Characterization and Application of Biodegradable Edible Films Produced by Casting, from Combinations of Rice, Cassava and Potato Starches with Gelatin, in Peanuts.

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Abstract*– The study of polysaccharides properties to make biodegradable films has been of research interest; due to its characteristics, these can be applied as an alternative material in the packaging industry. In the present study, films were produced by a casting method. For this investigation, dispersions of cassava (Y), potato (P), and rice (A) starch 2% (w/w) of total solids (TS) were combined (50:50) with gelatin, then, 30% (w/w) glycerol was added as a plasticizer. The formulas (YG, PG, AG) were characterized in thickness (µm) and water vapor permeability (WVP g mm kPa-1h-1m-2) at 10, 20, 40 and 60 days. The results suggest that PG and YG showed the highest thickness value, contrary to AG. On the other hand, the results of WVP suggested that the film YG at 60 days (2.15 g mm kPa-1h-1m-2) showed the lowest values for permeability, meanwhile, the PG was the most permeable film (3.8 g mm KPa-1h- 1m2). Thereafter, bags were made using the casted films, and used to pack peanuts; moisture levels were measured after 10, 20, 40 and 60 days. Results suggested that there was not significant difference among them, the peanuts shown a final moisture value of 7.4%, being the limit of moisture for peanuts 9%. This result justifies the application of the obtained film as a food packaging due to its characteristic of good barrier for water vapor therefore protecting the food stuff.

***Keywords*— biodegradable film, peanuts, water vapor permeability, thickness**

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I. Introduction

Worldwide annual consumption of plastic is about 275 million of metric tons (TM), 20% of this amount is used to manufacture non-recyclable packaging items such as bottles, containers, and films among others; which become environmental pollution. Biodegradable polymers (biopolymers) are seen as a valid alternative to replace the use of plastic in the food packaging industry. Research of biopolymers, suggests they may be an alternative that helps to lower the impact that food packaging items have on the environment [1, 2]. Proteins are amidst the most investigated biopolymers for this purpose, due to their tridimensional organization which is stabilized by their chain interactions, it contributes to obtain films with good mechanical and low water vapor permeability properties. The lifespan for using or application of these films are limited, owing to loss of their characteristics which are affected by environmental factors and time. Bovine gelatin is known for having a good gelling force resulting in stable, clear, shining films resistant to the oxygen [3, 4, 5, 6, and 7]. Another polymer with research interest are starches, that are widely applied in films, whose characteristics vary depending on the place of origin, raw material, obtaining method, and solids content. Starch films present a good barrier for water vapor, but their mechanical properties are limited [8, 9, and 10]. Curiously, blends of biopolymers have shown better properties than each of its components, in addition the use of plasticizers and nanoparticles improve their physical-chemical properties [11, 12, and 13]. Plasticizers are added with the objective of reduce the film brittleness and converting them into thermoplastics, this is caused due to the capacity of the plasticizer of reducing the internal hydrogen bonds in the polymer chains, resulting in an increment of the molecular volume of the film, facilitating in this way the molecular flux which ends in an improved flexibility and resistance to fail [14, 15, 16, 17].

The biopolymers can be processed using the same mechanisms used for traditional polymers such as extrusion, compression, thermoforming and casting. Films can be produced using the casting method, starting with a dispersion of the polymer which thereafter is pour in a mold, dried and cured or hardened with the objective of shaping a thermoplastic film at the cavity of the mold, this being the first step in pellet elaboration [18]. In this research, biodegradable films were produced from a biopolymer blend composed by starches of potato, cassava, and rice combined with bovine gelatin; glycerol was added as plasticizer. Water vapor permeability (WVP) was evaluated to determine which of the formulas shown better barrier to the WVP along the time set for this experiment. Finally, applying a "T" seal, bags were made from the films and used to pack low water activity foodstuff (peanuts). It was expected that the packed foodstuff keep its moisture content lower than quality standard specifications, avoiding the rancidity of the peanuts.

II. Materials and Methods

2.1. Materials

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The cassava starch used in this study was manufactured by "La pradera" in Ecuador, the potato and rice starches were manufactured by "Bob Red Mill" in the US. The bovine gelatin of 240° Bloom, the glycerol and the magnesium nitrate hexahydrate - Mg $(NO_3)_2.6(H2O)$ - were made by Merck. The roasted without peel, salt, or additives peanuts with an initial moisture of 1.55% (*Arachis Hypogae L.*) were purchased in the local produce market of Guayaquil-Ecuador.

