NANOSTRUCTURED BIODEGRADABLE POLYMERIC FILMS FOR FOOD PACKAGING APPLICATIONS

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***Abstract*—**

The present work aims to develop a bionanocomposite film from calcium caseinate and starch modified nanostructured with bentonite. The films were prepared by the solution casting method with the addition of glycerol as a plasticizer and PVA to improve their properties. A statistical analysis was performed to find the proportion to be used between casein and starch. Physical and chemical characterizations were performed using SEM, EDS and FTIR. From water vapor permeability analysis, a decrease in value was found with respect to casein and starch films without PVA, attributed to the addition of clay. Microbiological tests reported a total number of admissible bacteria in casein films and few yeast colonies due to their manipulation. The biodegradability test showed that films can be degraded at a rate of 13 weeks under ambient temperature and soil was not adjusted for any humidity conditions. It was concluded that the inclusion of PVA and modified starch with nanostructured bentonite suspension in the casein film formulation improves mechanical properties, compared to similar films with up to 30% increase in tensile strength with a value of 13.083± 2.1 MPa.

***Index Terms*—** bionanocomposite, casein, starch, bentonite, biopolymeric film, PVA.

I. INTRODUCTION

At present, the development of biodegradable materials has grown and the interest in creating alternatives to raw materials as a result of the production of plastics used for the food industry is increasing [1]. Every year more than 380 million tons of plastic are being produced that can end up as contaminants [2]. Many applications of polymers, such as those used for packaging, cause real environmental damage and take decades to hundreds of years to disintegrate [3]. The environmental impact also lies in the effects caused by plastics for marine species, since 43% of marine mammal species are susceptible to ingestion of these [4], while polymer exposure goes beyond the death of these species.

The management of waste in high amounts is expensive since 20-25% of plastics produced end up in landfills [4]. Although laws have been implemented to reduce plastic, recovery processes are limited due to the low budget allocated by cities for environmental regulation [3]. Recycling is one of the ways for the disposal of these wastes, but an alternative to increase this percentage of waste recovered in relation to those produced is the use of biodegradable polymers.

Biodegradable polymers can be obtained from biomass (polysaccharides, lipids, proteins), synthesized from monomers or petroleum mixtures; and also produced by microorganisms [5]. In recent years, due to the problems associated with obtaining conventional plastic materials and their accumulation [6], proteins have been explored as raw materials for the production of bio-plastics used for packaging materials [7].

Natural proteins are good candidates for producing food packaging films due to their excellent functional and structural properties [8] both for their film-forming qualities and their nutritional value [9]. For this reason, natural

materials have become a hot topic for research due to their biocompatibility and biodegradability [10], including casein and starch.

Casein is extracted from animal or vegetable proteins, and it is the protein that predominates in milk. Cow's milk proteins constitute approximately 80% casein and 20% whey proteins [11]. In milk, caseins exist as large colloidal particles of 50–600 nm in diameter, known as casein micelles where their 4 main components are αS1-, αS1-, β- and κ-caseins [12]. Previously, casein particles used to be called "calcium caseinate-calcium phosphate particles", however, it was discovered that casein particles dissociated if calcium was replaced by sodium there [10]. Today they are available in many different by-products. of the dairy industry, including as calcium and sodium caseinates [13].

Casein has excellent characteristics that make it very attractive for the preparation of edible films and coatings because it is available, non-toxic, and thermal stable [14]. It has the ability to bond ions and small molecules, a high degree of molecular flexibility [15] which makes it highly stable. Specifically, casein can easily form films due to its random coil nature, in addition, which has the ability to form electrostatic, hydrophobic [16] bonds and hydrogen bonds.

Casein films offer different advantages for a large number of applications, such as the plastics industry, medical and dental products, where the use of casein as a pharmaceutical tablet layer has been evaluated [17]; in the textile industry for synthesis of nano fibers [18][19]; in the food industry it has shown effects such as fruit surface coating [20] and for the packaging of cheddar cheese [21]. These films can form a good barrier to oxygen and other non-polar molecules, because casein provides the film matrix with many polar functional groups, such as hydroxyl and amino groups [22].

Some of the other advantageous properties are high moisture transfer capacity, good resistance against small insects and fungi, and UV resistance [23]. Despite their properties, casein films tend to become brittle during drying, so the use of edible plasticizers, such as glycerol, has been implemented to increase the free volume of the polymer network [22] and fabrication of flexible casein films. The addition of biodegradable polymers [5] can develop these limitations.

In the same way, starch films are gaining attention as substitutes for synthetic polymers due to their biodegradability and environmental compatibility [24], such that it could help to develop a biocomposite packaging material at a reasonable cost. According to [25], by 2020 the most important biological polymers will be from starch. Despite being an adequate substitute for polymers extracted from petroleum derivatives, starch-based films have poor mechanical properties and high rigidity [26] [27]; so, research focuses on using other polymers as additives [28].

On the other hand, polyvinyl alcohol (PVA) is the biodegradable synthetic polymer produced at the highest volume worldwide, due to its excellent physical properties and chemical resistance [26]. In addition, it has a good film formation, strong conglutination and high thermal stability [29]. However, PVA has a weak dimensional stability due to high water absorption [30]. The combination of PVA with natural sources is a reasonable option to compensate for the weakness of the mechanical properties, as well as the high cost of the PVA [31]. In this way, starch studies have been carried out to evaluate its properties [32][33], and even mixtured with casein to synthesize fibers [34].

