



Research article

Carnauba wax nanoemulsion applied as an edible coating on fresh tomato for postharvest quality evaluation



Marcela Miranda^{a,b}, Marilene De Mori M. Ribeiro^c, Poliana C. Spricigo^d, Lucimeire Pilon^e,
Milene C. Mitsuyuki^a, Daniel S. Correa^{a,b,**}, Marcos D. Ferreira^{a,b,*}

^a Embrapa Instrumentação, Rua XV de Novembro, 1452, São Carlos, SP, 13560-970, Brazil

^b PPGBiotech, Center for Exact Sciences and Technology, Federal University of São Carlos, São Carlos, SP, CEP 13565-905, Brazil

^c QGP/Tanquímica, Laranjal Paulista, SP, CEP 18500-000, Brazil

^d University of São Paulo - Luiz de Queiroz College of Agriculture, Piracicaba, SP, CEP 13418-900, Brazil

^e Embrapa Hortaliças, Rodovia BR060, Km 9, Caixa Postal 218, Brasília, DF, 70351-970, Brazil

ARTICLE INFO

Keywords:

Nanomaterials
GRAS-ingredients
Copernicia prunifera
Emerging processing technologies
Sustainable chemistry

ABSTRACT

Edible coatings to extend the shelf life and preserve the quality of fruit and vegetables are highly demanded nowadays. Recently, plant-based edible coatings have gained importance in the context of sustainability, which in combination with suitable top-down process can render “greener” nanoemulsions with optimized properties. Herein we developed a carnauba wax nanoemulsion (CWN) by using a high-pressure processing to be applied as an edible coating for fruit and vegetables. The as-developed nanoemulsion properties were compared to conventional carnauba wax emulsion (CWM), where CWN showed particle size diameter of 44 nm and narrow distribution, while CWM displayed larger particles and wider size distribution (from 200 to 1700 nm). For assessment of the postharvest quality, cv. ‘Debora’ tomatoes, employed here as a model, were coated with CWN or CWM, at concentrations of 9 and 18%, and then compared to uncoated fruit during storage at 23 °C for 15 days. Evaluation of fruit quality, including sugar, acids, pH, water vapor loss, firmness, gloss, color, ethylene and respiratory activity, were assessed at every 3 days, while sensory test were carried out at the end of storage. Uncoated tomatoes presented the highest water loss values, meanwhile, firmness, ethylene, and respiratory activity were not largely modified by the coatings during storage. Tomatoes coated with the CWN exhibited the highest instrumental gloss and were preferred by consumers in sensory evaluations, indicating the potential of the as-developed carnauba wax green nanoemulsion for postharvest applications.

1. Introduction

Fruit and vegetables play an essential function in human's nutrition, as they are rich sources of vitamins, minerals, fiber, and phytochemicals, reducing some disease risks and promoting health (Angelino et al., 2019). However, the relative short lifetime of fruit can be an obstacle to their widespread consumption while intensifying food losses. Therefore, novel technologies that can at the same time extend the shelf life while improving fruits appearance are important nowadays for inducing healthy food consumption and decrease food loss. In this context, edible coatings for fruits and vegetables are of utmost importance (Maringgal et al., 2020; Nor and Ding, 2020). They should be safe for consumers and follow countries' legislation, such as the Brazilian Health Regulatory

Agency (ANVISA, 2013) in Brazil, and international recommendations, including those of the Food and Drug Administration (FDA), Codex Alimentarius Commission (CODEX – INS 903, 1995) and European Union -E903 (EFSA, 2012).

Current commercial coating emulsions for fruit and vegetable are usually made up of different components, including polyethylene wax, shellac, beeswax, morpholine and candelilla, which many times are combined with carnauba wax (Bai and Plotto, 2011; Puttalingamma, 2014; Kumar and Kapur, 2016; De Freitas et al., 2019). Such a mix of components allows the coating to achieve proper permeability while increasing gloss, which is an important parameter for the consumer's purchase decision (Bai and Plotto 2011). It is important that the ingredients employed are Generally Recognized as Safe (GRAS),

* Corresponding author.

** Corresponding author.

E-mail addresses: daniel.correa@embrapa.br (D.S. Correa), marcos.david@embrapa.br (M.D. Ferreira).

considering countries legislations, since some are allowed to be used only for non-edible peel (Nor and Ding, 2020). For instance, morpholine are ordinary ingredients used as emulsifiers applied on fruits and vegetables, but it can be chemically nitrosated to form N-nitrosomorpholine (NMOR), a potential genotoxic compound, being not approved as an additive in the European Community and the United Kingdom (Kumar and Kapur 2016). Hagenmaier (2004) reported replacement of morpholine by ammonia-based anionic microemulsions for a number of waxes, especially carnauba.

Considering food safety, lipid-based edible coatings derived from natural sources can create a barrier to water vapor diffusion and provide gloss for fresh fruits, leading to increased shelf-life and quality maintenance during storage (Bai and Plotto 2011; Ncama et al., 2018). Carnauba wax is considered GRAS by FDA (FDA, 2018). Additionally, it delays color changes, maintains texture, improves fruit surface mechanical integrity and visual appearance (Puttalingamma 2014; De Freitas et al., 2019). However, conventional emulsions usually show a milky appearance (Prince, 1977) and lipid micelle diameter ranging from 200 nm to 200 μm , and turbidity and opacity due to their droplet size, which causes light dispersion (McClements and Rao 2011; Mayer et al., 2013). In contrast, nanoemulsions with particle diameters ranging from 10 to 100 nm (Tadros et al., 2004; McClements and Rao 2011) are less turbid and translucent than conventional emulsions because their average sizes are smaller than the visible light wavelength ($r \ll \lambda$). Besides, reducing particle diameter is also an interesting approach to produce more thermodynamically stable emulsions (McClements and Rao 2011; McClements 2011; Mayer et al., 2013).

Therefore, there are several benefits from using nanotechnology to develop plant-based coatings, as reported by De Oliveira Filho et al. (2021). In this context, the main goal of this work was to synthesize and characterize a carnauba wax-nanoemulsion based on GRAS ingredients and evaluate its beneficial features (including the increase of fruit gloss and reduction of weight loss) on the postharvest quality of tomatoes cv. 'Debora' during storage, and also compare the performance to conventional carnauba wax emulsion.

2. Material and methods

2.1. Preparation of CWM and CWN

Conventional carnauba wax emulsion (CWM) was prepared following the methodology proposed by Hagenmaier and Baker (1997). In an open cylindrical reactor, 150 g of carnauba wax Press 1 (classification according to Ministry of Agriculture, Livestock and Food Supply (MAPA), Normative Instruction No 35, November 30, 2004) was melted with 30 g of oleic acid (Sigma-Aldrich Chemical Co.), 20 g of ammonium hydroxide 8% and 75 mL of deionized water at 105 °C, under constant mechanical stirring (Fisatom, Model 713D, Brazil) at 100 rpm for 10 min, making a water/oil (W/O) emulsion. Afterward, the remaining water (700 mL) was heated at 100 °C and slowly added to the system, yielding an O/W system by phase inversion. The emulsion remained under mechanical stirring at 150 rpm for 20 min and then cooled to 24 °C.

