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Characterization and evaluation of the influence of an alginate, cocoa and a bilayer alginate-cocoa coating on the quality of fresh-cut oranges during storage

This is the submitted version (pre peer-review, preprint) of the following publication:

Published Version:

Glicerina, V., Siroli, L., Betoret, E., Canali, G., Dalla Rosa, M., Lanciotti, R., et al. (2022). Characterization and evaluation of the influence of an alginate, cocoa and a bilayer alginate-cocoa coating on the quality of fresh-cut oranges during storage. JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE, 102(11), 4454-4461 [10.1002/jsfa.11799].

Availability:

This version is available at: <https://hdl.handle.net/11585/893606> since: 2022-09-06

Published:

DOI: <http://doi.org/10.1002/jsfa.11799>

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(Article begins on next page)

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

Journal:	<i>Journal of the Science of Food and Agriculture</i>
Manuscript ID	JSFA-19-1604
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	23-May-2019
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Key Words:	edible coating, Double coating, cocoa, Alginate, Oranges, Shelf-life

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3 1 **Improvement of quality, sensory properties and shelf life of fresh cut oranges by**
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6 2 **using a bilayer cocoa-sodium alginate coating**
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9 3 **RUNNING TITLE: IMPROVEMENT OF QUALITY OF FRESH CUT**
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12 4 **ORANGES BY USING A BILAYER COATING**
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54 20 This is the preprint (submitted version) of the article *Characterization and evaluation of the influence of an*
55 21 *alginate, cocoa and a bilayer alginate-cocoa coating on the quality of fresh-cut oranges during storage*, by
56 22 *Virginia Glicerina, Lorenzo Siroli, Ester Betoret, Giada Canali, Marco Dalla Rosa, Rosalba Lanciotti,*
57
58 21 *Santina Romani*, which is published in its final version in JOURNAL OF THE SCIENCE OF FOOD AND
59
60 AGRICULTURE, Volume 102, Issue 11, pages 4454-4561, DOI <https://dx.doi.org/10.1002/jsfa.11799>

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

1
2
3 50 **Keywords:** edible coating, double coating, cocoa, alginate, oranges, rheology, shelf-life.
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8 53 **INTRODUCTION**

10
11 54 Fresh-cut products are ready-to-use goods which retain the fresh characteristics of raw produce.¹ Raw
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14 55 and minimally-processed fruits and vegetables are sold to consumers in a ready-to-use or ready-to-
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16 56 eat form. This type of product does not generally contain preservatives or antimicrobial substances
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18 57 and rarely undergoes any heat processing before consumption.² The food market evolves with new
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20
21 58 products and changing trends, but fresh-cut ones remain at the top of the list of products that meet the
22
23 59 needs of many consumers.³ However, numerous factors restrict the quality and shelf-life of fresh-cut
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25 60 products. During storage, in fact, they undergo significant deteriorations and as a consequence decay
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27
28 61 of their sensory (e.g. flavour, colour, texture) and nutritional value. Water loss is the primary factor
29
30 62 that involves deterioration of fruits and vegetables and may result in soft texture, translucency and
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32 63 loss of nutritional value and sensory attributes.^{4,5} Researches aimed to retard the quality loss of fresh-
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34
35 64 cut fruits, maintaining their safety in terms of microbial growth are of great interest for companies
36
37 65 involved in their production and distribution.^{6,7} Different approaches have been employed to preserve
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39 66 fresh-cut product quality during shelf-life, including modified atmosphere packaging, chemical
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41 67 treatments such as calcium dips and physical treatments such as gamma irradiation, pulse light, ozone,
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43
44 68 cold plasma, and high pressure pre-treatment combined with vacuum impregnation.^{8,9,10} One of the
45
46 69 most promising, convenient and safe technologies to preserve the quality and to prolong the shelf-life
47
48 70 of fresh fruits and vegetables is the application of edible coatings.¹¹ These coatings are formed from
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50
51 71 a suspension of a thickening agent, which after application on the product forms a film that acts as a
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53 72 barrier to gas exchange and water loss by modifying the atmosphere and slowing fruit ripening.¹²
54
55 73 Edible coatings can be classified in three categories with regards to the nature of their components:
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57 74 polysaccharides (alginate, gellan, etc...), lipids (paraffin, beeswax, chocolate, etc..), proteins (corn
58
59 75 zein, wheat gluten, etc...) and composites, made up from combining substance from previous

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2
3 76 categories (e.g. gelatin and fatty acids, methylcellulose and fatty acids, etc.). ¹³In recent years
4
5 77 researches are focused on the application of alginate as edible coating on fresh-cut fruits, for its
6
7 78 characteristics of good transparency and resistance to gas exchange. ¹²A lot of studies have been
8
9
10 79 performed on the influence of alginate coating alone or in combination with other substances on the
11
12 80 chemico-physical and microbiological characteristics of different type of fresh-cut fruit, such as
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14 81 pineapple,¹⁴ pears,¹⁵ apples,^{16,17,18} mango, ¹⁹papaya,²⁰ tomato fruit, ²¹melon^{22,23} and blueberries.²⁴

