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ORIGINAL ARTICLE

Shelf-life of green asparagus using cassava and chitosan blend coating

Validade de espargos verdes usando revestimento de mistura de mandioca e quitosana

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Abstract

Polysaccharide coating is a biodegradable alternative to conventional packings used for delaying senescence in vegetables. This work investigated the properties of cassava and chitosan biopolymeric film and its use as edible coatings to preserve the shelf-life (7 °C and 70% relative humidity (RH)) of green asparagus, with previous cold storage (1 °C and 90% RH) for 7 days. Based on a previous assessment of the films obtained from a) cassava (2.5%), b) chitosan (0.5%), and c) a blend of cassava-chitosan (Cassava+) (2.5 + 0.5%), it could be verified that cassava-chitosan blend films showed improved barrier property (water vapor transmission rate (WVTR) of 38.06 g/(m^2.h)) and mechanical properties, with tensile strength of 0.021 MPa, elongation at break of 1.93% and Young's module of 0.011 MPa. Cassava+ based coating resulted in a lower weight loss and colour L* parameter and minimized textural changes in basal-section of commercial green asparagus spears during a shelf-life of seven days (7 °C and 70% RH), with previous cold storage. All coated spears maintained overall higher carbohydrate levels than the control, except to sucrose which gradually decreased in basal sections and increased in the apical sections of asparagus spears, irrespective of treatment. Edible coating did not affect asparagine concentrations which steadily increased throughout the shelf-life of the spears. The blend (Cassava+) kept for a longer the green asparagus quality. However, spears under a shelf-life of seven days (7 °C and 70% RH), with previous cold storage under 1 °C and 90% RH conditions, developed tip-rot physiological disorder.

Keywords: *Asparagus officinalis*; Modified atmosphere; Biopolymer; Mechanical properties; Blend; Starch.

Resumo

Polímeros naturais, como o polissacarídeo, são uma alternativa biodegradável para a fabricação de novas embalagens, a fim de substituir as embalagens convencionais utilizadas para retardar a senescência em vegetais. Este trabalho investigou as propriedades de filmes biopoliméricos de amido de mandioca e quitosana, e seu uso como revestimentos comestíveis para preservar a vida útil (7 °C e 70% umidade relativa- UR) de aspargos verdes, com ou sem armazenamento prévio refrigerado (1 °C e 90% UR) por sete dias. Com base na avaliação prévia dos filmes obtidos a partir de a) fécula de mandioca (2,5%), b) quitosana (0,5%) e c) uma mistura de fécula de mandioca e quitosana (Cassava+) (2,5 + 0,5%), verificou-se que os filmes da mistura fécula e quitosana apresentaram propriedade de barreira melhorada (taxa de permeabilidade ao vapor d'água de 38,06 g/(m^2.h)) e propriedades mecânicas, com resistência à tração de 0,021 MPa, alongamento à ruptura de 1,93% e módulo de Young de 0,011 MPa. O revestimento à base de fécula de mandioca e quitosana resultou em menor perda de massa e valor L*, e minimizou as mudanças de textura na seção basal de hastes de aspargo verde comercial durante a vida útil de sete dias (7 °C e 70% UR),

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com armazenamento prévio refrigerado. Todas as hastes revestidas mantiveram níveis globais de carboidratos mais elevados do que o controle, exceto para a sacarose, que diminuiu gradualmente nas seções basais e aumentou nas seções apicais das lanças de aspargo, independentemente do tratamento. O revestimento comestível não teve efeito nas concentrações de asparagina, que aumentaram ao longo do período de vida útil. Todas as hastes com vida útil de sete dias (7 °C e 70% UR), com armazenamento prévio refrigerado (1 °C e 90% UR), desenvolveram o distúrbio fisiológico podridão das pontas.

Palavras-chave: *Asparagus officinalis*; Atmosfera modificada; Biopolímero; Propriedade mecânica; Blenda; Amido.

Highlights

- Cassava starch and chitosan based films were prepared and characterized
- Water vapor permeability of cassava starch and chitosan blend films were improved more than isolated film formulations
- Edible coating made the blend (Cassava starch and Chitosan) more effective in delaying senescence and preserving the quality of green asparagus
- The edible coating preserved the appearance and reduced the incidence of tip-rot for longer

1 Introduction

Asparagus (*Asparagus officinalis* L.) is a highly perishable vegetable widely appreciated by consumers due to its unique taste. Asparagus production was estimated at 8.451.689 tonnes worldwide in 2020 (Food and Agriculture Organization of the United Nations, 2022), with the majority consumed as a fresh product. However, asparagus has a relatively short shelf-life with quality deterioration taking place soon after harvest, including weight loss, textural modifications (lignification of pericyclic fibers), chlorophyll and carbohydrate degradation (Mastropasqua et al., 2016; Huyskens-Keil & Herppich, 2013; Qiu et al., 2013; Tzoumaki et al., 2009). Commercially, cold storage combined with either controlled atmosphere (CA) or modified atmosphere packaging (MAP) has been proposed as the most effective postharvest technology in prolonging the asparagus life span (Anastasiadi et al., 2022). Despite this, a physiological disorder named “tip rot”, can develop rapidly once spears have been cool stored for more than one week, characterized by the presence of water-soaked bracts at the tip of the spear and a foul odour (Lallu et al., 2000).

Innovative and economic strategies have been explored (Menezes et al., 2021) to prolong fruit and vegetable shelf-life and reduce postharvest waste (Huang et al., 2023), such as biodegradable packages (Vilvert et al., 2022; Bonilla et al., 2013). In this context, starch-based coating has been recommended for prolonging the shelf-life of fruit and vegetables (Vilvert et al., 2022; Paiva et al., 2020; Oliveira et al. 2018; Tzoumaki et al., 2009). However, the mechanical and barrier properties of the different biopolymer films used as a semi-barrier tend to vary widely according to the biopolymeric matrix (Menezes et al., 2021; Talón et al., 2017), which can adversely affect the postharvest quality of fruit and vegetables.