Carnauba wax nanoemulsion (CWN) was prepared following the methodology proposed by Hagenmaier and Baker (1997) with adaptations. 150 g of carnauba wax (Pontes Ltda, PI, Brazil), 30 g oleic acid (Sigma-Aldrich), 0.1 mL of dimethylpolysiloxane (Sigma-Aldrich), 20 g of ammonium hydroxide 8% (Sigma-Aldrich) and deionized water (775 mL) were heated at 100–120 °C in a closed cylindrical reactor (QGP/Tanquímica Ltda., Brazil), under mechanical stirring at 75 rpm for 30 min. Then, under mechanical stirring, the emulsion was cooled to 70–90 °C and subjected to high-pressure homogenization at 10–40 MPa at and rapidly cooled to room temperature (20–25 °C).

Final CWM and CWN were prepared at 18% of solid phase in suspension (concentrated emulsions). Then, the concentrated emulsions were diluted in distilled water to obtained CWN or CWM with 9% of solid

phase in suspension for postharvest assays. Additionally, the concentration was calculated by measuring solids content in an oven at 105 °C, 1g, 2 h.

2.2. Characterization of CWM and CWN

2.2.1. Particle size distribution, zeta potential, and polydispersity index (PDI)

Particle size distribution, zeta potential, and PDI of CWM and CWN were determined with suspensions (1:100) dispersed in deionized water at room temperature using a Zetasizer Nano ZS (Malvern Instruments Inc., Westborough, MA, USA). The data was acquired by 10 measurements and four runs each and 1s delay between the runs. All samples were analyzed in four replicates.

2.2.2. Stability at different environments over time

Twelve samples of each emulsion were poured into plastic bottles and exposed to four condition of light and temperature as follows: i) absence of light at 5 °C; ii) light at 24 °C; iii) absence of light at 24 °C and iv) absence of light at 40 °C. Zeta potential (equipment detailed in section 2.2.1) and viscosity values were measured (performed on a Brookfield[®] viscometer at 24 °C) at 1, 7, 14, 21, 28, 45, and 60 days in triplicate and expressed as average. Stability test was adapted from Isaac et al. (2008) and Da Silva Gündel et al. (2018) also used as reference.

2.3. Scanning electron microscopy

CWM and CWN were characterized using a field emission gun scanning electron microscope (JEOL, FEG-SEM JSM-6701F, USA) by dropping a diluted emulsion (1:1000) onto a silicon wafer. The material was allowed to dry for 24 h and then was carbon-coated using an SCD 050 sputter coater (Leica Microsystems, Germany). The acceleration voltage was 10 kV for CWM and 2 kV for CWN.

Scanning electron microscopy (MEV-SEM JEOL JSM-6701F) images of tomatoes peels coated and uncoated with CWM and CWN were collected (taken after immersing samples on the respective emulsions and drying period of 3 h), then frozen in liquid nitrogen and fractured. The samples were dried for 24 h in a desiccator and gold-coated.

2.4. Coating tomatoes with CWM and CWN

A cold room was previously washed and sanitized with 1 mL L⁻¹ quaternary ammonium, whereas utensils were washed and sanitized with 200 mg L⁻¹ sodium hypochlorite solution before coating and storage. Cv. 'Debora' tomatoes (*Solanum lycopersicum* L.) were harvested at breaker maturity stage (USDA, 2017) at a commercial tomatoes farm located in São Paulo State, Brazil. A total of 424 tomatoes were selected, discarding those with mechanical damages and pathogen decays. Before coating, tomatoes were washed, sanitized by immersion in a 200 mg L⁻¹ Sodium dichloroisocyanurate dihydrate (Sumaveg[®], Johnson Diversey Brazil Ltda.) solution for 15 min, rinsed, and dried.

Tomatoes were immersed for 3 min in CWM or CWN at concentrations of 9 and 18%, according to results optimized in preliminary tests (based on response variables: water vapor loss, fruit gloss, contact angle and overall appearance). Thus, the five treatments were: (i) control (tomatoes immersed in deionized water), (ii) CWM 9%, (iii) CWM 18%, (iv) CWN 9%, and (v) CWN 18%. After solvent evaporation (approximately 1h), the coating was formed on tomatoes, which were stored at 23 ± 1 °C and 80% RH for 15 days. Analyses were carried out at every 3 days. Physicochemical analyzes were performed at 0, 3, 6, 9, 12, and 15 days, and gas composition (ethylene, oxygen, and carbon dioxide) at 1, 4, 7, 10, 13, and 16 days. Experiments were conducted in a completely randomized factorial design composed of two factors, five treatments and six storage days. For destructive and non-destructive analysis, 234 and 190 fruits were used, respectively.

2.5. Physicochemical assessment

Surface wettability was determined by contact angle measurements using a CAM 101 system (KSV, Finland) equipment, following ASTM D7334-8 (2008). For the measurements, a water droplet (3 μL) was placed on the skin of uncoated and coated tomatoes (15 \times 4mm) for 60 s and the angles were recorded. Analyzes were performed in three replicates at 24 (± 1 °C). Additionally, a reflectometer (micro-TRI-gloss; BYK-Gardner, Silver Spring, MD) was used to evaluate the gloss of coated and uncoated tomatoes (Bai et al., 2003). For tomatoes skin assay, the reflectance was adjusted for an angle of 60°, and the results were expressed in gloss units (GU). A case with a circular 19 mm diameter orifice was attached to the equipment. Five tomatoes per treatment and three measures were done, totalizing 15 measurements per treatment.

Soluble solids (SS) were evaluated using a digital refractometer (ATAGO RX5000cx, Tokyo, Japan), following AOAC (1992). Titratable acidity (TA) was determined using 10 g of homogenized tomatoes diluted in 50 mL of distilled water by titration with NaOH 0.1 N until pH 8.1 (Goulas and Manganaris, 2011). The results were expressed in mg of citric acid $\times 100 \text{ g}^{-1}$ of pulp.

The ratio (SS/TA), an index used to indicate tomato quality (Beckles et al., 2012), was calculated by dividing Soluble Solids (SS) value by titratable acidity (TA) value. This index varies with fruit development, since TA during ripening decrease. The pH values of homogenized tomatoes were analyzed using a bench-top potentiometer (QUALSTRON, Model QX 1500, Brazil).

SS, TA, and pH were performed using nine tomatoes per treatment. Replicates were analyzed in triplicate. Weight loss was determined in a digital balance (Marte, Model AS2000C, São Paulo, Brazil) and expressed in percentage. For each treatment, 20 tomatoes were used. Water vapor loss was calculated by the following equation: $WL (\%) = [(w_0 - w_t]/w_0) \times 100$, in which WL represents water vapor loss (%), w_0 is the initial weight of tomatoes at day 0, and w_t is the weight of the analyzed day.

Flesh Firmness evaluation was performed in a TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK), which measured the maximum penetration force required for a 4-mm-diameter probe to penetrate into a tomato fruit without peel, until a depth of 5 mm at a rate of 5 mms^{-1} . Tomatoes were placed with equatorial region perpendicular to probe to allow the probe penetration and measurements were expressed in Newton (N). Per treatment, nine tomatoes were used, with three penetrations for fruit in each sampling time. Measurements were expressed in Newton (N).