16
17 82 However, to our knowledge, no studies have been performed on the influence of edible coating
18
19 83 alginate on the physico-chemical, sensory and microbiological properties of fresh cut orange
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21 84 products. Fresh cut oranges were selected as samples to cover, considering their high nutritional
22
23 85 values and quality that undergoes to significative loss, during storage, because of their sensitive to
24
25 86 microbiological growth, water loss and to low temperature, especially after cutting. For this reason,
26
27 87 in the first part of this study, the influence of a sodium alginate-based coatings on the main quality
28
29 88 characteristics of orange fresh-cut products is evaluated. However, the application of coatings without
30
31 89 compromising sensory attributes of fresh-cut fruits is not always achieved, and therefore needs further
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33 90 studies.²⁵ Moreover, companies requested innovative products, with improved sensorial
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35 91 characteristics, maintaining high quality parameters for longer storage times, for this reason, in
36
37 92 addition, cocoa as an alternative edible coating was examined. To our knowledge, few studies were
38
39 93 focused on the possibility to use cocoa based coatings, normally used in the bakery and confectionery
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41 94 industries, to preserve fresh cut fruits. In the 1988, Biquet and Labuza²⁶ performed a research with
42
43 95 the purpose to evaluate the moisture permeability properties of a cocoa coating without any kind of
44
45 96 applications on food system. Recently Khan²⁷ et al., and Meza et al. 2018²⁸ reported some studies
46
47 97 respectively focused on the efficacy and the deposition behaviour of a cocoa coating applied by
48
49 98 electro spraying, and on its rheological and adsorption properties. Only two **works** deal with the
50
51 99 application of cocoa coatings on fruits products, where in the first one Gounga et al. 2008,²⁹ applied
52
53 100 two cocoa coatings on dried chestnut and analysed them for nutritional and microbiological properties
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55 101 only after covering. While Glicerina et al., 2019³⁰ applied two cocoa based coating on fresh cut fruits

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3 102 (apples and grapes) and their influence on the main quality characteristics, during storage, was
4
5 103 evaluated. However, obtained results showed that in comparison to uncoated samples, cocoa based
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7 104 coatings gave a positive effect only on the sensory properties of fresh cut fruit samples during storage,
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9
10 105 showing a shelf-life very similar to uncoated ones; even if grapes maintained better quality properties
11
12 106 than apples probably because the presence of their natural skin that promoted a protective effect.

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15 107 The absence of an intermediate coating between fruit and cocoa did not contribute to extend the shelf
16
17 108 life; to fill the mentioned gap in the present research two layers of coating were used. For this reason,
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20 109 in this study, the effects of different coatings, alginate-based, cocoa-based and a combination of them
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23 110 (as double coating), on the physico-chemical, microbiological and sensory characteristics of fresh-
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25 111 cut oranges during storage were evaluated.

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31 113 **MATERIALS AND METHODS**

34 114 **Raw materials**

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37 115 Fresh-cut oranges were obtained from the consortium Agribologna (Bologna). The variety of orange
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39 116 was *Navel*. The oranges had a refractive index of 11.3 °Brix and an acidity of 0.83 ml/ 100 ml citric
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41
42 117 acid. Two types of coatings were employed to cover fresh-cut orange samples; one sodium alginate
43
44 118 based, the other cocoa based. The formulations of sodium alginate coating, obtained in laboratory by
45
46 119 adding calcium ascorbate and solved in distilled water, and that made with a commercial cocoa
47
48 120 realized with Cocoa Butter substitutes (CBS) are reported in Table 1. The sodium alginate formulation
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51 121 was realized according to Zhong et al.,³¹ and Fu et al.,³²

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125 **Sample preparation**

126 The orange fruits were manually peeled and subsequently obtained slices were separated and
127 subjected to a peroxiacetic acid solution dip (200 ppm) to prevent microbial contamination. After
128 drying at 4°C for 10 minutes, slices were divided in three parts of irregular shape. Subsequently, the
129 orange pieces were covered with the different coatings: cocoa cream (Co), sodium alginate (Al) and
130 both combined (Co+Al). This last coating type were made by covering each orange slice with a first
131 layer of alginate and after drying (at 4°C for 30 minutes) a second one of cocoa cream, preliminary
132 obtained by melting dark chocolate substitute in a microwave at 750 watts for 1 minute. These
133 conditions were chosen after preliminary trials, in order to avoid modification in the product structure,
134 in accordance with Stortz & Marangoni³³ and Glicerina et al.,³⁴ 2 grams of sodium alginate solution
135 plus 2 g of melted cocoa coating were used to cover each piece of fruit. In all coated fruits the single
136 coating layer had a thickness of 1.0 ± 0.1 mm. Each orange piece was completely dipped in each
137 coating type, removed with tweezers and left to dry in a cold room at 4°C for 15 minutes. After
138 cooling, approximately 100 g of each orange sample was packed in different PET trays closed with
139 polypropylene (PP) film at medium barrier to oxygen. In specific, the PET tray had a thickness of 3
140 mm, an oxygen transmission rate (OTR) of 60 cm³/m²/day/atm and a water transmission rate of 27
141 cm³/m²/ day/atm, while the PP film presented a thickness of 30 µm and an oxygen and a water
142 transmission rate respectively of 860 cm³/m²/ day/atm and 19 cm²/m²/ day/atm, in both cases
143 measured at 23°C. All samples were stored at 6°C for 10 days. Three trays of each sample were
144 analysed at six different times: after 0 (T0), 1 (T1), 2 (T2), 3 (T3), 6 (T6) and 8 (T8) days, chosen
145 after preliminary trials. Control sample was represented by orange pieces without coating, processed
146 and stored at the same conditions. Samples were named: Orange C, control without coating; Orange
147 Al, coated with sodium alginate; Orange Co, coated with cocoa cream; and Orange Al+Co, coated
148 with double layer of sodium alginate and cocoa cream.

