Fibers of calcium alginate produced by a microfluidic device and its mechanical properties

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ABSTRACT

Fibers are important microstructural elements in many foods and calcium alginate fibers may find applications in the structuring of many food analogues. Fiber properties depend largely on their uniform diameter and mechanical properties. The main objective of this research is to production of fibers calcium alginate of regular diameter and to present the effect of concentrations sodium alginate [Alg] and calcium chloride [CaCl\textsubscript{2}] on the elasticity properties of calcium alginate fibers.

The continuous production of calcium alginate fibers with a regular diameter is demonstrated by using a simple microfluidic device (MC). Mechanical properties of fibers were measured using a texturometer and a video camera. The concentrations [Alg] and [CaCl\textsubscript{2}] varied between 1.25 to 2.5\% and 0.5 to 2.5\% respectively. Results are presented as function of the tensile stress (\(\sigma_{\text{max}}\)) and tensile strain (\(\Delta L/L_0\)) and Young’s modulus (E). For the statistical analysis of the results was used the response surface (RS) method. A mathematical model showed a relationship between mechanical properties as a function of [Alg] and [CaCl\textsubscript{2}] concentrations.

As relevant results, for all [Alg] concentrations first a strengthening and then a weakening of fibers were observed. The tensile stress increased with the [CaCl\textsubscript{2}] concentration. Maximum fiber tensile stress was found for a [CaCl\textsubscript{2}] concentration around 1.4\%. Opposite to previous works the tensile stress decreased for higher [CaCl\textsubscript{2}] concentrations. This behavior suggest a model, first “egg-box” sites were filled when the concentration of calcium ions increased up to a saturation point (maximum tensile =1.4\% [CaCl\textsubscript{2}]). Then a weakness appeared at higher [CaCl\textsubscript{2}] concentration, this weakness was not reported in the literature. The tensile strain or percentage elongation at break (\(\Delta L/L_0\)) shown values over 100\%. Thus, it is necessary to understand the interaction of cation-polysaccharide-water and to take into account the concentration of sodium alginate and calcium chloride to predict the mechanical behavior of fibers. Alginate gelling properties can be considered as a model system for more complex food materials.

Keywords: alginate gel; diffusion; egg-box model; tensile stress; mechanical properties

INTRODUCTION

Structure formation in food materials is influenced by the ingredient properties and processing conditions. The use of well-defined flow, often simple shear, turned out to be essential to study and control the structure formation process in foods, such as processing of biopolimers systems [1]. Microfluidic devices allow to control microstructures in food engineering [2]. The rheological properties are a major influence on the acceptance of the product because of the texture properties of foods are linked to deformation, disintegration and flow of food subjected to strain. In particular, calcium alginate fibers find applications in the structuring of food analogues. Since alginate is an inexpensive and easily available natural biomaterials. Alginate fibers have an high moisture absorption, ion-exchange capabilities and perfect biodegradability. Alginate gels have the particular feature to be cold setting and heat stable. Properties of alginate gels can be considered as a model system for more complex food materials. In food engineering, alginate fibers are very used to encapsulate flavor, enzymes, proteins, drugs, active components [3-6]. Alginate gelation occurs when divalent cations (usually Ca\textsuperscript{2+}) interact with blocks of guluronic acid residues. Alginate is a family of linear copolymers of (1\(\rightarrow\)4)-linked \(\beta\)-D-mannuronic acid (M) and \(\alpha\)-L guluronic acid (G) residues, M and G residues are present in varying proportions and sequences depending on the alginic acid source. When forming alginate gels, two contiguous, diaxially linked guluronic residues form a cavity that acts as a binding site for calcium ions forming junction zones under electrostatic attraction; this behavior results in the formation of a three-dimensional gel network and is usually described by the “egg-box model” [7]. Characteristic affinities were shown to be a property exclusive to polyguluronate, while polymannuronate was almost without selectivity [8]. The diffusion method is characterized by letting a crosslinking ion (i.e. Ca\textsuperscript{2+}) diffuse from an outer reservoir into an
alginate solution, a gel immediate is formed at the interface when solutions of alginate and calcium ions are in contact each other. Mechanical properties are directly related to their chemical structure [9]. Values of tensile strength in calcium alginate fibers may be dependent of factors such as intra or intermolecular associations [10]. The fracture stress is a measure of the “strength” of a material [11]. This understanding suggests that material fracture is actually the stress overcoming the cohesive/adhesive forces within the network. For that reason, the fracture stress of alginate gels is proportional to the network crosslink density [12]. The change in density of gel depends on the concentration either of sodium alginate or calcium salt [13]. Contrary to engineering polymers, nowadays the fracture mechanisms for many biopolymer gels are still poorly understood. Despite of the wide use of alginate fibers, the studies on its mechanical properties are very scarce in the literature. The main objective of this research is to determine the effect of concentrations of sodium alginate and calcium chloride on the elasticity properties and to evaluate mechanical properties of alginate fibers. The diffusion method was used to prepare calcium alginate gel fibers continuous using a microfluidic device (MC).