Skin color was measured with a colorimeter Minolta® CR-400 Chroma Meter (Minolta Camera Co., Osaka, Japan), using the CIELAB system: L* (lightness), a* (green-red) values. Illuminant D65 and 10° observer angle were used. The instrument was calibrated using a standard white reflector plate. Values were obtained from 12 tomatoes per treatment at each sampling day (the same 12 fruits from water vapor loss were used) and three measurements for fruit.

Disease incidence was calculated as the number of infected tomatoes showing any disease symptoms out of the total amounts of tomatoes fruits stored, expressed in frequency percentage. To monitor the disease progress, 12 fruits were used.

2.6. Ethylene production and respiratory activity

Ethylene, CO₂ (carbon dioxide), and O₂ (oxygen) analyses were performed using 15 tomatoes per treatment at each sampling day (non-destructive sample). Three tomatoes from each treatment (in five replicates) were placed in 1.4 L hermetical glass jars and analyzed after 1 h at 22 °C and 85% RH (relativity humidity). CO₂ and O₂ in the headspace of the jar were analyzed using a respiration meter (Illinois Instrument Inc., Model 6600, USA). Results were expressed in $\text{mL kg}^{-1} \text{ h}^{-1}$, according to Martins et al. (2014). For ethylene, 1-mL samples from the headspace were withdrawn with a syringe (Gastight) through silicone septum adapted to the lid and measured by gas chromatography (Varian, CP-3800, CA, USA) equipped

with FID detector and Porapak N column. The column, injector, detector, and methanador were set at 50, 110, 200, and 350 °C, respectively. The hydrogen gas flow was 30 mL min^{-1} . Results were expressed in $\mu\text{L kg}^{-1} \text{ h}^{-1}$.

2.7. Sensory evaluation

Sensory analysis for affective acceptance tests, purchase intention, and discriminative tests of cv. 'Debora' tomatoes (after 10 days of storage at 23 ± 1 °C and RH = 80%) were evaluated by 53 panelists (composed of males and females, ages between 20 to 65). Sensory analysis was conducted in a room at 23 °C under fluorescent light. Samples were placed on trays containing 6 tomatoes per treatment. Each fruit were presented to the panel of judges with a 3-digit random code. Panelists were asked to rate (visually) color, gloss, and overall appearance as well as firmness by finger touch on an 11-point category scale, as follows: 1-dislike extremely, 2-dislike very much, 3-dislike considerably, 4-dislike moderately, 5-dislike slightly, 6-neither like nor dislike, 7-like slightly, 8-like moderately, 9-like considerably, 10-like very much or 11-like extremely (Lawless et al., 2010). For the purchase intention test, panelists were asked to rate tomatoes as fresh ready to eat fruit and ready to make tomato sauce based on appearance using a 5-point category scale as follows: "decidedly would not buy", "probably would not buy", "maybe yes/maybe no", "probably would buy", or "decidedly would buy." For the discriminative rank test, panelists were asked to rank tomatoes by increasing order of preference for appearance: 1 = ranked first (rated worst) to 5 = ranked last (most preferred). This study was done before covid-19 pandemic.

2.8. Statistical analyses

Test T student was employed to compare the results of zeta potential, viscosity, pH, and solid concentration of emulsions. Physical and chemical analyses were compared through analysis of variance (ANOVA) with measures repeated in time. In the cases where the sphericity condition of the variance and covariance matrix was not satisfied, the Geisser and Greenhouse correction for the degrees of freedom (Pereira et al., 2013) was used. In cases where an interaction between the factors (weight loss and titratable acidity) was observed, linear regressions were adjusted for each replicate in each treatment, and their angular coefficients (rates/day) were compared by Duncan multiple comparisons test.

Zeta Potential and viscosity analyses for CNW and CWM were compared by two-way analysis of variance and Duncan multiple comparisons test. Comparison among treatment scores for sensory analysis was performed through non-parametric ANOVA and multiple comparisons of the Kruskal-Wallis test due to the ordinal level of variables and five independent samples in the experiment. Statistical software R Core Team (2013) was used to perform the tests. A significance level of 0.05 was adopted for all analyses.

3. Results and discussion

3.1. Characterization of CWM and CWN

CW emulsions with different particle sizes were successfully produced by varying the preparation process (with or without high pressure). CWN showed a narrow size distribution, with an average particle size diameter of 44 nm (Table 1). The low PDI (0.28) indicates a uniform size distribution, which can also be evidenced by microscopy images (Figure 1a). In contrast, CWM showed a much broader size distribution (200–1700 nm) and high PDI (1.00), which is corroborated by SEM images in Figure 1b. Nanoemulsion showed significantly lower viscosity than conventional emulsion (Table 1), resulting in a thinner film (Figure 1d) than CWM (Figure 1e) on tomato skin (Figure 1c).

Emulsions were prepared with the same concentrations of the components, resulting in virtually the same pH (Table 1). CWN was less turbid and more translucent than CWM, which showed a milky appearance (Figure 2).

Table 1. Diameter size, polydispersity index (PDI), zeta Potential (ζ), viscosity (μ), pH, and solid concentration of CWM and CWN.

Emulsions	Size (nm) ^α	PDI ^α	ζ (mV) ^α	μ (cP) ^β	pH ^β	solid concentration ^β (%)
CWM	200 to 1700	1.00	-54.3 ^a (0.2)	6.9 ^a (0.5)	9.7 ^a (0.2)	18.0 ^a (0.4)
CWN	44 (7)	0.28	-43.8 ^b (0.6)	4.3 ^b (0.1)	10.1 ^a (0.1)	18.1 ^a (0.5)
-	-	-	0.138*	0.018*	0.328*	0.544*

Mean followed by the same letter in the column did not differ significantly from each other by T-test, * *p*-value. CWM: conventional carnauba wax, CWN: carnauba wax nanoemulsion. Means followed by (SD) standard deviation; ^α n = 4; ^β n = 3.

Hydrodynamic size, polydispersity index (PDI) and zeta potential provide information about the stability of an emulsion (Lemarchand et al., 2003). The zeta potential (ζ) is a measure that indicates the surface charge of the particles, and generally, values (in modulus) larger than 30 mV demonstrate stability in suspension, since the surface charge of the

particle prevents the formation of agglomerates (Mohanraj and Chen, 2006). According to our results, nano and micro-sized emulsions showed zeta potential values higher than |30| mV (Table 1), indicating colloidal stability (Attama et al., 2007).

Zeta potential and viscosity were significantly different between treatments for CWN and CWM (Tables 2 and 3), with a higher Zeta potential of CWM compared to CWN (Table 3). Phase separation or visual changes over time was not observed, corroborating the good stability of both CWN and CWM. The viscosity values of samples for different ambient conditions (Table 3) showed some differences for both treatments. The mean values for CWM were superior to CWN, and also presented some significant differences among treatments. Viscosity is an important parameter that can interfere in the final thickness of the coating, which influences water vapor changes (Hagenmaier and Baker, 1993).