Polysaccharides-based edible coatings have been extensively studied in recent years due to their biocompatibility, biodegradability, abundance, low cost and edibility (Vilvert et al., 2022). Cassava starch is the second source of starch produced worldwide, with 277.8 million tons (Food and Agriculture Organization of the United Nations, 2022), which is composed of amylose (20% to 30%) and amylopectin (70 to 80%), presenting α 1-4 and α 1-6 bonding, respectively (Masina et al., 2017). Chitosan is another popular biopolymer derived from chitin, a cationic polysaccharide, containing two functional groups (amine and hydroxyl) with antimicrobial and viscoelastic properties (Bonilla et al., 2013). Both cassava and chitosan are considered promising alternatives to synthetic-based polymers due to their ability to compose films. However, polysaccharides are hydrophilic (Deng et al., 2017), meaning that water vapor diffuses through in a manner faster than apolar gases, such as O_2, which leads to undesirable mechanical properties and high

water vapor permeability (Sun et al., 2016). In addition, coatings with higher gas exchange affect negatively respiratory activity, weight loss and, consequently, fruit metabolism steady-state and shelf-life (Huang et al., 2014; Deng et al., 2017).

Strategies to improve the edible starch-based coating layer properties are focused primarily on using combinations of different types of polysaccharides, which can repair probable failures in the polymeric matrix due to the interposition of chains and interactions between the NH_{3+} group from protonated chitosan and the hydroxyl groups of the starch, thus improving the mechanical characteristics (Menezes et al., 2021; Cazón & Vázquez, 2020; Bonilla et al., 2013).

Furthermore, the addition of natural waxes can further improve the barrier properties of polysaccharide films (Oliveira et al., 2018), by increasing their hydrophobicity and decreasing the polymer's water affinity (Klangmuang & Sothornvit, 2016). Examples include carnauba, a non-toxic wax, composed of esters and fatty acids that interact with film polysaccharide by forming microemulsions (Singh et al., 2016).

Several authors have reported that edible carbohydrate-based coating, such as carboxymethyl-cellulose (CMC) (Tzoumaki et al., 2009) and chitosan can extend asparagus shelf-life by decelerating weight loss, and lignification processes, and decreasing fungal activity (Qiu et al., 2013). However, to date, there have been no reports on the use of either edible cassava-based coating or blend with chitosan as a technology to prolong the shelf-life of asparagus spears, as well as investigations concerning their mechanical and physical-chemical properties.

Thus, we hypothesize that the postharvest waste of green asparagus can be reduced by using of cassava and chitosan-based edible coating as the interaction between the biopolymeric matrices can improve barrier properties of the film. Therefore, to reduce the impact of non-renewable packaging on the environment, this study aimed to evaluate the mechanical and barrier properties of cassava and chitosan-based edible coatings, and to assess their suitability for extending the shelf-life of green asparagus spears.

2 Material and methods

2.1 Materials

All High-Performance Liquid Chromatography (HPLC) grade solvents were obtained from Fisher Scientific (Loughborough, UK). D–fructose, L-asparagine, glycerol and powdered chitosan (medium molecular weight) were obtained from Sigma-Aldrich (Dorset, UK). D–glucose and sucrose were purchased from Fisher Scientific (Loughborough, UK). Cassava starch was obtained by Indústria Primícias do Brasil Ltda (Macaíba, Rio Grande do Norte, Brazil). Carnauba wax was provided by Indústria Ortal, (Mossoró, Rio Grande do Norte, Brazil). Tween 80 used as surfactant, was obtained by AppliChem (Darmstadt, Germany).

Green asparagus cultivars 'Gijnlim' were harvested, in June of 2018, from 13-year-old crowns. Asparagus was grown in the UK in sandy soil under commercial growing conditions in Cobrey Farm located in Herefordshire, UK (52°05′N, 2°45′W). Spears were hand-cut at ground level between 07:30 and 09:00h at relative field temperatures of 12 ± 4 °C and hydro-cooled within 2 h from harvest. Medium graded spears of ca. 15 mm diameter were stored overnight at 1°C before transportation to the Plant Science Laboratory within 3 h by refrigerated (at 4 °C) transport.

2.2 Methods

2.2.1 Coating solution preparation

To study the films properties three filmogenic solutions were carried out by Oliveira et al. (2018). The following solutions were prepared: **T1**: Cassava 2.5% (w/v), **T2**: Chitosan (0.5%) and **T3**: Cassava+ -

Cassava (2.5%) + Chitosan (0.5%). The cassava and chitosan solutions were obtained after mixing with deionized water and acetic acid solution (1%), respectively, in a glass beaker. In addition, all three solutions contained glycerol (20%) and carnauba wax (5%) over the dry mass of biopolymer (w/w) and Tween 80 (2.5% over the dry mass of wax, w/w). The three solutions were successfully homogenized with the application of heat (75 °C for T1 and 50 °C for both T2 and T3). The films properties were subsequently studied by casting methods in according to Oliveira et al. (2018) as described in the following section.

All the coating characterization was carried out in the Laboratory of Chemical Process of the Universidade Federal Rural do Semi-Árido – UFERSA, Mossoró, Rio Grande do Norte – Brazil. Objective colour and opacity measurements were performed according to Oliveira et al. (2018) with the use of a hand-held CR-10 Minolta colorimeter (Minolta Co. Ltd, Japan). The colorimeter was calibrated with a standard white background and measurements were performed at three points of trend of the films' surface. Data were expressed as red/green coordinate (a*), yellow/blue coordinate (b*) and lightness (L*).

