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Improvement of quality, sensory properties and shelf life of fresh cut oranges by using a bilayer cocoa-sodium alginate coating

RUNNING TITLE: IMPROVEMENT OF QUALITY OF FRESH CUT ORANGES BY USING A BILAYER COATING

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Abstract

BACKGROUND

Fresh-cut products are ready-to-use goods which retain the fresh characteristics of raw produce. However, numerous factors restrict the quality and shelf-life of fresh-cut products. One of the most promising, convenient and safe technologies to preserve the quality and to prolong the shelf-life of fresh fruits and vegetables is the application of edible coatings.

RESULTS

The aim of this study was to investigate the effects of different coatings (alginate-based, cocoa-based and a combination of them) on physico-chemical, microbiological and sensory characteristics of fresh-cut oranges during storage. Preliminary rheological analyses were performed on coatings in order to characterize them. The three different coated orange samples were packaged in polyethylene terephthalate trays under atmospheric conditions and stored for 10 days at 6°C. During storage, all samples were analysed for water activity, moisture, colour, texture, microbiological analyses and sensory quality. Orange samples coated with sodium alginate maintained the highest quality characteristics in terms of texture and microbiological properties, but not from a sensory point of view. Samples coated only with cocoa presented very high sensory attributes, but the lowest microbiological and textural quality. Samples covered in both alginate and cocoa demonstrated the best quality parameters throughout the whole storage period, including high sensory characteristics and the lowest microbiological cell loads (yeast and mesophilic aerobic bacteria under the threshold limit of 6.0 log cfu/g).

CONCLUSIONS

The bilayer coating represented the best solution in order to develop a new ready to eat fresh oranges with both high textural and sensory attributes and prolonged shelf life

Keywords: edible coating, double coating, cocoa, alginate, oranges, rheology, shelf-life.

INTRODUCTION

Fresh-cut products are ready-to-use goods which retain the fresh characteristics of raw produce.[1] Raw and minimally-processed fruits and vegetables are sold to consumers in a ready-to-use or ready-to-eat form. This type of product does not generally contain preservatives or antimicrobial substances and rarely undergoes any heat processing before consumption.[2] The food market evolves with new products and changing trends, but fresh-cut ones remain at the top of the list of products that meet the needs of many consumers.[3] However, numerous factors restrict the quality and shelf-life of fresh-cut products. During storage, in fact, they undergo significant deteriorations and as a consequence decay of their sensory (e.g. flavour, colour, texture) and nutritional value. Water loss is the primary factor that involves deterioration of fruits and vegetables and may result in soft texture, translucency and loss of nutritional value and sensory attributes.[4,5] Researches aimed to retard the quality loss of fresh-cut fruits, maintaining their safety in terms of microbial growth are of great interest for companies involved in their production and distribution .[6,7] Different approaches have been employed to preserve fresh-cut product quality during shelf-life, including modified atmosphere packaging, chemical treatments such as calcium dips and physical treatments such as gamma irradiation, pulse light, ozone, cold plasma, and high pressure pre-treatment combined with vacuum impregnation.[8,9,10] One of the most promising, convenient and safe technologies to preserve the quality and to prolong the shelf-life of fresh fruits and vegetables is the application of edible coatings.[11] These coatings are formed from a suspension of a thickening agent, which after application on the product forms a film that acts as a barrier to gas exchange and water loss by modifying the atmosphere and slowing fruit ripening.[12] Edible coatings can be classified in three categories with regards to the nature of their components: polysaccharides (alginate, gellan, etc…), lipids (paraffin, beeswax, chocolate, etc..), proteins (corn zein, wheat gluten, etc…) and composites, made up from combining substance from previous categories (e.g. gelatin and fatty acids, methylcellulose and fatty acids, etc.). [13]In recent years researches are focused on the application of alginate as edible coating on fresh-cut fruits, for its characteristics of good transparency and resistance to gas exchange. [12]A lot of studies have been performed on the influence of alginate coating alone or in combination with other substances on the chemico-physical and microbiological characteristics of different type of fresh-cut fruit, such as pineapple,[14] pears,[15] apples,[16,17,18] mango, [19]papaya,[20] tomato fruit, [21] melon[22,23] and blueberries.[24]

However, to our knowledge, no studies have been performed on the influence of edible coating alginate on the physico-chemical, sensory and microbiological properties of fresh cut orange products. Fresh cut oranges were selected as samples to cover, considering their high nutritional values and quality that undergoes to significative loss, during storage, because of their sensitive to microbiological growth, water loss and to low temperature, especially after cutting. For this reason, in the first part of this study, the influence of a sodium alginate-based coatings on the main quality characteristics of orange fresh-cut products is evaluated. However, the application of coatings without compromising sensory attributes of fresh-cut fruits is not always achieved, and therefore needs further studies.[25] Moreover, companies requested innovative products, with improved sensorial characteristics, maintaining high quality parameters for longer storage times, for this reason, in addition, cocoa as an alternative edible coating was examined. To our knowledge, few studies were focused on the possibility to use cocoa based coatings, normally used in the bakery and confectionery industries, to preserve fresh cut fruits. In the 1988, Biquet and Labuza[26] performed a research with the purpose to evaluate the moisture permeability properties of a cocoa coating without any kind of applications on food system. Recently Khan[27] et al., and Meza et al. 2018[28] reported some studies respectively focused on the efficacy and the deposition behaviour of a cocoa coating applied by electrospraying, and on its rheological and adsorption properties. Only two works deal with the application of cocoa coatings on fruits products, where in the first one Gounga et al. 2008,[29] applied two cocoa coatings on dried chestnut and analysed them for nutritional and microbiological properties only after covering. While Glicerina et al., 2019[30] applied two cocoa based coating on fresh cut fruits (apples and grapes) and their influence on the main quality characteristics, during storage, was evaluated. However, obtained results showed that in comparison to uncoated samples, cocoa based coatings gave a positive effect only on the sensory properties of fresh cut fruit samples during storage, showing a shelf-life very similar to uncoated ones; even if grapes maintained better quality properties than apples probably because the presence of their natural skin that promoted a protective effect.