PVA/casein films show good tensile strength and moderate elasticity, and starch also benefits this property but the presence of glycerol affects the barrier properties of the films [5]. Currently, nanocomposites that demonstrate improved barrier properties to water vapor, permanent nitrogen gases, oxygen, and carbon dioxide are being implemented for food packaging applications [35]. Clays are usually incorporated into polymer matrices to improve their physical properties [36], showing large improvements with very small amounts of clay fillers (<5%) [37]. Most clays are highly hydrophilic; therefore, they tend to absorb water molecules and coordinate them within their sheets, where positive counterions are usually found [35].

The most common materials included as inorganic fillers in the polymer nanocomposite composition are clays that belong to the group of magnesium-substituted and sodium-balanced silicates, also known as smectites [37]. Studies have implemented nanostructures with clay for starch [38] [39] [40] and for casein [41][42]. In view of the above, the present work was carried out to develop and characterize a biodegradable nanocomposite film based on calcium caseinate, starch and PVA with bentonite for packaging in the food industry.

2. MATERIALS AND METHODS

2.1. Materials

Calcium caseinate as a spray dried milk protein powder containing 92.1 % protein and having white to light cream color was purchased from Centro Agrolechero Group S.A.S. Modified starch Snow Flake (Roquette), bentonite (4,23 g/cm³) and poly(vinyl alcohol) (PVA) as a white powder were purchased in the local market.

2.2. Preparation of calcium casein solution

6% w / v solution of calcium casein was prepared in distilled water at room temperature. Then, the solution was heated at 35 °C and was added 1M NaOH solution (30% v / v solution of calcium casein) as a neutralizer mentioned by [21][43]. Prepared solution was continuously stirred with magnetic stirrer and glycerin (30% w/w of calcium casein) was added and mixed for 10 min.

2.3. Preparation of starch and bentonite solution

5% w / v of modified starch was prepared with distilled water at room temperature and heated at 90 ° C. Then, 40% glycerin was added and mixed for 20 min at 500 rpm until the starch was gelatinized. On the other hand, a bentonite suspension was prepared with distilled water (4% w / v) and mixed under magnetic stirring. Suddenly, it was placed in a ultrasonic bath 40 kHz to be dispersed in distilled water for 1 h at room temperature and then mixed with the starch solution.

2.4. Experimental statistical validation

Base solutions were prepared to choose the casein/starch ratio (Table I). A completely randomized design with repeated measurements ($P<0.05$) for tensile strength response variable was proposed. The data obtained from validation experiments of the study was subjected to analysis of variance (ANOVA) using software for statistical computing (R 3.6.1).

TABLE I
LEVELS OF DESIGN BASED ON THE MASS OF CASEIN AND STARCH

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Level	Casein [g]	Starch [g]
0S	0.9	0
1	1.8	0.9
0	0.9	0.9
-1	0.9	1.8
0C	0	0.9

2.5. Preparation of biodegradable film.

Casein solution was added to a starch solution with a specific volumetric ratio and stirred manually. 6% w / v of a PVA solution was prepared with distilled water at room temperature, and heated at 80 °C for 1 h under constant stirring. PVA solution was reserved for 24 h at room temperature. The polymer solution was prepared in a 30:70 ratio of casein/starch/bentonite solution to PVA, respectively. The final solution was heated at 35 ° C and was

degassed by applying vacuum to remove bubbles. Finally, the final solution was deposited in polystyrene Petri dishes corresponding to a volume of 15 mL to perform polymer extraction by molding. The samples were placed in a horn at 38 ° C for 24 h. Then, they were detached from the Petri dishes and placed in a desiccator for 48 h.

2.6. Viscosity of the final solution

BROOKFIELD DV-II+ Pro programmable viscometer was used to calculate the viscosity of the final solution. Viscosity value can greatly help the analysis of power requirements when the present film wants to be optimized by extrusion. The test was performed at 25 ° C with periodic speed increases. Viscosity was reported by average of samples that exceeded a torque percentage of 10%.

2.7. Characterization of bionanocomposite film.

2.7.1. Fourier transformed infrared (FTIR)

This technique was employed to study the molecular interactions that produced the combinations of polymers. Agilent Cary 630 FTIR was used to collect the spectra of each sample at room temperature and a wave number ranging from 4000 cm^{-1} to 400 cm^{-1}.

2.7.2. Surface morphology

The surface morphology of neat bentonite, CAS/STA film blends and nanostructured biodegredable film were examined by using TESCAN LYRA3 dual beam system that combines a high-resolution FE-SEM column with a versatile high-performance Ga ion source FIB at an accelerating voltage of 10 kV. Chemical analysis with coupled energy dispersive spectroscopy (EDS) was performed. All samples were fixed on aluminum stubs and coated to improve the contrast of each component.

2.7.3. Mechanical properties

The thicknesses of nanostructured biodegradable films were measured using a digital caliper (DC1004-6" Discover; 0.02 mm sensitivity) at five random places and the mean values were recorded for each sample (n=8). Mechanical properties were determined according to ASTM-D 882 standard. The test was applied by using electromechanical universal testing machine (Model: WDW-30, Jinan Testing Equipment IE corporation) programmed with 0.2 kN load. Samples were cut into 20 mm x 80 mm rectangle pieces and crosshead speed was set at 50 mm/min. The elongation at break and tensile strength values was determined (n=8).

2.7.4. Color analysis, light barrier properties and transparency

CR-410 Chroma Meter was used to realize color analysis of the films. The samples were placed in the measuring head and then covered to take each reading. The values L * (clarity), a * (red / green) and b * (yellow / blue) were measured for four repetitions. Two samples were evaluated, films with and without bentonite.

