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Application of natural coating improves the conservation of cut carnation flower

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ABSTRACT

Carnation (*Dianthus caryophyllus*) is one of the most important and popular cut flowers for the ornamental industry. However, it has a short decorative life, being water loss one of the main challenges. Water loss leads the petals to wither with a consequent loss of its commercial value. One of the techniques used in postharvest technology is the use of coatings and films, which interfere in gas exchange and water loss, maintaining the quality of cut flowers. The current study investigated the effects of a natural coating based on hydroxypropyl methylcellulose (HPMC) at 5% and beeswax (BW) in cut carnation flower cv. Delphi stored at 20°C and 70% RH. The application of HPMC + 40% BW treatment in flowers resulted in 30% higher fresh weight, 17% higher relative water content (RWC) and 80% higher flower opening compared to untreated flowers. The shelf life of untreated and HPMC + 40% BW carnations was 7 and 12 days, respectively, allowing a shelf-life extension of up to five days.

Keywords: *Dianthus caryophyllus*, hydroxypropyl methylcellulose, beeswax, cut flower, shelf-life extension, flower quality.

RESUMO

A aplicação de película natural melhora a conservação de flores de corte de cravos

O cravo (*Dianthus caryophyllus*) é uma das flores mais importantes e populares na indústria ornamental. No entanto, apresenta curta vida de prateleira, sendo a perda de água um dos principais problemas, resultando em murcha das pétalas e consequente perda de valor comercial. Uma das técnicas utilizadas na pós-colheita é a utilização de películas e filmes, que interferem nas trocas gasosas e na perda de água, conservando a qualidade das flores de corte. Este estudo investigou os efeitos da película natural à base de hidroxipropilmetilcelulose (HPMC) a 5% e cera de abelha (CA) em flores de corte de cravo cv. Delphi armazenadas a 20°C e 70% HR. As flores tratadas com HPMC + 40% CA apresentaram incremento de 30% na massa fresca, 17% no conteúdo relativo de água (CRA) e 80% na abertura floral. A vida útil dos cravos do controle e do HPMC + 40% CA foi de 7 e 12 dias, respectivamente, prolongando a vida de prateleira por até cinco dias.

Palavras-chave: *Dianthus caryophyllus*, hidroxipropilmetilcelulose, cera de abelha, flor de corte, vida de vaso, qualidade floral.

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The carnation is an important cut flower due to its high commercial and ornamental value, which has a relevant cultural purpose in many celebrations, such as weddings, proms, and festivals. Along with roses, orchids, and chrysanthemums, it is considered one of the most popular flowers, cultivated and exported worldwide with many flower colors and shapes (Astute Analytica, 2022).

The cut flower's longevity is a key aspect for commercialization, for most species, storage is short-term limited because of loss in quality. As well in carnation, flower senescence can be characterized by wilting and color modification as petal in-rolling and darkening, resulting from water loss, ethylene exposure, and carbohydrate depletion that happens after harvest (Gebremedhin, 2013; Lezoul *et al*., 2022).

Shelf-life is influenced by several factors, including on the one hand, preharvest and harvest conditions, such as genetic make-up, ethylene production, season, cultivation, and harvesting methods (Onozaki *et al*., 2018; Higashiura *et al*., 2020; Villagran & Bojacá, 2020), and on the other hand, postharvest conditions, as temperature, humidity, water relations, and storage methods (Shabanian *et al*., 2018; Ahmadi-Majd *et al*., 2021).

Additional postharvest treatments have been used to improve the quality and extend the cut flower shelf-life of many species by delaying flower metabolism.

The application of coatings is an option for cut flowers preservation (Pinsetta Junior *et al*., 2019). When placed on the plant surface, it forms a film on the plant structures (stomata, lenticels, and micropores), becoming a semipermeable barrier to gas exchange and water loss reduction, changing the metabolism mainly by the decrease of respiration and transpiration (Assis & Britto, 2014; Oliveira *et al*., 2018).

The use of natural coatings and films has shown significant reductions in weight loss, respiratory rate, ethylene production, firmness loss, soluble solids content, enabling an increase in postharvest conservation of vegetables (Pinsetta Junior, 2018), fruits (Formiga *et al*., 2019) and flowers (Pinsetta Junior *et al*., 2019). This convenient and feasible coating method could be used for industries as a postharvest treatment to be applied in packing houses or at the point of sale.