The absence of an intermediate coating between fruit and cocoa did not contribute to extend the shelf life; to fill the mentioned gap in the present research two layers of coating were used. For this reason, in this study, the effects of different coatings, alginate-based, cocoa-based and a combination of them (as double coating), on the physico-chemical, microbiological and sensory characteristics of fresh-cut oranges during storage were evaluated.

MATERIALS AND METHODS

Raw materials

Fresh-cut oranges were obtained from the consortium Agribologna (Bologna). The variety of orange was *Navel*. The oranges had a refractive index of 11.3 °Brix and an acidity of 0.83 ml/ 100 ml citric acid. Two types of coatings were employed to cover fresh-cut orange samples; one sodium alginate based, the other cocoa based. The formulations of sodium alginate coating, obtained in laboratory by adding calcium ascorbate and solved in distilled water, and that made with a commercial cocoa realized with Cocoa Butter substitutes (CBS) are reported in Table 1. The sodium alginate formulation was realized according to Zhong et al.,[31] and Fu et al.,.[32]

Sample preparation

The orange fruits were manually peeled and subsequently obtained slices were separated and subjected to a perossiacetic acid solution dip (200 ppm) to prevent microbial contamination. After drying at 4°C for 10 minutes, slices were divided in three parts of irregular shape. Subsequently, the orange pieces were covered with the different coatings: cocoa cream (Co), sodium alginate (Al) and both combined (Co+Al). This last coating type were made by covering each orange slice with a first layer of alginate and after drying (at 4°C for30 minutes) a second one of cocoa cream, preliminary obtained by melting dark chocolate substitute in a microwave at 750 watts for 1 minute. These conditions were chosen after preliminary trials, in order to avoid modification in the product structure, in accordance with Stortz & Marangoni[33] and Glicerina et al.,[34] 2 grams of sodium alginate solution plus 2 g of melted cocoa coating were used to cover each piece of fruit. In all coated fruits the single coating layer had a thickness of 1.0 ± 0.1 mm. Each orange piece was completely dipped in each coating type, removed with tweezers and left to dry in a cold room at 4°C for 15 minutes. After cooling, approximately 100 g of each orange sample was packed in different PET trays closed with polypropylene (PP) film at medium barrier to oxygen. In specific, the PET tray had a thickness of 3 mm, an oxygen transmission rate (OTR) of 60 cm3/m2/day/atm and a water transmission rate of 27 cm3/m2/ day/atm, while the PP film presented a thickness of 30 µm and an oxygen and a water transmission rate respectively of 860 cm3/m2/ day/atm and 19 cm2/m2/ day/atm, in both cases measured at 23°C. All samples were stored at 6°C for 10 days. Three trays of each sample were analysed at six different times: after 0 (T0), 1 (T1), 2 (T2), 3 (T3), 6 (T6) and 8 (T8) days, chosen after preliminary trials. Control sample was represented by orange pieces without coating, processed and stored at the same conditions. Samples were named: Orange C, control without coating; Orange Al, coated with sodium alginate; Orange Co, coated with cocoa cream; and Orange Al+Co, coated with double layer of sodium alginate and cocoa cream.

METHODS

Rheological analysis on fruit coatings

Rheological measurements were carried out on both cocoa and alginate coatings in order to characterize them. Measurements were performed at 40 °C using a controlled stress–strain rheometer (MCR 300, Physica/Anton Paar, Ostfildern, Germany) equipped with a system of coaxial cylinders (CC27). The rheological behaviour of coatings was analysed in steady state conditions. After a pre-shearing of 500 s at 5 s^{-1}, viscosity was measured by increasing shear rate from 2 to 50 s^{-1} within 180 s and taking 18 measurements at different points.[35] The obtained flow curves, on the basis of preliminary trials, were fitted according to the Casson model that showed the best fit, compared against other rheological models. The Casson model is a structure-based model derived from the analysis of a structure and its kinetic change, usually employed to study the rheological behaviour of food matrices characterized by the presence of a yield stress.[36,37]

This model is described by the following equation:

$$\tau^{0.5} = \tau_0^{0.5} + \eta_{PL}\gamma^{0.5} \qquad (1)$$

where τ_0 is the yield stress at the zero point and η_{PL} is the so-called plastic viscosity [34]

