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# Edible coating with coconut water to preserve probiotic strains and sensory characteristics of minimally processed carrots

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**Abstract**

The present work evaluated the protective effect of coconut water on the bacterium *Lactobacillus acidophilus* LA3 added to an edible sodium alginate-based coating, as well as its effect on the preservation of the sensory quality of minimally processed carrots. Treatments were employed based on alginate and the probiotic, with and without addition of coconut water to the coating applied on the carrots. The coconut water improved the bacterium's cell viability for seven days, rendering this coating three log cycles superior to the coating without coconut water. After 21 days, carrots with this coating had counts above log 6 CFU/g, which is a recommended level for health benefits, while the other treatment did not present viable cells within the detection limit of the method ($10^{-4}$ dilution). The panellists showed greater acceptance and purchase intention for the coating containing coconut water throughout the storage period of the samples than for the control carrots.



## Introduction

Innovations in food technology have become increasingly challenging as people's greater awareness of the relationship between adequate nutrition and good health has led to changes in their purchasing habits, thus increasing the demand for foods that are nourishing and boost their vitality (Burgain *et al.*, 2011). These include the so-called functional foods, which provide clinically proven health benefits, in addition to their known nutritional effects (Aboulfazli *et al.*, 2016). The component of a food that renders it functional may occur naturally, e.g., carotenoids in carrots, or may be added to a food matrix originally devoid of the component in question, such as the addition of probiotics to a food that contains very little of them or none at all (Tripathi and Giri, 2014).

Carrots can be transformed into minimally processed products, presenting characteristics of fresh, ready-to-eat, practical and nutritious products that meet people's daily needs. However, even minimal processing causes mechanical damage, rupturing the tissue and mixing the cell content, which can lead to numerous chemical and oxidation reactions (Pushkala *et al.*, 2012). Post-harvest treatments help reduce these processes and moisture loss, preserving the turgidity and firmness of the vegetable, thus reducing its respiration, maturation, colour modification and enzymatic browning, and extending its shelf life (Tesfay and Magwaza, 2017). Hence, the application of edible coatings on minimally processed carrots can prevent such undesirable changes, while increasing the protection and functionality of the food matrix through the addition of ingredients such as antioxidants (Pérez-Gago *et al.*, 2006; Ghidelli *et al.*, 2015), dyes, flavourings, antimicrobial substances and probiotics (Burgain *et al.*, 2011; Espitia *et al.*, 2016; Cazón *et al.*, 2017).

The materials most commonly used in the preparation of edible coatings are polymers, consisting of polysaccharides, proteins and lipids (Tavassoli-Kafrani *et al.*, 2016), that can be used

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alone or in combinations (AL-Hassan and Norziah, 2017). Sodium alginate polymers have been proven suitable for food coatings because of their colloidal properties and ability to form strong gels or insoluble polymers by reacting with polyvalent metal cations such as $Ca^{2+}$ (Song *et al.*, 2014; Nayik *et al.*, 2015). Previous studies demonstrated that alginate-based coatings applied to carrot slices were efficient as a support for freeze-dried *L. acidophilus*, thereby conserving its cell viability, besides to contribute for preservation of moisture content and the colour, reduce variations in acidity during storage and minimise the whitish surface discoloration of the sliced carrots (Shigematsu *et al.*, 2018).

Espitia *et al.* (2016) found that coatings containing probiotics can increase the stability of foods by controlling the growth of deteriorating microorganisms, contributing to the health of consumers, and can be considered potentially bioactive materials. Probiotics are defined as living organisms that are beneficial to the host's health when administered in adequate amounts (> log 6 to 7 CFU/g) (FAO and WHO, 2002). However, probiotic bacteria are nutritionally demanding, and *Lactobacillus acidophilus* is an example of this. This microorganism is a lactic acid bacterium which has complex nutritional needs, such as amino acids and growth factors such as riboflavin, folic acid, niacin and calcium pantothenate (Du Plessis *et al.*, 1996).

Most commercial probiotic products are derived from milk and are considered efficient matrices for probiotic microorganisms. However, other food matrices have also been studied as potential vehicles for these microorganisms. The growing number of individuals that are lactose intolerant and allergic to cow's milk protein underscores the importance of developing non-dairy probiotics products such as fruits and vegetables (Peres *et al.*, 2012; Lee *et al.*, 2013).

Even though the coatings based on alginate had been efficient as a support for freeze-dried *L. acidophilus* (Shigematsu *et al.*, 2018), the addition of non-dairy substances to the coating solution should be investigated in order to enhance the viability of the probiotics.