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8 9 152 **METHODS**

10 11 12 153 **Rheological analysis on fruit coatings**

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15 154 Rheological measurements were carried out on both cocoa and alginate coatings in order to
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17 155 characterize them. Measurements were performed at 40 °C using a controlled stress–strain rheometer
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20 156 (MCR 300, Physica/Anton Paar, Ostfildern, Germany) equipped with a system of coaxial cylinders
21
22 157 (CC27). The rheological behaviour of coatings was analysed in steady state conditions. After a pre-
23
24 158 shearing of 500 s at 5 s⁻¹, viscosity was measured by increasing shear rate from 2 to 50 s⁻¹ within 180
25
26 159 s and taking 18 measurements at different points.³⁵ The obtained flow curves, on the basis of
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28
29 160 preliminary trials, were fitted according to the Casson model that showed the best fit, compared
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31 161 against other rheological models. The Casson model is a structure-based model derived from the
32
33 162 analysis of a structure and its kinetic change, usually employed to study the rheological behaviour of
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36 163 food matrices characterized by the presence of a yield stress.^{36,37}

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39 164 This model is described by the following equation:

$$40
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42 165 \tau^{0.5} = \tau_0^{0.5} + \eta_{PL} \gamma^{0.5} \quad (1)$$

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45 166 where τ_0 is the yield stress at the zero point and η_{PL} is the so-called plastic viscosity³⁴

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49 50 51 168 **Moisture and Water Activity**

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53
54 169 Moisture content and water activity were determined at each storage time, separately on the fruit
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56 170 samples and cocoa coatings (after removing it from each fruit piece), in order to evaluate possible
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58 171 migration phenomena between them. In samples coated with sodium alginate analyses were
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60 172 performed on the whole fruit because of it was not possible to separate the coating from the fruit

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3 173 pieces because of the high tackiness of the sodium alginate. Moisture content was determined
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5 174 gravimetrically by difference in weight before and after oven drying at 70 °C, until constant weight
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8 175 was reached. The moisture content was calculated as follows:

$$11 \text{ 176 } \text{Moisture content \%} = 100 - \left(\frac{W_f - W_d}{W_f} \right) * 100$$

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14 177 W_f = weight fresh sample

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17 178 W_d = weight dry sample

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20 179 The water activity values of ground fruits and cocoa coatings were obtained by using a dew point
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22 180 hygrometer, AquaLab-Water Activity Meter (mod. SERIES 3TE. Decagon Device, Inc., Nelson
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24 181 Court, NE). Three measurements were carried out from each sample (Orange C, Orange AI, Orange
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26 182 Co, Orange AI+Co) and their respective cocoa coating, when present, after separation from fruit
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29 183 pieces, at each storage interval for both moisture and water activity analyses.

30 31 32 184 33 34 35 185 **Textural analysis**

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38 186 Evaluation of firmness and fracturability was conducted with a penetration test by means of a Texture
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40 187 Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, Godalming, UK), equipped with a 5 Kg
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42 188 load cell and a 6 mm diameter stainless steel probe. Test speed was 1 mm s⁻¹ with a 6 mm depth of
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44
45 189 penetration.³⁸ The maximum peak of the curve, obtained during penetration, was used as the firmness
46
47 190 value F (N). The distance between the origin of curve till the point until the end of the penetration, is
48
49 191 an index of the fracturability (N*s/mm). Results were expressed as the average of ten measurements
50
51 192 for each sample.

196 **Microbiological analysis**

197 Microbiological analyses were performed after 1, 3, 6 and 9 days of storage. For each type of sample,
198 30 g of whole product were placed in sterile bags and with 60 mL of sterile saline solution (0.9%
199 NaCl) and subsequently homogenized for 2 minutes in Stomacher (model Lab Blender Seward,
200 London, UK). Subsequently, the samples were serial diluted into sterile physiological solution
201 according to the expected microbial cell loads of the samples. The total loads of lactic bacteria, yeasts,
202 molds, total mesophilic aerobic bacteria, total psychrotrophic aerobic bacteria and total coliforms
203 were determined. In particular, yeasts were counted on Yeast extract Peptone Dextrose medium
204 (YPD) (Oxoid Ltd, Basingstoke, United Kingdom), total coliforms on Violet Red Bile Agar (Oxoid
205 Ltd, Basingstoke, United Kingdom), lactic acid bacteria on De Man Rogosa and Sharpe (MRS)
206 (Oxoid Ltd, Basingstoke, United Kingdom), total aerobic mesophilic and psychrotrophic bacteria on
207 Plate Count Agar (PCA) (Oxoid Ltd, Basingstoke, United Kingdom) and mold on Malt Extract Agar
208 (MEA) (Oxoid Ltd, Basingstoke, United Kingdom). In particular, yeasts, molds and total aerobic
209 mesophils were incubated at 30 °C for 48h, total coliforms and lactic bacteria were incubated at 37
210 °C for 24h, while psychrotrophic aerobic bacteria were incubate at 10 °C for 7 days.