The water vapor transmission rate (WVTR) of the films was determined, in triplicates, through the gravimetric method described by ASTM E96/ E96M-12, according to Oliveira et al. (2018). The mechanical properties such as Tensile strength (σ - MPa), Young's modulus (Y -MPa) and elongation at break (Є -%) were determined using a Testing Machine (DL5000/10000 Series EMIC 23).

2.2.2 Edible coating application

Prior to the shelf-life assessment, green asparagus spears were disinfected with 10 ppm of sodium hypochlorite and subsequently trimmed to 15 cm length according to commercial practices and divided into four batches by treatment. The edible coating treatment was carried out by dipping the asparagus spears for 5 min in the Cassava+ (T3) solution, prepared as described in section 2.2.1 and left to cool at room temperature (RT). Spears used as control were dipped in deionized water. Subsequently, all spears were left to dry inside a fume cupboard at ambient conditions (23 °C and 75% RH) for 2h (Figure 1).

2.3 Assessing the effect of cold storage and edible coating on asparagus shelf-life

After treatment with edible coating, a subset of spears (six spears) from each treatment (coated - T3 and uncoated - control) were randomly selected and packaged in trays wrapped with commercial polyvinyl chloride (12.7 µm thick) film (perforated with 9 small holes [ca. 1 mm diameter]) in triplicate. The trays were subsequently held under shelf-life conditions at 7 °C and 70% RH, in an environmental test chamber (Sanyo MLR-351H, Osaka, Japan) under artificial light (ca. 1358 lx) for up to seven days to simulate retail conditions.

Another random sub-sample of six spears per treatment was selected for physiological and biochemical analysis (baseline). The rest of the spears, approximately 36 spears, per treatment were placed inside Lock & Lock™ (12L) plastic storage boxes in a cold room set to 1 °C and 90% RH for 7 days. An ICA gas mixing system (Storage Control Systems Ltd, Kent, UK) was used to continuously supply synthetic air inside the boxes, which were fitted with an inlet and an outlet to allow continuous air exchange, preventing a build-up of CO_2. Tissue paper soaked in deionized water was placed underneath freshness trays inside the boxes to maintain a high RH (ca. ≥ 90%). Relative humidity and box temperature were continuously monitored using Gemini Tiny Tag Ultra 2 logging data (95% RH, 25 to 85 °C temperature, part number; TGU-4500). The boxes were opened after 7 days at 1 °C and the spears were assessed for subjective quality parameters, and subsequently six spears per treatment were sampled for physiological and biochemical assessment. Furthermore, six spears per treatment were randomly selected for shelf-life assessment, following cold storage.

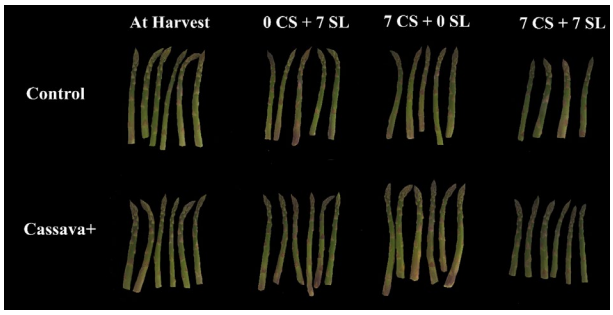


Figure 1. Appearance of uncoated (control) and coated (Cassava+) asparagus spears at harvest, with previous cold storage for seven days (7CS) at 1 °C and 90% RH, or without cold storage (0CS), followed by zero (0SL) or 7 days (7SL) of shelf-life condition at 7 °C and 70% RH.

2.4 Physical, chemical and biochemical analysis

Weight loss was determined periodically by weighting three trays containing 5-6 spears, for each treatment. The results were expressed as the percentage loss of initial weight.

Spears texture was determined as the cutting energy measured by a uniaxial testing machine (Instron 5542, Instron, Buckinghamshire, UK) as described in Anastasiadi et al. (2020). Cutting energy (mJ) defined as the force required for cutting the spears at a depth of 2 and 4 mm was used as a measure of firmness at two different sections: apical (4 cm from the tip) and basal (11 cm from the tip).

Objective colour assessments of the tip (0-4 cm from the top) and basal regions of the spears (11-15 cm from the top) were performed as described in Anastasiadi et al. (2020). Samples were placed inside a Photo-E-Box plus 1419 set at D65 (6500K) under LED lights (back, left and right) and images of the whole spears were captured using a Lumenera Infinity 3 high-definition digital camera with a CCD colour sensor (Lumenera Corporation, Ottawa, ON).

Non-structural carbohydrates and L-asparagine were extracted, after physiological assessments, which samples were immediately snap-frozen in liquid nitrogen and stored at 40 °C, before subsequent freeze drying and powdering in a homogenizer (Precellys 24, Stretton Scientific Ltd, UK) at 5000 rpm for 20 seconds using ceramic beads. The freeze-dried powder was stored at 40 °C. The analysis was performed according to the procedures proposed by Anastasiadi et al. (2020).

2.5 Statistical analyses

Statistical analyses were carried out using Statistic for Windows Version 13 as well as Genstat Version 12 (VSN International Ltd., Herts., UK). Analysis of variance (ANOVA) was used to demonstrate the main effects between treatments and storage time and their interaction to a probability of $p < 0.05$. The differences among the means of treatments were compared through Least Significance Difference (LSD), while Tukey's test was used to compare film properties. Principal components analysis (PCA) was performed to explain the relationship between the different measured variables using the statistical software R.

