a gac (*Momordica cochinchinensis* Spreng) aril product*

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Abstract

Gac aril has high contents of beta-carotenoids, lycopene, lipid and oil. This study aimed to delay the degradation of phytochemicals and avoid rancidification in gac aril by wrapping it with bacterial cellulose (BC). Fully ripe gac fruit were randomly sampled. The gac aril was removed and pasteurized by steaming at 100°C for 10 min and kept in the dark at 8°C. All experiments were done in triplicate. The conditions of wrapping were optimized. The functional, nutritional, physical and chemical properties, and specific sensory attributes of the wrapped gac aril were investigated. The results revealed that the gac aril was neatly wrapped over by *Rhodococcus* sp. MI 2 only under static conditions. A 10% seed culture gave the highest wet weight and dry weight of BC. The cellulose wrapping of gac aril by *Rhodococcus* sp. MI 2 could delay the degradation of phytochemicals and avoid rancidification for at least 14 days. The unwrapped and wrapped gac aril showed almost the same nutritive values. The main drivers of liking of the wrapped gac aril product were appearance, color and sweetness. Therefore, BC could be applied as an edible film in food wrapping and it may protect food against oxidation.

Keywords: lycopene, bacterial cellulose, food packaging, rancidity, just-about-right (JAR)

1. Introduction

Gac fruit (*Momordica cochinchinensis* Spreng or *Muricia cochinchinensis* Lour.) is typically round or oblong. The fruit becomes dark orange in color upon ripening. Its red aril contains phytochemicals: lycopene, beta-carotene, lutein and phenolic compounds. The proportion of aril is less than 31% of the total fruit weight (Kha, Nguyen, Roach, Parks, & Stathopoulos, 2013). Fully ripe gac fruit with dark red skin, dark yellow pulp and dark red aril has lower levels of carotenoids than fully ripe gac fruit with orange or red skin, yellow pulp and red aril (Tran, Parks, Roach, Golding, & Nguyen, 2016).

The aril is an oily red membrane that covers the black seeds of the fully ripe gac fruit. It shows high levels of essential fatty acids and very high levels of the carotenoids

lycopene and beta-carotene (Ishida, Turner, Chapman, & McKeon, 2004). The lipid in the aril helps the absorption of carotenes and other fat-soluble nutrients (Kuhnlein, 2004) and comprises about 70% unsaturated fats of which 50% are polyunsaturated fatty acids (Vuong, 2000). The most predominant fatty acids found in the aril are oleic, palmitic and linoleic acids (Ishida *et al.*, 2004). Oleic and linoleic acids can reduce LDL (low density lipoprotein)-cholesterol and have anti-atherogenic effects (Lopez-Huertas, 2010). Consumption of the aril improved plasma levels of retinol, α - and β -carotenes, and lycopene, in pre-school children (Vuong, Dueker, & Murphy, 2002). The carotenoids as antioxidants can prevent cancer (Fiedor, & Burda, 2014). Vitamin E (α -tocopherol) is also present in gac oil and aril (Vuong, & King, 2003). Therefore, the gac aril is a functional food, used to promote health and reduce the risks of various diseases. However, the aril is perishable and has a short shelf-life (Auisakchaiyoung, & Rojanakorn, 2015), especially the unsaturated fatty acids tend to quickly go rancid.

Rancidity is characterized not only by an unpleasant smell and taste, but also by the content of oxygen, especially hydro peroxides that imply the occurrence of oxidation (Kirk

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& Sawyer, 1991). Carotenoids are very sensitive to oxygen, light, temperature and microorganisms. Lycopene degrades rapidly when gac aril is encapsulated in polylactic acid (PLA) (Cao-Hoang, Phan-Thi, Osorio-Puentes, & Wache, 2011). Commercial gac products are available on the market as frozen gac aril, gac oil capsules and dried gac powder for traditional uses, as natural colorants and as medicinal supplements (Chuyen, Nguyen, Roach, Golding, & Parks, 2015). However, there have been no reports on the remaining phytochemicals in these commercial products.