Natural films and coatings can be used as physical protection against mechanical damage, modifying chemical and biological activities. Also, they can be edible, biocompatible, non-toxic, and food additives carriers. They are divided into categories according to the material type they were derived from: polysaccharides, proteins, and lipids (Dehghani *et al*., 2017).

The hydroxypropyl methylcellulose compound (HPMC) is a water-soluble cellulose hydrocolloid presenting good film-forming properties (Assis & Britto, 2014). Cellulose derivative coatings (such as HPMC) are specifically formulated to reduce O_2 and CO_2 exchanges, delaying fruit ripening (McGuire & Hallman, 1995). Lipid-based coatings are generally used to produce a moisture barrier. Among lipid materials, beeswax improves the water vapor barrier characteristics (Fagundes *et al*., 2013). Applying a coating that combines a material with a gas exchange barrier and another with a moisture barrier, enhances the beneficial effect of extending the postharvest conservation in plants.

A study carried out by Pinsetta Junior *et al*. (2019) demonstrated the potential of applying HPMC and beeswax-based coatings in 'Avalanche' cut roses conservation. The authors concluded that application of 3.0 mL/rose of the coating resulted in higher RWC and membrane stability index (MSI) throughout the storage time. Thus, the use of natural coatings can improve the conservation of other flowers, such as carnation.

The study aimed to test the use of natural coats, such as HPMC and beeswax, as alternative for the postharvest conservation of cut carnations cultivar Delphi stored at 20°C and 70% RH.

MATERIAL AND METHODS

Plant material

Cut flower carnation cv. Delphi, with white flowers, grown under irrigation, in greenhouse, were obtained from a commercial producer in the city of Holambra (22°37'59"S; 47°03'20"W, altitude 611 m), state of São Paulo, Brazil. The carnations were harvested in spring, with the flowers at the partially open stage the outer petals of the buds are open but not yet perpendicular to the stem (Faust & Dole, 2021) and transported to the Postharvest Technology Laboratory at UNESP, Jaboticabal, Brazil.

Reagents

The polysaccharide hydroxypropyl methylcellulose (HPMC), Methocel E15®, was obtained from Dow Chemical, USA. The beeswax was obtained from Synth©. The reagents stearic acid (CAS 57-11-4) and glycerol (CAS 56-81-5) were obtained from Merck KGaA (Darmstadt, DE).

Coating preparation

The coating was prepared by combining the hydrophilic phase (HPMC) and the lipid phase using beeswax (BW), both suspended in water, according to the methodology of Navarro-Tarazaga *et al*. (2011). HPMC was diluted in distilled water at 90°C with constant agitation in the proportion of 5 g/100 g. Then, it was cooled to 20°C in an ice bath for 30 minutes. BW was added at a concentration of 20 g and 40 g/100 g (dry base, db), glycerol (as plasticizer) in the proportion of HPMC: glycerol (2:1, w/w) and stearic acid (as an emulsifier) in the proportion of BW:stearic acid (5:1, w/w). At the end, enough distilled water was added to obtain a final solids concentration of 4 g/100 g (Table 1). All ingredients were mixed and heated to 90°C to melt the BW. The emulsion was formed with the aid of a mill with stirring for 1 minute at 968 x *g* followed by 3 minutes at 3871 x *g*. The emulsion was cooled in an ice bath to 20°C, under stirring for 30 minutes, to ensure complete hydration of the HPMC.

Coatings application and assessment

Before applying the treatments, the ends of the stem flowers were immersed in distilled water and cut to 45 cm in length. The stem flowers were standardized at 45 cm by cutting the stems inside containers with distilled water.

The treatments consisted of spraying with a spray gun (pressure of 30 Psi) the solutions on the carnations petals in a 3.0 mL/flower volume. After application of the treatment, the carnations were placed in Erlenmeyer flasks with 500 mL water with 0.33 mg/L of active chlorine. The carnations were kept in a room at 20.0 ± 0.7°C and 65-75% RH for up to 12 days.