3 Results and discussion

3.1 Characterization of biopolymeric films for coating in asparagus

Objective colour measurements of biopolymer films showed no significant differences between cassava (T1) and chitosan films (T2) (Table 1). In contrast, the colour parameters b* and L* were significantly different between cassava and Cassava+ films, with the latter exhibiting higher b* values and lower L* values than cassava alone (Table 1). This result could be explained by the natural yellow coloration of chitosan and has been reported elsewhere with other starch films after incorporation of chitosan (Ren et al., 2017).

Table 1. Colour parameter of the films obtained (Film 1: polymer base of Cassava starch; Film 2: Polymer- base of Chitosan; Film 3: polymer base of Cassava+ (Cassava-chitosan blend). Barrier and mechanical properties.

Film	Biopolimers	Colour parameter		
		a*	b*	L*
1	Cassava (2.5%)	2.23 ± 0.15[a]	11.63 ± 0.47[b]	83.17 ± 0.45[a]
2	Chitosan (0.5%)	2.23 ± 0.35[a]	12.56 ± 0.42[ab]	82.76 ± 0.15[a]
3	Cassava+	2.13 ± 0.23[a]	12.83 ± 0.46[a]	80.26 ± 0.63[b]
		Barrier and mechanical properties of films		
Film	**WVTR (g/(m².h))**	**σ (MPa)**	**Y (MPa)**	**Є (%)**
1	44.32 ± 0.94[b]	0.003 ± 0.002[b]	0.002 ± 0.001[b]	1.05 ± 0.58[c]
2	49.59 ± 1.14[a]	0.019 ± 0.002[a]	0.002 ± 0.000[b]	8.64 ± 0.27[a]
3	38.06 ± 1.58[c]	0.021 ± 0.005[a]	0.011 ± 0.001[a]	1.93 ± 0.35[b]

*Different letters in lines, "Colour parameter" and "Properties of films for coating in asparagus" indicate the statistical difference between treatments. All comparisons were performed by Tukey's test at 5% and ± deviation standard. WVTR – water vapor transmission rate; σ – Tensile Strength; Y – Young's module; Є – Elongation at break.

In this study, the Cassava+ presented better mechanical properties than neat starch films, displaying a decreased WVTR of 14.12% and 23.25% as compared to neat cassava and chitosan films, respectively (Table 1). The hydrophilicity reduction presented in this film may be a response to the uniform dispersion of chitosan over the polymeric matrix, which decreases the presence of hydrophilic groups, impairing their interactions with water molecules (Bonilla et al., 2013). Likewise, Ren et al. (2017) demonstrated such responses in films developed based on corn starch and chitosan. As a result, lower WVTR values can prevent moisture exchange between food and the surrounding atmosphere (Ren et al., 2017), reduce transpiration and extend the shelf-life of fruit and vegetables (Oliveira et al., 2018).

Furthermore, the Cassava+ exhibited a 7-, 5.5-, and 1.83-fold increase in tensile strength, Young's modulus and elongation at break values compared to neat cassava film, respectively (Table 1). The above parameters reflect the maximum tensile, stiffness and elongation that the film can achieve before rupture, respectively (Talón et al., 2017). The hydrogen bonds formed between the NH_3^+ groups in protonated chitosan and the starch OH^- groups confer greater stability to the polymer matrix, as compared to the neat starch matrix (Ren et al., 2017). The significant extensibility of neat chitosan (Є-%) film as compared to neat cassava or blend films occurs due to the higher plasticization level of chitosan (Talón et al., 2017).

Based on the above results it was concluded that the Cassava+ film possessed improved barrier and mechanical properties compared to the other biopolymers tested and it was selected to compose an edible coating solution to be applied on asparagus spears (Figure 1).

3.2 Effect of edible coating on asparagus quality parameters

Edible coating treatment in asparagus spears resulted in a lower weight loss than the control, only for the spears that were not placed in cold storage before shelf-life (Figure 2). These results are in agreement with previous research associating lower WVTR with reduced weight loss (Oliveira et al., 2018). However, this result was not replicated for the samples which had previously been stored at 1 °C for seven days, in which case the combination of edible coating and plastic packaging did not offer any additional benefit in retaining weight content, compared to plastic packaging alone. Similar results were reported by Tzoumaki et al. (2009), who studied the effect of combining plastic packaging films with edible coatings during cold storage of white asparagus.

Differences in vapor pressure between the atmosphere and the asparagus surface may lead to weight loss by transpiration (Oliveira et al., 2018). In this study, weight loss was less than 3% in all samples, irrespective of treatment, even after seven days of shelf-life. Also, Siomos et al. (2003) reported weight loss < 2% in white asparagus stored under MAP or air after six days for temperatures below 10 °C.

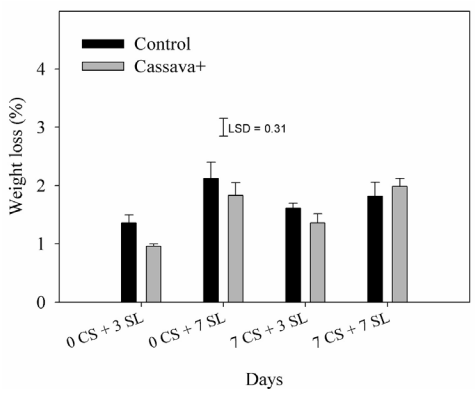


Figure 2. Weight loss (%) of coated (Cassava+) and uncoated (Control) asparagus spears, with previous cold storage for seven days (7CS) at 1 °C and 90% RH, or without cold storage (0CS), followed by 3 (3SL) or 7 (7SL) days of shelf-life condition at 7 °C and 70% RH.