Food packaging is designed to extend the shelf life and maintain the quality of the food or prevent its deterioration because of environmental influences (Restuccia *et al.*, 2010). Therefore, new materials and new packaging designs are needed in the development of packaging, such as edible films, active packaging, modified atmosphere packaging (MAP), and coatings.

Bacterial cellulose (BC) is cellulose produced by bacteria such as *Acetobacter*, *Pseudomonas*, *Agrobacterium*, *Rhizobium*, *Sarcina* (Cannon, & Anderson, 1991), and *Rhodococcus* sp. MI 2 (Tanskul, Amornthatree, & Jaturonlak, 2013). BC has been applied as an edible or biodegradable film in food packaging (Freitas, Alves, Reis, Crespo, & Coelho, 2014). It has been used as a prebiotic or as a type of dietary fiber. To the best of our knowledge, there is no report of food wrapping by BC. Gac aril wrapped by BC produced by microorganisms might delay the degradation of phytochemicals and help avoid the oxidation of lipid and oil. The aim of this study was to optimize the conditions of gac aril wrapped in BC by *Rhodococcus* sp. MI 2. The functional properties such as texture, softness retention and browning of the products were characterized. The nutritional, physical and chemical properties were investigated as well. In addition, just-about-right (JAR) tests can be used to investigate the connection between specific attributes of a product and consumer acceptance (Ortega-Heras, Gómez, de Pablos-Alcalde, & González-Sanjosé, 2019). Penalty analysis (PA) is a popular analysis method used for the JAR scale which can help in identifying whether attributes should be improved or adjusted.

2. Materials and Methods

2.1. Cultivation of bacteria producing cellulose

Each liter of the coconut juice medium used to cultivate *Rhodococcus* sp. MI 2 contained 50 g sucrose, 5 g ammonium sulfate, 10 mL of 1 N acetic acid and 1000 mL of coconut juice from a ripe fruit (Tanskul *et al.*, 2013). The pH of the media was adjusted to 3.5 with 0.5 N NaOH or HCl. All chemicals used were of analytical grade.

2.2 Gac aril preparation

Fully ripe gac fruit with orange or red skin were purchased from the local market in Hat Yai, Thailand. The gac aril was removed and pasteurized by steaming at 100°C for 10 min and kept in the dark at 8°C for further study.

2.3 Optimization of culture conditions

A 10% *Rhodococcus* sp. MI 2 seed culture or starter was inoculated in coconut juice medium and incubated under three alternative conditions: agitated (150 rpm), stirred (150 rpm by magnetic stirrer), and static (on the lab bench) conditions. The BC sheet appeared after 3 days of incubation. The pasteurized aril was placed on the BC sheet or added in the culture broth. The wrapped gac aril was observed after another 3 days of incubation.

2.4 Optimization of seed culture for bacterial cellulose production under static condition

Seed culture was inoculated into the coconut juice medium in proportions of 5, 10, 15 and 20% (v/v). The culture broth was incubated under static condition for 3 days. The wet weight, thickness and dry weight of the cellulose sheet were then determined. The experiments were done in triplicate.

2.5 Comparison of functional properties of sterilized and pasteurized products

The conditions of sterilization and pasteurization are at 121°C and 15 psi for 15 min and at 100° C for 10 min, respectively. The functional properties such as texture, softness retention and browning of the products were characterized. They were then kept in sterile plastic Petri dishes packed in aluminum foil laminate and stored in a refrigerator at 8° C for further study.

2.6 Determination of color coordinates

The color coordinates of unwrapped gac aril (before wrapping) and wrapped gac aril (after wrapping) were determined directly by using a colorimeter (Digital photo colorimeter, Punjab, India). The HunterLab Color Scale (1996) (L*, a* and b*) was used to report the color results (Sewall, 1996).

2.7 Determination of total acidity

A 3 g sample of each of unwrapped and wrapped gac aril was placed in 50 mL of distilled water. It was stirred well and filtered using a vacuum pump. A 25 mL aliquot of filtrate was poured into a 250-mL Erlenmeyer flask. Thereafter 2-3 drops of phenolphthalein were added. The filtrate was then titrated with 0.1 N standardized NaOH. The experiments were done in triplicate.

