Approaches for casein film uses in food stuff packaging

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Aplicaciones de biopelículas de caseína en el envasado de alimentos

Aplicacions de biopel·lícules de caseïna a l' envasat d'aliments

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SUMMARY

Films prepared with casein and its fractions were characterized by means of texture, SEM and microbiological analyses. Mechanical characterization showed that these films could have potential applications in packaging. Additionally, these materials proved to be a suitable physical barrier against microbiological contamination, which could be profited as protecting material for foods.

Keywords: Casein; films; texture; SEM; microbiology.

RESUMEN

Se prepararon biopelículas a partir de caseína y sus fracciones y los materiales desarrollados fueron caracterizados mediante texturometría, microscopía electrónica de barrido y análisis microbiológicos. De acuerdo a los resultados obtenidos, las propiedades mecánicas de las biopelículas demuestran que estos materiales pueden tener potenciales aplicaciones en el envasado. Por otra parte, y, aunque deben de ser desarrolladas más investigaciones en este aspecto, estos materiales actúan como barrera protectora frente a la contaminación microbiana.

Palabras clave: Caseína; biopelícula; textura; MEB; microbiología.

RESUM

Es van preparar biopel.lícules a partir de caseïna i les fraccions i els materials desenvolupats van se caracteritzats mitjançant texturometria, microscopia electrònica d'escombrat i anàlisi microbiològics. D'acord als resultats obtinguts, les propietats mecàniques de les biopel.lícules demostren que aquests materials poden tenir potencials aplicacions a l'envasat. Per una altre banda, tot i que han d'ésser desenvolupades més investigacions sobre aquest aspecte, aquests materials actuen com a barrera protectora front a la contaminació microbiana.

Paraules clau: Caseïna; biopel·lícules; textura; MBE; microbiologia.

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INTRODUCTION

Films have been obtained from proteins, polysaccharides, lipids or combinations of these components[1]. Applications of these materials on foodstuffs have been investigated since they could be an alternative to synthetic polymer films reducing the negative environmental impact and disposals costs[2]. Among their several advantages, non-toxicity, biodegradability, wide availability and biocompatibility are included[3].

However, formulation of protein-based films requires incorporation of plasticizer to reduce their brittleness, allow easier removal from the forming support and confer plastic properties. Moreover, the plasticizers improve flexibility, extensibility, toughness and tear resistance of the material. The most common plasticizers are polyols (e.g. glycerol, sorbitol and polyethylene glycol 400), mono-, di- or oligosaccharides, and lipids and derivatives thereof[4].

Milk proteins, including casein and whey proteins are considered exceptional candidates for biodegradable films. Specifically, casein can easily form films due to its random coil nature and its ability to form electrostatic, hydrophobic and hydrogen bonds[5]. Additionally, many studies reported on methods to improve the functional properties of films made from casein and casein derivatives[6]. However, there are few reports investigating the protector effect of these biopolymers regarding microorganism contamination.

Since every natural polymer has its own material-specific characteristics for packaging purposes, biopolymers are expected to satisfy the functional requirements of desirable barrier and mechanical (being tough, stress-resistant, elastic) properties. Hence, the aim of this work is to characterise the mechanical properties and the microstructure of films developed with casein and its fractions in order to evaluate the possible applications of these materials as physical barrier against microbiological contamination.

MATERIALS AND METHODS

Casein fractionation

Skim milk (0.25 g fat/100 ml) (CAPSA Food) was employed as raw material, pH was adjusted to 4.0 with 20% (v/v) HCl (Panreac) and after 15 min milk was centrifuged (2000 rpm, 10 min, 20°C) (Kubota High Speed Refrigerated Centrifuge 6500). The pellet was then washed with distilled water and it was centrifuged again. This pellet was freeze-dried at -70°C and 0.1 mBa in a Telstar Cryodos Lyophilizator, sample was frozen at -80°C previous to lyophilisation (sample FCAS). Different fractions of casein were obtained from the fresh pellet employing the method reported by Law and Leaver[8]. The obtained fractions were likewise lyophilised in the same conditions described above (samples αCAS, βCAS and κCAS). Additionally, commercial casein was employed as reference material (Sigma-Aldrich) (sample CCAS). The Kjeldahl method was carried out in triplicate to determine the protein content of different samples.