Moisture and Water Activity

Moisture content and water activity were determined at each storage time, separately on the fruit samples and cocoa coatings (after removing it from each fruit piece), in order to evaluate possible migration phenomena between them. In samples coated with sodium alginate analyses were performed on the whole fruit because of it was not possible to separate the coating from the fruit pieces because of the high tackiness of the sodium alginate. Moisture content was determined gravimetrically by difference in weight before and after oven drying at 70 °C, until constant weight was reached. The moisture content was calculated as follows:

$$\text{Moisture content } \% = 100-((W_f-W_d/W_f) * 100)$$

W_f= weight fresh sample

W_d= weight dry sample

The water activity values of ground fruits and cocoa coatings were obtained by using a dew point hygrometer, AcquaLab-Water Activity Meter (mod. SERIES 3TE. Decagon Device, Inc., Nelson Court, NE). Three measurements were carried out from each sample (Orange C, Orange Al, Orange Co, Orange Al+Co) and their respective cocoa coating, when present, after separation from fruit pieces, at each storage interval for both moisture and water activity analyses.

Textural analysis

Evaluation of firmness and fracturability was conducted with a penetration test by means of a Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, Godalming, UK), equipped with a 5 Kg load cell and a 6 mm diameter stainless steel probe. Test speed was 1 mm s^{-1} with a 6 mm depth of penetration.[38] The maximum peak of the curve, obtained during penetration, was used as the firmness value F (N). The distance between the origin of curve till the point until the end of the penetration, is an index of the fracturability (N*s/mm). Results were expressed as the average of ten measurements for each sample.

Microbiological analysis

Microbiological analyses were performed after 1, 3, 6 and 9 days of storage. For each type of sample, 30 g of whole product were placed in sterile bags and with 60 mL of sterile saline solution (0.9% NaCl) and subsequently homogenized for 2 minutes in Stomacher (model Lab BlenderSeward, London, UK). Subsequently, the samples were serial diluted into sterile physiological solution according to the expected microbial cell loads of the samples. The total loads of lactic bacteria, yeasts, molds, total mesophilic aerobic bacteria, total psychrotrophic aerobic bacteria and total coliforms were determined. In particular, yeasts were counted on Yeast extract Peptone Dextrose medium (YPD) (Oxoid Ltd, Basingstoke, United Kingdom), total coliforms on Violet Red Bile Agar (Oxoid Ltd, Basingstoke, United Kingdom), lactic acid bacteria on De Man Rogosa and Sharpe (MRS) (Oxoid Ltd, Basingstoke, United Kingdom), total aerobic mesophilic and psychrotrophic bacteria on Plate Count Agar (PCA) (Oxoid Ltd, Basingstoke, United Kingdom) and mold on Malt Extract Agar (MEA) (Oxoid Ltd, Basingstoke, United Kingdom). In particular, yeasts, molds and total aerobic mesophils were incubated at 30 °C for 48h, total coliforms and lactic bacteria were incubated at 37 °C for 24h, while psychrotrophic aerobic bacteria were incubate at 10 °C for 7 days.

Sensory analysis

A panel composed by 80 tasters (female and male, aged from 25 to 52 years) was asked to evaluate the four samples and to rate their preference using a 9-point hedonic scale (1 = extremely dislike; 9 = extremely like),[39] immediately after treatment (T0) and after 3 days of storage. The others storage times were not considered due to the limit of microbiological acceptability, according to microbiological analysis results. The attributes rated were: external and inner appearance, smell, firmness, flavour and overall acceptability. The test was performed in laboratory scale and conducted in individual booths.[40] Orange samples were served to the panellist in a randomized order.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and the test of mean comparisons according to Fisher's least significant difference (LSD) with a 0.05 level of significance were applied to find out significant differences among the different samples. The statistical package STSG Statistica for Windows, version 6.0 (Statsoft Inc., Tulsa, OK, USA) was used.

RESULTS AND DISCUSSION

Rheological characteristics of coatings

In Figure 1 the flow curves of the two different edible coatings, sodium alginate and cocoa, are showed. In both samples, apparent viscosity decreases with the increase of the shear rate, indicating pseudoplasticity. This behaviour can be explained by the structural breakdown of the molecules due to the hydrodynamic forces generated and to the increased alignment of the constituent molecules.[41] Alginate coating presents the highest viscosity, with initial values around 50.000 mPa, cocoa coating had an initial apparent viscosity values around 5.000 mPa. Moreover, in order to better explain the rheological values obtained by the flow curves, the Casson yield value and the Casson plastic viscosity parameters were calculated applying the Casson model, results are reported in Table 2. All data were well fitted by the Casson model, providing high determination coefficients (R^2), comprising between 0.98 and 0.99. Alginate coating presented highest yield stress and viscosity values compared to cocoa one, underlining how the amount of energy needed to start flow was the highest in the former. Sodium alginate is made up of d-mannuronic and l-guluronic acids and contains numerous hydrophilic molecular groups.[42, 31]When water is added to sodium alginate, strong bonds between molecules are created, giving arise to a tighter and more compact structure. High sodium alginate viscosity values may also be achieved through the addition of calcium ascorbate which, as has been demonstrated previously[32], causes high matrix aggregation due to its crosslinking effects that strengthen alginate solution bonds. Nevertheless, Skurtys et al., [13] and Zhong et al.[31] have shown that sodium alginate solutions with high viscosities, such as those observed in their study (around 100 mPa*s), may be appropriate for its use in edible coatings, especially if applied by a dipping method; while those with low viscosity can provide processing advantage during spraying methods. Cocoa coating showed lower yield stress and viscosity values than alginate one, this can be attributed to its lipid- based formulation.[43] However, according with literature,[44,28] the yield stress and viscosity values of the used cocoa coating make them suitable for coating purposes, being high enough to prevent gravity effects (sagging and dripping) but sufficiently low to allow capillarity-driven levelling.