The experiment was set in a completely randomized design, in a factorial scheme composed by two factors: the first factor the three postharvest solutions [Control (distilled water), HPMC + 20% BW and HPMC + 40% BW] and the second, the five evaluation dates (0, 3, 6, 9, and 12 days). For each combination of factors, four repetitions were used, with three flowers each. The experiment was performed only once.

The accumulated fresh weight percentage (FW%) was obtained by relating the difference between the initial fresh weight of each repetition and the weight obtained at each storage time (0, 3, 6, 9 and 12 days storage), by the use of equation 1:

$$FW(\%) = \left[\frac{(Initialmass - finalmass)}{(Initialmass)}\right] x100$$

The evaluation was carried out with an electronic scale with a measuring range of 0.01-1000 g ± 0.1 g (BEL, Piracicaba, Brazil). The results were expressed as percentage of accumulated weight loss.

The petal's relative water content

(RWC) was evaluated using petals from the 3rd layer, counted from the base. Ten 1 cm² discs were removed, weighed (fresh weight) and immersed in Petri dishes with deionized water for four hours at room temperature for complete hydration. The discs were removed from the water, dried in a paper towel, and weighed (turgid weight), and put in the drying oven at 105°C for 12 h. After the drying time, the discs were placed in a desiccator until they reached room temperature and weighed (dry weight). The RWC was obtained by the Kramer (1983) equation 2:

$$RWC(\%) = \frac{Freshmass - Drymass}{Turgidmass - Drymass} \times 100$$

The percentage of water absorption was assessed by measuring the solution absorbed from the Erlenmeyer flask in each evaluation day compared to the previous evaluation (Imsabai *et al.*, 2013).

The membrane stability index (MSI) was obtained following the methodology of Singh *et al.* (2008). From each carnation flower, five 1 cm² discs were cut from the petals of the 3rd layer, counted from the distal to the proximal region of the flower, and kept in 5 mL deionized water for 3 hours at room temperature. Then, the electrical conductivity of the solution was measured before (value A) and after heating at 100ºC (value B). The MSI was calculated using the equation 3:

$$MSI(\%) = 1 - \frac{ValueA}{ValueB} \times 100$$

The respiratory rate (RR) was obtained by placing the carnation flowers in hermetically sealed plastic containers with a volume of 15 L for 1 hour. A volume of 200 µL of the containers' internal air space and the standards was collected, being injected in a gas chromatograph (Trace GC Ultra, Thermo Scientific) equipped with a flame ionization detector (FID) and a Capillary Porapak N column 1.8 m long. The column temperature was adjusted to 80°C and hydrogen was used as the carrier gas (35 mL/min). The settings used were: oven at 140°C for 8 minutes. After that time, an increase of 20°C every minute until reaching 180°C, remaining at this temperature for 2 minutes to clean the column. Injector: 150°C; detector: 180°C; pressure: 190 kPa (constant) and N_2 flow of 70 mL/min. The results were expressed in mL CO_2/kg/h.

The floral opening was evaluated by the measurements of the diameter of the opened flowers, in the perpendicular direction, with the aid of a Mitutoyo digital caliper and expressed as percentage (compared to the initial diameter).

The vase life was assessed by calculating the number of days from the day when flowers were coated until the day that 50% flowers wilted or had bent-neck (bent-neck angle greater than 45°) (Li *et al.*, 2021).

Statistical analysis

Data were subjected to analysis of variance (ANOVA), using the software Agroestat, version 1.1 (Barbosa & Maldonado Júnior, 2015). The treatments (coatings) were compared by the Tukey test at 5% probability. To evaluate the effect of the coatings over the storage time, we carried out regression analyzes, when the F test was significant for the storage time. Polynomial models were selected, observing the significance of the F test, and the equations were adjusted according to the highest value of R^2.

