

CASSAVA STARCH-GELATIN BIO-BASED FILMS STRUCTURAL STABILITY AND COLOR DURING STORAGE

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The influence of additives (sucrose, propylene glycol and soybean oil) and process parameters (pH and temperature), on bio-based cassava starch-gelatin films stability during storage have been evaluated by a 2^{7-3} factorial experiment design. X-ray diffractograms have indicated that only sucrose influenced film stability resulting in crystallization during storage (60 days), which was also observed by scanning electron and light microscopy images. Light micrographs, besides indicated sucrose crystallization, also allowed observing phase separation. All biofilm samples presented a high lightness and low redness and yellowness, indicating to be almost colorless. Although temperature did not influence film microstructure and color, casting temperature of 60°C had completely destroyed samples, and it has not been recommended for further investigations.

KEY-WORDS: BIODEGRADABLE FILMS; ADDITIVES; CRYSTALLIZATION; MICROSTRUCTURE; COLOR.

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1 INTRODUCTION

Despite the biodegradable films environmental importance, starches bio-based materials also present low mechanical resistance and water vapor barrier when compared to traditional petroleum plastic films (KESTER & FENNEMA, 1986; KROCHTA & MULDER-JOHNSTON, 1997). However, the protective characteristics can be improved through incorporation of additives (ARVANITOYANNIS & BILIADERIS, 1998; GARCIA, MARTINO & ZARITZKI, 2000) and process parameters (GONTARD, GUILBERT & CUQ, 1992) modifications. Such additives and process parameter, however, can affect the bio-based stability during storage.

Soybean oil is normally added to bio-based materials to increased water vapor barrier properties (YANG & PAULSON, 2000; GARCIA, MARTINO & ZARITZKI, 2000) although lack of homogeneity has been reported (VEIGA-SANTOS et al., 2005). Gelatin (ARVANITOYANNIS et al., 1997) and propylene glycol (LACROIX et al., 1998) can affect interactions with the starch film matrix modifying the material mechanical properties and structure.

Although polyols are more frequently used as plasticizers (GAUDIN et al., 2000; ARVANITOYANNIS & BILIADERIS, 1998), sucrose demonstrated to have even more efficacy in plasticizing starch based materials (ARVANITOYANNIS, PSOMIADOU & NAKAYAMA 1996) although crystallization during storage may occur (VEIGA-SANTOS et al., 2005).

Sucrose crystallization is a known and well-studied phenomenon that starts with a crystalline nucleus and may be prevented by a humectant's agent such as propylene glycol (JACKSON & HOWLING, 1995).

With the aim to analyze the structure stability during storage of cassava starch bio-based materials, commonly used additives (gelatin, sucrose, propylene glycol and soybean oil) and process parameters (pH and temperature) have been investigated in a multi-component system.

2 MATERIAL AND METHODS

2.1 MATERIAL

Commercial cassava starch (Flor de Lotus, Brazil), gelatin type B (Liner Davis Gelatin, Brazil), soybean oil (Cargill Agricola S.A., Brazil), and analytically pure sucrose and propylene glycol (Sinth, Brazil) were used.

2.2 SAMPLE PREPARATION

Cassava starch (3-5%, w/w) was blended with water and the additives: gelatin (0-0.7%, w/w), sucrose (0-3%, w/w), propylene glycol (0-1.0%, w/w) and soybean oil (0-0.06%, w/w) considering the total weight of the film forming suspension. The film forming suspension pH was adjusted (4-8) with 50% citric acid solution or 5% sodium hydroxide solution, heated to 75°C for starch jellification, with constant stirring. The films were prepared using the casting technique. The film forming suspension (30 g) were dehydrated under renewable circulated air (30°C - 60°C \pm 2°C), over Petri plastic dishes. Films containing only cassava starch (5% w/w; no additives or pH adjustment), casted at 30°C, served as the control. Samples were stored (23°C, 75%RH with NaCl saturated solution) for at least 4 days prior testing (VEIGA-SANTOS, *et al.*, 2005).

2.3 MICROSTRUCTURE ANALYSES

2.3.1 Crystallinity

The relative crystallinity of the films was investigated with wide-angle X-ray diffraction (WAXS) (VEIGA-SANTOS et al., 2005). Measurements were carried out using a DMAX-2200 Rigaku International

Corporation $\theta/2\theta$ diffractometer, operating with 40 kV voltage and 20 mA amperage, with $\text{CuK}\alpha$ radiation. Samples were fixed at an aluminum sample holder, and analyzed from 5 to 60° (2 θ), with an angular step of 0.1° (2 θ), and a sampling interval time of 3 seconds. The films were analyzed every 15 days, during a storage period of 60 days (75% RH, 23°C), in duplicate. Crystallinity was also investigated (under the same conditions) for the raw materials cassava starch and sucrose in order to obtain crystallization standards.

2.3.2 Light Microscopy

Samples surface was observed by LEICA-DMLP light microscope, with no further preparation, by transmitted polarized or ordinary (not polarized) light. Images (200x-magnification) were collected with a CCD camera (VEIGA-SANTOS et al., 2005).