212 **Sensory analysis**

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

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56 222 **STATISTICAL ANALYSIS**
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9 223 Analysis of variance (ANOVA) and the test of mean comparisons according to Fisher's least
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11 224 significant difference (LSD) with a 0.05 level of significance were applied to find out significant
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13 differences among the different samples. The statistical package STSG Statistica for Windows,
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15 version 6.0 (Statsoft Inc., Tulsa, OK, USA) was used.
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2122 228 **RESULTS AND DISCUSSION**
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2425 229
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2728 230 **Rheological characteristics of coatings**
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31 231 In Figure 1 the flow curves of the two different edible coatings, sodium alginate and cocoa, are
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33 232 showed. In both samples, apparent viscosity decreases with the increase of the shear rate, indicating
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35 233 pseudoplasticity. This behaviour can be explained by the structural breakdown of the molecules due
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37 to the hydrodynamic forces generated and to the increased alignment of the constituent molecules.⁴¹
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39
40 235 Alginate coating presents the highest viscosity, with initial values around 50.000 mPa, cocoa coating
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42 236 had an initial apparent viscosity values around 5.000 mPa. Moreover, in order to better explain the
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44 237 rheological values obtained by the flow curves, the Casson yield value and the Casson plastic
45
46 viscosity parameters were calculated applying the Casson model, results are reported in Table 2. All
47 238
48 data were well fitted by the Casson model, providing high determination coefficients (R^2), comprising
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50 between 0.98 and 0.99. Alginate coating presented highest yield stress and viscosity values compared
51 240
52 to cocoa one, underlining how the amount of energy needed to start flow was the highest in the former.
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54 241 Sodium alginate is made up of d-mannuronic and l-guluronic acids and contains numerous
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56 242 hydrophilic molecular groups.^{42, 31}When water is added to sodium alginate, strong bonds between
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58 243 molecules are created, giving arise to a tighter and more compact structure. High sodium alginate
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3 245 viscosity values may also be achieved through the addition of calcium ascorbate which, as has been
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5 246 demonstrated previously³², causes high matrix aggregation due to its crosslinking effects that
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8 247 strengthen alginate solution bonds. Nevertheless, Skurtys et al.,¹³ and Zhong et al.³¹ have shown that
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10 248 sodium alginate solutions with high viscosities, such as those observed in their study (around 100
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12 249 mPa*s), may be appropriate for its use in edible coatings, especially if applied by a dipping method;
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14 250 while those with low viscosity can provide processing advantage during spraying methods. Cocoa
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17 251 coating showed lower yield stress and viscosity values than alginate one, this can be attributed to its
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19 252 lipid- based formulation.⁴³ However, according with literature,^{44,28} the yield stress and viscosity
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21 253 values of the used cocoa coating make them suitable for coating purposes, being high enough to
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24 254 prevent gravity effects (sagging and dripping) but sufficiently low to allow capillarity-driven levelling.
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30 256 **Moisture and Water Activity**

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33 257 In Figure 2 moisture changes during the storage of coated fresh-cut orange samples as well as the Co
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35 258 and Co+Al cocoa coatings alone (after removal from fruit pieces) are shown. In both mono and bilayer
36
37 259 alginate covered fruits, a constant trend with a slight reduction in moisture content at the end of
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39
40 260 storage was observed, while in C and Co samples a more pronounced moisture reduction was
41
42 261 highlighted during storage. Orange samples Al maintained the highest moisture content during the
43
44 262 entire storage period. This behaviour is probably due to the water barrier effect induced by sodium
45
46 263 alginate that limited the water migration, keeping the fruit pieces more hydrated than in the other
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48
49 264 samples.^{4,24} The calcium ascorbate, present in the sodium alginate coating formulations, caused a
50
51 265 molecular cross-linking effect, thereby strengthening the chemical bonds among sodium alginate
52
53 266 components and further promoting the water migration barrier effect of the coating.⁴⁵ The sodium
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55
56 267 alginate water barrier effect, which has also been reported in the studies of Meza et al.,²⁸ is
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58 268 underscored by the fact that the Orange Co sample underwent the highest moisture loss during
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60 269 storage, that was parallel to an increase in the moisture content of its cocoa coating (Coating Co).