3.2. Physicochemical assessment

CWM showed to be more hydrophobic than CWN (Figure 3a), which is probably related to the higher surface roughness (Jayasekara et al.,

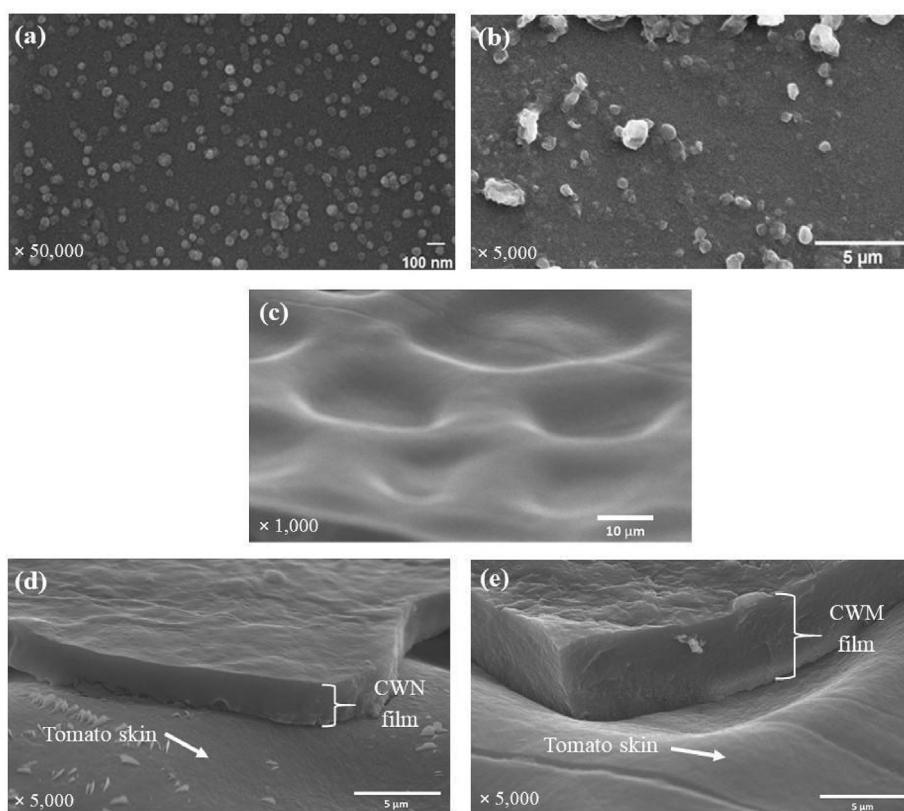


Figure 1. Field emission gun scanning electron microscopy (FEG-SEM) of diluted (1:1000) carnauba wax nanoemulsion-CWN18% (a) and conventional emulsion 18% (b) on silicon wafer. Scanning electron microscopy images of uncoated tomato surface (c), CWN18%-coated tomato (d), and CWM18%-coated tomato (e).



Figure 2. Detail of carnauba wax emulsion appearance for nanoemulsion (CWN) (a) and milky appearance for conventional carnauba wax (CWM) (b).

Table 2. P-values repeated measures analysis of variance for zeta Potential (ζ) and viscosity (μ).

Stability measures	Source of variation						
	Treatments (A)	Environment (B)	Period (C)	A*B	A*C	B*C	A*B*C
Viscosity	<0.001	0.038	<0.001	<0.001	0.111	0.132	0.605
Zeta	<0.001	0.642	<0.001	0.101	0.345	0.815	0.058

Table 3. Mean and standard deviation values for zeta Potential (ζ) and viscosity (μ).

(a)		
Emulsion	Environment	Viscosity
CWN	dark 5 °C	4.09 f (0.18)
	dark 40 °C	3.96 f (0.22)
	dark 24 °C	4.33 e (0.15)
	ligh 24 °C	4.69 d (0.57)
CWM	dark 5 °C	6.00 c (0.36)
	dark 40 °C	6.32 b (0.54)
	dark 24 °C	6.58 a (0.43)
	ligh 24 °C	6.27 b (0.16)

(b)	
Emulsion	Zeta potential
CWN	43.55 b (5.65)
CWM	53.66 a (3.54)

Mean values of (a) viscosity (μ) at different environments and (a) zeta potential (ζ), of carnauba wax nanoemulsion (CWN) and carnauba wax emulsion (CWM). The same letter's treatment did not differ significantly from each other by Repeated Measures Anova (a,b) and Duncan multiple comparisons test (a), n = 3. Standard deviation.

2004), as corroborated by SEM images in Figure 1 (d) and (e). CWN formed a more uniform and smoother coating due to the smaller particle size.

Fruit gloss decreased when coated with the 18% CWM, which resulted in opaque appearance. CWM coating at 9% exhibited similar gloss as uncoated tomatoes (Figures 3b and 3c). On the other hand, the 18% CWN-coated tomatoes attained the highest gloss value, superior to

control and fruits coated with CWM. CWN coating showed higher gloss due to the smaller particles (40 nm) compared to particles from conventional emulsions, which do not cause high light dispersion (McClements and Rao 2011; Prince 1977; Hagenmaier and Baker 1997; McClements 2011; Mayer et al., 2013). The higher gloss attained by CWN was possible due to the high-energy methodology employed to obtain nanoemulsions with smaller droplets, once the shear forces can reduce the particle size to nanoscale and allow more stabilization and optical transparency (Tadros et al., 2004; McClements 2011; Mayer et al., 2013). It is important to emphasize that the CWN developed here is different from standard commercial wax emulsion (usually made up of carnauba wax itself or mixed of other waxes, resins, and compounds), where the intense gloss was achieved by the nanostructured size of droplets composing the CWN, allowing to become shiner under visible light. Variation in lightness for all treatments was significant throughout storage, but there was no interaction among storage periods and treatments (Table 4). Mean lightness values for cv. 'Debora' tomatoes ranged from 57 (day 0) to 38 (day 15) (data not shown), indicating skin browning, which is an effect of ripening due to the synthesis of carotenoids and green color loss (Giuliano et al., 1993).

Weight loss significantly increased during storage for all treatments (Table 4). Tomatoes coated with CWN and CWM showed a significantly lower rate of daily weight losses (angular coefficients) compared to uncoated tomato (Table 5). R² of linear regressions adjusts were above 0.90. Tomatoes skin lacks stomata presence (Figure 1c), and weight loss in intact tomatoes (without cracking or mechanical injuries) occurs through stem scar, which allows transpiration and gas exchange (Calbo et al., 2007). Solid lipid structure (Chiumarelli and Ferreira 2006; Fagundes et al., 2014) adheres to fruit surface, which increases hydrophobicity, protecting the tomatoes surface and reducing water vapor loss, as exhibited by the fruits coated with CWM and CWN. Won and Min (2018) reported a Grapefruit seed extract (GSE) – incorporated carnauba wax

