Pomace-Cassava as Antioxidant Bio-Based Coating Polymers for Cheeses

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Abstract: Fruit and vegetable-based materials, rich in phenolic pigments, and especially anthocyanins, have attracted attention as promising sources for bio-based antioxidant coating polymers, being a non-toxic, natural, ecofriendly, and green label solution to lower oxidation degradation in oil-water emulsion food, such as cheeses. However, could their pomaces also be used in such materials? This work has investigated the use of jabuticaba peels and red cabbage stir pomace extracts as antioxidant additives for cheese coating polymers. The antioxidant capacity of the jabuticaba-red cabbage pomace cassava-based polymer was evaluated in vitro (total phenolic, total anthocyanin content and DPPH scavenging %) and in vivo (by coating Minas Frescal cheeses and monitoring their peroxide index increase during a 9-day shelf life, at 10 °C). An in vitro characterization has indicated a high antioxidant capacity for both pomace extracts, with a higher capacity observed for the jabuticaba peels. In vivo investigations indicated that the pomace-starch coatings have protected cheeses up to 8.5 times against oxidation when compared to the control, with a synergistic protector effect among pomaces. Physical–chemical characterizations (pH, acidity, total solids, ash, total protein, fat content and syneresis) have indicated no coating interference on the cheese’s development.

Keywords: dairy; active polymer; biodegradable; packaging; shelf-life

1. Introduction

Oil–water emulsion food, such as cheeses, are sensitive to light exposure; resulting in lipid, proteins, and vitamins autoxidation; causing undesired nutritional and sensory modifications [1,2] and harmful compounds development [3]. Antioxidant packaging and coating polymers appears as a safe and efficient alternative [1,4,5], not intending to substitute primary packaging materials, but offering an extra and sustainable preservation, and in some cases, even allowing these materials to have a lower protective capacity, with a consequent lower cost [6].

Most antioxidant active polymers use synthetic antioxidants; however, due to the potential long-term toxicological effect on human health and the environment [7,8], they have been replaced by natural, GRAS (generally recognized as safe), renewable antioxidants [9,10]. The antioxidant efficacy of fruit and vegetable-based ingredients is well known and has proven to be effective and safe as additives for antioxidant coating and packaging materials [11,12].

As the food industry aims for ways to increase sustainability, the use of fruit and vegetable pomaces, instead of the whole product, have attracted attention [1,2,7], despite the little exploration regarding antioxidant coatings for cheeses.

The utilization of fruit and vegetable pomaces in bio-based active polymers would not only be useful for cheeses, but for all food industries, also considering, besides its sustainable...
aspects, protection characteristics (ex. antioxidant, antimicrobial), smart ability potential (ex. pH, temperature sensitive) [1], and the possibility of achieving green label foods.

In this study, an antioxidant coating polymer was developed using the pomace extracts of jabuticaba peels [2,13] and red cabbage stirs [14,15], with well reported antioxidant capacity. The pomace extracts were added to starch coating polymers following a factorial design experiment. A cassava coating matrix was chosen for being a successfully reported renewable and biodegradable source for coating and packaging polymers, avoiding environmental issues related to solid waste [16–18] and maintaining its sustainable and edible characteristics. The extracts’ in vitro antioxidant capacity were characterized through total phenolic, total anthocyanin content and DPPH scavenging %. For an in vivo antioxidant evaluation, Minas Frescal cheeses were produced and recovered with the pomace coatings. The sheeses’ peroxide index increased after 9 days of storage (10 °C/9 days) and was evaluated to investigate the in vivo coating antioxidant effectiveness over a real product shelf life. Samples’ pH, acidity, total solids, ash, total protein, fat content and syneresis were evaluated to investigate the coatings’ effect over the cheeses’ normal development.

2. Materials and Methods

2.1. Jabuticaba and Red Cabbage Extract

Jabuticaba (Myrciaria cauliflora cv sabará, donated by professor Dr. Waldemar Gastosni Venturini Filho, from FCA-UNESP, Botucatu-BR) was smashed and heated in boiling mineral water (1:1) for 10 min, cooled to 25 °C and filtrated to separate peels from the first jabuticaba extract. Red cabbage stirs (Brassica oleracea cv capitata, purchased form Botucatu-BR, local market) were manually removed from cabbage leaves. Both jabuticaba peels and red cabbage stirs were blanched with water vapor (5 min), crushed, macerated with deionized water and stored in PEHD (high density polyethylene)-sealed films (−20 °C), until right before the starch forming coating production.