RESULTS AND DISCUSSION

Figure 1A shows a higher accumulation in the fresh weight of the carnations in the first 3 days, compared to the other storage days. The flowers treated with HPMC + 40% BW resulted in the highest accumulated fresh weight averages and differed statistically from flowers treated with HPMC + 20% BW and the control ($P<0.05$). The vertex (v) of the highest accumulated fresh weight regression equation was 18% at 8.2 days of storage. The carnations treated with HPMC + 20% BW (v = 15.4% at 7.6 days) and those untreated (v = 14.1% at 7.7 days) were similar ($P>0.05$) and presented lower fresh weight over the period. The treatment with HPMC + 40% BW allowed greater accumulation of fresh matter and greter durability under storage conditions. At the end of storage (12th day), the flowers treated with HPMC + 40% BW showed an accumulated fresh weight 30% higher than the HPMC + 20% BW treatment and untreated flowers. The HPMC and beeswax concentrations of the coating must be adjusted considering the surface characteristics of the plant before application. A hydrophobic surface establishes a strong interaction with a hydrophobic material, so the concentration of beeswax should be increased in relation to HPMC and vice versa. In this case, the 40% BW concentration was more efficient than the 20% BW concentration in accumulating fresh mass and increasing the shelf life of carnation flowers.

The volume of the solution is also a factor to be considered at the time of application, as the amount of solution should allow the polymer to anchor and settle accordingly on the plant surface. Pinsetta Junior *et al.* (2019) had observed a reduction of the fresh mass loss of the 'Avalanche' roses treated with the HPMC (5%) and beeswax-based (50%) coatings (3.0 mL coating/stem).

The increase in fresh weight is directly related to a positive balance between water absorption and losses due to transpiration. Water deficit symptoms in many cut flowers, including carnations, are the result of water loss by stomata that gradually exceeds the water uptake rate through the xylem vessels at the stem edges (Van Doorn, 2012; Mattos *et al.*, 2017). A deficit

Table 1. Coatings composition used in the experiment (g/100g on dry basis). Jaboticabal, UNESP, 2022.

Formulation	HPMC	Beeswax	Stearic acid	Glycerol
Distilled water	0	0	0	0
HPMC + 20% BW	50.7	20	4	25.3
HPMC + 40% BW	34.7	40	8	17.3

HPMC= hydroxypropyl methylcellulose; BW= beeswax.

water relationship, i.e., transpiration greater than water absorption leads to cell turgor reduction, one of the factors responsible for vase life reduction in gerbera which presented stem bending (Perik *et al*., 2012).

The relative water content (RWC) of the carnations treated with HPMC and beeswax showed a quadratic trend during storage, with an increase in the RWC values followed by a decrease, while the untreated carnations showed no adjustment (Figure 2A). The carnations RWC showed a similar trend to the fresh weight variable (Figure 1A), with the highest averages obtained in flowers covered with HPMC and beeswax. We highlight the HPMC + 40% BW treatment, which presented higher values and differed significantly (P<0.01) from the other treatments. The equations vertex for the two treatments was verified at 8.6 days for HPMC + 40% BW and 7.6 days HPMC + 20% BW. The average RWC value for the untreated flowers was 73.52%, i.e., 9.5% lower than the highest average obtained by treatment with HPMC + 40% BW and 6.6% lower compared to HPMC + 20% BW.

Coated flowers differed significantly compared to untreated ones (P<0,01) in terms of water absorption. We obtained an increasing linear trend for all treatments (Figure 2B), emphasizing the stems treated with HPMC + 20% BW, followed by treatment with HPMC + 40% BW that absorbed 47% and 19% more water than the untreated flowers, respectively.

The coatings improved the absorbed water maintenance, contributing to the maintenance of plant tissues turgidity in treated flowers, preventing commercial quality loss as verified by higher RWC in stems treated with HPMC + 40%BW.

Fresh weight loss is one of the most important physiological changes in flowers after harvest, leading to quality loss and vase life reduction (Saeed *et al*., 2016). Fresh weight loss is associated with water deficiency stress, resulting in symptoms such as wilted flowers and leaves, and pedicel bending (Mortensen & Gislerød, 2005).

From a physiological-functional point of view, maintaining a positive postharvest water relation is a critical aspect of the cut flowers vase life. In a system where water loss occurs by transpiration, through the leaf surface, alternatives to keep the water balance must be applied to compensate the absence of root system (Singh & Moore, 1992).