For the ultraviolet and visible light barrier properties film samples were cut into rectangles and placed on the inside of the spectrophotometer cell. The absorbance spectrum was definied in 400-800 nm. A Thermo Scientific Evolution 300 UV-Vis Spectrophotometer with Xenon lamp was used. According to methods proposed [44][45] the films were measured at 600 nm in duplicate and transparency was calculated based on the following equation:

$$Transparency = \frac{\log(\%T_{600})}{x} \quad \text{Eq(1)}$$

Additionally, according to the procedure [46] and ASTM D 1003-00 [47] recommendations, the opacity value was reported as absorbance units x nanometers (AU nm) defined as the area under the recorded curve.

2.7.5. Water Vapor Permeability tests

Water vapor permeability (WVP) was determined gravimetrically according to the standard methodology described in ASTM E96 [48]. The films were sealed in small beakers containing distilled water and were fixed on top of the test cells using a rubber and were taken to a desiccant that had silica gel inside. The samples in the desiccator were left at a relative humidity of 60% and a temperature of 21 ° C and weighed every 12 h for three days. The WVP was determined with the equation:

$$WVP = \frac{\left(\frac{g}{t}\right) * i}{A * \Delta P} \quad \text{Eq (2)}$$

where g/t was calculated from the slope of the linear regression of weight loss versus time (g/s); i, the film thickness; A, the exposed area of film, and ΔP, water vapor partial pressure difference across the respective film.

2.7.6. Microbiology test

In order to determine the possible protector effect of developed films as packaging material, films were subjected to 2 microbiological tests. For total germ and bacterial count according ASTM D 4635-86, samples were placed covering Petri dishes that contained TSA for triplicate. Then Petri dishes were incubated at 30ºC for 24-48 h and after this time, total colonies were counted. For the mold and vegetable count samples were placed covering Petri dishes that contained Potato Dextrose Agar (PDA) and films were incubated at 25ºC for 5 days.

2.7.7. Biodegradation rate

The biodegradability of the films was analyzed by burying dried films under indoor soil using method previously described in the literature [49] with some modification. Samples were cut into rectangular shape (2x3 cm^2) and then dried in a muffle furnace at 90 ºC for 2 h to record their initial weight (Wo). Each sample were buried in soil (6122 g) in a plastic box (30 ×11 × 13 cm^3) by placing them at 5 cm depth from the surface. The films were put into aluminum cups to permit an easy retrieval of the degraded samples. The test was performed at room temperature (21 ± °C) for 9 days.

In order to determine the average weight loss (Wt) [50] the samples were removed from the soil at an interval of 24 hours and cleaned to remove any soil from their surfaces. Then, the specimens were dried at 80ªC for 2 h to be weighted and calculated the biodegradation rate with the following equation:

$$\%WL = \left[\frac{W_o - W_t}{W_o}\right] x\ 100 \quad \text{Eq(3)}$$

3. RESULTS AND DISCUSSION

3.1. Statistical casein/starch ratio response.

The data taken report homocedasticity and the assumption of normality is validated, indicating that the data fit a normal distribution with constant variance. It is not necessary to perform non-parametric transformations.
Ho: There is no significant effect of the treatments on the response variable.
Hi: At least one treatment has a significant effect on the response variable.

Then, P value less than alpha is rejected *Ho* and concludes with a significance level of 0.05 that at least one treatment has a significant effect on the response variable. Tukey's multiple comparison test shows there is statistical evidence to state the treatments present significant differences by adding more glycerol. The effect of the treatments on the response variable was also estimated and it was found the one that had the greatest effect was level 1 of Table 1 (2 casein: 1 starch) Finally, it is concluded that the treatment chosen for the investigation optimizes tensile strength (MPa).

3.2. *Viscosity of the final solution.*

Polymeric liquid solution was analyzed to determine pseudoplastic behavior. By increasing the cutting speed, i.e. extruded faster [51], the viscosity is reduced as shown in the Fig.1 obtained by the viscometer. This viscosity reduction is due to molecular alignments [52] and the long chains of polymers included.

The behavior of the Newtonian fluid for the biodegradable solution was obtained. Increasing the cutting speed facilitates forcing polymers to flow through the dies and specific equipment. The average dynamic viscosity was 0.077 ±0.0018 Pa.s (n=3), a small value knowing that the viscosity of most molten polymers under extrusion conditions can vary from 102 (Pa.s) to 105 (Pa.s) [52]. However, it coincides with the viscosity of the PVA at that concentration [53] and also represents optimal information for future investigations of this polymer on nanofibers [54].

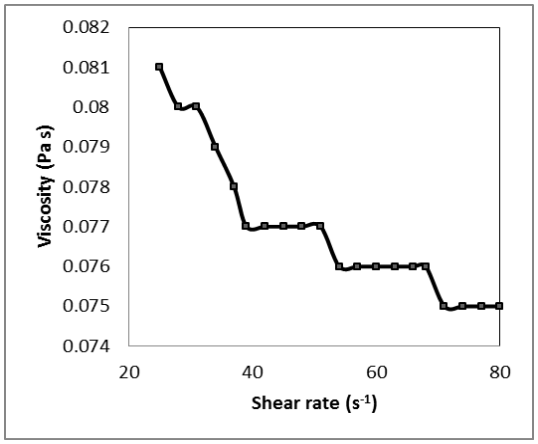


Fig. 1. Viscosity of biodegradable film solution as a function of shear rate.