2.2. *Film elaboration*

A 2% TS blend of starch and bovine gelatin were prepared using the dispersion method, for this purpose a 50:50 starch-bovine gelatin ratio was mixed and 30% (w/w) of glycerol was added as plasticizer. The starches were gelatinized by boiling them for 30 minutes (except rice starch where 45 minutes was used due to its high temperature of gelatinization) at constant temperature and agitation using a Thermo Scientific water bath model 18902A. The bovine gelatin was placed over a heating plate at 85 °C for 30 minutes using constant stirring. Then, both dispersions were placed at room temperature and let to cool at 40 ± 5°C; once both starch and bovine gelatin attain the desired temperature, blends of cassava starch – gelatin (YG), potato starch – gelatin (PG), rice starch – gelatin (AG) were made and a 30 % (w/w) of glycerol was added to the blend. These mixtures were homogenized for 4 minutes at 12400 rpm. Once homogenized, the mixtures, with an equivalent of 2% TS, were poured in Teflon molds at 25 °C and 53% of relative humidity (HR) and they were let drying for 48 hours by natural convection. Once dry, the films were separated from the molds (figure 1) and placed in a desiccator containing a saturated solution of Mg $(NO_3)_2.6(H_2O)$, where they were kept for 10, 20, 40, and 60 days; finally, characterization assays were practiced in the produced films.

Fig. 1. Starch and bovine gelatin film

2.3. *Film characterization*

2.3.1. *Thickness measurement*

The films were measured in 10 different locations along their surfaces (figure 2) using a 733M, Starrett electronic micrometer, precision ± 0.001 mm.

Fig. 2. Electronic micrometer

2.3.2. *Water vapor permeability (WVP)*

The water vapor transmission rates (WVTR) of the produced films were evaluated using the gravimetric method ASTM E96-95 used by other researchers [19]. Samples of the films were mounted between the base and cap of a glass cup with a diameter of 4.9 cm, and then 15 mL of distilled water was poured in the cup (100 % RH). Afterwards, the cups were placed inside a container with a saturated solution of Mg $(NO_3)_2.6(H_2O)$, a 12V S. Tech fan were placed on the top of the container with the objective of homogenize the atmosphere and securing a negligible resistance in the film Surface (Figure 3). The analysis was run at 25 °C and 58 – 60% RH. The weighing control was performed using an electronic Sartorius analytical scale model AZ214, precision range ± 0.00001g in a 120-minute interval by 24 hours. The water vapor transmission rates were calculated using a linear regression analysis of the plotted data (weight vs time) divided by the film surface, the results were expressed in g mm $kPa^{-1}h^{-1}m^{-2}$. The known blank for this analysis was a low-density polyethylene with a thickness of 60 μm.

Fig. 3. Equipment to measure water vapor permeability

2.3.3. *Packing of the foodstuff*

The produced films were let to stabilize for seven days, and then pieces of 11 x 6 cm were cut and were "T" sealed to form a bag, approximately 3 grams of peanuts were pour inside of each bag then completely sealed (figure 4). The bags containing 3 g of peanuts were stored at 25 °C and 75% RH. Finally, moisture analysis was performed in the peanuts after 10, 20, 40 and 60 days.

Fig. 4. Peanut packed using biodegradable films

2.3.4. Moisture of the packed foodstuff

To determine the moisture of the packed foodstuff ISO-712-2009 method was followed. For this effect, 2 g of the foodstuff was weighed using an electronic Sartorius analytical scale model AZ214, precision range ± 0.00001g. The weighed sample was placed into a Universal Memmert electric stove then placed in a desiccator and weighed again; the sample was heated, desiccated and weighed until reach constant weight. The moisture percentage was calculated using equation 1. The analysis was performed by triplicate.

$$lost\% = \frac{Po - Pf}{Po} x100 \qquad \text{(equation 1)}$$

P0 = boat weight + initial sample weight
Pf = boat weight + weight of dry sample

2.4 Statistical analysis

For the statistical analysis, Minitab ® software version 17.1.0 was utilized. The results were compared with a 95% confidence interval for control variables. For each variable mean, standard deviation and analysis of variance (ANOVA) was calculated; $p \leq 0.05$ relates to significant difference in sample population. The *p-values* for various biodegradable films for the similar storage times, are presented in tables with superscripts, a, b, c. Also, *p-values* for biodegradable films for different storage times, are presented in tables with superscripts, w, x, y, z.

III. Results and Discussion

3.1. Thickness

Results indicate that the film "PG" is 18% thicker than the less thick of the films "AG"; possibly due water lost by evaporation during drying is higher in "AG", diminishing the content of trapped water. These results relate to thickness range between 67 and 79 µm, as shown in figure 5.