Moisture and Water Activity

In Figure 2 moisture changes during the storage of coated fresh-cut orange samples as well as the Co and Co+Al cocoa coatings alone (after removal from fruit pieces) are shown. In both mono and bilayer alginate covered fruits, a constant trend with a slight reduction in moisture content at the end of storage was observed, while in C and Co samples a more pronounced moisture reduction was highlighted during storage. Orange samples Al maintained the highest moisture content during the entire storage period. This behaviour is probably due to the water barrier effect induced by sodium alginate that limited the water migration, keeping the fruit pieces more hydrated than in the other samples.[4,24] The calcium ascorbate, present in the sodium alginate coating formulations, caused a molecular cross-linking effect, thereby strengthening the chemical bonds among sodium alginate components and further promoting the water migration barrier effect of the coating.[45] The sodium alginate water barrier effect, which has also been reported in the studies of Meza et al.,[28] is underscored by the fact that the Orange Co sample underwent the highest moisture loss during storage, that was parallel to an increase in the moisture content of its cocoa coating (Coating Co).

This behaviour, according with Johansoon and Bergensthal,[46] can be probably attributed to a water exchange between the fruits and the cocoa characterized by different water amount. Furthermore (Figure 2) a similar behaviour was observed in Orange Co+Al, even if with lower intensity, thereby confirming the role of sodium alginate as moisture barrier. The control sample Orange C lost more water compared to samples Al and Co+Al. [47] but less than sample Orange Co, having only the fruit's natural skin as barrier against dehydration.

In Figure 3 results related to the water activity changes in fresh-cut orange samples and in the different cocoa coatings after their removal at each storage time are reported. Also in this case a reduction in water activity values was observed for all samples during storage. This reduction was parallel to an increase in the respective cocoa coating of samples Al+Co and Co, as previous observed for moisture. This trend may be a further confirmation of water exchange between fruit and coating, as previously seen in moisture results, and also of the barrier effect conferred by sodium alginate coating. Moreover, water activity results, showed how Al and Co+Al samples had the lowest a_w values compared to C and Co orange ones. These low values can be probably attributed to a water binding stronger in Al and Co+Al coatings than Co one, in fact Al and Co+Al coating formulations were rich in sodium alginate that promoted hydrogen bonds.[48]

Textural properties

In the Figures 4 and 5 the firmness and fracturability results of orange samples during storage are shown respectively. Coated Al sample presented the highest firmness values compared to the other ones, showing an increase of this parameter during storage. High firmness values in samples fruit coated with sodium alginate (Orange Al), can be attributed to the alginate network structure.[24] Uncoated samples firmness values were intermediate between Al and samples coated with Co (Orange Co+Al and Orange Co), showing a constant trend during all storage times. For what concern Co+Al and Co samples, lower firmness values were observed compared to Al and C ones, this trend can be probably attribute in part to the moisture exchange occurred between cocoa coatings and fruit and between coating and the surrounding environment as previous stated in the moisture section, that involved a softening effect. Moreover, a further softening effect can be attributed to the presence of fat in the cocoa coating formulation.

For what concern fracturability (Fig. 5), Al sample showed the statistically highest values compared to other samples at each storage time, while sample Co+Al presented intermediate values between Al and the other two samples (C and Co), even if not always statistically different from them. These values are a further confirmation of the barrier effect conferred by the sodium alginate coating, that maintained orange pieces more hydrated and more structured. Moreover, according with literature (Tapia et al., 2008)[16] the presence of Ca^{2++} improve the fruit resistance to the softening, probably because of the stabilization of membrane systems and the formation of Ca pectates, which increase rigidity of the middle lamella and cell wall.