2.3.3 Scanning Electron Microscopy (SEM)

SEM digital images of the samples surface and fracture were obtained by Jeol JSM-5900LV scanning electron microscope. Cross section images were obtained by cryogenic fracture of the films, using liquid nitrogen. Samples were sputtered with a 16 μg gold layer. Images were taken at 5-10KV, spot size 28-30, 1000x-magnification (VEIGA-SANTOS et al., 2005).

2.4 COLOR

The color of preconditioned (75% RH, 23°C) samples and control films were analyzed in duplicate by total transmittance, using a Color Quest II-Hunter lab equipment, CIELAB Ttran D65, 10° lecture angle, 2.54 cm² measuring area (1.0 square inch). Four measurements, taken at the different quadrants of each sample, were averaged and expressed as Hunter system “L” (lightness), “a” (redness) and “b” (yellowness) values (VEIGA-SANTOS et al., 2005). Total color difference (ΔE) was evaluated as the size of color difference between the control film and the experimental design samples, and calculated by Equation 1 (Francis, 1983, cited by HONG & PARK, 2000):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad \text{Equation 1}$$

2.5 STATISTICAL ANALYSIS

A 2⁷⁻³ experimental factorial design was performed in order to select additives and process parameters that ($p < 0.05$) affect cassava starch films stability during storage. The statistical block was designed with three central points, totalizing 19 experiments. The variables were cassava starch, gelatin, sucrose, soybean oil and propylene glycol concentration, temperature and pH modifications (Table 1). The independent variables significant ($p < 0.05$) influence was evaluated by the Pareto chart of standardized effect, considering the ANOVA pure error. The experimental data were generated and analyzed using the software Statistica for Windows 5.0. (STATISTICA..., 1995).

TABLE 1 - INDEPENDENT VARIABLES CODED AND REAL VALUES FOR FILM PREPARATION, CONSIDERING THE TOTAL WEIGHT OF THE FILM FORMING SUSPENSION

<i>Levels</i>	A Temperature (°C)	B pH	C Cassava starch (%)	D Sucrose (%)	E Propylene glycol (%)	F Soybean oil (%)	G Gelatin (%)
-1	30	4	3	0	0.00	0.00	0.00
0	45	6	4	1	0.25	0.03	0.35
+1	60	8	5	2	0.50	0.06	0.70

The experimental design demonstrated in Table 2 was used to estimate the main effects of independent variables on films stability during storage (60 days).

TABLE 2 - FACTORIAL 2⁷⁻³ DESIGN WITH CODED AND REAL VALUES OF THE EXPERIMENTAL BLOCK

Assays	Variables						
	A	B	C	D	E	F	G
1	-1 (30)	-1 (4.0)	-1 (3.0)	-1 (0.0)	-1 (0.00)	-1 (0.00)	-1 (0.00)
2	+1 (60)	-1 (4.0)	-1 (3.0)	-1 (0.0)	+1 (0.50)	-1 (0.00)	+1 (0.70)
3	-1 (30)	+1 (8.0)	-1 (3.0)	-1 (0.0)	+1 (0.50)	+1 (0.06)	-1 (0.00)
4	+1 (60)	+1 (8.0)	-1 (3.0)	-1 (0.0)	-1 (0.00)	+1 (0.06)	+1 (0.70)
5	-1 (30)	-1 (4.0)	+1 (5.0)	-1 (0.0)	+1 (0.50)	+1 (0.06)	+1 (0.70)
6	+1 (60)	-1 (4.0)	+1 (5.0)	-1 (0.0)	-1 (0.00)	+1 (0.06)	-1 (0.00)
7	-1 (30)	+1 (8.0)	+1 (5.0)	-1 (0.0)	-1 (0.00)	-1 (0.00)	+1 (0.70)
8	+1 (60)	+1 (8.0)	+1 (5.0)	-1 (0.0)	+1 (0.50)	-1 (0.00)	-1 (0.00)
9	-1 (30)	-1 (4.0)	-1 (3.0)	+1 (2.0)	-1 (0.00)	+1 (0.06)	+1 (0.70)
10	+1 (60)	-1 (4.0)	-1 (3.0)	+1 (2.0)	+1 (0.50)	+1 (0.06)	-1 (0.00)
11	-1 (30)	+1 (8.0)	-1 (3.0)	+1 (2.0)	+1 (0.50)	-1 (0.00)	+1 (0.70)
12	+1 (60)	+1 (8.0)	-1 (3.0)	+1 (2.0)	-1 (0.00)	-1 (0.00)	-1 (0.00)
13	-1 (30)	-1 (4.0)	+1 (5.0)	+1 (2.0)	+1 (0.50)	-1 (0.00)	-1 (0.00)
14	+1 (60)	-1 (4.0)	+1 (5.0)	+1 (2.0)	-1 (0.00)	-1 (0.00)	+1 (0.70)
15	-1 (30)	+1 (8.0)	+1 (5.0)	+1 (2.0)	-1 (0.00)	+1 (0.06)	-1 (0.00)
16	+1 (60)	+1 (8.0)	+1 (5.0)	+1 (2.0)	+1 (0.50)	+1 (0.06)	+1 (0.70)
17(c)*	0 (45)	0 (6.0)	0 (4.0)	0 (1.0)	0 (0.25)	0 (0.03)	0 (0.35)
18(c)*	0 (45)	0 (6.0)	0 (4.0)	0 (1.0)	0 (0.25)	0 (0.03)	0 (0.35)
19(c)*	0 (45)	0 (6.0)	0 (4.0)	0 (1.0)	0 (0.25)	0 (0.03)	0 (0.35)

*(c): central points.