MATERIALS & METHODS

Preparation of sodium alginate and calcium chloride solutions
Limit concentrations of sodium alginate were determined, the limit is the continuous production of calcium alginate fibers with MC. Solutions of sodium alginate (NaC₆H₇O₆) powder with 16% G and 38% M content at concentrations between 1.25 and 2.5% stirred up to complete dissolution. Then, a small amount of liposoluble red dye (red Laca FC-2030), 0.5% (w/w) was emulsified at each alginate solution, to distinguish the fiber at the outlet of the MC. Finally, let stand for 24 hours at 4°C before use. Calcium chloride [CaCl₂] solutions (CaCl₂.2H₂O p.a., CA-0520) were prepared at different concentrations (w/v).

Production of calcium alginate fibers
The MC used to produce calcium alginate fibers is presented (Fig. 1). It was fabricated by combining transparent polycarbonate plates using microfabrication technique and a metal needle. The calcium chloride-to-alginate flow rate ratio \( Q = Q_{CaCl₂}/Q_{Alg} = 5 \), where \( Q_{Alg} \) = 1. Measurements of single alginate calcium fiber diameters (Dₜ) were obtained using a stereo microscope (Olympus SZX7, Optical Co. Ltd., Tokyo, Japan). Images were recorded with a digital CCD camera Cool Snap Pro Color (Photometrics Roper, Division Inc, Tucson, AZ, USA) then processed and analyzed using Image-Pro Plus software (Media Cybernetics, Inc., Silver Spring, MD, USA).

Mechanical properties measurements
The apparatus used for measuring elasticity of calcium alginate fibers was a Universal Texture Analyser TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK), force-tension mode was selected. The calibration was carried out using 5 Kg load cell. Initial grip separation was set at 50 mm. A constant deformation speed of 0.1 mm/s, up to a tension strain ≥100% was applied. The force versus distance was recorded for each sample. A sample was constituted of a calcium alginate bundle or rod, i.e. a set of 20 calcium alginate fibers. Fibers were put together in parallel manner, i.e. without entanglement, to form a rod. Six replications for each sample (same concentration and same condition of production) were performed. The measurements were made at room temperature (25°C).

Maximum Tensile stress (\( \sigma_{max} \)) was calculate dividing the applied maximum force (at break) to the calcium alginate rod by the original cross-sectional area through which the force is applied (\( A_{rod} \)). The cross-sectional area calcium alginate rod is estimated from radius of the fiber, \( R_{fiber} \), multiplied by the number of rod’s fibers:

\[
A_{rod} = 20\pi R_{fiber}^2 = \pi R_{rod}^2 \quad \text{(Eq. 1)}
\]

Tensile strain (\( \Delta L/L_a \)) was calculated by dividing the elongated distance at break of the rod by the initial length of the sample (\( L_a = 50 \) mm). The Young’s modulus (E) was calculated using the relationship between tensile stress and tensile strain (\( \sigma \) vs \( \Delta L/L_a \)) in the linear viscoelastic range.

Experimental design and data analysis
The effect of [Alg] and [CaCl₂] concentrations, which were denoted as independent variables (X₁ and X₂ respectively) on the dependent variables, such as tensile stress (at break) (\( Y_1 \)), tensile strain (at break) (\( Y_2 \)), Young’s modulus (\( Y_3 \)), as well as a function of moisture content (\( Y_4 \)) of calcium alginate fibers has been studied by employing response surface (RS) methodology. A 3-levels factorial in 3 blocks design which studied the effects of 2 factors ([Alg] and [CaCl₂] concentrations), was executed in 06 blocks including 01 centerpoint per block, resulting in 24 experiments. All experiments were performed randomly, and data were treated with aid of Statgraphics Centurion XV Program, ANOVA 95% confidence (p<0.05).