Microbiological analysis

In Table 3, the cell loads of mesophilic aerobic bacteria, psychrotrophic aerobic bacteria and yeasts are respectively reported. The growth of mesophilic and psychotrophic aerobic bacteria resulted affected by the coating types of the samples. Since day 1 of storage, control samples C showed a significant higher cell loads, of both the microbial groups, than the other samples. Both mesophilic and psychotrophic aerobic bacteria rapidly increased their cell loads after 3 days of storage in controls and samples covered by cocoa cream (Co) that showed significantly higher cell loads than the other samples. After 6 days of storage also samples coated with alginate alone (Al) significantly increased the total aerobic loads, while samples coated by both cocoa and alginate showed significant lower cell loads that the other samples. The total microbial viable count represents an important criterion for the evaluation of food quality.[49,50] The international criteria of ready to eat fruit report as satisfactory levels of aerobic colony count cell loads below 6.0 log cfu/g, acceptable levels when the cell loads ranged between 6.0-7.0 log cfu/g and unsatisfactory levels when the cell loads are higher than 7.0 log cfu/g.[51,52] The results obtained showed that control sample overcame the level of 7.0 log cfu/g, both for mesophilic and psycotrophic bacteria, after 6 days of storage. The samples coated with alginate or cocoa alone never exceed the load of 6.0 log cfu/g after 9 days of storage with regard to mesophilic aerobic bacteria while both these samples exceed the acceptable limit of 7.0 log cfu/g for psycotrophic bacteria but only after 9 days of storage. The sample double coated (Co+Al) showed the best quality for the whole period of storage. In fact, the detected cell loads of mesophilic and psycotrophic bacteria after 9 days resulted 4.93 and 6.07 log cfu/g respectively. Yeasts load represents an important quality criterion for ready to eat fruits since they are one of the main spoilage in this food category.[53] Also in this case C sample showed a significant higher cell load compared to the other samples for the whole period of storage. Until the third day of storage the Al sample showed the lowest yeasts load. Nevertheless, from the sixth day both the samples Al and Al+Co showed the lowest yeasts loads. However, the international criteria on ready to eat fruit report as unsatisfactory yeast level when the cell load overcome 6 log cfu/g.[52] In this study, only control samples overcame the reported yeast limit after 9 days of storage. On the contrary, the other samples remained below this limit showing, after 9 days of storage, cell loads of 5.33, 4.77 and 4.70 log cfu/g respectively for the samples Co, Al and Al+Co. Furthermore, microbiological results agree with a_w values since Al and Co+Al samples, that showed lowest a_w values, highlighted the lowest cell load. [54] Lactic acid bacteria, total coliforms and moulds (data not shown) never exceed 3.5, 2.0 and 1.7 log cfu/g in all samples for the whole period of storage. However, they do not represent the main microbial category of microbial spoilage for this kind of product.[53] Overall obtained results are in agreement with literature data that report an antimicrobial effect of alginate coating on different fruit typology such as fresh-cut water melon [55-23] fresh-cut pineapple [14], pears, [15]apples [16,17] and blueberries.[24] Moreover, these results suggest a synergistic effect of alginate and cocoa coatings when combined, as demonstrated by the lowest growth kinetics of microorganisms in samples subject to this coating.

Sensory analysis

Results of sensory analysis carried out in all samples at 0 and 3 days of storage are reported in Table 4. At 0 day of storage all samples were judged quite similar, obtaining high scores for all evaluated attributes. Orange Al and Co+Al, were more appreciated for texture attribute, while the Orange Co obtained slightly higher scores than Co+Al for smell and flavour attributes; the others were judged similar. After 3 days of storage a reduction in the score of all sensory attributes was registered in all samples, but covered ones maintained scores over the acceptability limit, also after this time. In particular, after 3 days of storage, orange Co was the more appreciated from panellist compared to other samples, except that for the visual appearance and firmness, for which sample Al reached highest scores, followed by Co+Al one. In particular, double coated samples, presented intermediate flavour and overall acceptability values, between Co and Al. Control samples reached the lowest score compared to coated one except that for the visual appearance and firmness, that were judged higher than Co one.

CONCLUSIONS

The present study has demonstrated that a sodium alginate coating can preserve the firmness, moisture content, and product shelf-life of fresh-cut orange. After three days of storage, however, sodium alginate-coated fruits were deemed less appealing with regards to their flavour and smell. On the contrary, samples with cocoa coating maintained superior sensory attributes in terms of flavour, smell and overall acceptability, after three days of storage, but presented lower firmness and higher microbiological load than other coated samples. Control samples manifested the lowest quality of the study group across the main evaluated attributes.

Oranges with the double coating (sodium alginate and cocoa), seems to be the more promising solution in order to obtained fresh cut oranges with the lowest microbial load and at the same time high-quality characteristics. The results of this study suggest that the use of a double sodium alginate and cocoa coating would lead to both high sensory attributes and extended shelf-life of ready-to-eat, fresh-cut oranges.

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Table 1. Alginate and Cocoa based coating formulations.

Ingredients (g/100 g)	Alginate Coating	Cocoa Coating
Non -hydrogenated fats	-	45.0 g
Sugars	-	41.0 g
Cocoa powder	-	7.0 g
Skimmed milk powder	-	6.0 g
Soy lecithin	-	0.4g
Stabilizer	-	0.4g
Vanille flavour	-	0.2g
Distilled water	80.0 g	-
Calcium ascorbate	10.0 g	-
Sodium alginate powder	10.0 g	-

Table 2. Yield stress and plastic viscosity values of alginate and cocoa based coating obtained by applying the Casson model.

Samples	Yield Stress (mPa)	Plastic Viscosity (mPa)
Alginate Coating	98660.5±1817.9 [a]	951.2 ±4.90 [a]
Cocoa Coating	3344.5±209.5 [b]	519.5±13.8 [b]

[a-b]Values followed by different letters differ significantly at P<0.05 level.

Table 3. Cell loads expressed as log CFU/g of mesophilic and psychrotrophic aerobic bacteria and yeasts of non-coated sample (C), sample coated by sodium alginate (Al), by cocoa cream (Co) and covered by both coatings (Co+Al) during 9 days of refrigerated storage.