Film formation

The method used by Abu Diak et al. (9) was modified as follows. Aqueous solutions of ammonia solution (3M) and glycerol (Panreac) were prepared in distilled water at 2.25% and 1.50% (w/w), respectively. Then 7.5% (w/w) of casein powder was dispersed in this solution and the mixture was totally homogenized by magnetic stirring at 500 rpm. Subsequently, films were cast by pipetting 6, 12 or 15 g of the solution onto Petri plates (90 mm of diameter) and dried at room temperature for 24-48 h. Finally, the films obtained were peeled from the plates before any analysis.

Mechanical properties

A TA.XT*Plus* texture analyser (Stable Micro System, Surrey, U.K.) equipped with a 5 kg static load cell was used to carry out puncture tests. At least three samples of each film were prepared and tested. The films were fixed using the Film Support Rig (HDP/FSR) and punctured to breaking point with a spherical stainless steel plunger (5-mm diameter). Breaking force (gel strength) and deformation (elasticity/deformability) were calculated employing the *Exponent* software.

Scanning electron microscopy (SEM)

Previously to be characterized by scanning electron microscopy, films were frozen at -80°C and freeze-dried at -70°C and 0.1 mBa in a Telstar Cryodos Lyophilizator. Samples were subsequently critical point-dried with liquid CO_2 in a Bal-Tec CPD 030 Critical Point Dryer. After drying, the samples were mounted on small carbon-tape double-sided aluminum brackets and were bombarded with gold in a Sputtering Balzers SCD 004 vacuum evaporator. Finally, the films were observed on a JEOL-6610LV SEM.

Microbiological tests

In order to determine the possible protector effect of developed films as packaging material, under sterile conditions films were placed covering Petri dishes that contained PCA (Biokar). Then Petri dishes were exposed to the lab atmosphere during 24 h. Finally Petri dishes were incubated at 30°C for 24-48 h and, after this time, total colonies were counted. Control Petri dishes without films were also exposed to lab atmosphere during 24 h. All tests were carried out in duplicate.

RESULTS AND DISCUSSION

According to Kjeldalh analyses, commercial casein (CCAS) presented the major amount of yolk proteins (90.0%), while casein obtained in this work (FCAS) showed an amount of protein much lower (74.9%). Regarding casein fractions, values of protein content in α- (αCAS), β- (βCAS) and κ-casein (κCAS) were 71.9, 63.0 and 41.0%, respectively. In this context, it is important to point out that it only was possible to develop films with CCAS, FCAS and αCAS raw materials.

This can be due to the low protein content of βCAS and κCAS fractions which seems to be not enough to constitute a resistant film. So, only CCAS, FCAS and αCAS films were evaluated in the subsequently characterization.

Regarding the results of the texture analysis, the weight of the films is determinant on the mechanical properties. Hence, and as can be seen in Table 1, in all cases, the greater the weight of the film, the higher the strength required to break it. In addition, the greater the weight of the film, the greater the deformation produced before the rupture is achieved (the films were more elastic). However, it should be remark that, for the same weight, CCAS film showed the highest values of breaking force, FCAS film exhibited the lowest ones and αCAS films presented intermediate values. Taking into account that FCAS and αCAS contained a similar amount of proteins, regarding strength, not only the amount of proteins is determinant, but also its nature. In addition, deformation values for each weight were very similar in all cases.

Table 1. *Texture analysis results. Average values ± SD are reported.*

		Breaking force (kg)	Deformation (mm)
CCAS	6 g	1.29±0.38	3.25±0.47
	12 g	2.68±0.38	4.37±0.53
	15 g	4.13±0.96	4.68±0.36
FCAS	6 g	0.76±0.10	3.75±0.32
	12 g	1.68±0.30	4.39±0.30
	15 g	1.83±0.13	4.78±0.26
αCAS	6 g	0.99±0.34	3.21±0.43
	12 g	2.30±0.31	4.43±0.29
	15 g	2.96±0.59	4.42±0.55

Figure 1 shows the scanning electron micrographs of the films. FCAS and αCAS films showed heterogeneous surfaces where irregular particles were observed. On the contrary, CCAS films exhibited a very uniform soft appearance where particles were not appreciated. The smoothness and homogeneity of CCAS films bears a resemblance with casein films found in literature [6,10]. In addition, rough patches observed in FCAS and αCAS films can be attributed to protein complexes. Qualitative and quantitative differences in protein content of samples are probably responsible for differences between microstructure of films.