Based on our results, we infer that HPMC and beeswax-coating applied to carnations minimized transpiration by a film formation that partially blocked the stomata and micropores, becoming a semipermeable barrier to gas exchange (Assis & Britto, 2014; Oliveira *et al*., 2018). These barriers formed on the petal surface reduce the vapor pressure difference between the leaf mesophyll and the environment, resulting in greater water retention inside the plant for growth use. The same behavior was observed by Mujaffar & Sankat (2003), studying carnauba wax-based coating on roses, where it was found that the coating minimized transpiration, reducing the respiratory rate, and wilting.

The increase in beeswax concentration in the coating reduced the fresh weight loss of the carnations during storage by the same proportion. In other words, the coating with 40% beeswax allowed greater weight gain, the desired result and is considered one of the most important for maintaining commercial quality (Figure 1A). According to Oliveira *et al*. (2018), the beeswax

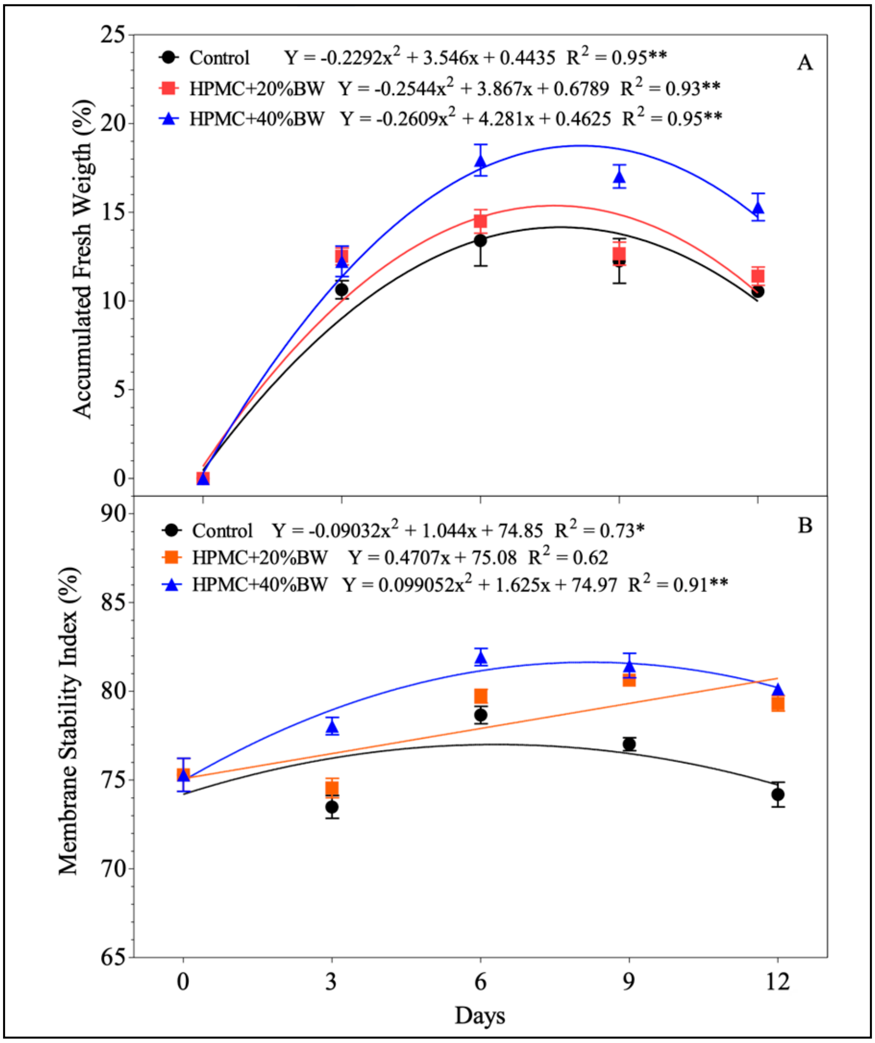

Figure 1. Accumulated fresh weight (A) and membrane stability index (B), in percentage, of carnations coated with hydroxypropyl methylcellulose (HPMC) and beeswax (BW) stored at 20ºC and 65-75% RH for 12 days. Bars indicate standard deviation (n=3). Jaboticabal, UNESP, 2022.

added to the coating has a hydrophobic characteristic, preventing the water vapor exchange by the coating, which reduces the plant fresh weight loss. However, Formiga *et al*. (2019) found that the increase in wax proportion on the coating did not alter the accumulated fresh weight loss of guavas during storage. Thus, it is possible to infer that vegetable surface morphology (presence of trichomes, thickness, and type of cuticle, stomata number, lenticels) and the physical properties of the coating, such as surface tension and viscosity, strongly influence the coated fruit mass transfer (Hagenmaier *et al*., 1993; Navarro-Tarazaga *et al*., 2011).