3 RESULTS AND DISCUSSION

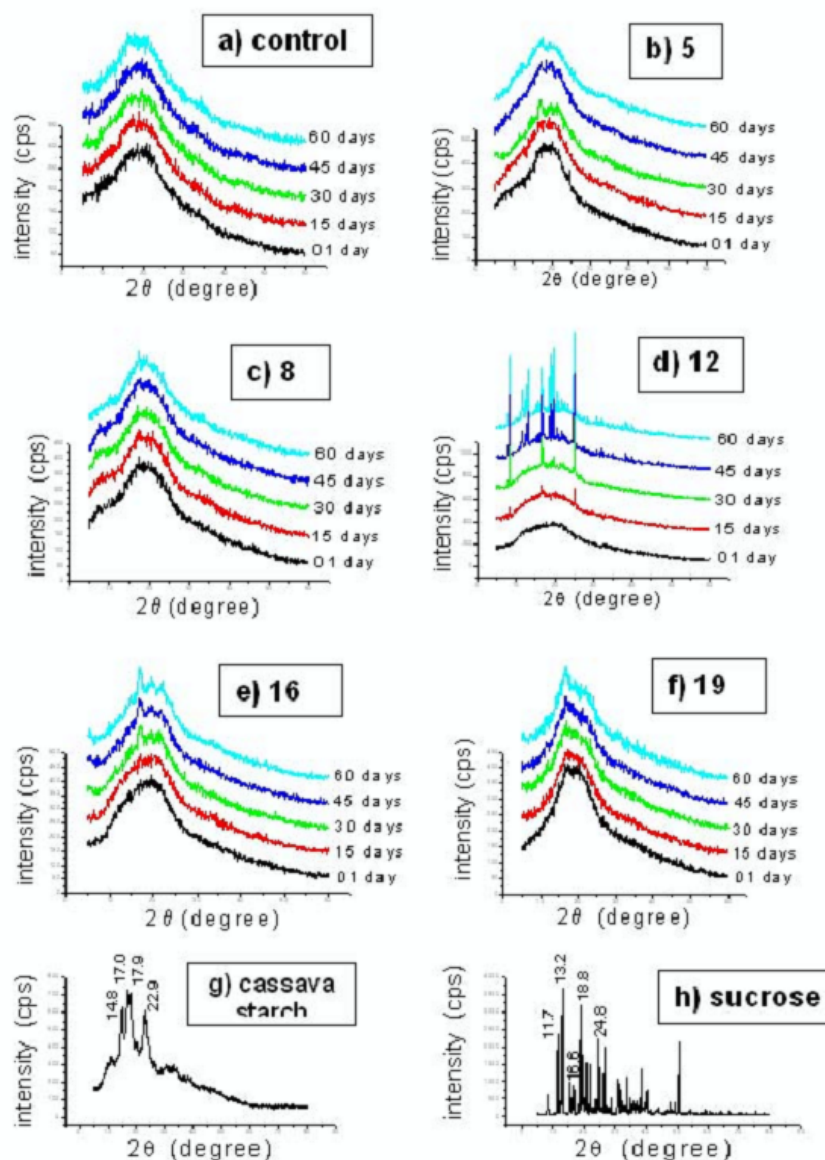
3.1 MICROSTRUCTURE ANALYSES

3.1.1 Crystallinity

WAXS crystallization diffractograms, collected during 60 days of storage, demonstrated an amorphous behavior for all samples at the first day of storage (Figure 1).

No crystallization was observed during the control sample storage period (Figure 1a), indicating that without additives or pH controlling (pH 5.78), the cassava starch film shows an amorphous behavior. The same amorphous behavior was also observed during storage of all samples formulated without sucrose, independent of the other additive, pH or casting temperature. Such results are exemplified by samples 5 (Figure 1b, containing all additives except sucrose, pH 4.0, 30°C casting temperature and 3% cassava starch) and 7 (Figure 1c, pH 8.0, 60°C casting temperature and 5% cassava starch). The X-ray diffractograms indicated that the additives gelatin, soybean oil and propylene glycol did not influence the amorphous behavior of cassava starch films. Also no influence was observed when modifying pH, casting temperature and cassava starch concentration.

FIGURE 1 - WAXS CRYSTALLINITY DIFFRACTOGRAMS OF AMORPHOUS: (a) CONTROL, (b) 5, (c) 8, AND CRYSTALLINE: (d) 12, (e) 16, (f) 19 SAMPLES BEHAVIOR DURING 60 DAYS OF STORAGE. WAXS CRYSTALLINITY DIFFRACTOGRAMS OF (g) NATIVE CASSAVA STARCH AND (h) SUCROSE STANDARDS



Cassava starch granules have a semi-crystalline structure, characterized by crystalline peaks (or crystallites) superposed to an amorphous halo (Katz, 1930, cited by RINDLAV, HULLEMAN & GATENHOLM, 1997). Native root starch semi-crystalline behavior is designated as C-type (ZOBEL & STEPHEN, 1995).