2.8 Extraction of gac aril for determination of lycopene content, total phenolics content and antioxidant activity

A 3 g sample of unwrapped and wrapped gac aril each was extracted in darkness for 1 h in a 15 mL mixture of hexane, ethanol and acetone at the volumetric proportions 2:1:1. The upper phase of the extract was separated and kept.

2.9 Determination of lycopene content

The aliquot of the extract from 2.8 was used for determination of lycopene content. Lycopene content was directly determined by using a spectrophotometer (Thermo, China) at 503 nm and calculated following Sadler, Davis, and Dezman (1990) as follows.

$$\text{mg lycopene/kg fresh weight} = (A_{503} \times 537 \times 8 \times 0.55) / (0.1 \times 172) \text{ or } A_{503} \times 137.4$$

2.10 Analysis of carbohydrate, protein, lipid, fiber and ash

The carbohydrate (crude fiber and nitrogen-free extract), protein, lipid, energy, fiber and ash of the gac aril before and after wrapping were analyzed. The protein and lipid contents were determined by the AOAC Kjeldahl method (1995) and the AOAC Soxhlet extraction method (1973), respectively. The carbohydrate and energy contents were calculated. The fiber content was investigated by fiber analyzer (ANKOM²⁰⁰, ANKOM Technology, New York, USA). The ash and moisture content AOAC (1995) were determined by burning at 550-600° C, and heating at 135° C, respectively.

2.11 Determination of peroxide using iodometric titration

Peroxide was determined using the method of Pearson (Pearson, 1976) as follows. Unwrapped and wrapped gac aril samples were weighed and placed in 250 mL conical flasks containing 30 mL of acetic acid-chloroform (3:2 v/v). They were then mixed well. A 0.5 mL aliquot of saturated potassium iodide solution was added. The mixture was kept in darkness for 1 min with occasional shaking. After 1 min, 30 mL of distilled water were added. The mixture solution was then titrated with 0.1 N sodium thiosulfate solution and standardized against standard potassium dichromate solution. When the mixture solution presented a light yellow color, it was titrated again using 0.5 mL of starch solution as an indicator until the mixture reached the end point (colorless). Peroxide values were calculated from the following formula:

$$\text{Peroxide value} = \text{titre} \times N \times 100 / \text{weight of sample},$$

where titre was the volume of sodium thiosulfate used in mL and N was the normality of the sodium thiosulfate solution.

2.12 Determination of total phenolics content

Total phenolics were determined using Folin-Ciocalteu reagent following the method of Abu Bakar *et al.* (2009). The total phenolics content was calculated using the standard curve of gallic acid and is expressed as gallic acid equivalents per gram of dry weight.

2.13 Determination of total antioxidant capacity

The method for determination of antioxidant capacity was adopted from Dasgupta and Bratati (2004). The total antioxidant capacity was calculated using the standard

curve of gallic acid and expressed as gallic acid equivalents per gram of dry weight.

2.14 DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

DPPH free radical scavenging activity was determined using the procedure described by Braca *et al.* (2001). A 0.1 mL aliquot of the extract from 2.8 was mixed with 3 mL of 0.004% DPPH and the mixture was kept in darkness for 30 min. Absorbance was determined at 517 nm with a spectrophotometer (Thermo, China). The capacity to scavenge the DPPH radical (DPPH•) was calculated as follows.

$$\text{DPPH}\cdot \text{ scavenging effect (\%)} = (1 - A_s/A_c) \times 100$$

where A_c is the absorbance of the control containing DPPH• solution and A_s is the absorbance in the presence of gac aril extract (Elmastas, Gülcin, Beydemir, Kufrevioglu, & Aboul Enein, 2006). The DPPH scavenging effect was then calculated as the Trolox equivalent antioxidant capacity from the calibration curve.

2.15 Ferric reducing/antioxidant power (FRAP)

The reducing power of gac aril was determined using the FRAP assay (Oyaizu, 1986). The absorbance was measured at 700 nm with a spectrophotometer (Thermo, China).