Samples	Mesophilic aerobic bacteria (log CFU/g)			
	day 1	**day 3**	**day 6**	**day 9**
C	4.82 ± 0.14^{a}	5.93 ± 0.11^{a}	7.14 ± 0.09^{a}	7.39 ± 0.04^{a}
Al	3.05 ± 0.45^{bc}	3.93 ± 0.41^{c}	5.14 ± 0.05^{b}	5.83 ± 0.11^{b}
Co	3.24 ± 0.13^{c}	4.61 ± 0.14^{b}	4.83 ± 0.08^{c}	5.51 ± 0.20^{b}
Co+Al	3.66 ± 0.18^{b}	3.86 ± 0.16^{c}	4.22 ± 0.25^{d}	4.93 ± 0.13^{c}
	Psychrotrophic aerobic bacteria (log CFU/g)			
C	5.15 ± 0.09^{a}	6.85 ± 0.10^{a}	8.06 ± 0.17^{a}	8.66 ± 0.12^{a}
Al	3.49 ± 0.35^{c}	5.16 ± 0.39^{c}	6.22 ± 0.15^{c}	7.02 ± 0.08^{b}
Co	4.65 ± 0.12^{b}	5.91 ± 0.16^{b}	6.76 ± 0.22^{b}	7.05 ± 0.17^{b}
Co+Al	4.71 ± 0.19^{b}	4.95 ± 0.60^{c}	5.20 ± 0.26^{d}	6.07 ± 0.18^{c}
	Yeasts (log CFU/g)			
C	3.49 ± 0.12^{a}	4.83 ± 0.83^{a}	5.51 ± 0.14^{a}	6.13 ± 0.12^{a}
Al	2.36 ± 0.13^{c}	2.61 ± 0.08^{c}	3.71 ± 0.13^{c}	4.77 ± 0.29^{c}
Co	3.05 ± 0.11^{b}	3.68 ± 0.31^{ab}	4.82 ± 0.14^{b}	5.33 ± 0.12^{b}
Co+Al	3.02 ± 0.06^{b}	3.15 ± 0.53^{b}	3.27 ± 0.27^{c}	4.70 ± 0.14^{c}

$^{a-d}$Values followed by different letters differ significantly at $P<0.05$ level.

Table 4. Sensory attributes of non-coated sample (C), sample coated by sodium alginate (Al), by cocoa cream (Co) and covered by both coatings (Co+Al) at 0 and 3 days of storage.

Time (days)	Sample	External visual appearance	Inner visual appearance	Smell	Flavour	Firmness	Overall Acceptability
0	**C**	8.11 ± 0.29^{a}	8.46 ± 0.35^{a}	8.23 ± 0.22^{c}	8.25 ± 0.06^{c}	8.21 ± 0.26^{b}	8.19 ± 0.31^{b}
	Al	8.43 ± 0.32^{a}	8.64 ± 0.23^{a}	8.00 ± 0.14^{c}	8.15 ± 0.09^{c}	8.83 ± 0.18^{a}	8.13 ± 0.15^{b}
	Co	8.50 ± 0.23^{a}	8.80 ± 0.29^{a}	9.00 ± 0.16^{a}	8.70 ± 0.13^{a}	7.82 ± 0.31^{c}	8.95 ± 0.13^{a}
	Co+Al	8.35 ± 0.38^{a}	8.70 ± 0.24^{a}	8.66 ± 0.11^{b}	8.48 ± 0.09^{b}	9.00 ± 0.36^{a}	8.73 ± 0.17^{a}
3	**C**	6.02 ± 0.10^{b}	6.04 ± 0.07^{b}	4.90 ± 0.09^{c}	4.94 ± 0.21^{d}	6.00 ± 0.06^{c}	5.41 ± 0.31^{d}
	Al	6.42 ± 0.20^{a}	6.47 ± 0.15^{a}	6.46 ± 0.17^{b}	5.70 ± 0.10^{c}	6.56 ± 0.17^{a}	6.23 ± 0.07^{c}
	Co	5.83 ± 0.16^{c}	5.66 ± 0.19^{c}	6.85 ± 0.15^{a}	6.80 ± 0.19^{a}	5.65 ± 0.11^{d}	6.65 ± 0.16^{a}
	Co+Al	6.13 ± 0.18^{b}	6.33 ± 0.17^{a}	6.57 ± 0.13^{b}	6.58 ± 0.11^{b}	6.35 ± 0.16^{b}	6.44 ± 0.12^{b}

$^{a-d}$ Values followed by different letters differ significantly at $P<0.05$ level.