Microorganism counts were 76.3 ± 6.2, 2.5 ±0.5, 2.0±1.0 and 21.0±8.0 UFC/Petri dish for CONTROL, CCAS, FCAS and αCAS, respectively. As can be seen, in all cases the number of colonies was lower than in control. In fact, in CCAS and FCAS films, values were very similar, whereas in αCAS the amount of colonies was ten times higher than in case of the other films. Microstructure is a key parameter regarding film characterization, since structural defects will affect the films' mechanical and barrier properties [10]. So, the differences observed in microorganism counts are presumably due to microstructure characteristics. As it is shown in Figure 1, CCAS and FCAS films, despite their different surface, showed no voids, on the contrary in αCAS surface many tiny holes can be observed (see arrows in Figure 1).

Mechanical properties of developed films have proved these materials as suitable for being used in food packaging. Furthermore, the notable property of the films developed in this study was the possibility to work as physical barrier against microbiological contamination.

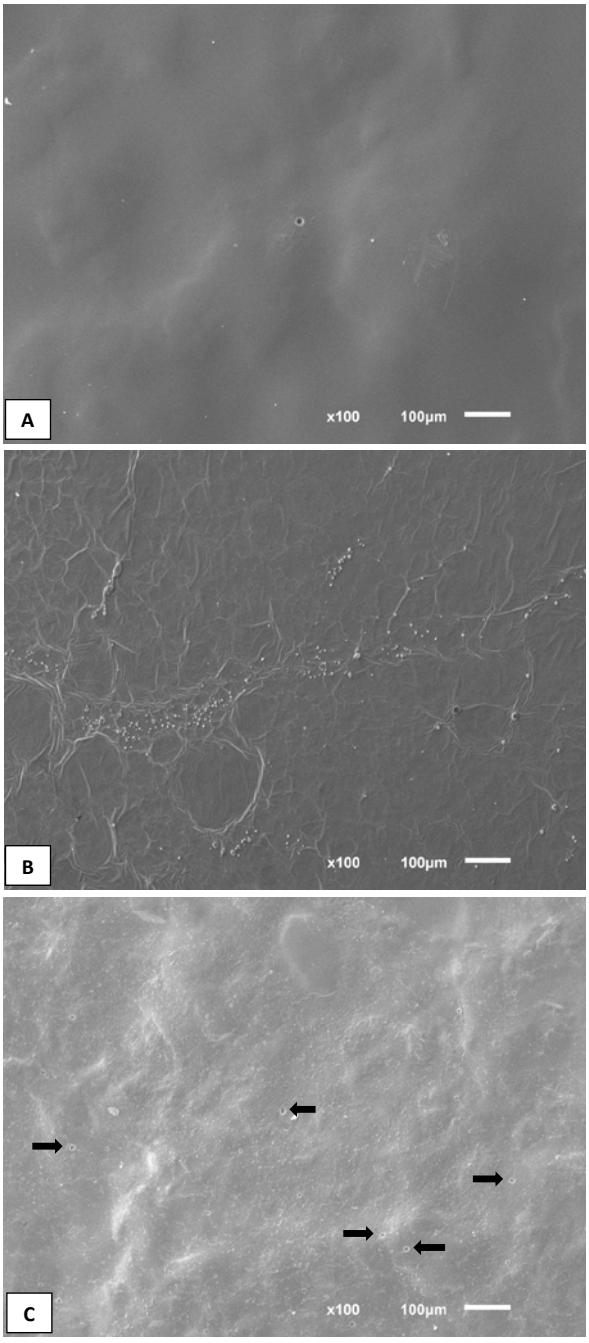

Figure 1. SEM micrographs of CCAS film (A), FCAS film (B), αCAS film (C). Magnification: 100x. Bar: 100µm.

CONCLUSIONS

Results showed that casein-based films exhibited accurate mechanical properties to be potentially employed in different areas of biocompatible and/or biodegradable coatings. Moreover, these films showed interesting properties against microbial contamination. That is the reason to highlight the importance of casein films to be used in food stuff packaging. Further investigations are needed to test the effectiveness of these films on specific food products, particularly those that are highly microbial-sensitive.

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