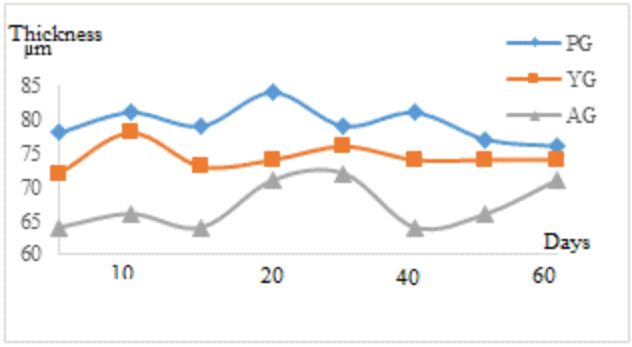

Fig. 5. Thickness of PG, YG, AG biodegradable films

A study on films made of 5% w/w of bovine gelatin with 30% of glycerol as plasticizer reported thickness of about 51 µm [20]; another study using 3% TS dispersion of cassava starch with 30% (w/w) glycerol as plasticizer reported thickness of 30 µm [21]. These values are less than the thickness values obtained in this experiment. This may be due to its matrix possess only one structure, so there are no other bonds that resist water lost by evaporation. These results contrast with the 180 µm thickness film reported in a study using potato starch 6% (w/w) dispersion with 33% (w/w) of glycerol [22]; which is a higher value than the reported in the present study, may be due to its higher solids content. Researchers working in 2% TS cassava – bovine gelatin matrix and 25% (w/w) of glycerol reported film thickness of 74 µm, which are in the range reported in this study.

3.2. Water vapor permeability (WVP)

The samples after 10 and 20 days are significantly different, however, from day 40 the films stabilize with no significant differece among them. The least permeable film along the time was the YG, with similar values than the AG film, being the PG the most permeable with an 83% higher WVP than the other two films (table 1). Figure 6 shows the comparison of the biodegradable films against a film of similar thickness made from low density polyethylene (LDPE). We can conclude that the PE film initial WVP values are low, and shows a slightly increment with the time; having a final value of WVP (after 60 days) considerably less than the biodegradable films.

Table 1. Water vapor permeability (WVP) of PG, YG, AG y PE films along storage time.

	WVP ($g \cdot mm \cdot KPa^{-1} \cdot h^{-1} \cdot m^{-2}$)			
	10 Days	20 Days	40 Days	60 Days
PE	$0,03(0,01)^{(y)}$	$0,05(0,02)^{(y)}$	$0,09(0,01)^{(z)}$	$0,11(0,01)^{(z)}$
PG	$3,32(0,11)^{(a)(x)}$	$3,53(0,06)^{(a)(xy)}$	$3,54(0,18)^{(a)(xy)}$	$3,80(0,06)^{(a)(y)}$
YG	$2,25(0,07)^{(b)(x)}$	$2,35(0,08)^{(b)(x)}$	$2,27(0,35)^{(b)(x)}$	$2,15(0,31)^{(b)(x)}$
AG	$1,40(0,04)^{(c)(x)}$	$1,67(0,25)^{(c)(y)}$	$2,03(0,04)^{(b)(y)}$	$2,86(0,21)^{(b)(y)}$