3.3. FTIR.

Fig. 2 shows the FTIR spectrum of biodegradable final film and others components of the mix. The band between 3700 cm^{-1} and 3000 cm^{-1} are attributed to the stretching vibration regions of hydroxyl (OH) groups [55]. This -OH band is sensitive to intermolecular and intramolecular hydrogen bonds [34] (observed at 3273.82 cm^{-1}). It is known the mechanical properties of the developed films can be affected by the strength the hydrogen bonding [45]. However, the spectra of the biodegradable film in Fig. 2(I) and Fig. 2(II) suggest bonding interactions between the CAS/STA/GLY and PVA.

Specifically, casein is characterized by some vibrational bands at 2920 cm^{-1} and 2850 cm^{-1} of CH groups, i.e. symmetric bonds [56]. Those vibrations in the Fig. 2(I) indicate the presence of amino acids with a higher concentration of CH2 group [56]. Intense vibrational band at 1733.18 cm^{-1} in the biodegradable film indicate the presence of carbonyl groups (C=O). Additionally, there are bands from casein observed at 1634.43 cm^{-1}, and 1515

cm^{-1} can be attributed to the stretching of the amides [20] [57] which are determined in the proteins present in the polymer.

The angular stretching of C-H is observed at 1418 cm^{-1}, but in the polymer is not, confirming a physical interaction, while the C-O is at 1034 cm^{-1} e 990 cm^{-1} [58] and is observed at 1030.83 cm^{-1} and 996.23 cm^{-1} for final film and starch, respectively. For the additional reactive, sodium hydroxide has its common peaks to confirm the presence of OH groups showing adsorption at 3292.87 cm^{-1} and 1637.015 cm^{-1}. The glycerol showed vibrations at 3273.04 cm^{-1} (OH groups), 2936 cm^{-1} and 2881.97 cm^{-1} for CH groups [59], and it is observed that the -OH peak (1030.9 cm^{-1}) is maintained at the polymer wavelength. This value corresponds to the functional group because there would be a variation towards a region of greater wavelength without glycerol. This plasticizer plays an important role because it is attributed to the regions of crystallization and rearrangement of the chain in the final matrix of the polymer [5].

By last, the IR spectrum of the bentonite is in Fig. 2(II) whose initial band is presented at 3622.97 cm^{-1} OH stretching but in the polymer spectra are small brands at 3611.18 cm^{-1}. For this reason, it has been indicated in literature [60] a possible interaction among starch, glycerol and clay molecules as a characteristic of formation of hydrogen bond [59]. Peak at 1633.59 cm^{-1} represents H-O-H bending region and 1089.8 cm^{-1} indicates the symmetric Si-O and Si-O- [61] bonds in the clay structure. Final film was shifted to 1652.604 cm^{-1} and 1087.39 cm^{-1} and was an indication of the formation of nanocomposites [42] as reported.

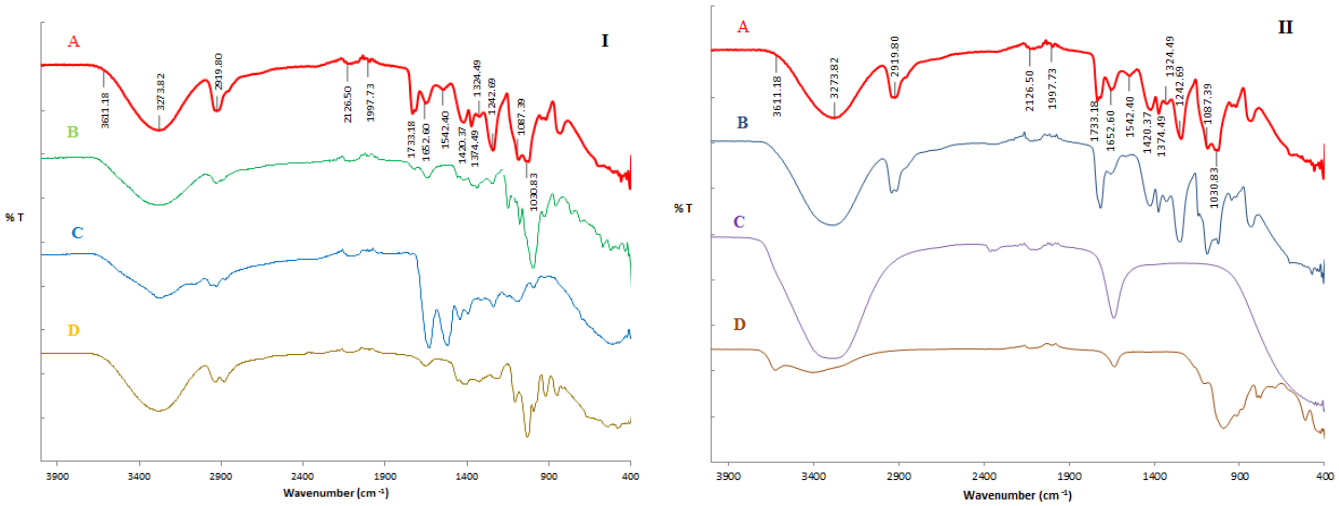


Fig. 2 I) FTIR spectra for (A) biodegradable final film, pure modified starch (B), calcium caseinate (C) and glycerol (D). II) FTIR spectra for (A) biodegradable final film, PVA (B), NaOH (C) and bentonite (D).

3.3. Surface morphology

Morphological aspects of the bentonite and films are outlined in Fig. 3. Micrograph shows a smooth surface for the film without clay Fig. 3 (B) despite the presence of some white particles of casein, which means that not all casein can be dissolved in the starch matrix as had happened in another study [62]. Air bubbles can also be attributed to insufficient degassing of the final solution at extrusion molding.

