Effect of a gelatin-based edible coating containing cellulose nanocrystals (CNC) on the quality and nutrient retention of fresh strawberries during storage

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Abstract. Strawberry is a non-climacteric fruit with a very short postharvest shelf-life. Loss of quality in this fruit is mostly due to its relatively high metabolic activity and sensitivity to fungal decay, meanly grey mold (Botrytis cinerea). In this study, the ability of gelatin coatings containing cellulose nanocrystals (CNC) to extend the shelf-life of strawberry fruit (Fragaria ananassa) over 8 days were studied. The filmogenic solution was obtained by the hydration of 5 g of gelatin (GEL) in 100 mL of distillated water containing different amounts of CNC dispersion (10 mg CNC/g of GEL or 50 mg of CNC/g of GEL) for 1 hour at room temperature. After this period, the solution was heated to 70 ºC and maintained at this temperature for 10 minutes. The plasticizer (glycerol) (10g/100g of the GEL) was then added with constant, gentle stirring in order to avoid forming air bubbles and also to avoid gelatin denaturation until complete homogenization. Strawberries (purchased at the local market) were immersed in the filmogenic solution for 1 minute and after coated were dried at 15 ºC by 24 hours. The strawberries were then kept under refrigeration and characterized in terms of their properties (weight loss, ascorbic acid content, titratable acidity, water content). The results have shown that samples covered with GEL/CNC had a significant improvement in its shelf-life. For instance, for the control sample (without coating) the weight loss after 8 days of storage was around 65%, while covered samples loss in the range of 31-36%. Edible coating was also effective in the retention of ascorbic acid (AA) in the strawberries, while control sample presented a fast decay in the AA content, covered samples showed a slow decay in the AA concentration. Moreover, the use of GEL/CNC edible coating had an antimicrobial effect in the fruits.

1. Introduction

In the last two decades, many studies were presented in scientific literature, using edible films, and coatings to extend shelf life and enhance the quality of fresh, frozen and processed food. A variety of polysaccharides, proteins and lipids were used either singularly or combined to produce compound films (KESTER, FENNEMA, [1]).

Edible coating can be defined as a layer of edible material formed around food or placed between its components (KROCHTA, MULDER-JOHNSTON, [2]). According to Krochta et al. (1994), edible films are different from edible coatings for being formed before its application on the product, whereas the coatings are formed during their application [3].

This technology is used to inhibit the migration of moisture, oxygen, carbon dioxide, aroma, lipids and can also introduce additives to food, such as antioxidants, antimicrobial agents and aroma, enhancing the mechanical integrity and/or the handling characteristics of a food product (KROCHTA, JOHNSTON, [2]). Many deteriorating chemical and enzymatic reactions such as lipid oxidation, browning due to Maillard reactions and enzymatic browning, are directly influenced by humidity and water activity on food, which cause changes in color, odor, texture and nutritional value of food.

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Consequently, in order to extend the storage period of many different kinds of food, it is necessary to slow the migration of moisture (KESTER, FENNEMA,1989a [4]).

The permeability to gases like oxygen and carbonic gas can also influence, in a significant way, food stability during the storage period. A primary way of deterioration due to oxygen permeability, which occurs in many food products involves the oxidation of lipids, vitamins, composite of flavor and pigments (KESTER, FENNEMA b [5]).

The ripening of the fruit is accompanied by a series of physical and biochemical processes, which result in synthesis and degradation of pigments, conversion of starch into sugar, loss of firmness, production of volatiles and increase of respiration of climacteric fruits (Andrews and Li [6]). Physico-chemical parameters and sensorial alterations are usually employed to accompany the biochemistry of ripening or to evaluate the processes of preservation.

Recent studies are exploring the potential of surface edible coatings to maintain and extend the quality and the shelf-life of fresh products, as well as to reduce the quantity of non-degradable disposable packaging. Among the studied fruits are: bananas (Banks [7], citric fruits (Hagenmaier and Baker [8]), apples (Banks et al. [9]), mangoes (Dhalla and Hanson [10]), pears (Amarante et al. [11]), avocados (Johnston and Banks [12]), strawberries (Garcia et al. [13] [14], Tanada-Palmu [15] and guavas (Fakhouri and Grosso, [16], Oliveira and Cereda [17], Tanada-Palmu et al. [18]).

The use of surface coatings can exert effects similar to the storage under controlled atmosphere. Usually, the effect of reducing the quantity of O2 and elevating the content of CO2 over the respiration rate, as well as the synthesis of C2H4, are pointed out as the primary causes for the beneficial effects of coatings on the storage of fruits and vegetables (Kader [19]). The coatings exert effect on the permeability of the peel to gases by blocking the pores on the surface, in greater or smaller proportions.

The modification of the internal atmosphere of the fruits and leafy vegetables, obtained from the coatings, depends on the permeability of the film, its thickness and the covering on the surface of the fruit, (Cisneros – Zevallos and Krochta [20]). Besides, the permeability throughout the coating and the film is influenced by several factors, including morphology, density, chemical structure and polymeric orientation. The kind of solvent, the plastifier and the drying rate also influence the coefficient of permeability (Mehugh et al. [21]).