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3 270 This behaviour, according with Johanson and Bergensthal,⁴⁶ can be probably attributed to a water
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5 271 exchange between the fruits and the cocoa characterized by different water amount. Furthermore
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8 272 (Figure 2) a similar behaviour was observed in Orange Co+Al, even if with lower intensity, thereby
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10 273 confirming the role of sodium alginate as moisture barrier. The control sample Orange C lost more
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12 274 water compared to samples Al and Co+Al.⁴⁷ but less than sample Orange Co, having only the fruit's
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15 275 natural skin as barrier against dehydration.

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18 276 In Figure 3 results related to the water activity changes in fresh-cut orange samples and in the different
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20 277 cocoa coatings after their removal at each storage time are reported. Also in this case a reduction in
21
22 278 water activity values was observed for all samples during storage. This reduction was parallel to an
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24
25 279 increase in the respective cocoa coating of samples Al+Co and Co, as previous observed for moisture.
26
27 280 This trend may be a further confirmation of water exchange between fruit and coating, as previously
28
29 281 seen in moisture results, and also of the barrier effect conferred by sodium alginate coating. Moreover,
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31 282 water activity results, showed how Al and Co+Al samples had the lowest a_w values compared to C
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33
34 283 and Co orange ones. These low values can be probably attributed to a water binding stronger in Al
35
36 284 and Co+Al coatings than Co one, in fact Al and Co+Al coating formulations were rich in sodium
37
38 285 alginate that promoted hydrogen bonds.⁴⁸

44 287 **Textural properties**

46
47 288 In the Figures 4 and 5 the firmness and fracturability results of orange samples during storage are
48
49 289 shown respectively. Coated Al sample presented the highest firmness values compared to the other
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51
52 290 ones, showing an increase of this parameter during storage. High firmness values in samples fruit
53
54 291 coated with sodium alginate (Orange Al), can be attributed to the alginate network structure.²⁴
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56 292 Uncoated samples firmness values were intermediate between Al and samples coated with Co
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59 293 (Orange Co+Al and Orange Co), showing a constant trend during all storage times. For what concern
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294 Co+Al and Co samples, lower firmness values were observed compared to Al and C ones, this trend

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3 295 can be probably attribute in part to the moisture exchange occurred between cocoa coatings and fruit
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5 296 and between coating and the surrounding environment as previous stated in the moisture section, that
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7 297 involved a softening effect. Moreover, a further softening effect can be attributed to the presence of
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10 298 fat in the cocoa coating formulation.

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13 299 For what concern fracturability (Fig. 5), Al sample showed the statistically highest values compared
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15 300 to other samples at each storage time, while sample Co+Al presented intermediate values between Al
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17 301 and the other two samples (C and Co), even if not always statistically different from them. These
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19 302 values are a further confirmation of the barrier effect conferred by the sodium alginate coating, that
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21 303 maintained orange pieces more hydrated and more structured. Moreover, according with literature
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23 304 (Tapia et al., 2008)¹⁶ the presence of Ca²⁺⁺ improve the fruit resistance to the softening, probably
24
25 305 because of the stabilization of membrane systems and the formation of Ca pectates, which increase
26
27 306 rigidity of the middle lamella and cell wall.

35 308 **Microbiological analysis**

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37
38 309 In Table 3, the cell loads of mesophilic aerobic bacteria, psychrotrophic aerobic bacteria and yeasts
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40 310 are respectively reported. The growth of mesophilic and psychotrophic aerobic bacteria resulted
41
42 311 affected by the coating types of the samples. Since day 1 of storage, control samples C showed a
43
44 312 significant higher cell loads, of both the microbial groups, than the other samples. Both mesophilic
45
46 313 and psychotrophic aerobic bacteria rapidly increased their cell loads after 3 days of storage in controls
47
48 314 and samples covered by cocoa cream (Co) that showed significantly higher cell loads than the other
49
50 315 samples. After 6 days of storage also samples coated with alginate alone (Al) significantly increased
51
52 316 the total aerobic loads, while samples coated by both cocoa and alginate showed significant lower
53
54 317 cell loads that the other samples. The total microbial viable count represents an important criterion
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56 318 for the evaluation of food quality.^{49,50} The international criteria of ready to eat fruit report as
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58 319 satisfactory levels of aerobic colony count cell loads below 6.0 log cfu/g, acceptable levels when the