Coconut (*Cocos nucifera* L.) water is the aqueous part of the coconut endosperm. Known as a refreshing and nutritious beverage, its broad range of applications can be justified by its composition of minerals, sugars, amino acids, vitamins and phytohormones (Yong *et al.*, 2009; Yuliana *et al.*, 2010; Giri *et al.*, 2018). Coconut water is of cytoplasmic origin and some of its most important components are the cytokinins, which are a class of phytohormones (Yong *et al.*, 2009). According to Leshem (1988), their strong antioxidant properties suggest that they can prevent oxidative damage of unsaturated fatty acids in cell membranes. In addition, inorganic ions and vitamins also play an important role in mitigating the oxidative stress of cells (Yong *et al.*, 2009). Therefore, these compounds found in coconut water may have positive effects on *L. acidophilus*, as it is a Gram-positive and microaerophilic bacterium and, consequently, susceptible to oxidative stress generated by the dissolved oxygen in the medium. Besides, its micronutrient composition could supply the nutritional needs of the *L. acidophilus*, since coconut water is extensively used as a growth supplement in tissue culture medium formulation (Yong *et al.*, 2009).

Considering the increasing demand for foods that combine quality, practicality and healthiness, the purpose of the present work was to evaluate the performance of coconut water in a food coating as a carrier of *L. acidophilus* LA3 and its sensory characteristics, using minimally processed carrots as the food matrix.

## Materials and methods

### *Materials*

The coatings were composed of sodium alginate (Dinâmica®, Diadema, Brazil), glycerol (Rioquímica, São José do Rio Preto, Brazil), sunflower oil (Cargill Agrícola S.A, Brazil), Tween 80 (Cooperativa Agroindustrial Alegrete, Brazil), and green coconut water (fresh coconut purchased in the retail market in Marilia, Brazil). Calcium chloride was used as a gel former to cross-link with alginate (Synth, Diadema, Brazil). The probiotic culture *L. acidophilus* LA3 (Sacco®, Campinas, Brazil) was used.

The carrots (Daucus carota L., cv. Verano) used in the present work, were harvested in August 2016 from a commercial vegetable garden located in São Gotardo, in the state of Minas Gerais, Brazil (19°18'40''N, 46° 02'56''), and stored at 8 ± 2°C until further processing.

### *Preparation of the probiotic inoculum*

The probiotic strain of *L. acidophilus* LA3 was activated following inoculation in whole milk (Molico, São Paulo, SP, Brazil), reconstituted (12%) with yeast extract (0.3%) and L-cysteine (0.05%) for 20 h at 37°C. Loopfuls of the bacteria were then reseeded twice in a formulated medium enriched with green coconut water (6%), soy extract (10%), sucrose (3%), yeast extract (0.5%), L-cysteine (0.05%), zinc sulphate (0.008%) and magnesium sulphate

(0.005%), which was then incubated at 37°C for 24 h each. At the end of incubation period, 3.2% of the second reseeding was inoculated into the edible coating solution.

*Edible coating and addition of probiotic culture*

The components used in the formulation of the edible coating of sodium alginate were sodium alginate (1.50 g), glycerol (0.75 g), sunflower oil (0.04 g), Tween 80 (0.05 g) and water (100 g). The sodium alginate was dissolved in water heated to 70°C and mechanically stirred (MA 259, MARCONI, Piracicaba, SP, Brazil) at 2,400 rpm for 10 min. The glycerol, sunflower oil and Tween 80 were then added, after which the pH of the solution was adjusted to 5.5 by adding 30% m/v of acetic acid.

The alginate and coconut water coating were prepared following the same procedure as the sodium alginate coating, substituting 70% of the distilled water with coconut water, and it was termed alginate/coconut water treatment ($T_{AW}$).

For the treatments with the probiotic addition, after preparing the sodium alginate coating solutions, the probiotic culture of the culture medium activated at log 9 CFU/.mL was added. They were termed alginate/probiotic ($T_{AP}$) and alginate/coconut water/probiotic ($T_{AWP}$) treatments.

*Application of the coating and storage of the minimally processed carrot*

The coatings were applied to the carrots using the dipping technique (Costa *et al.*, 2012). The 4 mm thick carrot slices were immersed in the sodium alginate based filmogenic solution for 2 min and then dipped in the calcium chloride solution (2.0 g/100 g water) for 1 min to promote the ionic gelation of the alginate by its reticulation with the bivalent $Ca^{2+}$ ion (Krasaekoopt *et al.*, 2004).