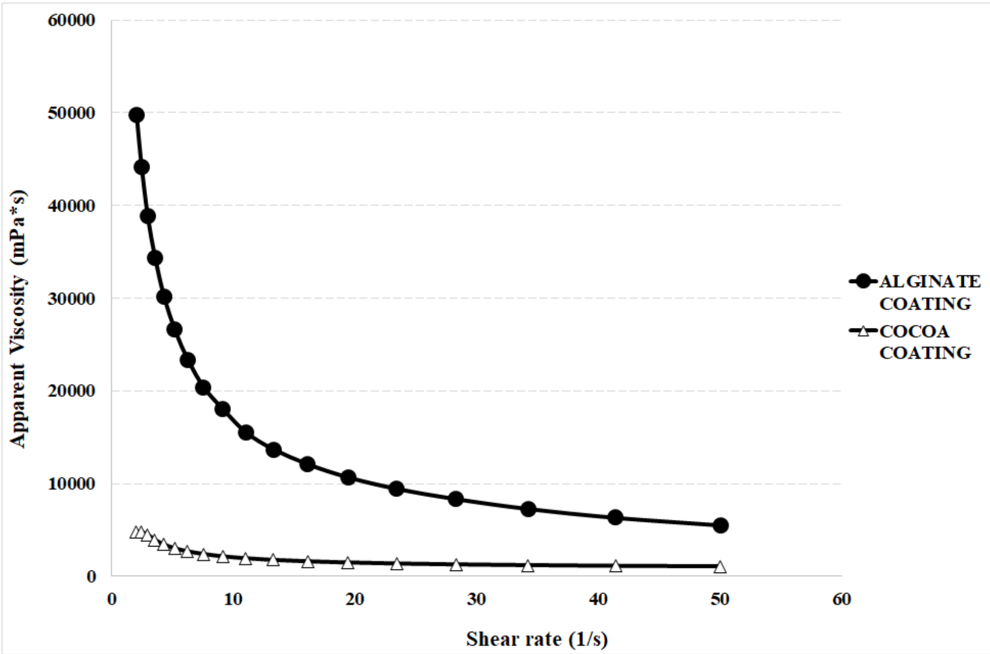


Figure 1. Flow curves of alginate and cocoa based coating evaluated by increasing the shear rate from 2 to 50 s-1.

258x169mm (96 x 96 DPI)

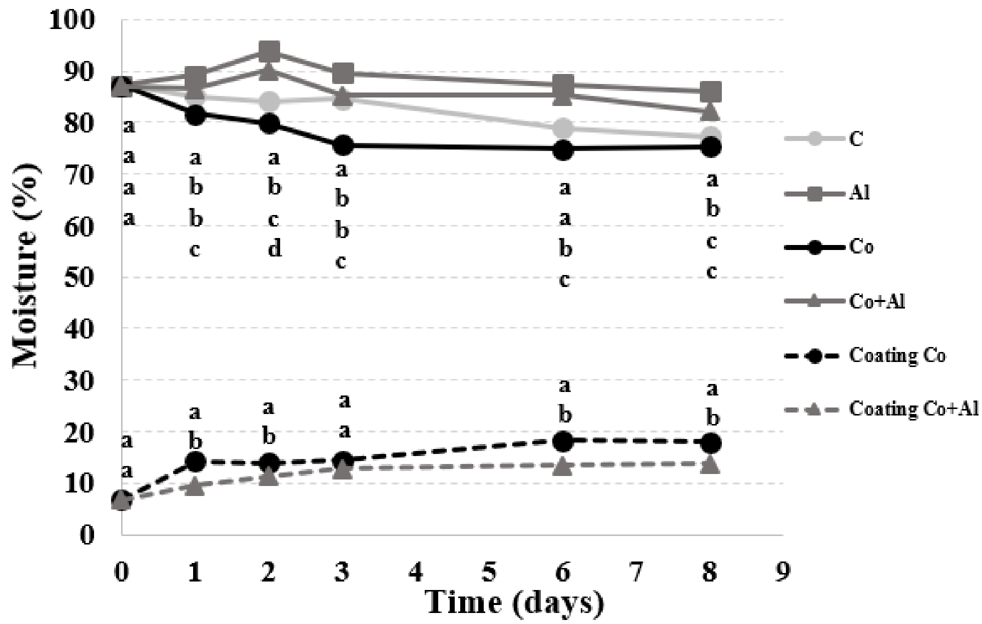
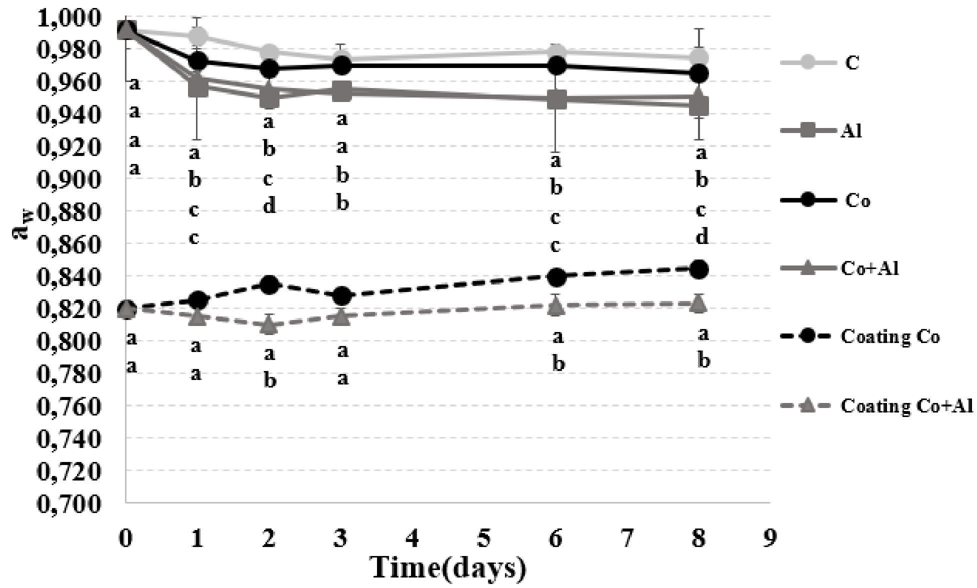