This research corroborates with other cut flowers studies that report dehydration as the main leading factor to flowers and leaves deterioration. In a study carried out with two gerbera cultivars (*Gerbera jamesonii*), it was found that the cultivar Bayadère, which presented the longest vase life, was also the one that was able to maintain an adequate hydration condition for the longest time, with fresh weight maintenance (Shabanian *et al*., 2018).

Although the coated carnations flowers presented a similar (P>0.05) respiratory rate compared to the untreated flowers, presenting an average of 209±3 mg of CO_2/kg/h and a reduction in the flower respiratory rate from 225.71 to 209.49 mg of CO_2/kg/h (data not shown), this remained higher than that obtained by Matsushima *et al*. (1999) whose work carried out with two carnations cultivars presented a reduction in respiratory rate near to 50% at 12 days of storage.

A remarked reduction in respiration indicates the consumption of energy reserves prematurely, leading the respiratory rate to decrease until the vegetable senescence. Therefore, maintaining the respiratory rate at adequate levels means maintaining the plant's metabolism, delaying senescence, and extending commercial availability. When subjected to stressful situations, vegetables can accumulate free radicals that cause the peroxidation of lipids constituting cell membranes and, consequently, the leakage of

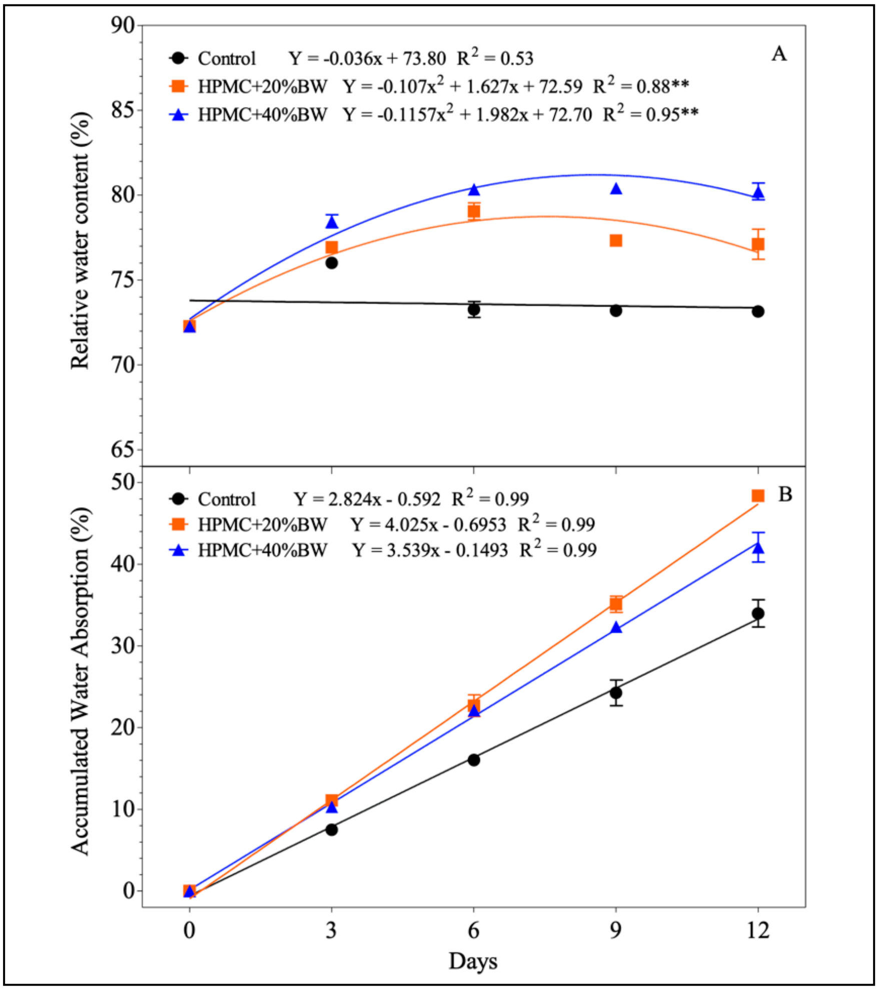

Figure 2. Relative water content (A) and accumulated water absorption (B), in percentage, of carnations coated with hydroxypropyl methylcellulose (HPMC) and beeswax (BW), stored at 20ºC and 65-75% RH, for 12 days. Bars indicate standard deviation (n=3). Jaboticabal, UNESP, 2022.

