Effect of collagen fiber and gelatin on gelling properties of alginate gels
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ABSTRACT
Mixed gelatin-alginate gels are widely studied as encapsulating matrices. However, the dissolution of gelatin at mild temperatures (close to body temperature) imparts low stability for these systems, particularly as delivery matrices. In this context, collagen fiber arises as an alternative due to its water binding properties. Thus, the main objective of this work was to evaluate the effect of substituting gelatin for collagen fiber in alginate mixed gels. Thereunto, alginate and gelatin/collagen fiber were firstly mixed before added to water at 45°C. Solutions were magnetically stirred during 90 minutes and were set in dialysis membrane. The membranes were sealed and put into a vessel containing a calcium chloride solution. The influence of biopolymer gelling conditions on the characteristics of the formed gel was also evaluated by inducing the gelation of alginate prior to the protein (dialysis prior to cooling) or simultaneously. Gels were evaluated according to its visual aspect, compression tests (mechanical properties) and shrinkage after 24-hours at room temperature. Generally, collagen-mixed gels were larger than gelatin ones, with lower shrinkage observed after the 24-hour storage. Mechanical properties results showed that gelatin-alginate mixed gels were quite similar to pure alginate gels. Similar behaviour was observed for collagen-mixed systems in which biopolymers gelation occurred simultaneously, indicating that these systems may be a good alternative to the substitute gelatin in delivery systems.

Keywords: biopolymers; mixed gels; mechanical properties

INTRODUCTION
Gelling biopolymers are widely used in food industry once they play an important role as texture modifiers and, more recently, as encapsulating matrices. Although biopolymer mixtures have been widely studied in recent years, their behavior is quite complex. Among the great variability of proteins and polysaccharides, the combination of alginate and gelatin for mixed gels has already been reported in literature. Alginate is a linear polysaccharide extracted from seaweeds that gels in the presence of calcium in the form of egg-box structures. It is already used in food and pharmaceutical industry as thickener, gelling agent and/or as coatings. Its gel is relatively stable to acidic pH (p.e. gastric digestion), swelling in weakly basic solutions (intestinal fluids) and thus this biopolymer is widely explored as encapsulation matrix for controlled release systems. However, alginate shows low cell-adhesiveness which is essential for adequate delivery systems. To improve such characteristic, alginate coating of gelatin microspheres have already been studied as well as the gelation behavior of gelatin and alginate mixtures [1, 2]. Gelatin is a protein obtained from the denaturation of the triple helix of collagen obtained from animal tissues that is widely known by its property of gelling with heating and further cooling. However, mild temperatures around 40°C promotes the dissolution of such gel, which imparts low stability of gelatin systems in temperatures close to human body (T~37°C). To suppress this dissolution, cross-linking agents have been explored, but the toxicity of these compounds usually hampers the applicability of the gels in food systems. Collagen fiber, is an ingredient produced from collagen that is not submitted to the drastic hydrolysis reaction carried out in gelatin production. This ingredient is already used in meat industry due to its water binding properties, although its use in other fields is still scarce. Although the aminoacid composition of collagen-fiber and gelatin is the same, their functional properties are quite diverse. It has already been reported that less drastic treatments leads to products with higher moisture absorbing and gelling activities as compared to gelatin [3]. Thus the main objective of this work was to evaluate the effect of substituting gelatin for collagen-fiber in alginate mixed gels.
MATERIALS & METHODS

Alginate, gelatine and collagen fiber used in the present research were donated by Danisco (Copenhagen, Denmark), Gelita (Eberbach, Germany) and NovaProm Food Ingredients (Guaiçara, Brazil), respectively. Alginate concentration was fixed in 1% (w/v) and gelatin in 1.5% (w/v). Collagen fiber concentration was fixed in 5% (w/v), once preliminary assays showed that its pure gel at this concentration exhibits mechanical properties more similar to the 1.5% gelatin system.

The biopolymers (alginate and gelatin or collagen-fiber) were firstly mixed before added to water at 45°C. Solutions were magnetically stirred during 90 minutes and were set in dialysis membrane. The membranes were sealed and put into a vessel containing a calcium chloride solution (150mM). In order to evaluate the influence of biopolymer gelling conditions on the characteristics of the formed gel, the samples were identified (Table 1) and stored at 2 different conditions: 1) at 45°C during 72 hours to induce the gelation of alginate by the slow migration of calcium ions, with a further storage at 10°C during 24 hours to induce thermal gelation of gelatin and collagen-fiber; 2) at 10°C during 72 hours in order to induce the gelation of both biopolymers simultaneously. After storage, gels were removed from dialysis membrane and cut into cylinders for mechanical properties tests. Samples were also stored at room temperature (25°C) during 24 hours in order to evaluate the shrinkage of the samples, which was associated to syneresis due to the amount of water released within the evaluated period.