2.16 Sensory evaluation

Panelists who were sick, had a cold or those with some kind of allergy were not allowed to participate. There were 107 panelists, 38 males and 69 females at ages from 18 to over 40. The basic technique and the specific vocabulary were explained to the panelists. The appearance, color, aroma of fermentation, sweetness and texture (firmness and cohesion), and overall-liking were analyzed on a 3-point hedonic scale ranging from 1 (like extremely) to 3 (dislike extremely).

2.17 Statistical analyses

Means and standard deviations of three replications of each treatment were calculated using SPSS 11.5 software (SPSS Inc., Chicago, H., USA). The differences of the mean values were compared by t-tests. Differences at 95% confidence level were considered to be significant.

3. Results and Discussion

The results revealed that BC wrapped the gac aril neatly only under static condition and was about 3 mm thick. Under agitated and stirred conditions, the gac aril was dispersed in the culture broth. The results correlated with our previous report that BC produced in SH medium is formed mostly in small granules or like tapioca-pearls and a few irregular shapes under agitated condition, and is formed into feather-like shape under stirred condition (Tanskul *et al.*, 2003). Thus, under agitated and stirred conditions the BC

could not form a wrapping on the fruit. Yordshahi *et al.* (2020) use the impregnation method with a BC sheet dipped into a postbiotic cell free supernatant of lactic acid bacteria (Aguilar-Toalá, 2018) containing different concentrations of bacteriocins, organic acids, hydrogen peroxide, fatty acids, carbon dioxide and di-acetylene for antimicrobial activity (Moradi, Mardani, & Tajik, 2019). Impregnation times varied with constant agitation at 200 rpm (Yordshahi *et al.*, 2020). The results emphasize that the concentration of postbiotics is more effective than impregnation time because the postbiotics are not all adsorbed during the time period. Yang *et al.* prepare cellulose acetate resulting from modification of plant cellulose for packaging production which could extend shelf life of foods (Yang *et al.*, 2017). Encapsulation strategy is one of the most prevalent methods for protection of antioxidant properties (Akbarbaglu *et al.*, 2019) and electrohydrodynamic processes including electrospraying and electrospinning have been recently applied to encapsulate bioactive compounds (Assadpour, & Jafari, 2018). The aims of these reports were similar to this study but the methods are different.

The percentage of seed culture (5, 10, 15 and 20%) had no significant influence of thickness of BC. The 5% (v/v) seed culture gave the lowest wet weight of BC. In contrast, 10, 15 and 20% (v/v) seed cultures showed the highest wet weight of BC and had no significant difference. The 10% (v/v) seed culture produced the highest dry weight of BC, whereas 5, 15 and 20% (v/v) seed culture produced slightly lower dry weights of BC than that of 10% (v/v) seed culture. Regarding our previous report (Tanskul *et al.*, 2013), we have found that a 5% starter shows the highest BC yield in SH medium. In contrast, 5% starter gives the lowest BC yield in coconut juice medium. Starters of 10-20% produce the highest yields of BC but there is no significant difference among them in the wet weight of BC. Zeng, Small and Wan have reported an optimum starter of 6% (v/v) for BC production by *Acetobacter xylinum* BPR 2001 under agitation in maple syrup (Zeng, Small, & Wan, 2011). Therefore, a 10% (v/v) seed culture was chosen for further study.

The wrapped gac aril products were harvested, and either sterilized or pasteurized to extend shelf-life. The results showed that the product became hard, lumpy and brown after sterilization (Figure 1a) while the product was still soft and smooth with softness retention after pasteurization (Figure 1b). The browning, or Maillard reaction occurred during sterilization at high temperature for a long time but the pasteurized product was transparent and the aril inside clearly visible. Although the pasteurized product had an attractive red color, antioxidant activity had to be considered because heat, oxygen, and light affect the stability of lycopene (Xianquan, Shi, Kakuda, & Yueming, 2005). Therefore, the product in this study was packaged in aluminum foil laminate and kept in a refrigerator.

Regarding the HunterLab Color coordinates, L* a* and b* represent brightness, green-red and blue-yellow, respectively. L* of unwrapped gac aril slightly increased from day 0 to 56 (Figure 2a). L* of wrapped gac aril were higher than those of unwrapped gac aril, and a* (green-red) (Figure 2b) and b* (blue-yellow) (Figure 2c) of wrapped gac aril were lower than those of unwrapped gac aril.