Unlike the spears tip section, there was a slow change in coated basal-section texture (Figure 3) over the shelf-life, with a significant difference between treatments after cold storage (7CS+3SL and 7CS+7SL). The specific characteristic of the spears basal-section surface may have provided a better adherence with the film-forming solution, as compared to the tip section (presence of leaf bracts). This consequently improved the barrier layer to gases, reducing O_2 and increasing CO_2 levels, which provided less alteration in texture characteristics. Generally, stiffness and toughness determine the texture of asparagus (Herppich et al., 2005). Furthermore, an increase in cutting energy is a consequence of increased toughness and decreased stiffness tissue, which leads to more elastic spears during storage (Hassenberg et al., 2012).

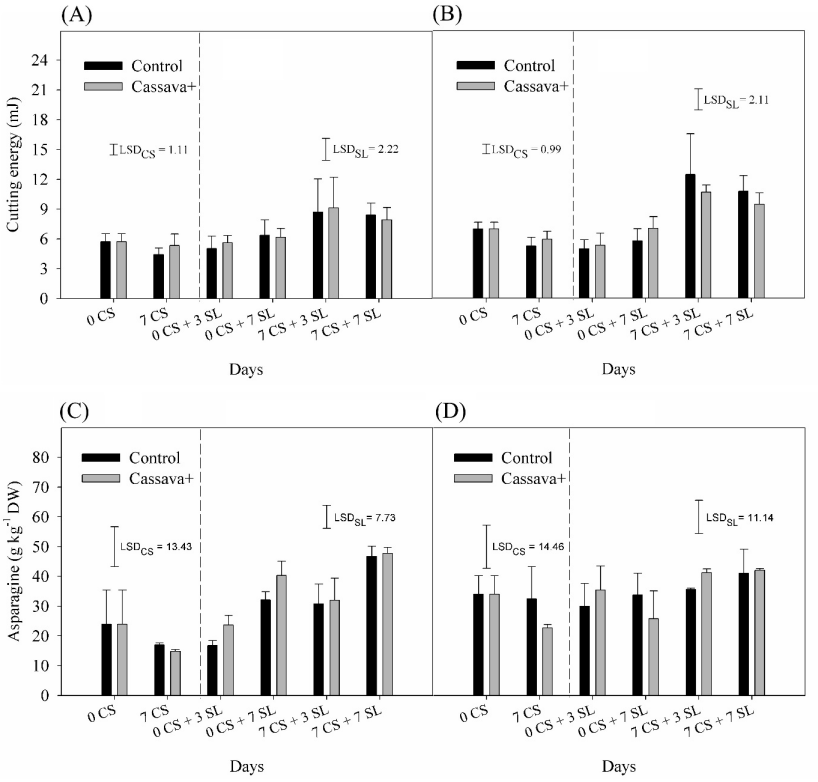


Figure 3. Texture in tip (A) and basal-sections (B) and Asparagine content in tip (C) and basal-sections (D) of the coated (Cassava+) and uncoated (Control) asparagus spears with previous cold storage for seven days (7CS) at 1 °C and 90% RH, or without cold storage (0CS), followed by 3 (3SL) or 7 (7SL) days of shelf-life condition at 7°C and 70% RH.

Likewise, previous studies concerning the use of packaging associated with the modified atmosphere on green asparagus (Villanueva et al., 2005), CMC based coating on white asparagus (Tzoumaki et al., 2009) and chitosan-based coating on green asparagus (Qiu et al., 2013) have already demonstrated delays in the toughness, mainly, in spears basal-sections.

Cold storage (1 °C) for seven days, before shelf-life condition damaged the spears' texture irrespective of either treatment or sections, which was observed in an increase in cutting energy in spears (Figure 3). The texture is an important attribute to verifying asparagus quality (Barrett et al., 2010) by the consumers. Changes in texture of asparagus spears can be linked to the storage atmosphere conditions, such as gas concentration, especially CO_2 (Huyskens-Keil & Herppich, 2013) and lower air humidity (Anastasiadi et al., 2020), causing an undesirable alteration in the cell wall and increasing lignification activity (Mastropasqua et al., 2016; Hassenberg et al., 2012; Siomos, 2003).

The edible coating showed no direct effect on the asparagine content since significant differences ($p < 0.05$) were observed only at shelf-life 0CS+7SL, where the tip-section and basal-section of coated and control asparagus had a higher content of asparagine, respectively (Figure 3). Despite the edible coating providing an additional barrier, after cold storage both the coated and uncoated spears were wrapped in another package, with similar atmospheric and temperature conditions, which may explain the same behavior concerning the asparagine content during the shelf-life.

Organs harvested while still growing are exposed to several stresses, including wounding, dehydration, and separation from nutrient supply, which triggers asparagine synthesis in the tissues (Davies et al., 1996). This is a consequence of an ammonium detoxification activity, as a way to store nitrogen when protein synthesis is inhibited under biotic and abiotic stress conditions (Herrera-Rodríguez et al., 2007; Lea et al., 2007; Gaufichon et al., 2010). The increase in asparagine content observed in this study is in agreement with the results reported by King et al. (1990), in which glutamine levels declined rapidly after postharvest, while asparagine content increased 200% in asparagus spears upon storage for 24 h under 20 °C.

At the end of shelf-life (7CS + 7SL), asparagine content was higher in the spear tip section (11.7%) than in basal-sections, which may be explained due to the presence of intense meristematic activity in the tip section. It was also reported by Eason et al. (2002) and Davies et al. (1996), since an increase in asparagine synthetase transcription was detected during the senescence of asparagus.

During cold storage, there was a decrease in L* and C* values on the tip section of spears, unlike the hue parameter. Despite that, there was no difference between treatments (Table 2). Also, on the basal-sections, there was a significant decrease in L* and C* values, but there was no difference between treatments for value L*. In addition, only coated samples maintained the initial value of C* after cold storage. Changes in chroma value can be a consequence of weight loss as well as pigment synthesis or degradation during shelf-life conditions (Mastropasqua et al., 2016).