<table>
<thead>
<tr>
<th>System</th>
<th>Composition</th>
<th>Gelling condition</th>
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<tbody>
<tr>
<td>A</td>
<td>1.5% gelatin + 1% alginate</td>
<td>1) alginate gelation prior to protein gelation</td>
</tr>
<tr>
<td>B</td>
<td>1.5% gelatin + 1% alginate</td>
<td>2) simultaneous gelation of both biopolymers</td>
</tr>
<tr>
<td>C</td>
<td>5% collagen fiber +1% alginate</td>
<td>1) alginate gelation prior to protein gelation</td>
</tr>
<tr>
<td>D</td>
<td>5% collagen fiber +1% alginate</td>
<td>2) simultaneous gelation of both biopolymers</td>
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RESULTS & DISCUSSION

Visual aspect and shrinkage

The formation of an opaque white gel was observed for all conditions with an outer transparent layer (gelled or not) observed around them (Figure 1). The characteristics of such layer depended on the formulation and storage conditions for the gel formation.

Generally mixed collagen gels showed a thinner outer layer, ~60% thinner than the outer layer from mixed gelatin gels, and were stable after the 24-hour storage with no shrinkage observed. Such result was related to the high water holding capacity of this ingredient [4]. On the other hand, the 24 hour storage led to a reduction of 5.5 – 7.7% on the diameter of mixed gelatin gels due to shrinkage, which was associated to water loss (syneresis) at room temperature.

Considering the gelling conditions, an outer transparent gel was observed in samples in which gelatin/collagen fiber gelation was induced after alginate gel formation (gels A and C). In this case, it was assumed that a strong alginate network was firstly formed, expelling more water and part of the protein from the network, that gelled in outer layer. On the other hand, simultaneous gelation of both biopolymers (gels B and D) led to a thinner and liquid outer layer, indicating the higher influence of protein on the structure of the formed gel.

Figure 1. Visual aspect of formulated gels: a) A; b) B; c) C; d) D.
**Mechanical properties**

Generally, depending on the protein (gelatin or collagen fiber), fracture curves showed distinct behaviours. The rupture of mixed gelatin gels was similar to pure alginate gels behaviour (abrupt fracture), presenting a more brittle characteristic, while the rupture of mixed collagen gels was smoother, independently of the gelling condition (Figure 2).

**Figure 2. Typical rupture curves from gels: a) mixed; b) pure alginate; c) pure collagen; d) pure gelatin.**

Mechanical properties of gelled systems are shown in Figure 3, and are compared to pure biopolymer gels. As observed in the fracture curves, rupture properties (stress and strain) of gelatin mixed gels were more similar to pure alginate gels, independently the gelling condition at which the gel was submitted. Generally mixed collagen fiber gels showed intermediate properties between pure gels of both biopolymers, although in this case, differences in the responses were observed for systems gelled in different conditions. In the case in which protein (collagen fiber) gelation was induced simultaneously with the polysaccharide, mechanical properties were more similar to pure alginate gels, indicated by the harder (higher $\sigma_{rup}$) and more deformable (higher $\varepsilon_{rup}$) responses.

Considering the low deformation property (elasticity modulus) (Figure 3c), it can be observed that gels in which the gelation of alginate occurred prior to the protein (samples A and C) were more elastic (reversible deformation), even as compared to pure gels. According to this property, the simultaneous gelation of protein and polyssacharide led to more fragile gel or that supports lower stress within the reversible range (lower E).
CONCLUSION

Collagen-fiber leads to a mixed gel with lower water loss leading to a higher stability to shrinkage (associated to syneresis) as compared to gelatin-alginate mixture. Moreover, the results presented in this paper showed that simultaneous gelation of both biopolymers on collagen-fiber mixed gels led to the formation of systems with mechanical properties similar to pure alginate and gelatin-mixed gels, indicating that this ingredient may be a good alternative for the development of delivery systems.

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REFERENCES