Fig. 3 (A) reveals the surface of the bentonite for reference when included in the material. It is possible to observe a good dispersion of the clay in the polymer matrix that can be attributed to the PVA-clay interactions [63]. However, a rougher surface is shown in Fig. 3 (C) and studies clearly show the difference in surface roughness between films [64]. There are only visible heterogeneities on the surfaces that are attributed to agglomerates and/or tactides of clay particles [65] dispersed in the polymer matrix. The presence of these tactides may indicate that an exfoliated structure is not achieved in the nanocomposite samples but it does confirm the existence of silicate layers [66].

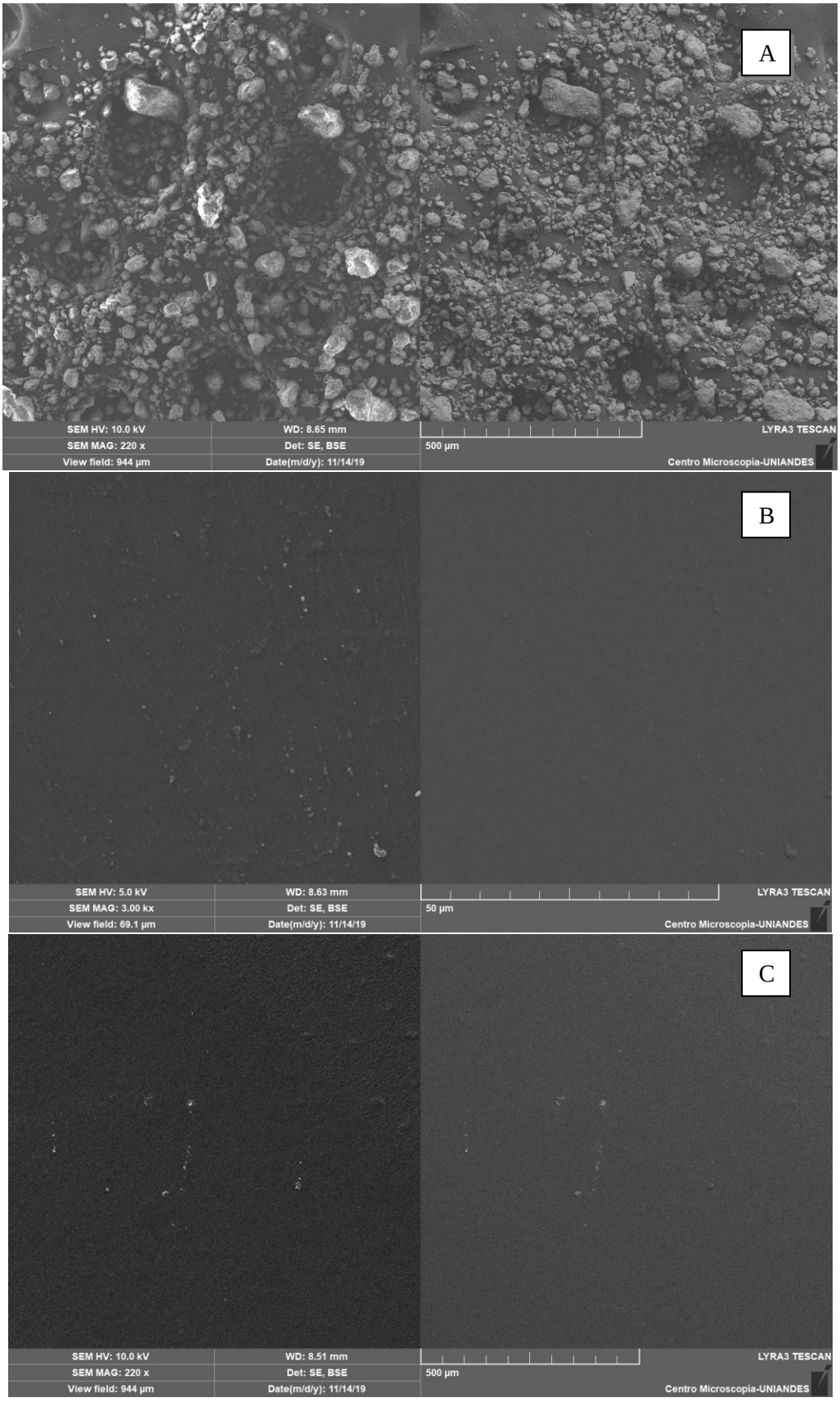
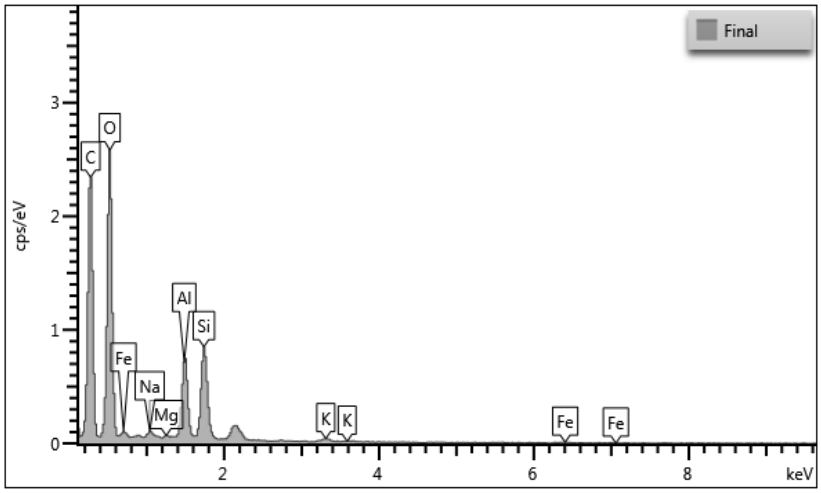


Fig. 3 SEM images on the left correspond Back-scattered electrons (BSE) and on the right Secondary electrons (SE) of (A) pure bentonite, (B) CAS/STA/GLY/PVA film and (C) nanostructured CAS/STA/GLY/PVA/BENT biodegradable film.