Although cassava starch is the main component on the studied films, the amorphous behavior observed for all films at the first day of storage (Figure 1a, 1b, 1c, 1d, 1e) can be explained by the jellification step used for film preparation. During jellification, the ordered crystalline regions undergo melting (ZOBEL & STEPHEN, 1995), which according to JENKINS & DONALD (1998) could decrease the crystallization ratio until zero, resulting in an amorphous structure. Also the low casting temperature

(30°C) can be responsible for the amorphous structure. According to JENKINS & DONALD (1998), a casting temperature below 50°C results in amorphous behavior for starch films. However, when sucrose was added (samples 9 to 19), with (Figures 1e, 1f) or without (Figure 1d) the presence of the other additives and process parameters, the cassava starch films developed a crystalline structure during storage. Such results indicate that sucrose addition is the responsible for film crystallization during storage.

Such a theory is reinforced when comparing the X-ray diffractograms of crystalline samples with sucrose standard. The 2θ angle and d -spacing (distance between lattice planes) of the crystalline peaks observed on the cassava starch-sucrose films, matches with the sucrose standard peaks (Figure 1h), as can be observed in Table 3.

TABLE 3 - 2θ ANGLE AND D -SPACING (d) MATCHES FOR MAIN PEAKS PRESENTED BY CRYSTALLINE SAMPLES WAXS DIFFRACTOGRAMS

Sample / [sucrose]	2θ	d (Å)	2θ	d (Å)	2θ	d (Å)	2θ	d (Å)	dc**
Sucrose Standard/ (99.72%)	11.7	(7.56)	16.6	(5.34)	18.8	(4.72)	24.8	(3.59)	na***
Assays									
9 / (2.00%)	11.6	(7.62)	16.6	(5.34)	18.8	(4.72)	24.7	(3.60)	15
10 / (2.00%)	11.6	(7.62)	---	---	18.8	(4.72)	24.8	(3.59)	15
11 / (2.00%)	11.6	(7.62)	16.7	(5.31)	18.8	(4.72)	---	---	30
12 / (2.00%)	11.6	(7.62)	---	---	18.8	(4.72)	24.7	(3.60)	15
13 / (2.00%)	---	---	16.6	(5.34)	---	---	---	---	45
14 / (2.00%)	---	---	16.6	(5.34)	---	---	---	---	45
15 / (2.00%)	---	---	16.6	(5.31)	---	---	---	---	30
16 / (2.00%)	---	---	16.7	(5.31)	18.8	(4.72)	---	---	30
17(c)* / (1.00%)	---	---	16.7	(5.31)	---	---	---	---	45
18(c)* / (1.00%)	11.6	(7.62)	16.7	(5.31)	---	---	---	---	45
19(c)* / (1.00%)	---	---	16.7	(5.31)	---	---	24.7	(3.60)	30

(c)*: central points; dc**: days that samples remained amorphous; na***: not applicable.

Sucrose crystallization is a known phenomenon that starts with a crystallinity nucleus (JACKSON & HOWLING, 1995). When in supersaturated conditions, spontaneous nucleation can occur. Nucleation and crystal formation could arise from rearrangement of randomly bound sugar molecules (STANSELL, 1995).