The total acidity of unwrapped and wrapped gac aril was implied by the molarity of citric acid content. Citric acid

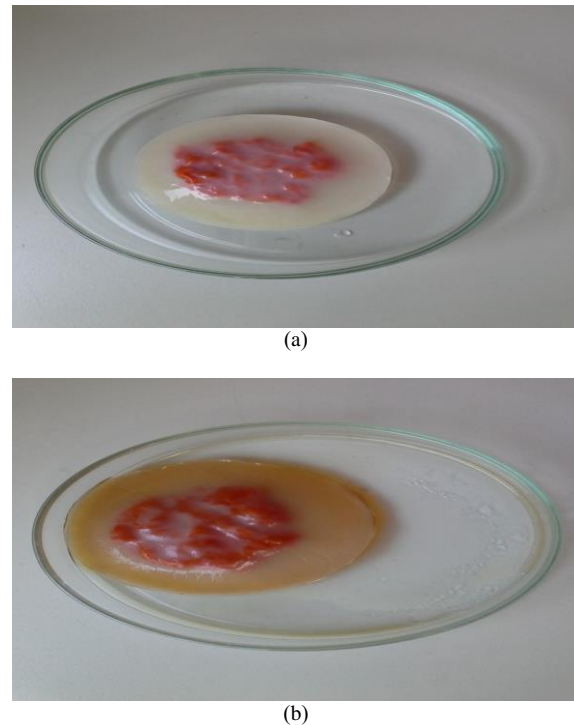


Figure 1. Comparison of functional properties of the wrapped gac aril product after (a) sterilization at 121°C, 15 psi for 15 min, and (b) pasteurization by steaming at 100° C for 10 min.

concentrations of gac aril were much higher (1.5-2 M citric acid) across the whole study period (0-56 days) after wrapping than before wrapping (0.35-0.65 M citric acid). Acidity was formed during the development of the product.

The lycopene content of unwrapped gac aril was higher than that of wrapped gac aril across the whole study period except on day 0 (Figure 3). The lycopene content of unwrapped gac aril was not highest on day 0. Xianquan *et al.* (2005) have reported that reisomerization of lycopene occurs during storage under optimal moisture and temperature (Xianquan *et al.*, 2005). Shi and Le Maguer (2000) have proposed that lycopene content may have increased because food processing weakens or breaks bonds between lycopene and the tissue matrix (Shi & Le Maguer, 2000). Regarding the color values, the lower a* and b* of wrapped gac aril might result from the oxidation of lycopene. The loss of color and an off-flavor can occur after oxidation of lycopene (Xianquan *et al.*, 2005). Color values at the beginning showed no correlation with phenolics content and antioxidant activity. The results did not agree with the positive correlation between phenolics content, antioxidant activities and color of fruit wines (Yildirim, 2009).

The nutritional compositions of unwrapped and wrapped gac aril were investigated. A 100 g sample of gac aril contained 1.44 g of protein, 2.70 g of lipid, 11.90 g of carbohydrate, 2.98 g of fiber, and 1.47 g of ash and had a nutritional energy value of 77.68 kcal (Table 1). A 100 g of wrapped gac aril contained 1.83 g of protein, 3.08 g of lipid, 9.90 g of carbohydrate, 2.49 g of fiber, and 2.73 g of ash and had a nutritional energy value of 74.614 kcal (Table 1). The