2.2. Starch Coating Forming Solution

Zhong, Cavender and Zhao [19] have reported no difference in cheeses’ morphology and basic quality changes when coated through different methods (dipping, enrobing, spraying and electrostatic spraying). For that matter, due to its simplicity, low cost and good coverage ability, the antioxidant coating forming solution were applied directly to cheeses through a dipping process. For the coating forming polymer, jabuticaba and red cabbage (0.0–6.2%, w/w and 0.0–4.0%, w/w total solids, respectively) extracts were added to corn starch (5%, w/w), with sucrose (1.4%, w/w) as a plasticizing additive [20] and heated (73 °C) for gelatinization, following the experimental design presented in Table 1. The maximum level for incorporation of the extracts was determined in preliminary tests considering the capacity of incorporation of the extracts into the polymeric matrix and the antioxidant capacity presented.

Table 1. Factors and respective levels (−1 for the minimum concentration, +1 for the maximum and zero for the center point) of the variables of red cabbage stirs and jabuticaba peels extracts, coded and real values (in total solids %).

<table>
<thead>
<tr>
<th>Assays</th>
<th>Coded Values</th>
<th>Cabbage (%)</th>
<th>Jabuticaba (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−1</td>
<td>−1</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>−1</td>
<td>4.02</td>
</tr>
<tr>
<td>3</td>
<td>−1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4.02</td>
</tr>
<tr>
<td>5 (C)</td>
<td>0</td>
<td>0</td>
<td>2.01</td>
</tr>
<tr>
<td>6 (C) *</td>
<td>0</td>
<td>0</td>
<td>2.01</td>
</tr>
</tbody>
</table>

(C): Central points.
2.3. Minas Frescal Cheese Processing and Coating

Raw whole milk (donated from the Veterinary Medicine Faculty of UNESP, Botucatu-BR, just after milking) was immediately pasteurized (65 °C/30 min) and cooled to 35 °C. Reconstituted freeze-dried lactic culture for direct vat set (DVS) (donated from CHR HANSEN, BR), 0.09% commercial rennet (Coalho Estrela, BR), and 250 ppm calcium chloride (Synth, BR) was added for milk coagulation during a 40 min/35 °C period. The curd was gently square cut (2 cm²), drained under refrigeration (4 ± 1 °C), placed in cylindrical perforated containers (500 g capacity), drained again for 60 min in each side and dry salted with sodium chloride (1 g/kg). Cheeses were refrigerated (4 ± 1 °C) overnight and dipped in starch coating film, forming solutions containing different jabuticaba: red cabbage extract with total solids concentrations (as defined by a factorial design experiment, Table 1) at 4 ± 1 °C, for 5 min. Cheeses samples were drained on stainless steel screens at 4 ± 1 °C and stored (10 °C/9-days).

2.4. Experimental Design

A factorial design 2² experiment was used to investigate the relationship between the experimental variables of red cabbage (%) and jabuticaba (%) pomace extract concentrations and corresponding responses. The factors and their respective levels were presented in Table 1. The independent variables were red cabbage (0.00 to 4.02%, w/w) and jabuticaba (0.00 to 6.27%, w/w) extract concentrations. The central points were investigated in duplicate to estimate the experimental pure error.

2.5. Physico-Chemical Analysis

Syneresis were evaluated through the weight of whey (g) released from each coated cheese during storage (1 and 9 days) divided by the total weight of the same cheese (g) at day 1 and multiplied by 100 [21]. Jabuticaba peels and red cabbage extracts: Total solids and ash content (gravimetric method) [22]. Minas Frescal cheeses: pH, dornic acidity, total solids, ash, total protein and fat content [22].

2.6. In Vitro Antioxidant Capacity

Jabuticaba peels and red cabbage stir extract in vitro antioxidant capacity was given through the following characterization analysis: 1,1-Diphenyl-2-picryl-hydrazyl-DPPH scavenging % [23]. Total phenolic (determined spectrophotometric ally, according to [24], 1965; with results expressed as catching equivalent—CE). Total anthocyanin content with pH-differential method; absorbance read at 510 and 700 nm, expressed as mg/L of cyanidin-3-glucoside equivalent) [25].