Fifteen carrot slices were then placed on expanded polystyrene trays (70 mm × 110 mm × 30 mm) lined with acetate sheets, and stored at 8°C ± 2°C without air circulation to partially dehydrate the coatings for 12 h. After this period, the trays were covered and sealed with vinyl polychloride film (WYDA, Sorocaba, SP, Brazil) at 8.7 g/m$^2$, calculated by weighing a delimited area of the film (Henrique *et al.*, 2008). The coated samples of the two treatments ($T_{AP}$ and $T_{AWP}$) were stored for 21 days at 8°C ± 2°C to evaluate the cell viability. Some of the carrot slices were processed in parallel without coating, for use as the control (C), and a few carrots were coated with sodium alginate and coconut water without probiotics ($T_{AW}$) to assess the probiotic effects on sensory analysis.

*Cell viability of the Lactobacillus acidophilus LA3*

Cell viability analyses of the edible coatings $T_{AP}$ and $T_{AWP}$ were performed under aseptic conditions after days 1, 7, 14 and 21 of storage at 8°C ± 2°C. To this end, serial dilutions of up to 108 of the samples were made in a solution of sodium citrate (2% m/v), yeast extract (0.1% m/v) and L-cysteine (0.05%), and the dilutions were then spread-plated on MRS agar following the official method (Frank and Yousef, 2004) with slight modifications. Incubation was performed in a BOD incubator (NI 1704, NOVA Instruments) for 72 h at 37°C.

*Analysis of contaminating microorganisms*

The microbiological quality of the samples ($T_{AP}$, $T_{AWP}$ and C) was controlled based on an analysis of thermotolerant coliforms and *Salmonella* spp., following the guidelines of Resolution No. 12 of January 2, 2001 of ANVISA, Brazil's National Health Surveillance Agency (ANVISA, 2001). A study of moulds and yeasts was also performed to verify possible spoilage. After homogenising the samples, serial dilution up to $10^4$ was prepared. Total counts were made of moulds and yeasts by spread-plating on potato dextrose agar (PDA) following the methodology of the American Public Health Association (Taniwaki *et al.*, 1999). The method validated by AFNOR 3M 01/2 – 09/89C was used to check for thermotolerant coliforms, using 3M™ Petrifilm™ Coliform Count Plates (3M Company, St. Paul, MN, USA), which involved placing 1 mL of each diluted sample onto the plates and incubating them at 44°C for 24 h. *Salmonella* spp. were identified using the traditional method described by ISO 6579-1:2017 (ISO, 2007).

*Sensory analysis*

The samples of the treatment with alginate/coconut water/probiotic ($T_{AWP}$), which showed a higher cell viability of the probiotic culture than the treatment with alginate/probiotic, as well as the samples containing sodium alginate coating and coconut water without probiotic ($T_{AW}$) and the control samples (C) were subjected to an affective acceptance test. For this, the minimally processed carrots ($T_{AWP}$, $T_{AW}$ and C) were subjected to a sensory analysis by 44 untrained panellists, of both sexes, on days 1, 7 and 14 of storage at 8°C ± 2°C. The samples were presented monadically, in complete blocks, and the evaluation was performed according to a structured 9-point hedonic scale, where (1) corresponded to "disliked extremely" and (9) to "liked extremely" for the attributes of appearance, aroma, texture, colour, taste and overall assessment. The purchase

intention was evaluated based on the aforementioned scale, ranging from "would certainly buy" to "would certainly not buy". The sensory analysis was approved by the Research Ethics Committee of the Institute of Biosciences, Letters and Exact Sciences (IBILCE/UNESP) at São José do Rio Preto (Opinion No. 2.252.081).

*Statistical analysis*

Using Student's t-test, a comparison was made of the mean values of viability of *L. acidophilus* LA3 in the two samples with edible coatings ($T_{AWP}$ and $T_{AP}$). An analysis of variance (ANOVA), followed by Tukey's Test, were used to compare the treatments in the sensory analysis. The results were considered significant at a *p*-value of < 0.05, using BioStat statistical software (Ayres *et al.*, 2007).