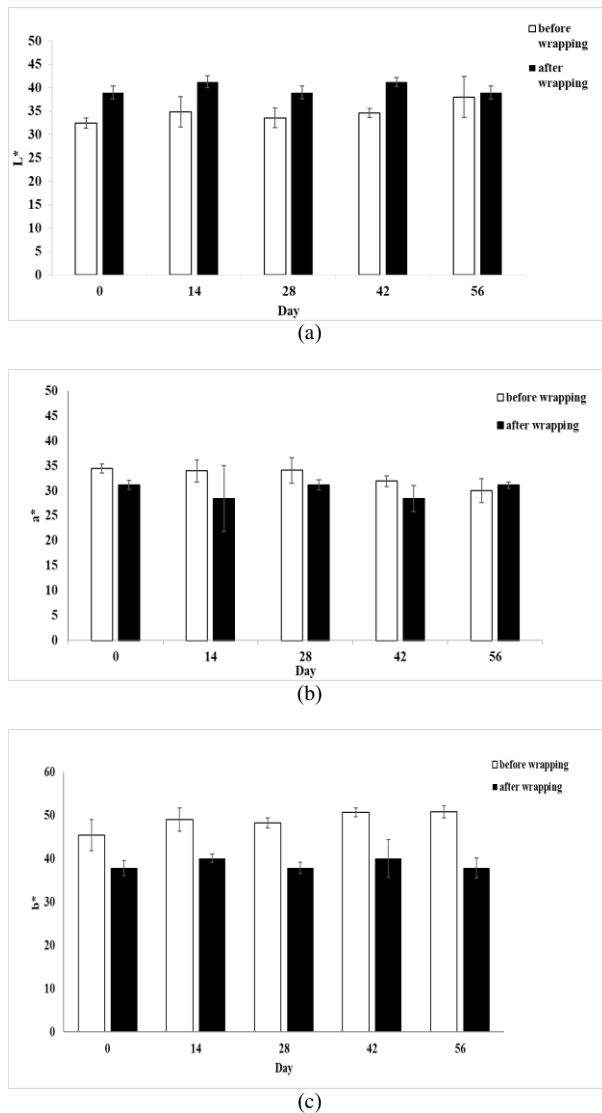


Figure 2. Color coordinates (a) L*, (b) a*, and (c) b* of gac aril before wrapping and after wrapping

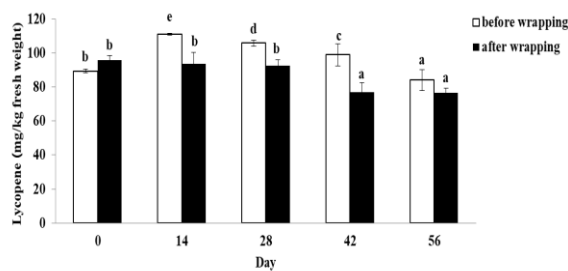


Figure 3. Lycopene content of gac aril before and after wrapping from day 0 to 56

nutritive values showed that the nutritional quality of the aril before and after wrapping were almost the same. The presence of ash in the aril implied residual inorganic mineral content in the food.

Table 1. The nutritional compositions of 100 g unwrapped and wrapped gac aril

Nutrient	Unwrapped gac aril		Wrapped gac aril	
	Mean	SD	Mean	SD
protein (g)	1.44	0.159	1.83	0.111
lipid (g)	2.7	0.115	3.08	0.13
carbohydrate (g)	11.87	0.702	9.9	0.208
fiber (g)	2.98	0.222	2.49	0.049
ash (g)	1.47	0.036	2.73	0.194
energy (kcal)	77.68	1.863	74.61	2.095

The peroxide values of unwrapped gac aril and wrapped gac aril from day 14 were determined. The peroxide value of gac aril could not be detected either before or after wrapping. In addition, no unpleasant smell or taste was found in either unwrapped or wrapped gac aril. Riaz and Rokey (2012) reported that gac oil reaches the highest PV after five days of incubation, and heat treatment during extrusion can destroy lipases (Riaz & Rokey, 2012). Thus, lipase in gac aril product might be not oxidized during 14 days of storage.

The phenolic compounds of unwrapped gac aril were high from day 0 to 14 (72 gallic/g dry weight) and fluctuated from day 14 to 56 (Figure 4a). The phenolic compounds of wrapped gac aril were constant from day 0 to 14 and significantly decreased from day 14 to 28. They had no change from day 28 to 42 and significantly decreased from day 42 to 56.