EDS (Energy Dispersive X-Ray Spectroscopy) in Fig.4 reveals the chemical composition of final film showing principal components for clay such as silica and iron in a low proportion, knowing that the clay consists mainly of alumina and silica in large quantities and different oxides [67][68]. Significant peaks of silica, aluminum, iron and a large amount of oxides that can be attributed to the addition of bentonite at only 4% representing the effects of nanostructure at very low rates [69].



Element	Wt%
C	48.06
O	37.63
Na	0.40
Mg	0.09
Al	5.34
Si	6.79
K	0.52
Fe	1.18

Fig. 4. EDS of nanostructured CAS/STA/GLY/PVA/BENT biodegradable film.showing the Chemical Composition

3.4. *Mechanical properties*

TABLE II
MECHANICAL PROPERTIES OF THE NANOSTRUCTURED BIODEGRADABLE FILM AND THE COMPARISON WITH OTHER ELABORATED FILMS*

Sample	Thickness (mm)	Tensile strength, (MPa)	Tensile strain, %	Young's modulus (MPa)
CAS/STA/GLY/PVA/BENT	0.093 ± 0.01	13.083 ± 2.1	109.296 ± 0.08	11.727 ± 0.96
CAS/STA/GLY/CLAY	0.1307 ± 4.83	9.92 ± 1.27	92.33 ± 19.21	NA
CAS/GLY/PVA	NA	19	275	NA
STA/GLY/PVA/CLAY	NA	12.41 ± 4.19	3.2 ± 0.81	NA
CAS/GLY/WPC	0.19 ± 0.05	3.4 ± 0.59	55.3 ± 1.83	5.5± 1.13

*MEAN ± SD (N = 8)
NA: NOT AVAILABLE.

Table II shows the thickness, tensile strength, tensile strain and Young's modulus of film elaborated and the most recently studies [5][42][70][71] with these components. The incorporation of clay significantly affected the mechanical properties, as reported in other nanocomposite films in different clay contents [71], even with proportions less than 0.05.

The addition of plasticizer reduces the tensile strength but increases the percentage of elongation. Smaller plasticizers such as glycerol have the ability to interact with protein molecules [72] to decrease these mechanical properties in films. On the other hand, the addition of PVA improves by 32% in samples made without this polymer, due to the plasticizing effect of PVA since it is easy to combine with several polymers [45][73] increasing the

mechanical properties of the compounds. The behavior of the test performed for an average sample is shown in Fig 2.

In this study a higher Young's modulus value was presented, in addition, tensile strength values were improved. This behavior is likely to be the result of the resistance exerted by the montmorillonite structure, this provides specific properties of the bentonite clays [74] such as the large surface area, and the aspect ratios of the interleaved silicate layers [75].

3.5. Color analysis, light barrier properties and transparency

Color is a very important factor to consider in food packaging, since it will influence consumer acceptance and commercial success of a product in the market. The significant change in the color analysis is the film samples with bentonite had a color change towards yellow as seen in Table III due to the yellowish color of Cloisite Na+ [76] in this kind of clay. Studies based on nanoclay nanocomposition films also revealed increases in yellowish color [42][77].

TABLE III
INSTRUMENTAL COLOR ANALYSIS *

Sample	L*	a*	b*	C*	h
CAS/STA/GLY/PVA	39.74 ± 0.44	0.395 ± 0.1	-1.118 ±1.55	1.21 ± 1.77	282.932 ± 2.95
CAS/STA/GLY/PVA/BENT	38.937 ± 0.77	0.42 ± 0.18	-2.76 ±0.09	2.8 ±0.08	278.65 ±3.77

*MEAN ± SD (n = 6)

The films without clay showed lower values of b *, which indicates a tendency towards the blue color attributed to the PVA. Adding clay also shows a slight decrease in the luminosity values (L) also reported as the different clay contents increase [78] as opposed to the chroma value of the films that increased significantly. With respect to parameter a*, it means that the red color of the samples decreased and the value of the green color increases as a result the nature of the clay according to the visual observation.

TABLE IV
LIGHT BARRIER PROPERTIES AND OPACITY *

Sample	Absorbance	%T	Thickness (mm)	Transparency	Opacity (AU nm)
CAS/STA/GLY/PVA	0.25	60.206	0.1016	17.52 ±0.77	134.48 ± 0.52
CAS/STA/GLY/PVA/BENT	0.36	44.370	0.1016	16.21 ±0.43	181 ± 2

*MEAN ± SD (n = 2)

As for the light barrier properties, opacity is an established measure of the transparency of an informed film, such as the calculation of the area under the curve by an integral procedure in Fig.5. Higher opacity values were reported for films with content of clay as shown in Table IV and according to other research [46][77] it is possible to determine that a higher opacity value means less transparency. The film with betonite had a low transmittance percentage (% T) for UV radiation (44.37%) with respect to the other sample that improves the light barrier properties.