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3 320 cell loads ranged between 6.0-7.0 log cfu/g and unsatisfactory levels when the cell loads are higher
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5 321 than 7.0 log cfu/g.^{51,52} The results obtained showed that control sample overcame the level of 7.0 log
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7 322 cfu/g, both for mesophilic and psychotrophic bacteria, after 6 days of storage. The samples coated with
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10 323 alginate or cocoa alone never exceed the load of 6.0 log cfu/g after 9 days of storage with regard to
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12 324 mesophilic aerobic bacteria while both these samples exceed the acceptable limit of 7.0 log cfu/g for
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14 325 psychotrophic bacteria but only after 9 days of storage. The sample double coated (Co+Al) showed the
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17 326 best quality for the whole period of storage. In fact, the detected cell loads of mesophilic and
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19 327 psychotrophic bacteria after 9 days resulted 4.93 and 6.07 log cfu/g respectively. Yeasts load represents
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21 328 an important quality criterion for ready to eat fruits since they are one of the main spoilage in this
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23 329 food category.⁵³ Also in this case C sample showed a significant higher cell load compared to the
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25 330 other samples for the whole period of storage. Until the third day of storage the Al sample showed
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27 331 the lowest yeasts load. Nevertheless, from the sixth day both the samples Al and Al+Co showed the
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29 332 lowest yeasts loads. However, the international criteria on ready to eat fruit report as unsatisfactory
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31 333 yeast level when the cell load overcome 6 log cfu/g.⁵² In this study, only control samples overcame
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33 334 the reported yeast limit after 9 days of storage. On the contrary, the other samples remained below
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35 335 this limit showing, after 9 days of storage, cell loads of 5.33, 4.77 and 4.70 log cfu/g respectively for
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37 336 the samples Co, Al and Al+Co. Furthermore, microbiological results agree with a_w values since Al
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39 337 and Co+Al samples, that showed lowest a_w values, highlighted the lowest cell load. ⁵⁴ Lactic acid
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41 338 bacteria, total coliforms and moulds (data not shown) never exceed 3.5, 2.0 and 1.7 log cfu/g in all
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43 339 samples for the whole period of storage. However, they do not represent the main microbial category
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45 340 of microbial spoilage for this kind of product.⁵³ Overall obtained results are in agreement with
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47 341 literature data that report an antimicrobial effect of alginate coating on different fruit typology such
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49 342 as fresh-cut water melon ⁵⁵⁻²³ fresh-cut pineapple ¹⁴, pears, ¹⁵apples ^{16,17} and blueberries.²⁴ Moreover,
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51 343 these results suggest a synergistic effect of alginate and cocoa coatings when combined, as
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53 344 demonstrated by the lowest growth kinetics of microorganisms in samples subject to this coating.
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345 Sensory analysis

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3 346 Results of sensory analysis carried out in all samples at 0 and 3 days of storage are reported in Table
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5 347 4. At 0 day of storage all samples were judged quite similar, obtaining high scores for all evaluated
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8 348 attributes. Orange Al and Co+Al, were more appreciated for texture attribute, while the Orange Co
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10 349 obtained slightly higher scores than Co+Al for smell and flavour attributes; the others were judged
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12 350 similar. After 3 days of storage a reduction in the score of all sensory attributes was registered in all
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15 351 samples, but covered ones maintained scores over the acceptability limit, also after this time. In
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17 352 particular, after 3 days of storage, orange Co was the more appreciated from panellist compared to
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19 353 other samples, except that for the visual appearance and firmness, for which sample Al reached
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22 354 highest scores, followed by Co+Al one. In particular, double coated samples, presented intermediate
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24 355 flavour and overall acceptability values, between Co and Al. Control samples reached the lowest
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26 356 score compared to coated one except that for the visual appearance and firmness, that were judged
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29 357 higher than Co one.

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34 359 CONCLUSIONS

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38 361 The present study has demonstrated that a sodium alginate coating can preserve the firmness, moisture
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40 362 content, and product shelf-life of fresh-cut orange. After three days of storage, however, sodium
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42 363 alginate-coated fruits were deemed less appealing with regards to their flavour and smell. On the
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45 364 contrary, samples with cocoa coating maintained superior sensory attributes in terms of flavour, smell
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47 365 and overall acceptability, after three days of storage, but presented lower firmness and higher
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49 366 microbiological load than other coated samples. Control samples manifested the lowest quality of the
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52 367 study group across the main evaluated attributes.

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54 368 Oranges with the double coating (sodium alginate and cocoa), seems to be the more promising
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56 369 solution in order to obtained fresh cut oranges with the lowest microbial load and at the same time
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59 370 high-quality characteristics. The results of this study suggest that the use of a double sodium alginate
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3 371 and cocoa coating would lead to both high sensory attributes and extended shelf-life of ready-to-eat,
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5 372 fresh-cut oranges.

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12 13 14 375 **ACKNOWLEDGEMENTS**

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16
17 376 This research did not receive any specific grant from funding agencies in the public, commercial, or
18
19 not-for-profit sectors. We would like to thank Consortium Agribologna and in particular Dott.
20 377
21
22 378 Emiliano Cocci for providing raw materials and for technical supports

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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	-

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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
Samples	Psychrotrophic aerobic bacteria (log CFU/g)			
	day 1	day 3	day 6	day 9
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
Samples	Yeasts (log CFU/g)			
	day 1	day 3	day 6	day 9
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.