Table 2. Colour parameters, lightness (L*), hue angle (^{0}h) and chroma (C*) on the tip and basal-sections of the coated (Cassava+) and uncoated (Control) asparagus spears with previous cold storage for seven days (7CS) at 1 °C and 90% RH, or without cold storage (0CS), followed by 3 (3SL) or 7 (7SL) days of shelf-life condition at 7 °C and 70% RH. Means with different superscript letters are significantly different ($p < 0.05$) for each sampling time.

Section	Days	Treatment	Colour parameter		
			L*	^{0}h	C*
Tip	0CS	Control	91.73 ^{ab}	92.43 ^{a}	25.40 ^{ab}
		Cassava+	93.36 ^{a}	95.55 ^{a}	27.02 ^{a}
	7CS	Control	86.83 ^{b}	92.68 ^{a}	19.90 ^{b}
		Cassava+	88.84 ^{ab}	96.44 ^{a}	22.21 ^{ab}

Table 2. Continued…

Section	Days	Treatment	Colour parameter		
			L*	^{0}h	C*
	LSD ($p \leq 0.05$)		4.48	4.40	5.95
Basal	0CS	Control	105.48 ^{a}	104.97 ^{a}	41.58 ^{a}
		Cassava+	102.33 ^{a}	108.39 ^{a}	44.92 ^{a}
	7CS	Control	96.65 ^{b}	105.91 ^{a}	33.12 ^{b}
		Cassava+	97.71 ^{b}	109.60 ^{a}	39.24 ^{a}
	LSD ($p \leq 0.05$)		3.38	6.01	5.82
Tip	0 CS + 3 SL	Control	90.94 ^{a}	95.20 ^{a}	20.20 ^{ab}
		Cassava+	89.55 ^{ab}	96.91 ^{a}	18.83 ^{b}
	0 CS + 7 SL	Control	84.24 ^{d}	94.13 ^{a}	17.89 ^{b}
		Cassava+	88.61 ^{abc}	96.17 ^{a}	21.33 ^{ab}
	7 CS + 3 SL	Control	91.11 ^{a}	95.28 ^{a}	24.62 ^{a}
		Cassava+	85.90 ^{bcd}	96.81 ^{a}	19.69 ^{b}
	7 CS + 7 SL	Control	86.77 ^{abcd}	94.78 ^{a}	18.20 ^{b}
		Cassava+	84.66 ^{cd}	100.07 ^{a}	18.66 ^{b}
	LSD ($p \leq 0.05$)		4.35	6.06	4.80
Basal	0 CS + 3 SL	Control	102.14 ^{a}	105.27 ^{b}	31.89 ^{ab}
		Cassava+	99.35 ^{ab}	110.41 ^{ab}	32.58 ^{ab}
	0 CS + 7 SL	Control	99.72 ^{ab}	111.03 ^{ab}	39.45 ^{a}
		Cassava+	96.31 ^{bc}	109.92 ^{ab}	36.17 ^{ab}
	7 CS + 3 SL	Control	102.62 ^{a}	106.17 ^{ab}	39.35 ^{a}
		Cassava+	92.29 ^{cd}	111.34 ^{ab}	35.49 ^{ab}
	7 CS + 7 SL	Control	92.73 ^{c}	111.34 ^{ab}	30.53 ^{b}
		Cassava+	87.67 ^{d}	113.51 ^{a}	31.20 ^{ab}
	LSD ($p \leq 0.05$)		4.98	8.16	8.35

In this research work, coated spears maintained a lower value L* for basal-sections over the shelf-life of 7CS + 7SL. Studies have reported that a modified atmosphere can delay the chlorophyll decay in asparagus (Lee, 1981). On the other hand, there was no difference between treatments for hue angle on both tip and basal-sections, over the shelf-life (7CS + 3SL; 7CS + 7SL). This may be a response to the light incidence on asparagus since the coated and control spears remained under the light during the shelf-life condition. In green asparagus, for instance, anthocyanins were induced by light only on the basal section according to the study of Mastropasqua et al. (2016). These facts could explain the observed no difference, in the value C* and hue angle, between treatments at end of shelf-life (7CS + 7SL).

After cold storage (1 °C, for seven days), the coated asparagus spears presented similar or highest carbohydrate levels in both tip and basal-sections as compared to time zero. In addition, the control spears showed lower glucose content (Figure 4A and 4B). On the other hand, there was no difference between treatments regarding glucose and fructose (Figure 4C and 4D) content in the tip spears section over the shelf-life conditions, before or after cold storage. Fructose content increased in the tip section and concomitantly decreased in coated spears basal-sections in 0CS+7SL shelf-life. Likewise, Sergio et al. (2019) verified a decrease in glucose and fructose content in asparagus spears submitted to modified atmosphere packing during the shelf-life of 7 days at 4 °C.

Particularly in coated spears basal-sections, fructose levels were 17% and 14% higher than in control spears at shelf-life 7CS + 3SL and 7CS + 7SL, respectively. The most important property of this sugar is its sweetening power, which is nearly twice that of sucrose (Periche et al., 2014). These results are in agreement with those described previously on asparagus spears under edible polysaccharide-based coating conditions (Qiu et al., 2013; Tzoumaki et al., 2009) or conventional packaging (Sergio et al., 2019), which linked the use of package to a reduced metabolic activity in asparagus spears.