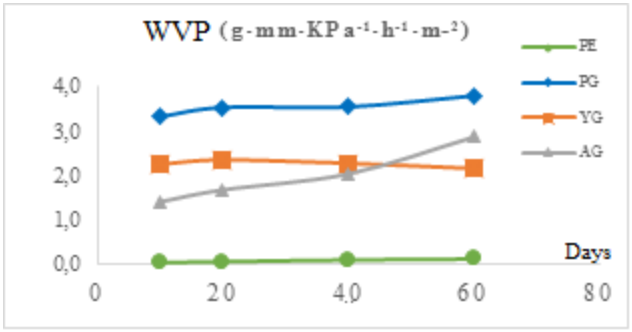

Fig. 6. WVP in PG, YG, AG y PE films along storage time

Studies using dispersions of potato starch with agar in different proportion and 25% (w/w) glycerol reported that the mechanical properties and WVP of the film improved with a 30% (w/w) agar, having a WVP value of 1,66 (g.mm/KPa.h.m^2) [24], lower than that reported in this study. The possible reason may be the increment in agar content. Another studies using potato starch reported WVP values of 0,864 (g.mm/KPa.h.m^2) when using nanoparticles, and value of 1,62 (g.mm/KPa.h.m^2) when nanoparticles are not added [25]; these results suggest that nanoparticles made the film less permeable due to its inclusion in the carbon bonds. Research using 5% (w/w) of cassava starch and 30 % (w/w) of glycerol exposed to different drying temperatures, reported film thickness of 200 µm with a WVP ranging from 2.30 to 1.80 g.mm/KPa.h.m^2[26] these values are similar to the reported in this study. Another research using 3.5% (w/w) rice starch and 25% (w/w) sorbitol reported a WVP value of 2.98 (g.mm/KPa.h.m2) [27], this difference is may be due to the use of different plasticizer. There is being reported a WVP value of 35 (g.mm/KPa.h.m^2) using a dispersion of 4% (w/w) of rice starch – bovine gelatin with a 50:50 ratio and 30% (w/w) of glycerol [28], these values are higher than the reported in this study, maybe due to the higher solids concentration and a higher amount of internal water.

3.3. Moisture in the foodstuff

The moisture in the peanuts packed with biodegradable films are shown in table 2. It can be seen that the films stabilize on day 40 and there is no significant difference among them, AG film being the more stable during the time duration. The results obtained at the end of the study shown that the PG film preserves the packed peanuts better due to its lower moisture content. Despite of gain 27% more moisture than the PE reference film, its moisture remained below the 9% limit required for food safety.

The results suggest that the films preserve the foodstuff in an acceptable moisture range along the 60 days required as shelf life by the market, as shown in figure 7.

Table 2. Relative humidity of the peanut packed using PG, YG, AG y PE films along storage time

	Peanut moisture (%)			
	10 Days	20 Days	40 Days	60 Days
PE	1,66(0,01)$^{(a)(w)}$	3,91(0,19)$^{(a)(x)}$	4,50(0,03)$^{(a)(y)}$	5,42(0,22)$^{(a)(z)}$
PG	3,01(0,05)$^{(b)(w)}$	5,92(0,09)$^{(b)(x)}$	6,50(0,22)$^{(b)(y)}$	6,99(0,28)$^{(b)(z)}$
YG	3,52(0,07)$^{(c)(x)}$	6,15(0,13)$^{(b)(y)}$	6,58(0,34)$^{(b)(y)}$	7,43(0,22)$^{(b)(z)}$
AG	3,80(0,06)$^{(d)(y)}$	6,66(0,07)$^{(c)(z)}$	6,95(0,44)$^{(b)(z)}$	7,00(0,09)$^{(b)(z)}$

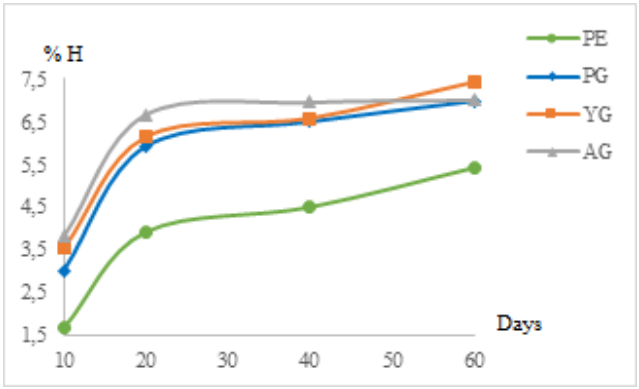

Fig. 7. Moisture of the packed peanuts using PG, YG, AG y PE films along storage time

III. Conclusions

The biodegradable films were made by 2% (w/w) dispersion from cassava, potato and rice starches and the combined with gelatin in a 50:50 ratio; 30% (w/w) of glycerol was added as plasticizer. The method selected for the film elaboration was casting, the parameters controlled in the films were thickness and water vapor permeability (WVP); performed at 10, 20,40 and 60 days. Results obtained for thickness parameter shows that the PG film has higher thickness (73 µm) and AG film, the one with lower thickness (67 µm), this indicates that the potato starch structure retains a higher amount of internal water, than the YG film, which showed only slight change during the essay time. In terms of WVP parameter, the YG film was more stable with a value of 2.25 to 2.15 g.mm/KPa.h.m2. On the contrary, the PG and AG films showed gradual increase from day 10 to 60, PG being the most permeable, these results allow us to relate the thickness with the higher amount of trapped water.

For the film application, bags were prepared from YG, AG, PG films and peanuts were packed in the bags. The moisture content of the peanuts during the control time and at the end of the experiment (60 days) was measured. The packed peanuts in all the biodegradable films bags (PG, AG, YG) did not show significant differences. Despite of the higher water vapor permeability of PG film, the peanut moisture content was kept under 9%, maximum limit for this product to be safe for human

consumption. Comparing these results with the PE film, we can conclude that biodegradable films preserve the foodstuff excellently and can be considered as an alternative material to LDPE packaging.

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