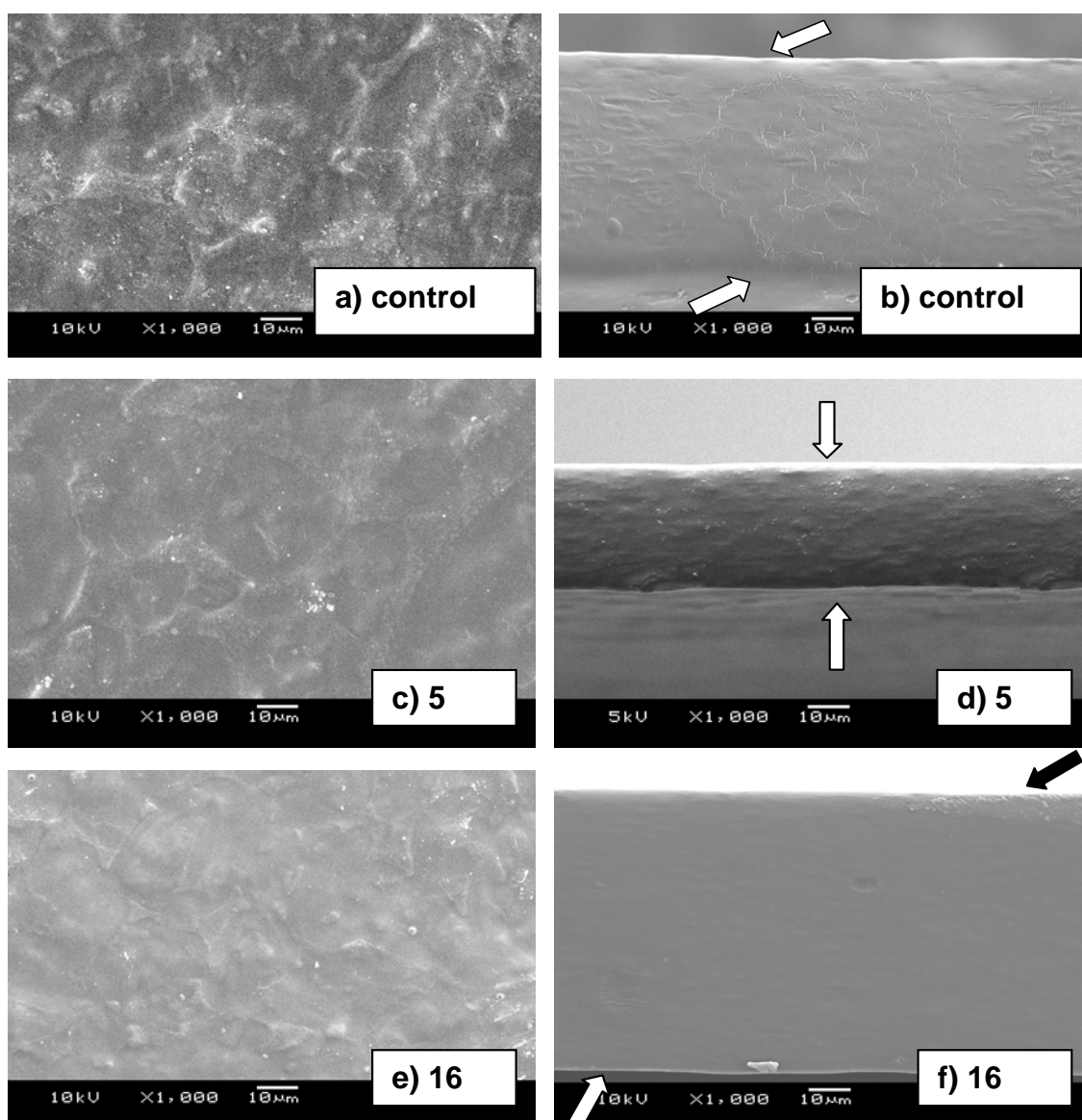
Statistical Pareto analyses were performed to evaluate the additives and process pH influence on the time required for crystallization beginning. For that matter, it was considered the total of days that the material remained amorphous (Table 3). In order to present values for the statistical calculations, for the films that did not crystallize during storage, the time required for crystallization to begin was considered the total storage period (60 days) evaluated in this study. Only sucrose has affected ($p < 0.05$) ($R^2: 0.86$) films crystallization during the storage period evaluated, affecting the material stability.

The sucrose crystallization observed resulted in brittle materials. As mechanical resistance is essential to a packaging material, the sucrose crystallization during storage is undesired and determines the biomaterial shelf life expiration.

3.1.2 Scanning Electron Microscopy

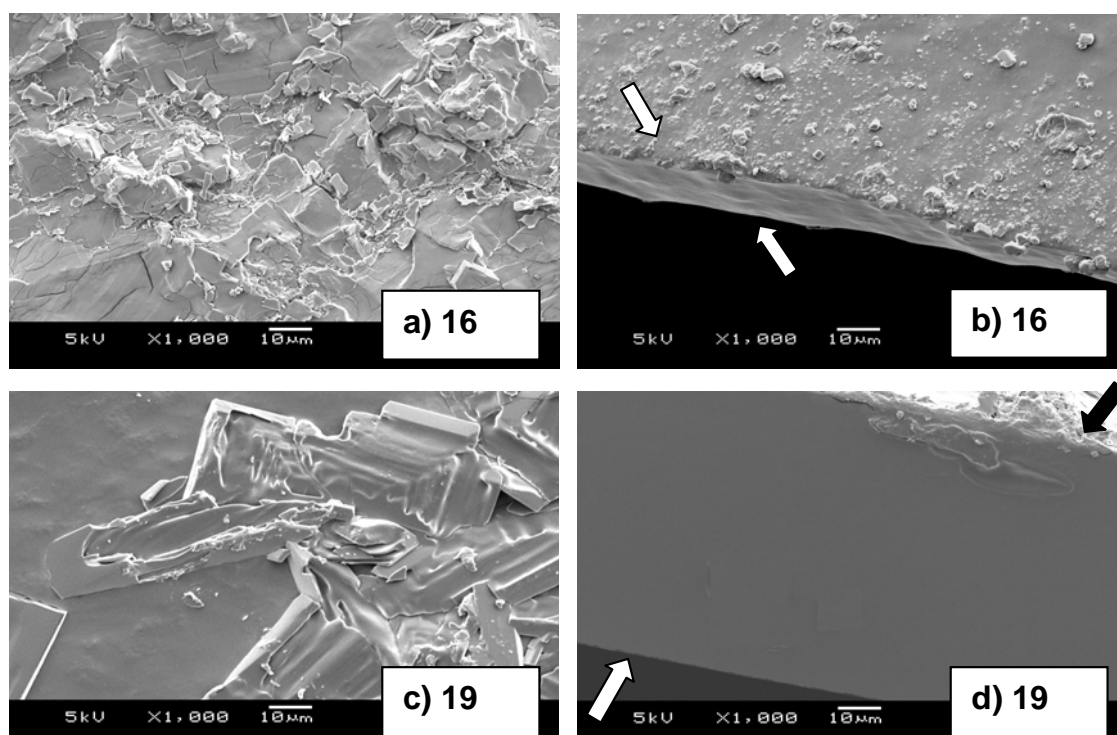
Samples surface and cross section SEM micrographs were recorded at 5 and 60 days (± 4) of storage. At the first week storage period, all samples presented a compact and smooth structure, both for surface and cross section, as exemplified by the control and samples 5 (containing all additives except sucrose) and 16 (containing the maximum level of all additives, pH and temperature) (Figure 2).