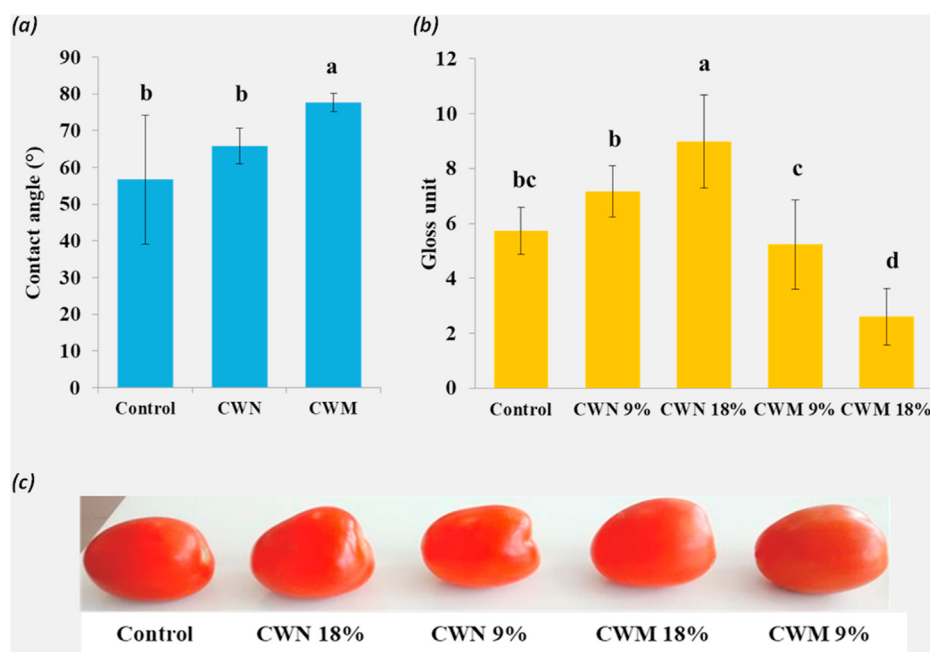


Figure 3. a) Water contact angle in tomato skin coated with conventional carnauba wax microemulsion (18% CWM), nanoemulsion (18% CWN) and uncoated tomatoes (mean ± SD, n = 3), b) Fruit gloss of uncoated tomatoes (control); CWM and CWN coatings by reflectometer instrument (mean ± SD, n = 5). Treatments followed by the same letter did not differ significantly by Repeated Measures Anova (a) or One Way Anova (b) and Duncan post hoc test (p < 0.05); c) Digital images illustrating the overall appearance of uncoated and coated tomatoes with CNW and CNM.

Table 4. Repeated measures analysis of variance for weight loss (WL), total titratable acidity (TA), soluble solids (SS), ratio (SS/TA), pH, firmness, lightness (L*), a* value, ethylene production, and respiratory activity (CO₂ production and O₂ consumption).

Physicochemical assessment measures	Source of variation		
	Treatments	Storage period	Period x Treatments
WL ¹	<0.001	<0.001	<0.001
TA ¹	0.969	<0.001	0.582
SS	0.241	<0.001	0.330
Ratio	0.014	<0.001	0.866
pH	0.233	<0.001	0.398
Firmness	0.367	<0.001	0.984
L* ¹	0.653	<0.001	0.909
a* ¹	0.998	<0.001	0.428
Ethylene ¹	0.852	<0.001	0.481
CO ₂ ¹	0.215	<0.001	0.782
O ₂ ¹	0.429	<0.001	0.622

¹ Geisser and Greenhouse correction for degrees of freedom due to noun sphericity.

Table 5. Rate weight loss/day mean of 'Deborá' tomatoes stored at 23 ± 1 °C and 80% UR.

Treatments	Rate Weight Loss/Day (%)	
Control	0.170 a	(0.030)
CWN 9%	0.118 b	(0.020)
CWN 18%	0.120 b	(0.010)
CWM 9%	0.104 b	(0.015)
CWM 18%	0.101 b	(0.019)

Mean values of rate weight loss/day and standard deviation. Means with the same letter did not differ significantly from each other by Duncan multiple comparisons test.

(CW) coating applied to mandarin fruits showing fruit preservation, reducing significantly weight loss.

Treatments did not affect acid (TA) concentration, soluble solids (SS), ratio (SS/TA), and pH, although the storage period was significant ($p < 0.001$, Table 4). Uncoated tomatoes and tomatoes coated with CWM or CWN exhibited similar SS (4.5°–4.0 °Brix), SS/TA ratio (13.3–24.3), and pH (4.0–4.3) ranges during storage (data not shown), which demonstrated that the coatings did not affect those variables. Frequently, coated tomatoes show slight ratio (SS/TA) changes for all harvesting stages, including mature green, turning, and light red. SS/TA above 10 can be used to determine the degree of ripeness for tomatoes (Abebe et al., 2017), indicating that all treatments achieved similar maturity in this study.

Fruit firmness was not affected by treatments ($p > 0.05$, Table 4), and only the storage period was significant ($p < 0.001$, Table 4). Tomatoes' firmness was reduced from 18 to 6 N during storage (data not shown). Although coatings could influence tomatoes' weight loss, they were not sufficient to affect the firmness of coated cv. 'Deborá' tomatoes.

The a* values (green to red) significantly increased over the storage period (Table 4) similarly for all treatments. Coated tomatoes showed no substantial delay in red color development, regardless of treatment (with CWM or CWN). It can be attributed to the loss of chlorophyll and concomitant synthesis of lycopene (Giuliano et al., 1993).

The nanoemulsions slowed the development of tomato disease. Fruits coated with 18% CWN and 18% CWM showed incidence of 26% and 32%, respectively, and those coated with 9% CWN and 9% CWM, 31% and 28%, respectively. Uncoated tomatoes showed the highest decay incidence (41%).

3.3. Ethylene production and respiration rate

Ethylene production by the fruits significantly decreased during storage ($p < 0.001$) similarly for all treatments, (8.26–0.78 $\mu\text{L kg}^{-1} \text{h}^{-1}$), since fruits had passed the climacteric peak of ethylene production. Neither treatment nor interaction between treatment and storage was observed ($p > 0.05$, Table 4) (Kim et al., 2013), indicating conventional and nanoemulsion coatings did not exhibit strong control of gas exchange during the experiments.

CO₂ production and O₂ consumption were significantly influenced by storage time but they were not affected by any of the treatments (Table 4). Coatings based on lipids and waxes, such as CW, are hydrophobic and usually effective as moisture barriers (De Freitas et al., 2019). However, they are less effective as gas barriers than polysaccharide, protein, or resin materials (Baldwin 1994). One likely reason for not being able to notice differences between treatments CWN and CWM can be related to the storage conditions, once fruits were stored at 23 ± 1 °C and 80% RH for 15 days, which high humidity might give ideal conditions to keep quality.

CWM and CWN demonstrated different characteristics size, coating formation, contact angle measurements, as well as shining features to the fruit peel. However, they did not show considerable differences for respiration rate, ethylene production, and the other physicochemical parameters for tomatoes. These results may be explained by the fact that tomato gas exchange occurs through a small gas exchange area - stem scar pores- (Calbo et al., 2007). However, coating this area with CW was not enough to cause differences in permeability for tomatoes (Hagenmaier and Baker (1993), unlike to tangerines (Miranda et al., 2021) and papayas (Miranda et al., 2019 and Miranda et al., 2022), in which the gas exchange area is relatively larger than tomatoes.

3.4. Sensory evaluation

Color, gloss, firmness, and overall appearance of tomatoes were statistically affected by the treatments. The 11-point category scale was grouped in five categories, as follow: (A) 1-dislike extremely, 2- dislike very much, 3-dislike considerably; (B) 4-dislike moderately, 5- dislike slightly; (C) 6- neither like nor dislike; (D) 7- like slightly, 8-like moderately; and (E) 9- like considerably, 10- like very much or 11-like extremely, as shown in Figure 4. Tomatoes coated with 18% CWN showed the highest frequency of *like extremely to considerably* for color (55%) and gloss (60%), significantly different from the other CWM treatments. For firmness the highest percentages for *like extremely to considerably* were for CWN 9% (43%) and CWN 18% (42%), not significant different from CWM treatments, but all significantly different from control. CWN 18 and 9% presented for the category *like extremely to considerably*, high frequency values for overall appearance (19 and 40% respectively), significantly different from CWM treatments and control. It is important to notice that CWN treatments were less rejected, with overall lower frequency on the category *dislike extremely to considerably* and on *dislike moderately or slightly* when compared to CWM treatments and control.