2.7. In Vivo Antioxidant Capacity

The antioxidant efficacy of the pomace–starch coatings was investigated by monitoring the coated Minas Frescal cheeses’ increasing peroxide index content [18,22], given by the difference observed between the peroxide index obtained at days 1 and 9 of storage (10 °C).

3. Results and Discussion

3.1. Cheese Coating

The dipping process has been demonstrated to be feasible, resulting in homogeneous and evenly covered cheeses, with a thin layer over the cheeses’ surface.

The coatings had no effect (p < 0.05) on cheeses’ total solids, ash, fat, pH, acidity, total protein or syneresis (Table 2), indicating no effect on cheese humidity exchange and on the microorganism’s normal development. We expected these results because of the hydrophilic nature of starch polymers, leading to higher water vapor permeability and oxygen permeability [26], which are necessary to microorganism maintenance in cheeses [27,28]. Such results are in agreement with other studies that have observed that the coatings did not interfere with the cheeses’ development, and in some cases, even protected them against humidity loss during storage [5,6].
Table 2. Coated cheese samples’ physicochemical analysis: Total solids (TS), ash, fat, pH and acidity \( (^\circ D) \) and total protein (TP) at 1 day-storage. Syneresis (S, g/100 g\(^{-1}\)) at 9 days-storage.

<table>
<thead>
<tr>
<th>Assays</th>
<th>TS (%)</th>
<th>Ash (%)</th>
<th>Fat (%)</th>
<th>pH</th>
<th>Acidity ( (^\circ D) )</th>
<th>TP (%)</th>
<th>S (g/100 g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.47</td>
<td>1.58</td>
<td>13.66</td>
<td>6.34</td>
<td>12</td>
<td>19.8</td>
<td>2.09</td>
</tr>
<tr>
<td>2</td>
<td>2.45</td>
<td>1.47</td>
<td>15.22</td>
<td>6.30</td>
<td>12</td>
<td>20.1</td>
<td>2.10</td>
</tr>
<tr>
<td>3</td>
<td>2.46</td>
<td>1.50</td>
<td>14.96</td>
<td>6.45</td>
<td>13</td>
<td>20.0</td>
<td>2.09</td>
</tr>
<tr>
<td>4</td>
<td>2.51</td>
<td>1.48</td>
<td>14.01</td>
<td>6.42</td>
<td>14</td>
<td>20.3</td>
<td>2.08</td>
</tr>
<tr>
<td>5 (C)</td>
<td>2.45</td>
<td>1.50</td>
<td>14.19</td>
<td>6.33</td>
<td>13</td>
<td>20.6</td>
<td>2.11</td>
</tr>
<tr>
<td>6 (C)</td>
<td>2.46</td>
<td>1.50</td>
<td>14.19</td>
<td>6.34</td>
<td>13</td>
<td>20.5</td>
<td>2.10</td>
</tr>
</tbody>
</table>

3.2. In Vitro Antioxidant Evaluation

In vitro results (Table 3) testified the well reported antioxidant capacity of the jabuticaba peels [13,29] and red cabbage stirs [30], with the jabuticaba peels extract DPPH scavenging % higher (93.5) than the presented red cabbage stirs (36.3%). The observed antioxidant capacity can probably be explained by the presence of antioxidant components [31] evaluated in this study through the jabuticaba and cabbage pomaces’ total phenolic (12.9 and 4.4 CE mM, respectively) and anthocyanin content (8.4 and 2.1 mg/L, respectively) (Table 3).

Table 3. Extracts’ antioxidant capacity (total phenolic content (TPC) in catechin equivalent (CE), total anthocyanin content (TAC): TAC, DPPH: scavenging %, Equivalent Trolox and TEC: antioxidant capacity in TROLOX equivalent).