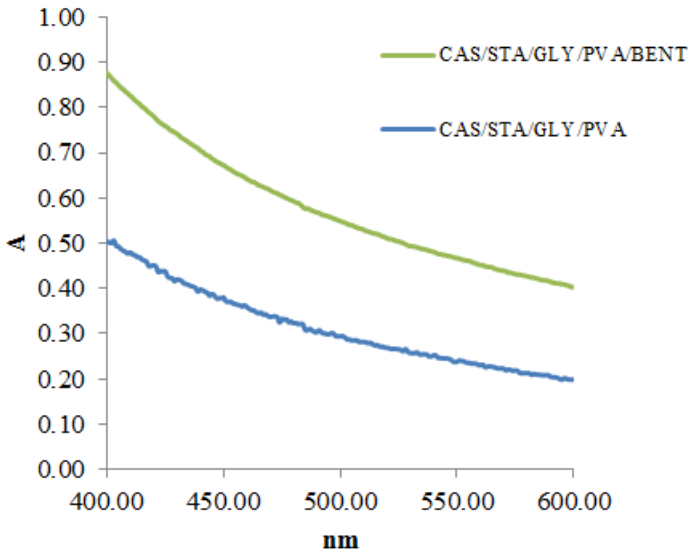


Fig. 5. Absorbance units x nanometers to define film opacity.

3.6. Water Vapor Permeability

WVP is one of the most studied properties of edible and packaging films. The weight loss with respect to time is observed in Fig.6 and a percentage of weight loss of 4.7% is determined. High WVP values are reported for PVA [5] films because in this polymer the water molecules easily penetrate through the water phase.

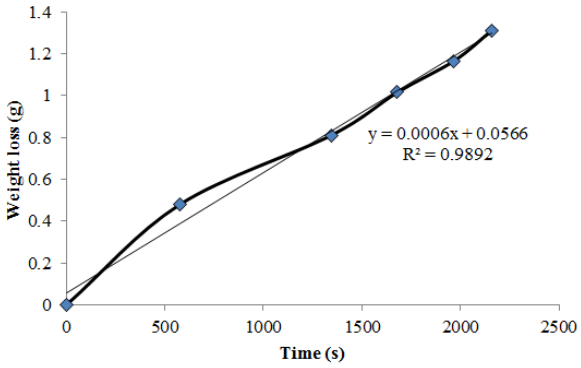


Fig. 6. Monitored weight changes against time for water vapor transmission rate.

TABLE V
WATER VAPOR PERMEABILITY OF NANOSTRUCTURED BIODEGRADABLE FILM *

SLOPE(g/s)	i(m)	A(m´2)	P (Pa)	WVP(g/m*s*Pa)
0.0006	0.0001016	0.000314	2488.19	8.23E-08

In Table V the calculated WVP values for the nanostructured polymer with bentonite are reported, these values are three orders of magnitude smaller than those reported for casein starch and glycerin films [62]. This can be attributed to the fact that a small amount of plasticizer is implemented and the increase in plasticizer concentration caused an increase in the WVP of the hygroscopic films [21]. The differences between these results may be related to the differences in polymer matrix structure and in the free volume between polymer chains when using a large

number of components. Other studies covered the effect of clay in nano compositions and demonstrated the decrease in WVP [46][70].

3.7. Microbiology test

Microorganisms counted for the total bacterial count was 70 ± 7.07 UFC/cm^2 (n=3) Fig.7A a very similar value in aerobic mesophilic bacteria reported for a control of casein films [16]. It is important to maintain sterile conditions when placing films that cover Petri dishes because the manipulation of each sample can affect the result. In the same way, a fungus was presented in test 2 and 7 ± 2.3 UFC/cm^2 (n=3) Fig.7B attributed to yeast colonies.

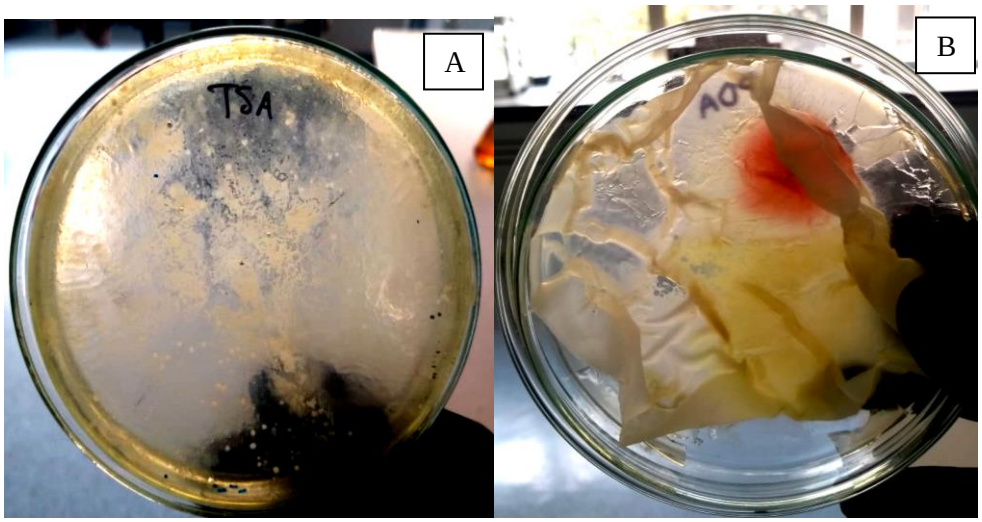


Fig. 7. (A) TSA total germ and bacterial count by 48h and (B) PDA mold and vegetable count by 120h.

3.8. Biodegradation rate

The biodegradability rate was measured in samples of the nanostructured film for 9 days. Weights were taken periodically and it was observed that the samples, in addition to losing weight, deteriorated and became opaque (Fig. 8). A large growth in the biodegradability rate was observed up to 72 h, after which a linear behavior is presented. Recent studies showed that the biodegrability of films with PVA and starch [40] showed lower rates of 20% from 3 weeks of study. The presence of casein improves the solubility of the film, an improvement in biodegradability is obtained by burying this film.