FIGURE 2 - SCANNING ELECTRON MICROGRAPHS OF SAMPLES SURFACES (a) CONTROL, (c) 5, (e) 16, AND CROSS SECTIONS (b) CONTROL, (d) 5, (f) 16, AT ONE-WEEK STORAGE



After 60 days of storage, a visible crystallization was observed on the surface and the cross-section SEM micrographs of samples containing sucrose (Figure 3), as exemplified by samples 19 (containing intermediate values of all additives and process parameters) and 16 (containing the maximum level of all additives and process parameters).

**FIGURE 3 - SCANNING ELECTRON MICROGRAPHS OF SAMPLES SURFACES
(a) 16, (c) 19, AND CROSS SECTIONS (b) 16, (d) 19, AFTER 60
DAYS OF STORAGE**



The crystalline surfaces observed in Figure 3 micrographs are in agreement with the crystallization behavior observed by X-ray diffraction analyses, confirming the crystalline behavior presented by cassava starch films with the addition of sucrose.

Cassava starch-sucrose films cross-section micrographs (Figures 3b, 3d) also demonstrate that the crystallization began at the surface and not at the interior of the material. Such a result indicates that the crystallization probably occurred by spontaneous nucleation of sucrose crystals and not due to residual non-dissolved sucrose crystals. If non-dissolvable sucrose grains were present along the interior of the film structure, they would have acted as seeding agents. The other sucrose molecules would lean and stick to this nucleus beginning crystallization (JACKSON & HOWLING, 1995) all over the film, and not only at the surface.

The fact that the film surface area is a more susceptible to water vapor exchange with the environment could be an explanation for the sucrose crystallization began at the film surface. According to LEES (1995), outside disturbance such as supersaturated level modifications, may start the crystallization process.

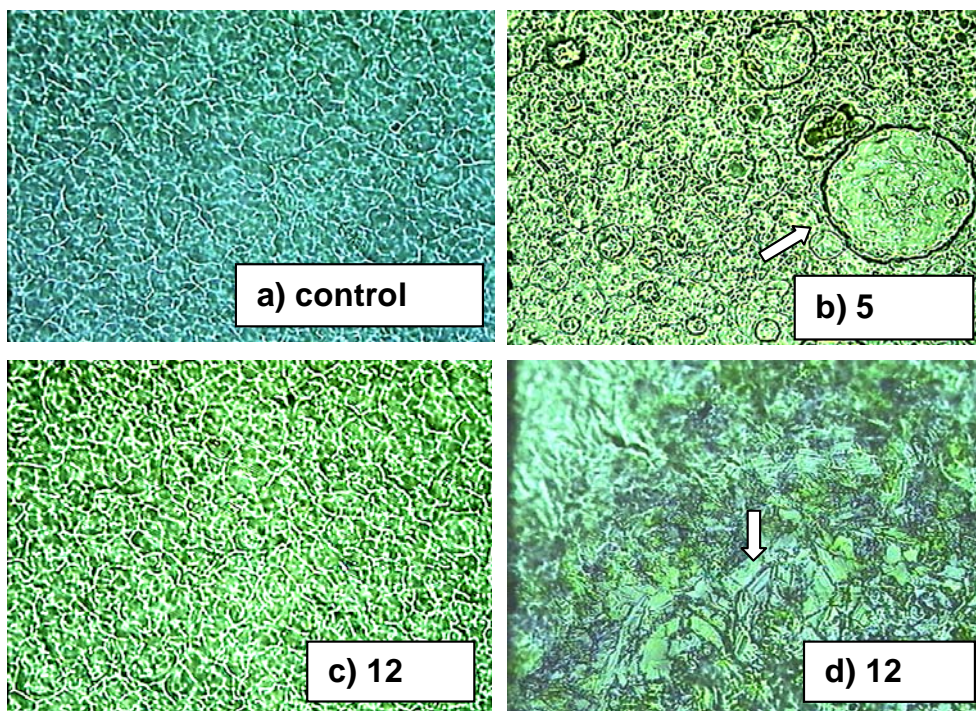
3.1.3 Optical Microscopy

Although not often utilized for analyzing biodegradable films structure, light microscopy demonstrated to be a very important tool in characterizing the component interaction of the samples. Light microscopy images allowed observing film homogeneity and crystallization during storage (Figure 4).

Light micrographs have demonstrated phase separation for films added with soybean oil (0.06%), as demonstrated by sample 5, showing dispersed oil drops along the material structure (Figure 4b), since the first day of storage. Such phenomenon was not possible to observe by SEM micrographs,

indicating that light microscopy should be used in ensemble with SEM for characterizing biofilms microstructure.