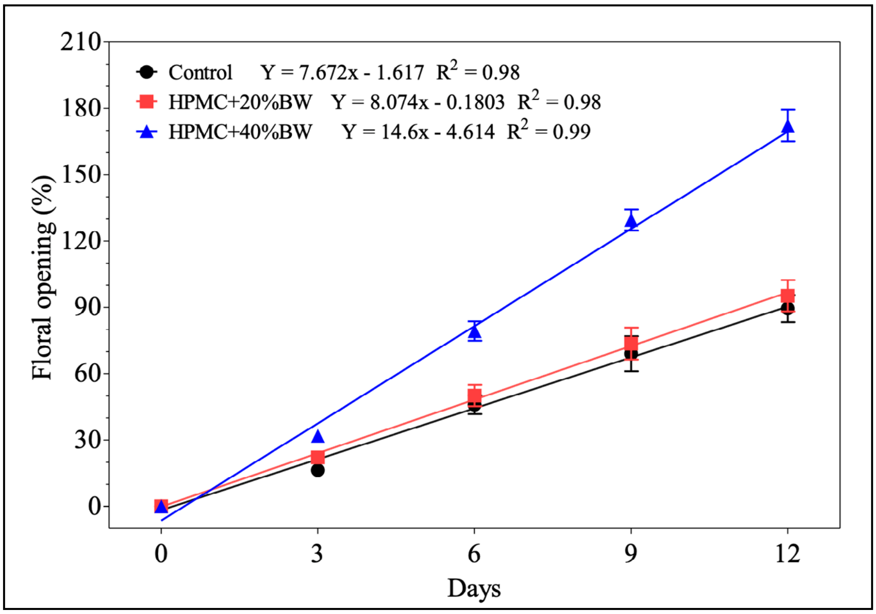

Figure 3. Floral opening, in percentage, of carnations coated with hydroxypropyl methylcellulose (HPMC) and beeswax (BW), stored at 20ºC and 65-75% RH, for 12 days. Bars indicate standard deviation (n=3). Jaboticabal, UNESP, 2022.

the electrolytes as a result of their degradation (Sen & Alikamanoglu, 2013).

The carnation flowers covered with HPMC + 20% BW and HPMC + 40% BW presented mean values of Membrane Stability Index (MSI) of 77.81% and 78.42%, respectively, and differed significantly (P<0.05) compared to untreated flowers.

The MSI values of untreated carnations showed a regular trend over the storage period, with an average of 76.46%. The treatments with HPMC and beeswax showed an increase in the MSI over the period (Figure 1B). The equation vertex for the HPMC + 40% BW treatment was at 8.2 days with an MSI of 82%, which is in line with that obtained for the fresh weight percentage (Figure 1A) and the RWC (Figure 2A).

Vegetables with a positive water balance have a higher stress tolerance and, consequently, greater membrane stability, as can be evidenced by the results of MSI and RWC. Our result corroborates with that obtained by Slabbert & Krüger (2014) when studying amaranth leaves under water stress, which found a lower MSI for water-stressed leaves. In general, senescence of ethylene-sensitive flowers, such as carnations, is associated with membrane integrity loss, increased respiration, and ethylene production (Ebrahimzadeh *et al*., 2008). The maintenance of the vegetable turgidity and, a high MSI can lead to delayed senescence. Therefore, increased cut flowers vase life.