Therefore, the results indicate that the nanoemulsion coatings were generally preferred by the panelists. In contrast, uncoated tomatoes presented the lowest frequency for *like extremely to considerably*. Panel results for gloss are consistent with the fruit gloss measurements by reflectometer, where CWN coatings presented the highest values, which result is advantageous compared to other edible coatings reported in the literature (Fagundes et al., 2014) which did not improved gloss compared to uncoated fruits.

Tomatoes coated with CWN yielded the highest frequency for *like extremely to considerably*, which result is compatible with the lower-water vapor loss compared to uncoated fruits. Considering consumers usually choose fresh tomatoes by their appearance and flavor (Kader et al., 1978), the greater acceptance of overall appearance led by the

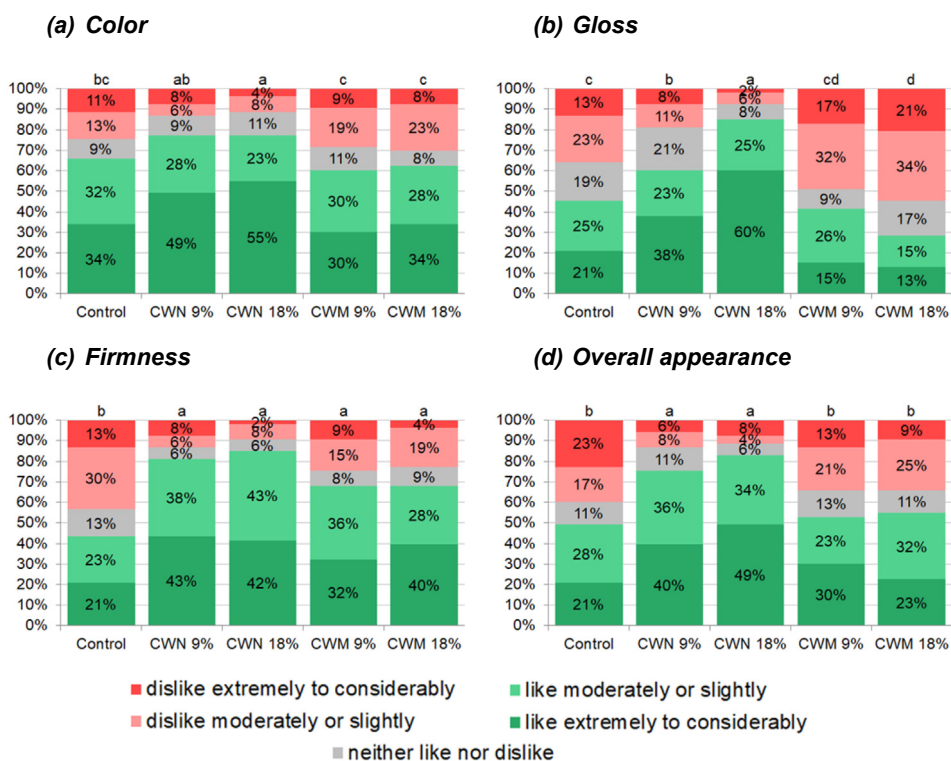


Figure 4. Relative frequency for sensory evaluation (color, gloss, firmness, and overall appearance) of ‘Debora’ tomatoes stored at 23 ± 1 °C and 80% UR. Treatments followed by the same letter did not differ significantly from each other by Kruskal-Wallis multiple comparisons test. 53 panelists per treatment. CWN: carnauba wax nanoemulsion and CWM: carnauba wax emulsion.

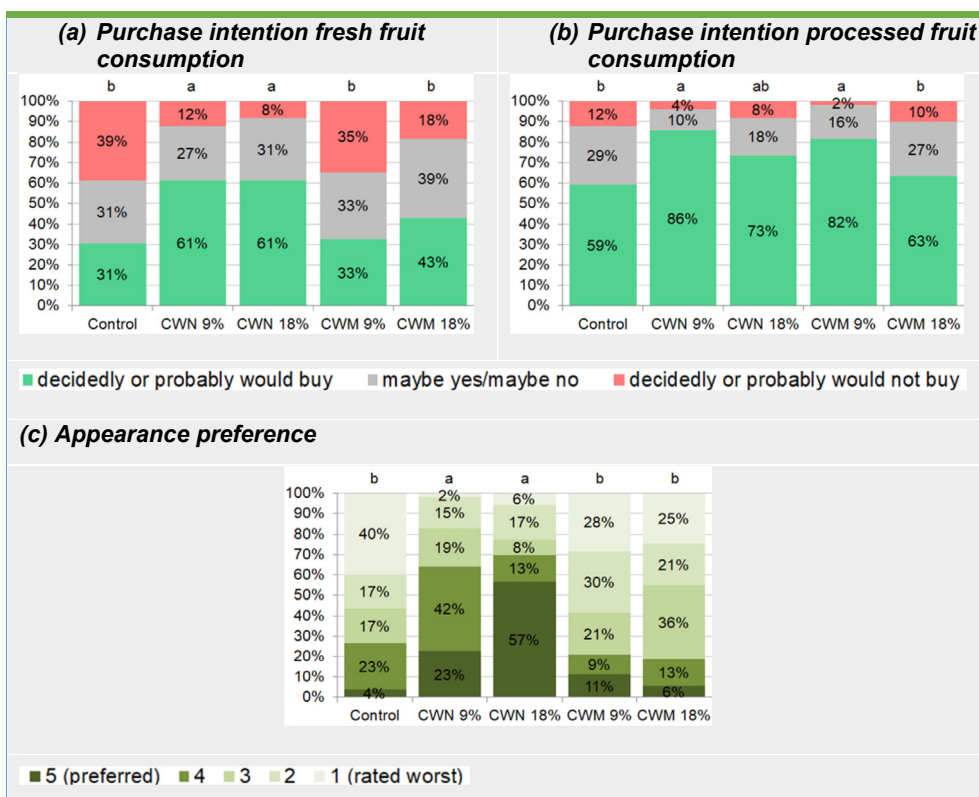


Figure 5. Frequency of tomatoes purchase intention by consumers for fresh (a) or processed tomatoes consumption (b), and appearance preference (c). Treatments followed by the same letter did not differ significantly from each by Kruskal-Wallis multiple comparisons test (p < 0.05), n = 53 panelists.

nanoemulsion coatings may positively influence consumers' purchase decisions.

The sensory test scores of purchase intention for fresh tomatoes and processed tomatoes were also grouped in three categories, as follows: *decidedly or probably would buy*; *maybe yes/maybe no*; and *decidedly or probably would not buy*, shown in Figure 5.