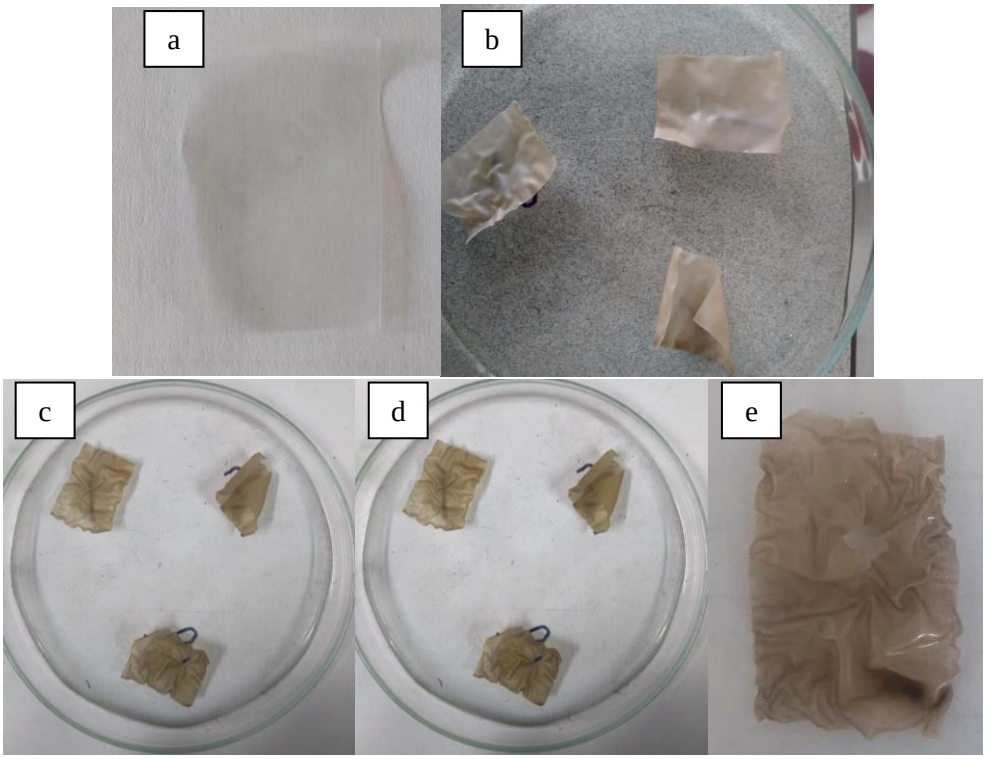


Fig. 8. Biodegradation samples in different time measurements a) 0 hours b) 24h c) 168h d) 192h and e) 216h.

The linear regression (R^2 = 0.988) Fig. 9 that was adjusted allows to predict the time it will take to complete a 100% weight loss. The estimated time was 13 weeks, a longer time than those reported for casein and starch but affirms the potential for biodegradability including PVA. The crosslinking effect of these protein-based films can be attributed to the decrease in the availability of water within the matrices, so that several crosslinked polymeric materials are resistant to hydrolysis, proteolytic action and microbial attack [42] [50] present in this study of burial in interior ground.

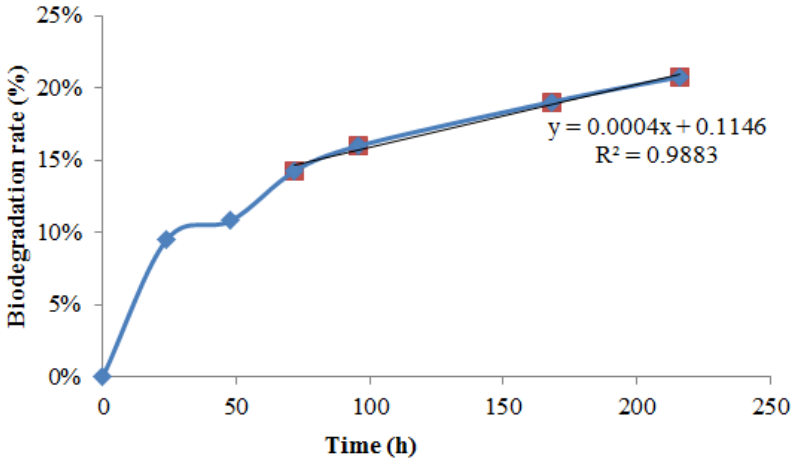


Fig. 9. Biodegradation rate and linear regression for degradation time (n=3).

4. CONCLUSION

In this study, a film composed of calcium caseinate and modified starch was prepared for a ratio of 2;1, respectively. A value of 13.083± 2.1 MPa was found in tensile strength and an elongation percentage of 109.3± 0.08 where mechanical properties conclude a resistant and better film with respect to films reported without the addition of PVA. FTIR showed the stretching vibration regions of characteristic hydroxyl (OH) groups in each component, peak 1087.39 cm-1 confirms the interaction of clay in the film and a non-significant effect of the presence of NaOH. SEM analysis showed the rough surface of the film as an effect of clay. Transparency was less in the films but this increase in opacity was not significant to the naked eye. Water vapor permeation was benefited with the decrease in orders of magnitude up to 2. Microbiological tests indicate a total allowable bacterial count for casein films to be implemented as food packaging. In addition, biodegradability test confirms film potential to disintegrate in soil in an estimated time of 13 weeks as a good alternative for conventional plastics.

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