**Table 1.** Log CFU/g of Lactobacillus acidophilus in coated carrots obtained when using different coatings; alginate/probiotic ($T_{AP}$) and alginate/coconut water/probiotic ($T_{AWP}$).

| Treatment | Storage (day) | | | |
|---|---|---|---|---|
| | 1 | 7 | 14 | 21 |
| TAP | 8.9 ± 0.25[b] | 4.0 ± 1.36[a] | < 4,0[a] | < 4,0[a] |
| TAWP | 8.1 ± 0.14[a] | 7.5 ± 0.21[b] | 6.7 ± 0.45[b] | 6.3 ± 0.06[b] |

Data are means ± standard deviation of triplicate (n = 3) determination. Means with different letters in each column differed significantly ($p < 0.05$).

## Results and discussion

*Cell viability of Lactobacillus acidophilus LA3 in the edible coatings*

In terms of the probiotic therapeutic quantities in food, a concentration of log 6 to 7 CFU/g is recommended for its action, considering daily portions of 100 g (Cruz *et al.*, 2009).

Evaluation of the viability of *L. acidophilus* LA3 added to the sodium alginate-based edible coatings, with and without coconut water ($T_{AWP}$ and $T_{AP}$, respectively), was carried out with minimally processed carrots during 21 days of refrigerated storage. This evaluation revealed loss of viability higher than 4.0 log cycles in 7 days of storage in the coated samples treated without coconut water ($T_{AP}$) while this loss in the samples treated with coconut water ($T_{AWP}$) did not exceed 0.6 log cycles (Table 1). After 14 days of storage, the viable cells in the samples of $T_{AP}$ treatment could not be quantified because they were below the sampled minimum dilution factor ($10^{-4}$), indicating that this coating was inefficient as a carrier of *L. acidophilus* LA3.

The treatment with alginate and coconut water coating ($T_{AWP)}$ showed a desirable count of viable probiotic cells (log 6.3 CFU/g of product) until storage day 21 (Table 1). These results indicate that the addition of coconut water improved the viability of this probiotic when compared with the other treatment.

Camargo Prado *et al.* (2015) evaluated a beverage based on fermented coconut water and demonstrated that this raw material can be used as a substrate for the growth of *L. plantarum* AC-1, a probiotic, whose count remained at log 8.7 CFU/mL for 28 days in refrigerated storage. Lee *et al.* (2013) prepared two coconut water-based beverages, and added the probiotics *L. acidophilus* L10 and *L. casei* L26. The two probiotic cultures showed a similar growth capacity; reaching approximately log 8 CFU/mL of viable cells after two days of fermentation at 37°C, and maintained a viability of log 7 to 8 CFU/mL for 26 days at 4°C.

Tapia *et al.* (2007) used *Bifidobacterium lactis* Bb-12 as a probiotic culture in sodium alginate (2% w/v) or gellan gum (0.5% w/v) coatings on apple and papaya slices, and reported a viability rate higher than log 6 CFU/g in 10 days of storage at 2°C. Rößle *et al.* (2010) demonstrated the symbiotic potential of fresh-cut apple wedges with sodium alginate-based edible coating (1% w/v) containing the prebiotics oligofructose (35% w/v) or inulin (15% w/v) with added L. rhamnosus GG containing log 8 CFU/g of viable cells at the end of 14 days of storage at 2 to 4°C.

It should be kept in mind that the composition of the coating, the probiotic strains and the coated food could behave synergistically in a way that may be positive or antagonistic to bacterial cell viability. In the present work, the addition of coconut water had a positive effect on the tested probiotic culture, while the carrots and sodium alginate had unfavourable effects.

*Microbiological quality of the samples*

The samples collected from the different treatments ($T_{AWP}$, $T_{AW}$ and C) were shown to be safe within microbiological parameters established by the legislation (ANVISA, 2001), given the absence of thermotolerant coliforms and *Salmonella* spp. Moulds and yeasts, which are possible causes of deterioration, were also absent throughout the storage period, indicating that hygienic procedures were followed in the processing and storage of the samples.