For purchase intention for fresh fruit consumption, CWN-coated tomatoes, regardless of concentration, yielded the highest frequency (61%) of "*decidedly and probably would buy*." Uncoated- and CWM-coated tomatoes exhibited a high percentage of "*probably or decidedly would not buy*" (Figure 5a). For the purchase intention of tomatoes *as ready to make tomato sauce*, 9% CWN coating obtained 86% (Figure 5b) of "*decidedly and probably would buy*." The lowest purchase intentions were found for the 18% CWM-coated and uncoated tomatoes (63 and 59%, respectively). There were effects for treatment in *appearance preference rank*, in which fruits coated with 18% and 9% CWN were rated the highest (57 and 23%, respectively), although not different from each other; and tomatoes coated with 18 and 9% CWM and uncoated were rated lowest (11, 6, and 4%, respectively) (Figure 5c). Motamedi et al. (2018) found similar results with carnauba wax-nanoclay emulsion applied in postharvest treatments on orange, which showed sensory acceptability than commercial ones, with additional extension of postharvest shelf life.

4. Conclusion

A carnauba wax nanoemulsion (CWN) was developed and characterized in terms of physical-chemical properties and compared to conventional carnauba wax emulsion (CWM). CWN increased fruit gloss and improved tomatoes appearance, as demonstrated by sensory evaluations, compared to CWM or uncoated fruit. Neither CWM nor CWN significantly affected gas permeability or oxygen and carbon dioxide production, color, or sugars while reducing weight (water) loss. The CWN developed and produced with GRAS ingredients (i.e. without morpholine neither other synthetic resins and waxes) meet the current requirements and regulations for consumption. Thus, the CWN developed here shows the potential for maintaining tomatoes quality, reducing decay, and promoting consumption of fruits, helping to extend the postharvest shelf life of fruits and vegetables.

Declarations

Author contribution statement

Marcela Miranda: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Marilene De Mori M. Ribeiro: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Poliana C. Spricigo, Lucimeire Pilon: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Milene C. Mitsuyuki: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Daniel S. Correa, Marcos D. Ferreira: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Dr Marcos David Ferreira was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico [482535/2012-1].

Daniel S. Correa was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico [402.287/2013-4].

Marcela Miranda was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior [001].

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Abebe, Z., Tola, Y.B., Mohammed, A., 2017. Effects of edible coating materials and stages of maturity at harvest on storage life and quality of tomatoes (*Lycopersicon Esculentum Mill.*) fruits. *Afr. J. Agric. Res.* 18, 550–565.
- Angelino, D., Godos, J., Ghelfi, F., Tieri, M., Titta, L., Lafranconi, A., Marventano, S., Alonzo, E., Gambera, A., Sciacca, S., Buscemi, S., Sumantra, R., Galvano, F., Del Rio, D., Grosso, G., 2019. Fruit and vegetable consumption and health outcomes: an umbrella review of observational studies. *Int. J. Food Sci. Nutr.* 70 (6), 652–667.
- ANVISA. Agência Nacional de Vigilância Sanitária, 2013. Resolução da Diretoria Colegiada – RDC Nº 8, de 06 de março de 2013. Aditivos frutas e vegetais. http://port.al.anvisa.gov.br/documents/10181/3352026/RDC_08_2013_COMP.pdf/ea34430b-4774-450c-bcc8-73919315b132/. (Accessed 11 June 2019).
- AOAC - Association of Analytical Chemists International, 1992. Official Methods of Analysis of the Association of Analytical Chemists International, fourteenth ed. AOAC, Washington.
- ASTM - International. American Society for Testing and Materials, 2008. Standard Practice for Surface Wettability of Coatings, Substrates and Pigments by Advancing Contact Angle Measurement. ASTM, West Conshohocken (PA). <https://www.astm.org/Standards/D7334.htm>.
- Attama, A.A., Schicke, B.C., Paepenmüller, T., Müller-Goymann, C.C., 2007. Solid lipid nanodispersions containing mixed lipid core and a polar heterolipid: characterization. *Eur. J. Pharm. Biopharm.* 67, 48–57.
- Bai, J., Plotto, A., 2011. Coatings for fresh fruits and vegetables. In: Baldwin, E.A., Hagenmaier, R., Bai, J. (Eds.), *Edible Coatings and Films to Improve Food Quality*. CRC Press, Boca Raton, pp. 185–242.
- Bai, J., Hagenmaier, R.D., Baldwin, E.A., 2003. Coating selection for 'Delicious' and other apples. *Postharvest Biol. Technol.* 28, 381–390.
- Baldwin, E.A., 1994. Edible coatings for fresh fruits and vegetables: past, present, and future. In: Krochta, J.M., Baldwin, E.A., Nisperos Carriedo, M. (Eds.), *Edible Coatings and Films to Improve Food Quality*. Technomic Publishing Co, Pennsylvania, pp. 25–56.
- Beckles, D.M., 2012. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum L.*) fruit. *Postharvest Biol. Technol.* 63 (1), 129–140.
- Calbo, A.G., Moretti, C.L., Henz, G.P., 2007. Respiração de frutas e hortaliças. Technical communication. Embrapa Hortaliças. Brasília: Embrapa. ISSN 1414-9850. <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/103079/1/cot-46.pdf/>. Accessed 12 June 2019.
- Chiumarelli, M., Ferreira, M.D., 2006. Qualidade pós-colheita de tomates "Debra" com utilização de diferentes coberturas comestíveis e temperaturas de armazenamento. *Hortic. Bras.* 24, 381–385. Accessed 12 June 2019. <http://taurus.unicamp.br/bitstream/REPOSIP/24702/1/S0102-05362006000300023.pdf>.
- Codex Alimentarius, 1995. General Standard for Food Additives CODEX STAN 192-1995. Revision 1997, 1999, 2001, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2018. http://www.fao.org/gsaonline/docs/CXS_192e.pdf. Accessed 12 June 2019.
- R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>. Accessed 1 July de 2018.
- Da Silva Gündel, S., De Souza, M.E., Quatrin, P.M., Klein, B., Wagner, R., Gündel, A., Vaucher, R.A., Santos, R.C.V., Ourique, A.F., 2018. Nanoemulsions containing *Cymbopogon flexuosus* essential oil: development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. *Microb. Pathog.* 118, 268–276.
- De Freitas, C.A.S., de Sousa, P.H.M., Soares, D.J., da Silva, J.Y.G., Rathinaraj Benjamin, S., Guedes, M.I.F., 2019. Carnauba wax uses in food-a review. *Food Chem.* 291, 38–48.
- De Oliveira Filho, J.G., Miranda, M., Ferreira, M.D., Plotto, A., 2021. Nanoemulsions as edible coatings: a potential strategy for fresh fruits and vegetables preservation. *Foods* 10, 2438.
- EFSA. European Food Safety Authority, 2012. Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific Opinion on the re-evaluation of carnauba wax (E 903) as a food additive. *EFSA J.* 10 (10), 2880.
- Fagundes, C., Palou, L., Monteiro, A.R., Pérez-Gago, M.B., 2014. Effect of antifungal hydroxypropyl methylcellulose-beeswax edible coatings on gray mold development and quality attributes of cold-stored cherry tomato fruit. *Postharvest Biol. Technol.* 92, 1–8.
- FDA - Food and Drugs Administration, 2018. Chapter I- federal food, drug, and cosmetic act, part 184, direct food substances affirmed as generally recognized as safe, Sec. 184.1978 - carnauba wax. In: Code of Federal Regulations Title 21, 3. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=184.1978>. (Accessed 1 June 2019).