Thus, it is observed that among the fundamental parameters for maintaining the carnations quality, the fresh weight, RWC, and MSI presented their highest values between 8.2 to 8.6 days, for carnations treated with HPMC + 40% BW. That means, this treatment conferred the best water balance compared to the others. Despite the lower water absorption by the stems compared to 20% BW treatment, the greater amount of wax present in the HPMC + 40% BW treatment enabled greater water retention of the absorbed water by the cells and petal tissues, a physiologically favorable condition which provided at the end a greater floral opening and greater maintenance of decorative and commercial quality.

There is a directly proportional relationship between the storage time and the floral opening, for all treatments, emphasizing the treatment with HPMC + 40% BW, which obtained the highest averages for the floral opening in the entire studied period (Figure 3). The carnations treated with HPMC + 40% BW differed statistically from the others (P<0.01) and, at the end of the evaluation period, representing an increase of 80% compared to untreated flowers and the HPMC + 20% BW, in the total opened flowers.

At the end of 12 days of storage, 86.5% of the total carnations treated with the HPMC + 40% BW solution maintained the quality considered decorative and commercial, while untreated flowers and HPMC + 20% BW treatments presented values much lower, with only 45% and 50%, respectively, of commercially viable flowers (Figure 4).

The decorative quality is one of the main factors in the product ornamental value loss, which characterizes the end of its vase life (Boxriker *et al*., 2017). The coating application on 'Delphi' carnations resulted in vase life of 10, 12, and 15 days for untreated flowers, HPMC + 20% BW and HPMC + 40% BW treatments, respectively. Thus, the treatment with a higher beeswax concentration allowed an extension in the vase life of 5 days, compared to untreated flowers, representing a longer commercialization period and postharvest loss reduction. In our study, we showed that the coated carnation flower with HPMC + 40%BW presented a longer vase life.

Thus, the natural coating based on hydroxypropyl methylcellulose (HPMC) and beeswax (BW) at 40% applied on cut carnation cv. Delphi promoted a positive water balance and cellular membrane integrity, resulting in greater floral opening, longevity, and quality, allowing a 5 days increase in vase life.

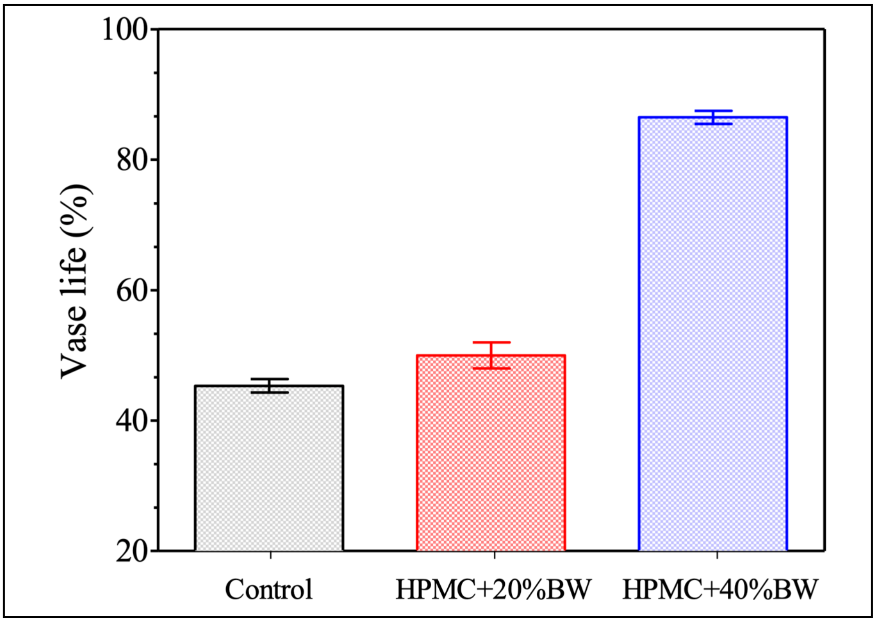

Figure 4. Vase life, in percentage, of carnations coated with hydroxypropyl methylcellulose (HPMC) and beeswax (BW), stored at 20ºC and 65-75% RH, for 12 days. Bars indicate standard deviation (n = 3). Jaboticabal, UNESP, 2022.

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