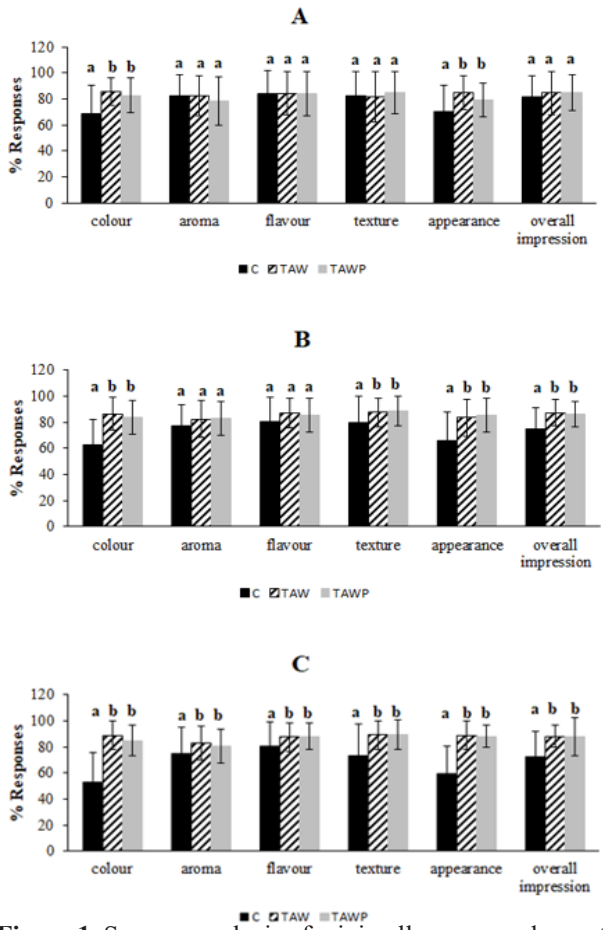


**Figure 1.** Sensory analysis of minimally processed carrots without coating (C), with alginate and coconut water coating without probiotic ($T_{AW}$) and with probiotic ($T_{AWP}$), evaluated on days 1 (A), 7 (B) and 14 (C). Means with different letters in each column differed significantly ($p < 0.05$).

*Sensory analysis*

The coating on the minimally processed carrots containing alginate and coconut water was efficient in preserving the viability of the probiotic culture of *L. acidophilus* LA3 ($T_{AWP}$) throughout 21 days of refrigerated storage (Table 1). Therefore, this sample was selected for the sensory analysis on days 1, 7 and 14, together with the control sample (C, without coating), and the sample coated with alginate and coconut water without probiotics ($T_{AW}$).

In the three days of sensory analysis, the $T_{AW}$ and $T_{AWP}$ samples attained higher scores than control in all the evaluated attributes, except for aroma on the first day of storage (Figure 1). The minimally processed carrots that were coated ($T_{AW}$ and $T_{AWP}$) attained higher than 80% acceptance rates on days 7 and 14, indicating that the coatings had a positive influence on the sensory attributes evaluated in the present work (Figures 1B and 1C). Control lost sensory quality of all its attributes, the most strongly affected being colour, followed by appearance (Figure 1). The colour and appearance of minimally processed carrots are critical attributes at the moment of purchase because they rapidly lose its characteristic bright orange colour due to dehydration, developing a white blush on the surface, which reduces its acceptability (Mastromatteo *et al.*, 2012). This fact was illustrated by the comments of the tasters, who stated that the development of a whitish colour was the item that they least liked on days 7 and 14 of sensory evaluation. According to Bourtoom (2008), polysaccharide-based edible coatings can delay the loss of water from fruits and vegetables thereby extending the shelf life of fresh products.

A comparison between the two treatments with coatings indicated that the addition of the probiotic to the coating formulation ($T_{AWP}$) did not modify the product's sensory characteristics, i.e., it did not interfere in the acceptability index of the evaluated attributes ($p > 0.05$). Unlike the uncoated sample (C), the mean values of the $T_{AW}$ and $T_{AWP}$ treatments showed an increase as the storage time progressed (Figure 1). These results are in agreement with those reported by Rößle *et al.* (2010), who evaluated the addition of *L. rhamnosus* GG to several coatings on sliced apples, namely sodium alginate (1% m/v) and the prebiotic oligofructose (35% m/v); sodium alginate (1% m/v) and the prebiotic inulin (15% m/v), and a control sample containing only the probiotic microorganism. The authors found that addition of both prebiotics and probiotics did not significantly interfere in the product's acceptability during 14 days of storage at 2 to 4°C.

Martins *et al.* (2016) also did not find a significant difference ($p > 0.05$) in their evaluation of the attributes of colour, taste and overall acceptability in their analysis of fruit salad (pineapple, guava, banana, apple, mango and papaya) immersed in a solution with and without *L. rhamnosus* HN001. These samples were stored from 0 to 120 h at 5°C, and the probiotic did not interfere in the quality of the attributes.