- Giuliano, G., Bartley, G.E., Scolnik, P.A., 1993. Regulation of carotenoid biosynthesis during tomato development. *Plant Cell* 5, 379–387.
- Goulas, V., Manganaris, G.A., 2011. The effect of postharvest ripening on strawberry bioactive composition and antioxidant potential. *J. Sci. Food Agric.* 91 (10), 1907–1914.
- Hagenmaier, R.D., 2004, December. Fruit coatings containing ammonia instead of morpholine. *Proc. Fla. State Hortic. Soc.* 117, 396–402.
- Hagenmaier, R.D., Baker, R.A., 1993. Reduction in gas exchange of citrus fruit by wax coatings. *J. Agric. Food Chem.* 41, 283–287.
- Hagenmaier, R.D., Baker, R.A., 1997. Edible coatings from morpholine-free wax microemulsions. *J. Agric. Food Chem.* 45, 349–352.
- Isaac, V.L.B., Cefali, B.G., Chiari, B.G., Oliveira, C.C.L.G., Salgado, H.R.N., Corrêa, M.A., 2008. Protocolo para ensaios físico-químicos de estabilidade de fitocosméticos. *Revista de Ciências Farmacêuticas Básica e Aplicada* 29 (1), 81–96.
- Jayasekara, R., Harding, I., Bowater, I., Christie, G.B.Y., Lonergan, G.T., 2004. Preparation, surface modification and characterization of solution cast starch PVA blended films. *Polym. Test.* 23, 17–27.
- Kader, A.A., Morris, L.L., Stevens, M.A., Albright-Holton, M., 1978. Composition and flavor quality of fresh market tomatoes as influenced by some postharvest handling procedures. *J. Am. Soc. Hortic. Sci.* 103, 6–13. <http://ucce.ucdavis.edu/files/datastore/234-519.pdf/>. (Accessed 5 January 2019).
- Kim, I.H., Lee, H., Kim, J.E., Song, K.B., Lee, Y.S., Chung, D.S., Min, S.C., 2013. Plum coatings of lemongrass oil-incorporating carnauba wax-based nanoemulsion. *J. Food Sci.* 78 (10), E1551–E1559.
- Kumar, R., Kapur, S., 2016. Morpholine: a glazing agent for fruits and vegetables coating/waxing. *Int. J. Sci. Technol. Eng.* 2 (11), 694–697. ISSN (online): 2349-784X.
- Lawless, H.T., Popper, R., Kroll, B.J., 2010. A comparison of the labeled magnitude (LAM) scale, an 11-point category scale and the traditional 9-point hedonic scale. *Food Qual. Prefer.* 21 (1), 4–12.
- Lemarchand, C., Couvreur, P., Vauthier, C., Costantini, D., Gref, R., 2003. Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. *Int. J. Pharm.* 254, 77–82.
- Maringgal, B., Hashim, N., Tawakkal, I.S.M.A., Mohamed, M.T.M., 2020. Recent advance in edible coating and its effect on fresh/fresh-cut fruits quality. *Trends Food Sci. Technol.* 96, 253–267.
- Martins, D.R., Barbosa, N.C., Resende, E.D.D., 2014. Respiration rate of Golden papaya stored under refrigeration and with different controlled atmospheres. *Sci. Agric.* 71, 369–373.
- Mayer, S., Weiss, J., McClements, D.J., 2013. Vitamin E-enriched nanoemulsions formed by emulsion phase inversion: factors influencing droplet size and stability. *J. Colloid Interface Sci.* 402, 122–130.
- McClements, D.J., 2011. Edible nanoemulsions: fabrication, properties, and functional performance. *Soft Matter* 7, 2297–2316.
- McClements, D.J., Rao, J., 2011. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Crit. Rev. Food Sci. Nutr.* 51, 285–330.
- Miranda, M., Gozalbo, A.M., Sun, X., Plotto, A., Bai, J., Assis, O.B.G., Ferreira, M.D., Baldwin, E.A., 2019. Effect of mono and bilayer of carnauba wax-based nano-emulsion and HPMC coatings on postharvest quality of 'Redtainung' papaya. In: *Simpósio Nacional De Instrumentação Agropecuária, 2019. Anais SIAGRO: ciência, inovação e mercado, São Carlos*, pp. 705–708. Available in: <https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/1116606/1/PEFFECTOFMONOANDBILAYEROFCARNAUBAWAXBASEDNANOEMULSION.pdf>.
- Miranda, M., Sun, X., Ference, C., Plotto, A., Bai, J., Wood, D., Benedito Garrido Assis, O., Ferreira, M.D., Baldwin, E., 2021. Nano- and micro- carnauba wax emulsions versus shellac protective coatings on postharvest citrus quality. *J. Am. Soc. Hortic. Sci.* 146 (1), 40–49.
- Miranda, M., Sun, X., Marín, A., Dos Santos, L.C., Plotto, A., Bai, J., Benedito Garrido Assis, O., Ferreira, M.D., Baldwin, E., 2022. Nano- and micro-sized carnauba wax emulsions-based coatings incorporated with ginger essential oil and hydroxypropyl methylcellulose on papaya: Preservation of quality and delay of post-harvest fruit decay. *Food Chem. X* 13 (March 2022-100249), 1–13.
- Mohanraj, V.J., Chen, Y., 2006. Nanoparticles – a review. *Trop. J. Pharmaceut. Res.* 5, 561–573.
- Motamedi, E., Nasiri, J., Malidarreh, T.R., Kalantari, S., Naghavi, M.R., Safari, M., 2018. Performance of carnauba wax-nanoclay emulsion coatings on postharvest quality of 'Valencia' orange fruit. *Sci. Hortic.* 240, 170–178.
- Ncama, K., Magwaza, L.S., Mditshwa, A., Tesfay, S.Z., 2018. Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: a review. *Food Packag. Shelf Life* 16, 157–167.
- Nor, S.M., Ding, P., 2020. Trends and advances in edible biopolymer coating for tropical fruit: a review. *Food Res. Int.* 134, 109208.
- Pereira, M.C., Jesus, D., Martorelli, S., Vieira, A., Bottaro, M., 2013. Effects of wearing arm progressive compression sleeves on upper-body muscular performance in trained men. *Motricidade* 9, 33–39.
- Prince, L.M., 1977. Schuman's microemulsion. In: Prince, L.M. (Ed.), *Microemulsion: Theory and Practice*. Academic Press, New York, pp. 1–19, 192.
- Puttalingamma, V., 2014. Edible coatings of carnauba wax - a novel method for preservation and extending longevity of fruits and vegetables - a review. *Int. J. Food Safety* 16, 1–5.
- Tadros, T., Izquierdo, P., Esquena, J., Solans, C., 2004. Formation and stability of nano-emulsions. *Adv. Cand Interface Sci.* 108, 303–318.
- USDA - United States Department of Agriculture, 2017. *Tomato Color Chart Color Chart for Classification Requirements of Surface Color for Tomatoes Described as green, Breakers, Turning, Pink, Light Red, and Red (February 1975). Index of Official Visual Aids. USDA Visual Aid TM-L-1.* <https://www.ams.usda.gov/sites/default/files/media/Official%20Inventory%20of%20FV%20Inspection%20Aids.pdf>.
- Won, M.Y., Min, S.C., 2018. Coating Satsuma Mandarin using grapefruit seed extract-incorporated carnauba wax for its preservation. *Food Sci. Biotechnol.* 27 (6), 1649–1658.