FIGURE 4 - SAMPLES LIGHT MICROGRAPHS (a) CONTROL, (a) 12, AT THE ONE-WEEK STORAGE; AND AT (b) 5, (d) 12, 60 DAYS OF STORAGE



The heterogeneity observed indicates that the cassava starch films network structure was unable to embody the soybean oil, although added in low concentration (0.06%), even in the presence of gelatin (Figure 4b).

The sucrose crystallization was also clearly observed by light micrographs images, as exemplified by sample 12 (added with 2% sucrose) after 60 days of storage (Figure 4d). The crystal growths observed are in agreement with SEM images and X-ray diffractograms.

Light micrographs demonstrated to be a feasible, fast and low cost tool for observing sucrose content film crystallinity during storage and phase separation in oil-starch systems.

3.2 COLOR

The Pareto chart of standardized effect, considering ANOVA pure error has indicated that no additives and process parameters affected ($p < 0.05$) the films lightness ("L"), redness ("a") and yellowness ("b").

All samples presented high lightness (above 97.9%), and low color values for "a" redness (< 0.24) and "b" yellowness (< 4.14), as can be observed in Table 4. Such results indicate that, independent of the additives, pH or casting temperature investigated in this study, the materials were almost colorless, with a high brilliancy. When compared to the control ($L=98.5$; $a=0.25$; $b=3.37$), samples presented similar results, indicating that the additives and pH modifications investigated in this study have little effect on the cassava starch films color parameters and that all materials were almost colorless, with a high brilliancy.

The total color difference between samples and the control (ΔE) is a good indicative to measure how much the additives affected the total color of the films in relation to the control.

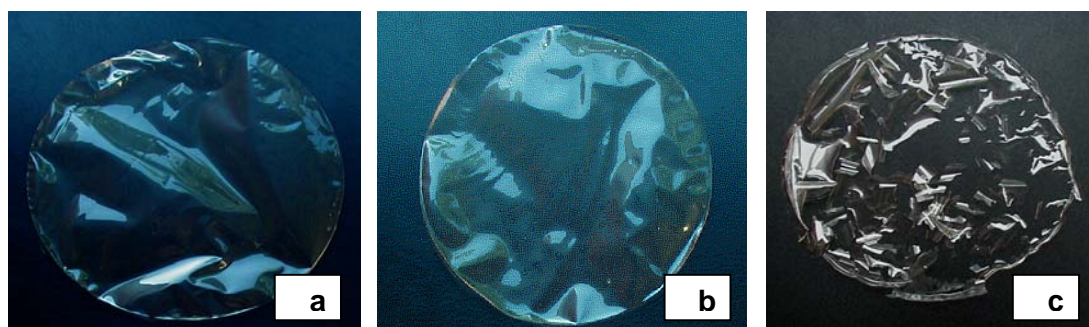
TABLE 4 - SAMPLES HUNTER COLOR PARAMETERS “L”, “a” AND “b” AND TOTAL COLOR DIFFERENCE AMONG SAMPLES AND THE CONTROL (ΔE %)

Assays	“L”	“a”	“b”	ΔE (control)
control	98.5	0.25	3.37	na**
1	98.60	0.24	3.38	0.11
2	98.70	0.17	3.41	0.23
3	98.72	0.27	4.14	0.80
4	98.12	0.19	3.40	0.38
5	98.34	0.15	3.19	0.25
6	98.05	-0.01	2.29	1.19
7	97.97	0.22	4.05	0.86
8	97.98	-0.10	2.41	1.14
9	98.35	0.21	3.59	0.26
10	98.39	0.11	3.13	0.30
11	98.14	0.19	3.48	0.37
12	98.38	0.09	3.64	0.33
13	98.48	0.07	2.29	1.09
14	98.15	0.00	2.91	0.62
15	98.39	0.12	2.65	0.74
16	98.23	-0.01	3.59	0.43
17 (c)*	98.59	0.01	2.66	0.81
18 (c)*	98.56	-0.01	2.47	0.94
19 (c)*	98.61	0.03	2.59	0.82
R^{2***}	0.33	0.59	0.52	0.28

(c)*: central points; na**: not applicable, R^{2***} : correlation coefficient.

Temperature has not influenced the films microstructure and color parameters. Although, when using process temperature of 60°C, samples were completely destroyed during casting, and it has not been recommended in future experiments (Figure 5).

FIGURE 5 - SAMPLES PREPARED AT (a) 30°C, (b) 45°C AND (c) 60°C CASTING TEMPERATURES



4 CONCLUSION

Among the additives and process parameters investigated, cassava starch film stability can only be affected by sucrose, although soybean oil addition resulted in heterogeneity. Color parameters were not affected by the additives or the process parameters investigated. Although temperature did not influence cassava starch film stability or color, casting temperature of 60°C has completely destroyed the films, and it has not been recommended for further investigations.