<table>
<thead>
<tr>
<th>Extracts</th>
<th>DPPH %</th>
<th>TROLOX ((\mu)mols/g)</th>
<th>TEC</th>
<th>TPC (CE mM)</th>
<th>TAC (mg/L)</th>
<th>Total Solids (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cabbage stirs</td>
<td>36.3027</td>
<td>0.0105</td>
<td>0.4345</td>
<td>4.4</td>
<td>2.1</td>
<td>4.0213</td>
<td>1.0363</td>
</tr>
<tr>
<td>Jabuticaba peels</td>
<td>93.5079</td>
<td>0.0262</td>
<td>3.8217</td>
<td>12.9</td>
<td>8.4</td>
<td>6.2717</td>
<td>1.4764</td>
</tr>
</tbody>
</table>

3.3. In Vivo Antioxidant Evaluation

Coatings added with both pomace extracts, and their interaction, have negatively affected \((p < 0.05)\) the cheeses’ peroxide index increase % (Figure 1), as indicated by the Pareto graphic (Figure 2). As peroxide is a product generated in the first steps of the autoxidation reaction [1,31], the results have indicated that the pomace–starch coatings have protected the cheeses up to 8.5 times against oxidation (Table 3) when compared to the control (sample 1).

The highest antioxidant in vivo protective capacity was observed for the coatings added with the higher jabuticaba extract % (assays 3 and 4), (Figures 1 and 2, Table 1), which was expected due to the higher in vitro antioxidant capacity observed (Table 3) in this study.
Figure 1. Coated cheeses’ (assays 1–6) peroxide index increase (%) after 9 days-storage indicating the in vivo antioxidant protection of the active coatings.

Figure 2. The Pareto graphic indicating the jabuticaba and cabbage extracts effect \((p < 0.05)\) on cheeses’ (assays 1–6) peroxide index increase (%) after 9 days-storage.

4. Discussion

Since the dipping process resulted in homogeneously coated cheeses, it is suggested that the extracts be incorporated into the cheeses using the wet salting step, composing the brine, as already used for incorporating bioactive compounds to cheeses [32]. In this sense, in the way it was proposed the coating formation is advantageous when compared to other proposed cheese coating processes, which utilize processes and/or steps that are not usual in dairies, such as those that involve the coating formation in a previous step [33] or applying nanotechnology [6].
Independently of the combination of extracts, the cheese coatings’ pigmentation was visibly noticed, the jabuticaba pomace coatings (assay 3) being responsible for the most intense purple coloration when compared to the red cabbage samples (assay 2). This pigmentation was expected due to the rose-to-violet anthocyanin pigmentation present in both extracts [34]. Taking advantage of the chemo chromic nature of anthocyanins [10], future studies should investigate a correlation between changes in the coloration of these pomace-based coatings with changes in cheese acidity during storage, and also evaluate the acidity-indicating capacity of these polymers.

The jabuticaba pomace extract presented higher in vitro antioxidant capacity, with 2.6 times more DPPH%, 2.9 more total phenolic and 4.0 times more anthocyanin content when compared to the red cabbage pomace extract capacity (Table 2). The higher jabuticaba extract antioxidant capacity observed over the red cabbage extract agrees with other literature reports [11,13,29]. New studies should address the integral use of the jabuticaba peels as flour [35]; seeking, besides the antioxidant capacity, an increased fiber content and antimicrobial [36] characteristics.

When comparing to most antioxidant coatings for cheeses that use essential oils as active ingredients, the pomace extracts as used in this study have the advantage of not requiring encapsulation and not interfering so heavily with the cheese’s natural flavors [11,33,37].

Regardless of the higher in vivo antioxidant capacity presented by the jabuticaba coating materials, an interesting synergistic linear protection (p < 0.05; Figures 1 and 2) occurred when using both pomaces together, as indicated by sample 4 (highest jabuticaba and red cabbage extracts %). It is important to observe that using both pomaces could be an excellent alternative regarding cost and seasonality, as well as a sustainable food waste exploitation tool and a green label allegation possibility.

5. Conclusions

Grape and cabbage pomaces extracts can be used in active coating polymers to lower oxidation degradation in cheeses during storage. Applying such antioxidant coatings could be a way to reduce direct antioxidant addition to cheeses and increase its sustainability.

The coating forming process proposed is a feasible process for dairy industries that already use a dipping process for the brining procedure.

Considering the pH color sensitivity of the anthocyanin presented in the studied pomaces, new studies should investigate whether during storage a color modification could indicate an undesirable pH drop due to microbial growth.

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