Studies have reported that rapid glucose and fructose metabolization in stored asparagus spears occurs due to the rapid activation of carbohydrate metabolism enzymes (Irving & Hurst, 1993), whose activity can be decreased depending on the temperature and O_2 and CO_2 concentrations under the storage environment (Huyskens-Keil & Herppich, 2013). Also, the activity of several asparagus sugars metabolizing enzymes can be still maintained under a controlled atmosphere with a high CO_2 level (5% O_2, 10% CO_2) argues McKenzie et al. (2004).

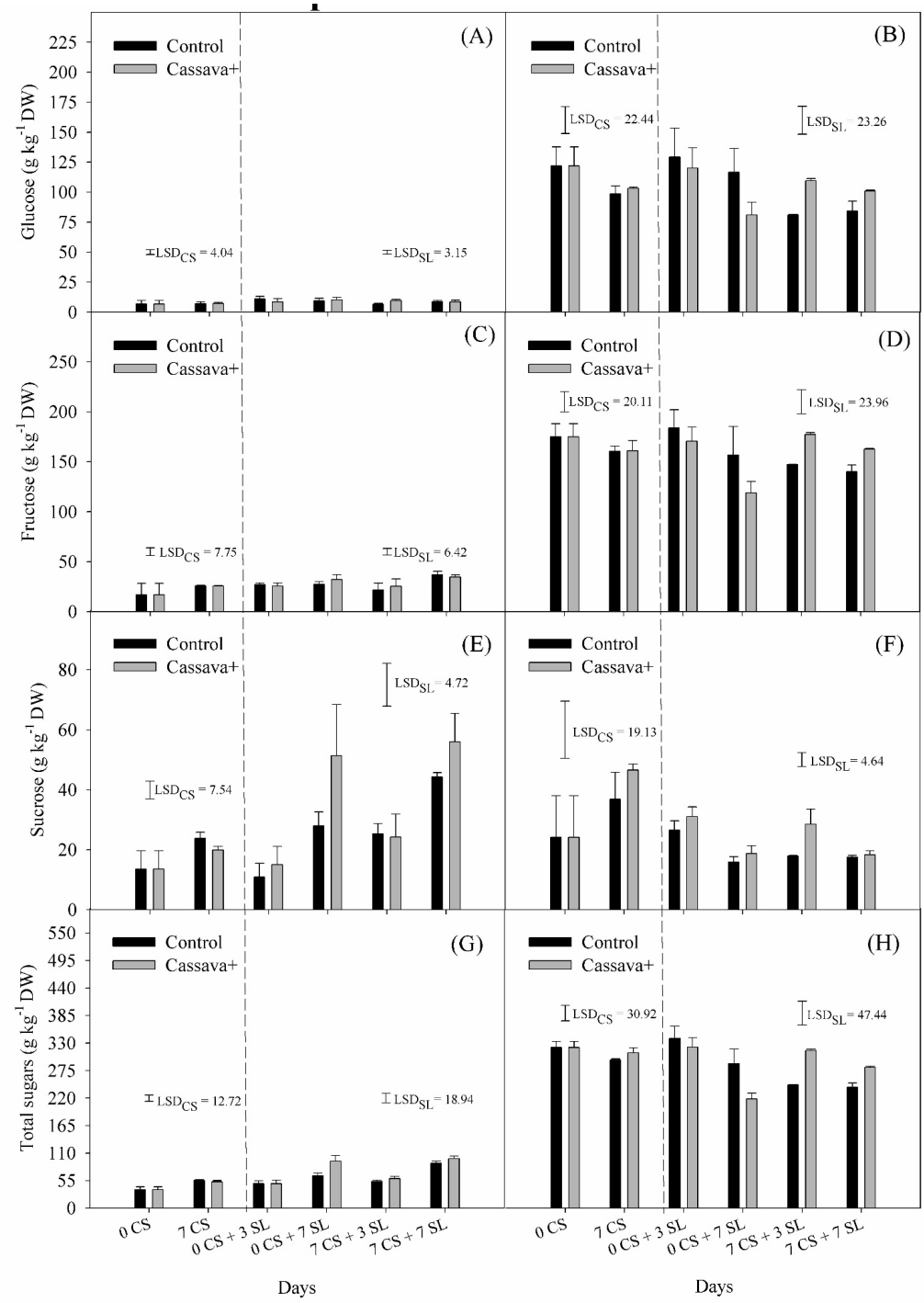


Figure 4. Glucose (A, B), Fructose (C, D), Sucrose (E, F) and total sugars (G, H) in tip (A) and basal sections (B) of the coated (Cassava+) and uncoated (Control) asparagus spears with previous cold storage for seven days (7CS) at 1°C and 90% RH or without cold storage (0CS), followed by 3 (3SL) or 7 (7SL) days of shelf-life condition at 7 °C and 70% RH (right side).

Coated spears presented sucrose levels similar to or higher than control spears, in both tip and basal-sections, under 7CS + 7SL conditions, before or after cold storage (Figures 4E and 4F). Comparing total sugar content in time zero with 7CS + 7SL time, there was an increase of 62.02% and 87.9% in the tip section in control and coated spears, respectively. In addition, there was a decrease of 18.25% and 9.4%, respectively, in basal-sections (Figure 4G and 4H).

All these results suggest that the modified atmosphere provided by edible coating was positive in maintaining carbohydrate content in asparagus as well as delaying its senescence. In close agreement with this, the mechanical properties of the cassava-chitosan (Cassava+) films were evidenced by the lower WVTR (Table 1). This response was also reported to preserve guava fruit using an edible starch-based coating (Oliveira et al., 2018).

Asparagus spears presented higher carbohydrate content in basal-sections than in tip sections. Nevertheless, carbohydrates significantly increased in the tip section over the shelf-life, while simultaneously decreasing in basal-sections, mainly sucrose content. Also, it was evidenced in green asparagus during storage by Mastropasqua et al. (2016). According to Benkeblia & Shiomi (2010), a high activity of invertase in the apical segment occurs in green asparagus spears.