The total antioxidant capacity of unwrapped gac aril was highest from day 0 to 14 and dramatically decreased from day 14 to 28 (Figure 4b). It significantly increased from day 28 to 42 and had no change from day 42 to 56. The total antioxidant capacity of the wrapped gac aril was highest from day 0 to 14, significantly decreased from day 14 to 42, and had no change from day 42 to 56 (Figure 4b). The results correlated with determination of total acidity, as the antioxidant activity decreased as pH decreased. The antioxidant activity increased substantially with increasing pH (Amorati, Pedulli, & Cabrini, 2006).

DPPH free radical scavenging activity (mg Trolox equivalent/g extract) of unwrapped gac aril was highest on day 0 but decreased significantly from day 0 to 14 (from 480 to 350 mg Trolox equivalent/ g extract) (Figure 4c). It decreased gradually from day 14 to day 56. DPPH free radical scavenging activity of wrapped gac aril was highest on day 0 (320 mg Trolox equivalent/ g extract) and gradually decreased from day 0 to 28 (from 320 to 260 mg Trolox equivalent/ g extract). It significantly decreased from day 28 to 56.

The FRAP value of the unwrapped gac aril was highest from day 0 to day 14 (14.8 μ M ferrous sulfate equivalent/ g extract), significantly decreased from day 14 to 42, and then significantly increased from day 42 to 56 (Figure 4d). The FRAP values of wrapped gac aril significantly decreased from day 0 to 14 (from 13.8 to 12.8 μ M ferrous sulfate equivalent/ g extract) and significantly decreased from day 14 to 56 (Figure 4d).

Regarding the results, the DPPH values correlated with the total phenolic contents and FRAP values. They were in agreement with the finding of Win *et al.* that the loss of antioxidant activity can be evaluated by phenolics and FRAP (Win, Buanong, Kanlayanarat, & Wongs-Aree, 2015).

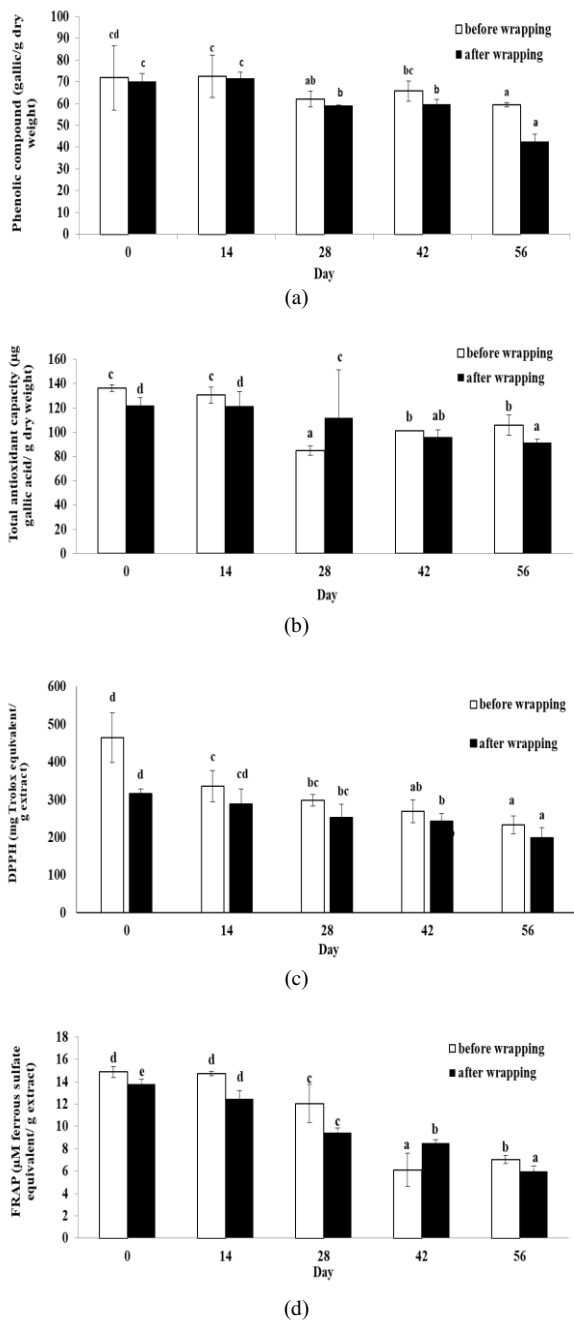


Figure 4. Comparison of (a) phenolics content, (b) total antioxidant capacity, (c) DPPH, and (d) FRAP values of gac aril before and after wrapping from day 0 to 56