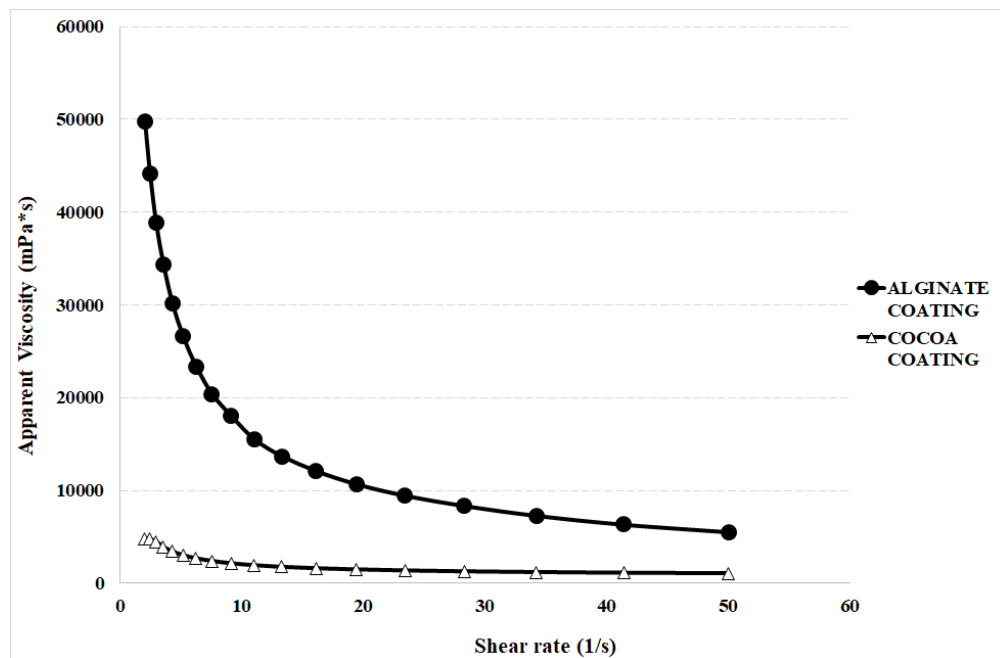


Figure 1. Flow curves of alginate and cocoa based coating evaluated by increasing the shear rate from 2 to 50 s⁻¹.

258x169mm (96 x 96 DPI)

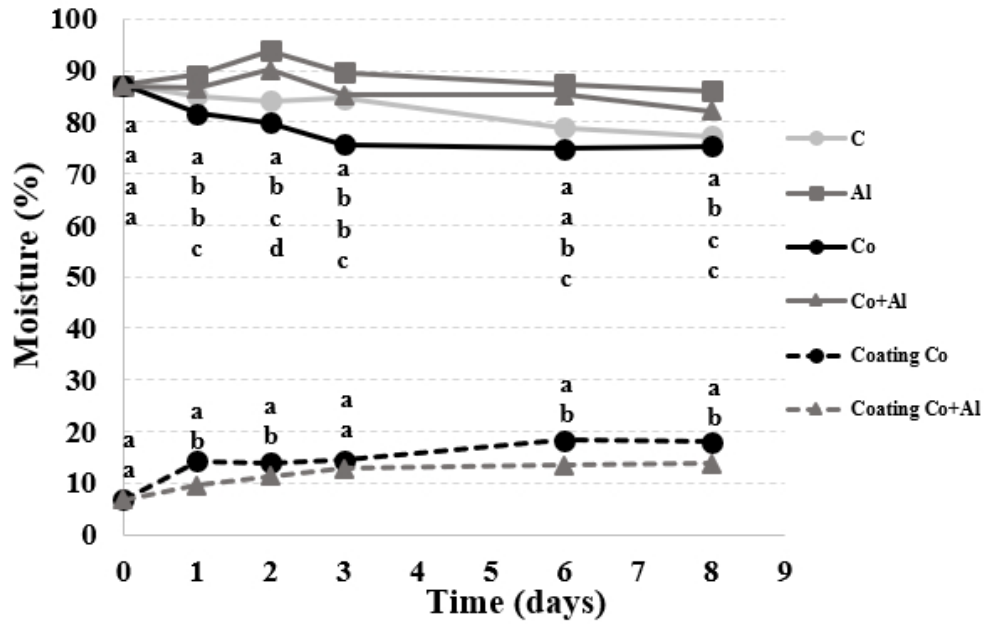
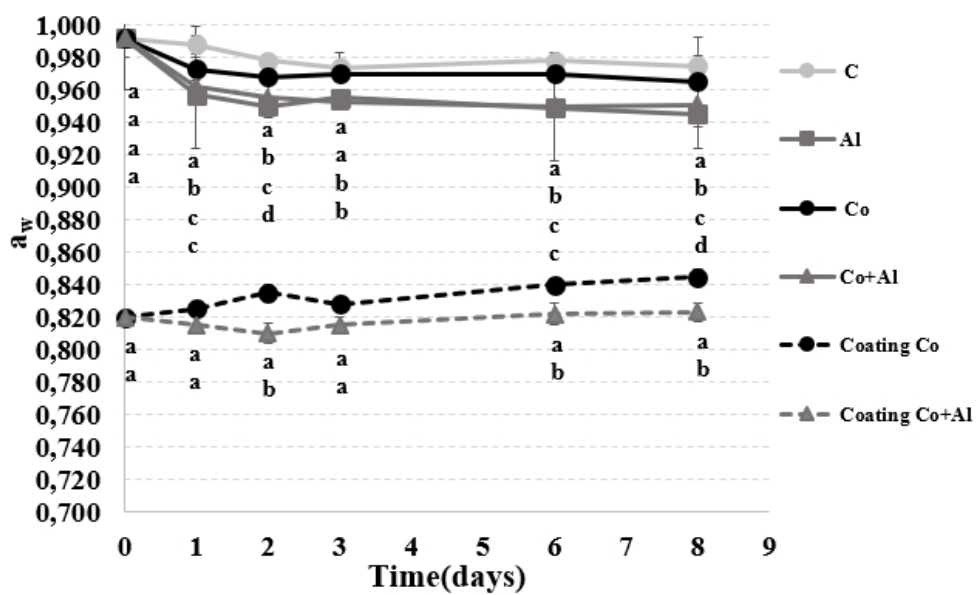