Results from at least six samples were used for the stress-strain relationships, a minimum of ten samples to the diameter and three samples to the moisture content of the fibers. The dependent variables were
expressed individually as a function of the aforementioned independent variables using the following polynomial (Eq. 2). The coded levels of variables are -1, 0 and +1 ([Alg] = 1.25 -2.5%; [CaCl₂] = 0.5 – 2.5%).

\[ Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_1X_1^2 + \beta_2X_2^2 + \beta_1X_1X_2 \]  
(Eq. 2)

RESULTS & DISCUSSION

Elasticity behavior for various residence time
Residence time is defined as the wetting time of the calcium alginate rods in the collection bath fill of [CaCl₂] solution. The residence time ensures that the formation of calcium alginate gel has ended or calcium ion diffusion through the fiber has stopped. In Figure 2, the fibers quickly reaches equilibrium (or gelation) with the surrounding solution and are stable over time (>=15 min). This result is consistent with other studies that suggest time of gelation varied a few seconds to several hours [14], e.g. up to 24 h for pieces of 3.18 cm diameter of restructured scallops (Suklim and others 2004).

Fiber diameters
In Figure 3, fiber diameters are plotted versus [CaCl₂] concentration for four [Alg] concentrations. Fiber diameters showed an oscillatory behavior when [CaCl₂] concentration varied; the amplitude of the fluctuation in diameter is of approximately 150 µm.

Effect of the factors on response surface (RS)
The dependent variable measures experimental design, such as tensile stress, tensile strain are shown in plots RS. As well as plots for Young’s modulus and moisture content.

Maximum tensile stress (σ_max)
Contour plots of response surface for maximum tensile strength (σ_max) are shown in Figure 4. Strengthening and weakening of fibers are observed.

Figure 1. Sketch of calcium alginate fibers generator.

Figure 2. For various residence time (t), the tensile stress (MPa) vs its strain (%). In this case, the concentrations were fixed: [Alg]=2% and [CaCl₂]=0.5%

The reinforcement shows that the tensile stress increases with increasing calcium concentration up to a maximum area near [CaCl₂]=1.4%, then the values of tensile stress decreases (weakening) as the
concentration [CaCl₂] increases. Mathematical model coded for maximum tensile stress represented by Eq. (3) was statistically significant (p<0.05; R²=0.84), using the notation of equation (2) the model is as follows:

\[ Y_1 = 0.4709 + 0.1256X_1 - 0.0237X_2 - 0.1114X_2^2 \quad \text{: Eq. (3)} \]

A similar pattern with maximum tensile stress was observed about 30 mM (0.33%) [CaCl₂] concentration for mixed gellan gels [15]. In another study on the contrary [16] concluded that the tensile stress increased with [CaCl2] concentration or [Alg] concentration.

![Figure 4. Response surface for maximum tensile stress (σ_max) as function of sodium alginate and chloride calcium concentrations produced by the microfluidic device con Q=5](image)

**Tensile strain (ΔL/L₀)**

Figure 5 shows values of tensile strain for calcium alginate fibers above 100%, being the maximum value of elongation the experimental values of 116% record for [Alg] = 2.5% and [CaCl₂] = 0.5%. Coded mathematical model was statistically significant (p<0.05; R²=0.78) and is shown in equation (4):

\[ Y_2 = 0.8870 + 0.1896X_1 + 0.0622X_2 + 0.1436X_1^2 + 0.0517X_2^2 - 0.2543X_1X_2 \quad \text{: Eq. (4)} \]

An investigation reported that in the case of wet calcium alginate fibers, it was impossible to measure the elasticity because of difficulties in handling the sample [17], in this study were carried out such measures. Other research concluded that the tensile strain was insensitive to [CaCl₂] or [Alg] composition [12]. Moreover, the tensile strain values obtained are more higher than those reported in another study [14] for curves two-cycle compression in cylindrical samples of alginate gels (40 mm in diameter and 10 mm in height), whose values were between 17.6% and 38.8% ([Alg] varied 0-3%). A material with very low tensile strain is usually undesirable, as it appears to be too soft or soggy to the consumers. However, the values of tensile strain found in this study shows a high elasticity of this material, in some cases over 100%. This could be because the fibers were produced in other way. The shape of the gel produced (fibers) allowed to determine elongation correctly, test in tension. Therefore, the resulting elongation values may correspond to their actual values.