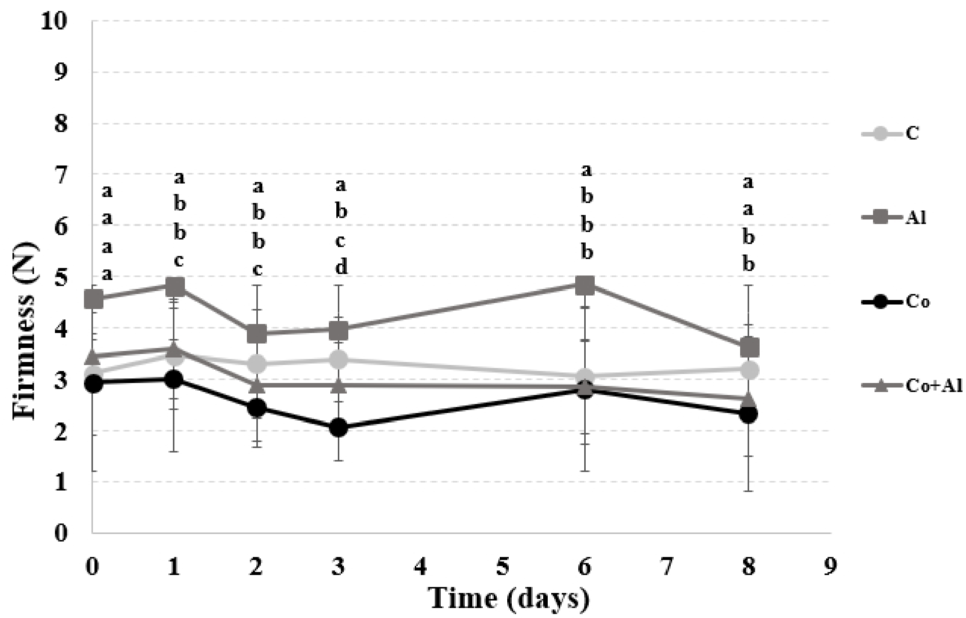
Figure 2. Moisture of orange samples evaluated during storage.
a–d Values followed by different letters differ significantly at P<0.05 level

157x101mm (96 x 96 DPI)



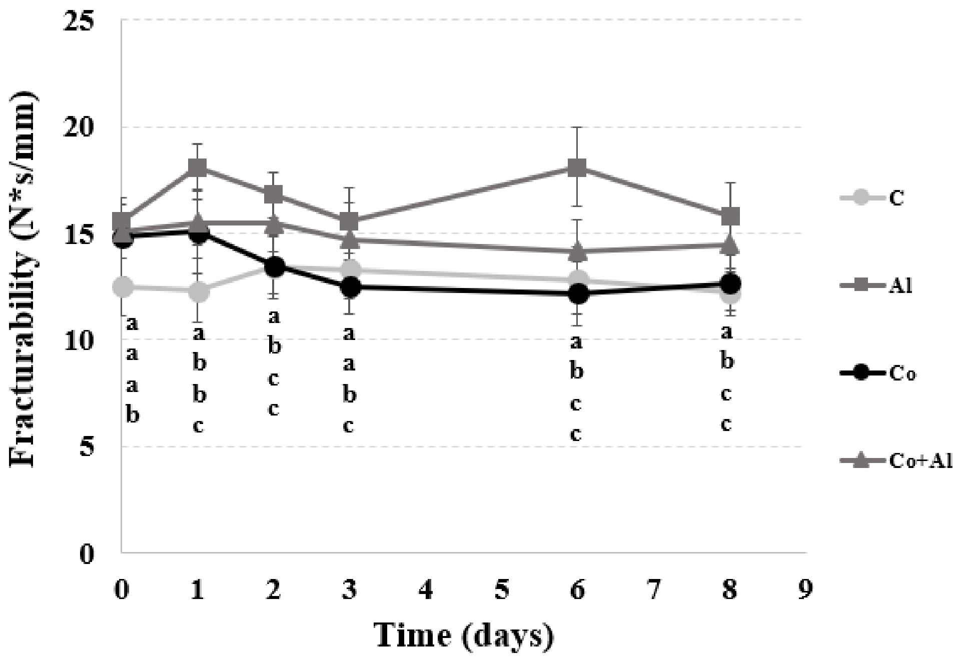
Water activity (aw) of orange samples evaluated during storage.
a–d Values followed by different letters differ significantly at P<0.05 level

171x106mm (96 x 96 DPI)



Firmness of orange samples evaluated during storage.
a–d Values followed by different letters differ significantly at P<0.05 level

174x114mm (96 x 96 DPI)



Fracturability values of orange samples during storage
a–c Values followed by different letters differ significantly at P<0.05 level

156x111mm (96 x 96 DPI)