Glucose and fructose levels in control spears decreased 29.7% and 17.6%, respectively, as compared to the time zero and shelf-life of 7CS + 7SL. It was higher than in coated spears. These differences between treatments may have a significant impact on how sugar profiles change during shelf-life. From a marketing perspective, the preservation of the carbohydrate over the shelf-life is required to maintain the asparagus sensory quality.

According to Mastropasqua et al. (2016), the significant decrease in the total sugar content in the basal part, during the storage of green asparagus, could be associated with the translocation of hexoses from the basal to the apical part.

3.3 Multivariate Analysis

As seen by instrumental analysis, PCA also revealed unequivocal segregation of cold storage and shelf-life conditions between tip and basal-sections (Figure 5A and 5B). At cold storage, the first principal component (PC1) and the second principal component (PC2) variations were 81.9 and 13.9%, respectively. This means that 95.8% of the total variation was explained by both PCs, which is sufficient to explain much of the variation in the original data, since that is higher than 50.0% (Rencher, 2002). PC1 separates basal-section, which exhibits differences in the instrumental analysis (sugar, firmness, asparagine, glucose, fructose, and colour parameter) from the tip section; while PC2 separates time zero (irrespective of treatments) to cold storage for seven days (Figure 5A).

On the other hand, for shelf-life, the PCA showed that the first and second principal components explained 63.7% (PC1) and 19.4% (PC2) of the observed variation (84.1%) (Figure 5B). PC1 also separated the basal section from the tip section; while shelf-life three and seven, mainly in the tip section, explained the variance of principal component 2. In addition, for a better understanding of the association between variables and the treatments, we also examined the loadings of physicals and biochemical parameters in the first two components. Highly weighted variables in PC1 included CE, Asp and Suc for basal-sections under cold storage; while for shelf-life it was included Suc for tip sections and Asp, CE, gluc, fruct, and colour parameters (L, h and C) for basal-sections. Notably, the sharp angle between their vectors suggests a positive correlation between these parameters. Under shelf-life, in PC2, the Suc was a highly weighted variable. Since the angle between the non-reducing sugar (Suc) and reducing sugars (Gluc or fruct) vectors were obtuse, these variables present a negative correlation for tip and basal-sections.

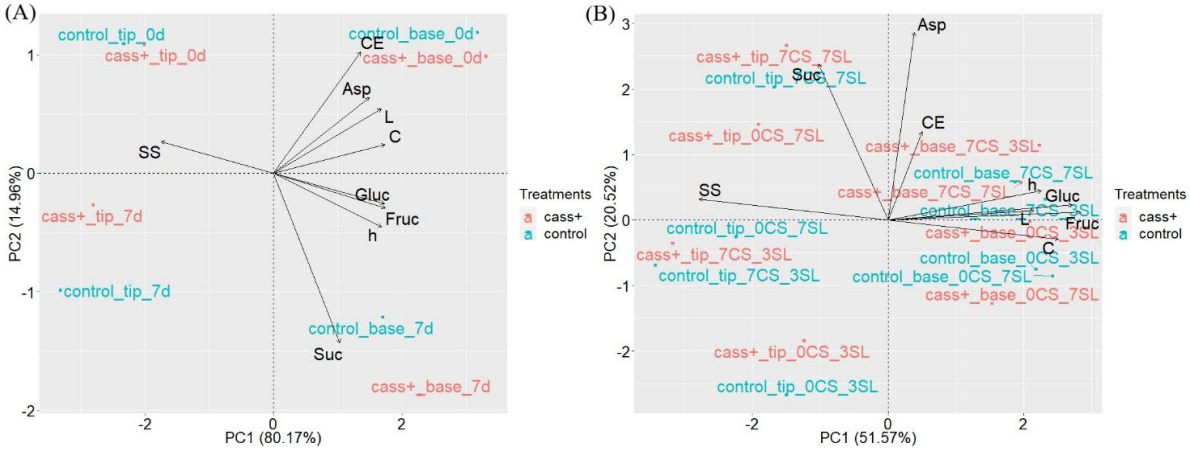


Figure 5. Biplot projection of the tip and basal-sections (base) of coated (Cassava+) and uncoated (Control) asparagus spears without cold storage (0CS), followed by 7 (7SL) days of shelf-life condition at 7 °C and 70% RH (A) or with previous cold storage for seven days (7CS) at 1 °C and 90% RH, followed by 3 (3SL) or 7 (7SL) days of shelf-life condition at 7 °C and 70% RH (B). Glucose (Gluc), fructose (Fruc), sucrose (Suc), texture (CE), asparagine (Asp), colour parameter, lightness (L), hue angle (h) and chroma (C).

4 Conclusion

Cassava starch, chitosan and the blend of cassava-chitosan (Cassava+) films were developed, and the Cassava+ film showed improved mechanical and physical barrier properties. Furthermore, there was a reduction in the film WVTR and an increase of the Young's module as compared to the isolated polysaccharides. However, the coating containing biopolymer blend (Cassava+) promoted delays of green asparagus senescence, characterized by weight loss reduction, which was associated with the maintenance of firmness, green colour, carbohydrate levels (in both the tip or basal-sections) for a longer period. Therefore, asparagus coated with this renewable and biodegradable packaging is kept green asparagus with a suitable market quality for up to seven days under 7 °C and 70 ± 1% RH conditions, without previous cold storage or for up to three days, with previous cold storage of seven days under 1 °C and 90 ± 1% RH conditions. Coated and uncoated spears presented tip-rot physiological disorder upon seven days at 7 °C and 90 ± 1% RH conditions, with previous cold storage.

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