**Young's modulus**

Contour plots of response surface for maximum Young's modulus (E) are shown in Figure 6. Young’s modulus or the elastic modulus is fundamental because is a measure of material stiffness, such that the higher the value E, stiffer is the material. The model was statistically significantly (p<0.05; R²=0.863). The behavior of Young's modulus values were similar to the maximum tensile stress, with maximum values around 0.5 MPa. The elastic modulus are relatively low (i.e., below about 10⁶ Pa) which indicates can be predominantly elastic on deformation during a relevant time-scale.

**Moisture content (MO)**

Moisture content is another parameter to take account in the mechanical properties of alginate gels. In Figure 7, RS plot indicate that the moisture content (MO) decreased slightly with an increase in the concentration [Alg] and decreases drastically with increasing the concentration [CaCl₂]. Values MO corresponding to the higher values of tensile strain (elasticity ≥100% for [Alg] ≥ 1.8%) were minor to the 95%. These values can be related to the fact that the plasticizers reduce the interactions between the adjacent chains in the biopolymers. An important variable in mechanical properties is the moisture content of calcium alginate fibers, so whether the presence of excess calcium ions may affect the hydration of the alginate fibers.
Proposed Model

One step that complements the egg-box model, can be used to explain the effect of increasing concentration [CaCl₂] on mechanical and microstructural properties of fibers calcium alginate. The model is presented using a sketch in Figure 8. In freshly prepared gel fibres, junction zones are assumed to consist of dimeric units (“egg-box dimers”) [18]. Strengthening and weakening of fibers are observed. First “egg-box” sites were filled when the concentration of calcium ions increased up to a saturation point (maximum tensile stress ≈ 1.4% [CaCl₂]). Then a weakness appeared at higher [CaCl₂] concentration. This weakness was not reported in the literature. The decrease of the tensile stress could be related to a possible partial collapse of the network. The decline which continues, is also mentioned in another study [19], suggested that lateral chain association reduced the number of junction zones thus leading to a decrease in the modulus. This behavior, the presence of a maximum indicates a determined presence in number and size of binding sites along the fibers of calcium alginate. Other studies, with small-angle X-ray scattering and rheological characterization of calcium alginate gels, mentioned that lateral association of chain segments depends on several factors resulting in well-defined association numbers [13]. The results of this study are contrary to previous reported studies, at given concentration of alginate, the number and size of junctions zones increased with increasing Ca²⁺ concentration, resulting in high crosslink density and short network chain segments between junction zones [13], the increase in network crosslink density gave rise to increasing fracture stress [12]. However, the literature indicates that, it is still unclear whether the increase in modulus is caused by a higher strength of the junction or if it is caused by a larger number or junction zones [8]. These results are a tentative extension model of the “egg-box” model used to describe ionotropic gelation of alginate and hence enhance the understanding of the depth of interaction cation-polysaccharide-water and the structure-function relationship of alginate gels. Alginate gelling properties can be considered as a model system for more complex food materials.
CONCLUSION

Calcium alginate fibers continuous and uniform in diameter and stable over time were successfully fabricated. The tensile stress increased with the Ca$^{2+}$ concentration. Maximum tensile stress of the fiber was found for a calcium chloride concentration around 1.4%. Opposite to previous works the tensile stress decreased for higher calcium chloride concentrations. Similarly, concentrations sodium alginate ≥1.8%, reported tensile strain or elasticity values near 100%, which correspond to moisture content values underneath 95%. The values of Young's modulus shows a maximum around of 0.5 MPa for concentrations calcium chloride around 1.4%. Thus, it is necessary to understand the interaction of cation-polysaccharide-water and to take into account the concentration of sodium alginate and calcium chloride to predict the mechanical behavior of fibers. Alginate gelling properties can be considered as a model system for more complex food materials.

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