Most panelists showed differences between treatments in the sensory attributes describing appearance, color and sweetness for the “JAR” level (Table 2, Figure 5). The panelists strongly penalized “too much” for the aroma of fermentation and for the cohesion of the product. The evaluation results for the firmness of the product were not significant. The mean drops value was -0.151 because the liking mean for the “JAR” level was less than the mean for the “too little” level. The panelists strongly penalized the aroma

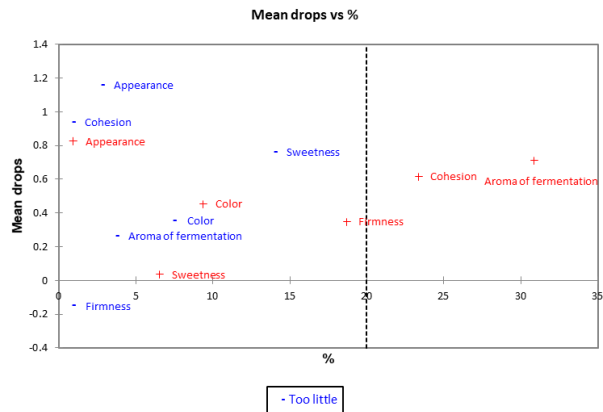


Figure 5. Mean drops of the sensory attributes appearance, color, aroma of fermentation, sweetness and texture (firmness and cohesion) of the wrapped gac aril product

of the product which they considered to smell too much of fermentation. They also strongly penalized the cohesion of the product which they considered too hard to bite. Therefore, the aroma of fermentation and cohesion of the wrapped gac aril should be developed. For example, pandan extract might be used to decrease the aroma of fermentation and size reduction of the product can help in biting or chewing. The evaluation results for the firmness of the product were not significant. The firmness of the product did matter for the panelists.

4. Conclusions

The wrapped gac aril exhibited stability of color, lycopene content, phenolics content and antioxidant activity for at least 14 days. The main drivers of liking of the wrapped gac aril product were appearance, color and sweetness. Thus, bacterial cellulose could be applied as an edible film in food wrapping and it may protect food from oxidation. It is the most environmentally friendly food packaging.

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Table 2. Sensory evaluation of wrapped gac aril product.

Variable	Level	Frequencies	%	Sum (Liking scores)	Mean (Liking scores)	Mean drops	Standardized difference	p-value	Significant	Penalties	Standardized difference	p-value	Significant
Appearance	Too little	3	2.80	8.000	2.667	1.159							
	JAR	103	96.26	394.000	3.825					1.075	2.800	0.006	Yes
	Too much	1	0.93	3.000	3.000	0.825							
Color	Too little	8	7.48	28.000	3.500	0.354							
	JAR	89	83.18	343.000	3.854					0.409	2.070	0.041	Yes
	Too much	10	9.35	34.000	3.400	0.454							
Aroma of fermentation	Too little	4	3.74	15.000	3.750	0.264							
	JAR	70	65.42	281.000	4.014					0.663	4.573	<0.0001	Yes
	Too much	33	30.84	109.000	3.303	0.711	4.696	<0.0001	Yes				
Sweetness	Too little	15	14.02	47.000	3.133	0.761							
	JAR	85	79.44	331.000	3.894					0.530	2.955	0.004	Yes
	Too much	7	6.54	27.000	3.857	0.037							
Firmness	Too little	1	0.93	4.000	4.000	-0.151							
	JAR	86	80.37	331.000	3.849					0.325	1.734	0.086	No
	Too much	20	18.69	70.000	3.500	0.349							
Cohesion	Too little	1	0.93	3.000	3.000	0.938							
	JAR	81	75.70	319.000	3.938					0.631	3.823	0.000	Yes
	Too much	25	23.36	83.000	3.320	0.618	3.678	0.000	Yes				

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