The samples with and without coating showed no significant difference ($p > 0.05$) in texture on the first day of storage, and $T_{AWP}$ was the treatment with the highest acceptance index (85.1%). This value increased to 89.4% on day 14, and sample $T_{AW}$ was similar, increasing from 84.4% of acceptance on day 1 to 89.1% on day 14 of storage, while the acceptance rate of control decreased from 82.2% on day 1 to 73.2% on day 14 of storage. The observed decrease in the firmness of the minimally processed carrots may be associated with the action of pectinolytic enzymes, but may also be due to the increased activity of other enzymes such as glycolytic enzymes (glucanases), which participate in the hydrolysis of

hemicellulose and other cell wall components that are present in carrots and that can be activated as a defence mechanism against a microbiological attack and/or injury, such as during minimum processing (Kurosaki *et al.*, 1992; Taiz and Zeiger, 2010). The edible coatings applied to the carrots probably involved these injuries, diminishing the performance of these enzymes and preserving the firmness of the samples from $T_{AW}$ and $T_{AWP}$ treatments for at least 14 days. This fact can be confirmed by the reports of the panellists who stated that the coated samples were crisp during the three days of sensory analysis, which indicated that the coatings were very efficient in keeping the texture of the product. Allegra *et al.* (2017) also achieved positive results in the visual score values, maintaining brightness and fruit firmness, with *Opuntia ficus-indica* mucilage edible coating on Breba figs, contributing to extending the product's shelf life for up to 10 days.

A comparison on the acceptability indexes determined by sensory evaluation from day 1 to 14 of storage revealed that the colour of control decreased to 15.6%, showing the lowest index of acceptability (Figures 1A and 1C).

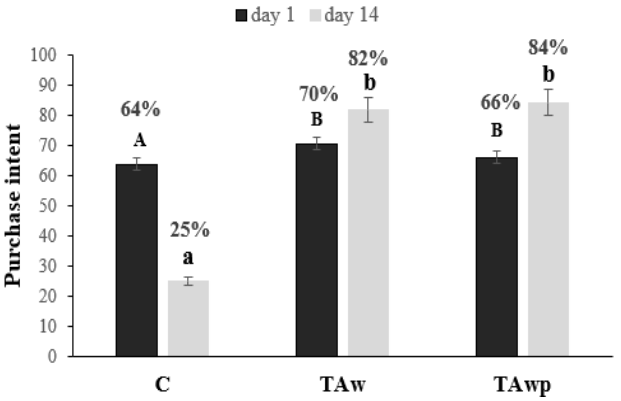


**Figure 2.** Graphic representation of the sum of the frequencies of the alternatives "would certainly buy" and "would probably buy" indicating the purchase intention of the panellists of minimally processed carrots with alginate/coconut water coating with probiotic ($T_{AWP}$), alginate/coconut water coating ($T_{AW}$) and no coating (C) after 1 day and 14 days of storage. Different letters indicate a significant difference ($p < 0.05$).

The purchase intention of the three samples was also evaluated (Figure 2), and at the first day no significant differences ($p > 0.05$) were found between control and treated samples $T_{AW}$ and $T_{AWP}$ in terms of sum of the two alternatives: "would certainly buy" plus "would probably buy" (64%, 70% and 66%, respectively). On days 7 and 14 of evaluation, the purchase intention for the control sample was lower than 50%, and dropped to 25% on the last day, while for coated samples this rate increased, reaching 82% for sample TAW and 84% for sample $T_{AWP}$.

In general, the acceptability rates of the attributes and the overall evaluation of treated samples $T_{AW}$ and $T_{AWP}$ did not present significant differences ($p > 0.05$) at any of the time intervals, and the purchase intention of two samples was also approximately the same. Sample $T_{AWP}$ did not score in the purchase intentions of "would probably not buy" and would certainly not buy" on day 14, demonstrating the panellists' satisfaction with the sample (Figure 2).

## Conclusion

The presence of coconut water in the alginate coating positively contributed to preserving the viability of the probiotic *L. acidophilus* LA3 in coated carrots during 21 days of refrigerated storage, at recommended levels for health benefits. The use of coatings with and without the addition of probiotics favoured the sensory quality of minimally processed carrots evaluated over a storage period of 14 days, when compared to the uncoated sample. The coatings improved the panellists' scores of all the evaluated attributes, particularly the colour, appearance and texture of the product after 14 days. These are crucial attributes for the marketability of minimally processed carrots. All the samples were still microbiologically stable after 21 days of storage at 8°C ± 2°C. In view of these results, it can be stated that the sodium alginate and coconut water coating were efficient both as a probiotic carrier and for extending the shelf life of minimally processed carrots with good sensory quality.

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