In the present work, were developed biofilms formed from filmogenic solutions of gelatin and nanocrystals. The filmogenic solutions were later applied as coatings in strawberries, which is a fragile and of ripening fruit and also a highly nutritious food (Poling [22]). In addition, this fruit was chosen due to its (i) easiness of handling and storage (due to its reduced volume); (ii) wide surface of transpiration in relation to its weight; (iii) frailty; and (iv) the strawberry does not have a protective membrane, so the use of coatings would make difficult the loss of water, noting that it possesses 85% of water in its composition.

2. Material and Methods

2.1 Materials

Gelatin, the biopolymer used to obtain the coatings, was supplied by Leiner Davis Gelatin, Cellulose nano crystals (CNC) were produced from eucalyptus fiber and glycerol (Synth) was used as plastifier. The strawberries used were supplied by a small-scale producer in the borough of Dourados-MS, Brazil.

2.2 Methods

2.2.1. Production of the filmogenic solution:
The filmogenic solution of gelatin was elaborated through the hydration of 10g of gelatin in 100 mL of distilled water with 0, 1 and 5g of CNC/ 100 g GEL. The mixture was heated at 70°C for 10 minutes. Afterwards, the glycerol was added under smooth mechanical agitation (10g/100 g ).
Production of CNC from eucalyptus fibers: The material has been provided in milled form.

i) Step 1: 10 grams of fibers have been submerged in 200 mL of NaOH solution (10% w/w) for a period of 4 hours at 60°C. After this period, the solution was filtered and washed until a pH of 7.0 was achieved. Then the material was dried at 40°C.

ii) Step 2: 300 mL of a combined solution of NaOH (5% w/w) + CaO(Cl)\textsubscript{2} (2.5% w/w) was added to the dried fiber obtained in step 1. The material was submitted to a rest of 4 hours at 45°C. After this period, the solution was filtered and washed until a pH of 7.0. Then the material was dried at 40°C.

iii) Step 3: The extraction of the CNC was done using the treated fiber material. For that, the fiber obtained from step 2 was treated with 250 ml of sulphuric acid 60% for 30 minutes at 55°C under vigorous stirring. After 30 minutes, hydrolysis reaction was stopped and the sample was centrifuged until the CNC were in suspension (Figure 1).

iv) Step 4: The suspended solution went through dialysis for 72 hours.

2.2.2. Strawberry coating:
For the application of edible coatings, strawberries were picked ripe and immediately transported to the department of Food Technology Department of FEA/UNICAMP. The strawberries were then selected as to for a homogeneous lot. Afterwards, were hygienized with sodium hypochlorite solution and then submitted to the recoating with filmogenic solutions. Then four lots were formed: (i) LC – control lot (without coating), (ii) AC1 – strawberries coated with filmogenic solution containing 1% of nanocrystals, (iii) AC2 – strawberries coated with 5% of nanocrystals. All the dried strawberries were dried for 12 hours at 25°C.

2.2.3. Physical Chemical characteristics

2.2.3.1 Mass Loss
Strawberries were weighed in semi-analytical weighing machines

2.2.3.2. Soluble solids, pH, Total Titrable Acidity, Humidity and Total Solids
Determined by the utilization of AOAC Methods, 1997

2.4. Microorganism grown.
The dilution plating was used to detect yeasts and molds in the strawberries. This method provides maximum exposure of the cells to atmospheric oxygen and allows greater uniformity in the development of the colonies increasing accuracy of the results and facilitating the subsequent isolation of pure cultures.

2.5. Transmission electron microscopy.
The shape and dimensions of the CNC were assessed through transmission electron microscopy (TEM). To perform this, a drop of the nanocrystal suspension to be analyzed was diluted in 2 ml of iso-propyl alcohol and placed in an ultrasonic bath (UltraCleaner 1600 A) for 10 minutes. Then, the alcoholic dispersion was deposited on a special grade for 24 hs. After, each film was covered with a 2% solution of uranyl acetate (for coloring) and dried again at room temperature. The images were obtained using a machine model JEM-1011 TEM.

2.6. Statistical Analysis
Made using Statistica® 5.5 (Stasoft, USA).

3. Results and Discussion
All the filmogenic solutions presented themselves transparent and homogeneous. The filmogenic solution obtained presented nanocrystals in solution (Figure 1).
Visually the coatings adhered uniformly to the fruits, presenting a natural bright, without rupture zones. Moreover, the use of coating kept the natural flavor of fruits. The appearance of fruits can be seen in Table 1.

**Table 1.** Images of strawberries (coated and control) during storage

| Storage time (days) | Control | 1% CNC | 5% CNC |
|--------------------|---------|--------|--------|
| 1                  | ![Image](image1) | ![Image](image2) | ![Image](image3) |
| 8                  | ![Image](image4) | ![Image](image5) | ![Image](image6) |

In all coated fruits, the weight loss was lower than in control fruits (Figure 2). While control fruits lost 65.8 % of total weight, the maximum loss in coated fruits was 35%, observed in trial 3, coated with films based on gelatin and CNC. On the control fruit, the loss of 35% was observed on the 5th day of storage.
4. Conclusion

The ability of gelatin containing CNC coatings to extend the shelf-life of strawberry fruit (Fragaria ananassa) were studied, mainly for industrial applications. The coating was effective in protect fresh strawberries during the storage period, the aspect of coated fruits was uniform aspect. This type of treatment could be used to extend the conservation period of strawberries to support long periods of time between the fruit harvest and consumer.

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