Figure 2. Moisture of orange samples evaluated during storage. a-d Values followed by different letters differ significantly at P<0.05 level

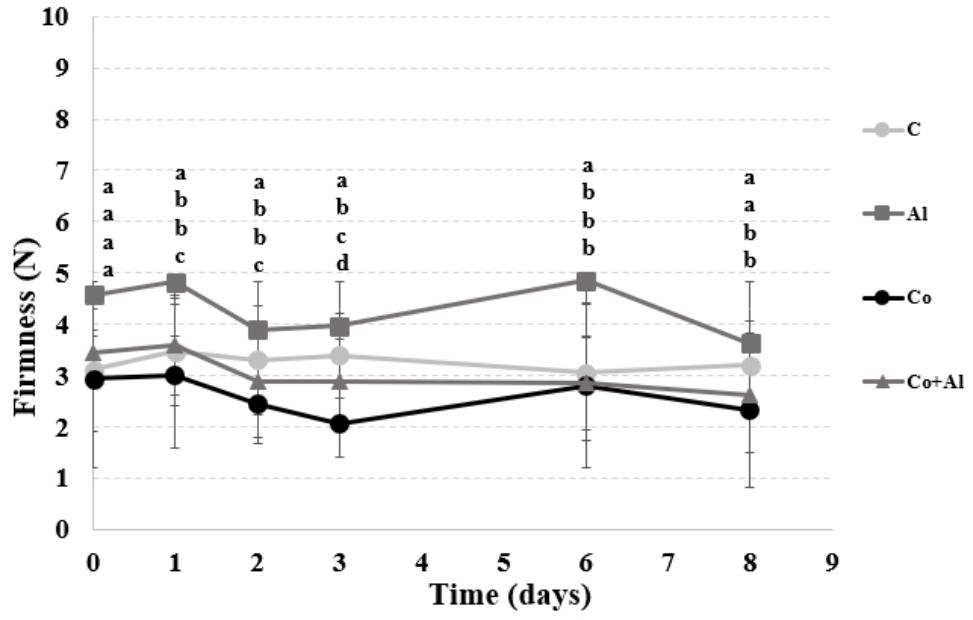
157x101mm (96 x 96 DPI)



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

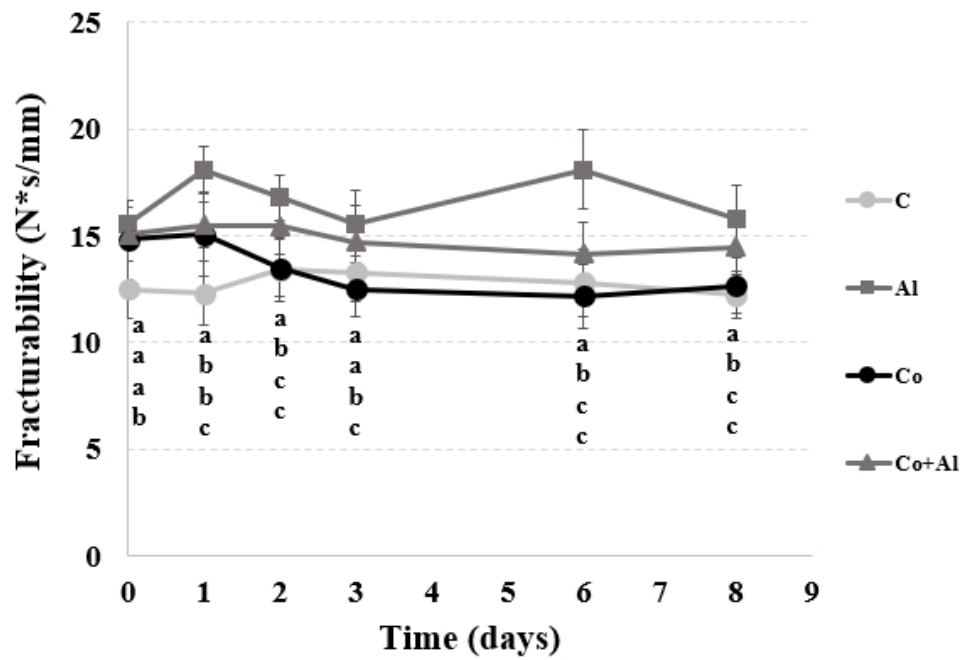
171x106mm (96 x 96 DPI)

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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)