RESUMO

ESTABILIDADE ESTRUTURAL E COLORAÇÃO DE BIOFILMES FEITOS A BASE DE FÉCULA DE MANDIOCA-GELATINA DURANTE ESTOCAGEM

A influência de aditivos (sacarose, propileno glicol e óleo de soja) e parâmetros de processo (pH e temperatura) na estabilidade durante a estocagem de filmes feitos a base de fécula de mandioca e gelatina foi avaliada mediante planejamento fatorial 2^{7-3} . Difrátogramas de raios-X indicaram que somente a sacarose influenciou a estabilidade dos filmes, resultando em cristalização durante estocagem (60 dias), o que também foi observado por meio de microscopias óptica e eletrônica de varredura. A microscopia óptica, além de ter indicado cristalização, permitiu observar a separação de fase. Todos os biofilmes apresentaram alta luminosidade e baixos valores para tons vermelho e amarelo, sendo quase incolores. Embora a temperatura não tenha influenciado a microestrutura e coloração dos filmes, a secagem feita a 60°C destruiu completamente as amostras, não sendo recomendada para futuras investigações.

PALAVRAS-CHAVE: BIOFILMES; ADITIVOS; CRISTALIZAÇÃO; MICROESTRUTURA; COR.

REFERENCES

- 1 ARVANITOYANNIS, I.; PSOMIADOU, E.; NAKAYAMA, A. Edible films made from sodium caseinate, starches, sugar or glycerol. Part 1. **Carbohydrate Polymers**, v. 31, p. 179-192, 1996.
- 2 ARVANITOYANNIS, I.; PSOMIADOU, E.; NAKAYAMA, A.; AIBA, S.; YAMAMOTO, N. Edible films made from gelatin, soluble starch and polyols. Part 3. **Food Chemistry**, v. 60, n. 4, p. 593-604, 1997.
- 3 ARVANITOYANNIS, I.; BILIADERIS, C. G. Physical properties of polyol-plasticized edible films made from sodium caseinate and soluble starch blends. **Food Chemistry**, v. 62, n. 3, p. 333-342, 1998.
- 4 GARCIA, M. A.; MARTINO, M. N.; ZARITZKI, N. E. Lipid addition to improve barrier properties of starch-based films and coatings. **Food Chemistry and Toxicology**, v. 65, n. 6, p. 941-947, 2000.
- 5 GAUDIN, S.; LOURDIN, D.; FORSELL, P. M.; COLONNA, P. Antiplasticisation and oxygen permeability of starch-sorbitol films. **Carbohydrate Polymers**, v. 43, n. 1, p. 33-37, 2000.
- 6 GONTARD, N.; GUILBERT, S.; CUQ, J. L. Edible wheat film: influence of the main process variables on film properties using Response Surface Methodology. **Journal of Food Science**, v. 57, n. 1, p. 190-199, 1992.
- 7 HONG, S.; PARK, W. Use of color indicators as an indicative packaging system for evaluating kimchi fermentation. **Journal of Food Engineering**, v. 46, p. 67-72, 2000.
- 8 JACKSON, E.B.; HOWLING, D. Glucose syrups and starch hydrolysates. In: JACKSON, E. B. (Ed.). **Sugar confectionary manufacture**. 2nd ed. London: Blackie Academic & Professional, 1995. 200 p.
- 9 JENKINS, P. J.; DONALD, A. M. Gelatinization of starch: a combined SAXS/WAXS/DSC and SANS study. **Carbohydrate Research**, v. 308, n. 1-2, p. 133-147, 1998.
- 10 KESTER, J. J.; FENNEMA, O. R. Edible films and coatings: a review. **Food Technology**, v. 10, n. 12, p. 47-59, 1986.
- 11 KROCHTA, J. M.; MULDER-JOHNSTON, C. Edible and biodegradable polymer films: challenges and opportunities. **Food Technology**, v. 51, n. 2, p. 61-73, 1997.
- 12 LACROIX, M.; JOBIN, M.; MEZGHENI, E.; SROUR, M.; BOILEAU, S. Polymerization of calcium caseinates solutions induced by gamma irradiation. **Radiation Physical Chemistry**, v. 52, n. 1-6, p. 223-227, 1998.
- 13 LEES, R. General technical aspects of industrial sugar confectionery manufacture. In: JACKSON E. B. (ed.). 2nd ed.

Sugar confectionary manufacture. London: Blackie Academic & Professional, 1995. 200 p.

- 14 RINDLAV, A.; HULLEMAN, H. D.; GATENHOLM, P. Formation of starch films with varying crystallinity. **Carbohydrate Polymers**, v. 34, n. 1, p. 25-30, 1997.
- 15 STANSELL, D. The composition and structure of confectionery. JACKSON, E. B. (Ed.). **Sugar confectionary manufacture**. 2nd ed. London: Blackie Academic & Professional, 1995. 200 p.
- 16 STATISTICA for Windows software. v.5.0. Tulsa: StatSoft Inc., 1995.
- 17 VEIGA-SANTOS, P.; SUZUKI, C. K.; CEREDA, M. P.; SCAMPARINI, A. R. P. Microstructure and color of starch-gum films: effect of gum deacetylation and additives. Part 2. **Food Hydrocolloids**, v. 19, n. 6, p. 1064–1073, 2005.
- 18 YANG, L.; PAULSON, A. T. Effects of lipids on mechanical and moisture barriers properties of edible gellan film. **Food Research International**, v. 55, p. 571-578, 2000.
- 19 ZOBEL, H. F.; STEPHEN, A. M. Starch: structure, analysis and application. In: STEPHEN, A. M. (Ed.). **Food polysaccharides and their applications**. New York: Marcel Decker, 1995. p. 19-66.

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