http://dx.doi.org/10.22161/ijeab/3.5.38 ISSN: 2456-1878

Starch films for agronomic applications: comparative study of urea and glycerol as plasticizers

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Abstract*—This work aims to study the effects of urea, glycerol and their mixture as plasticizers for cassava starch films, regarding their impact on the material structure, water susceptibility, barrier and mechanical properties. All plasticizers were compatible with starch-based matrices, without detecting migration at the plasticizers level tested. In general water related properties were not affected. Plasticizer-polymer interactions as well as those involving water molecules were evidenced by ATR-FTIR spectra. Urea resulted the most efficient plasticizer, since it lowers glass transition temperature values and enhances mechanical properties. The co-plasticization of the starch films with glycerol and urea mixture resulted in poorer mechanical performance, though with higher light absorption which is relevant considering the potential film applications as mulching functionalized cover material.*

***Keywords*—cassava starch, barrier properties, plasticizer interaction, mechanical properties, urea.**

I. INTRODUCTION

Research related to functionalised biodegradable materials with active compounds is highly demanded for biomedical and pharmaceutical uses. However, the applications reported in the agronomic area are scarce, being these mainly focused on fertilisers' encapsulation [1, 2]. Likewise, the great plastics consumption for agronomic purposes has triggered the development of biodegradable materials [3-5]. In this regard, the inclusion of fertilisers to biodegradable films could help diminishing pollution as well as increasing crop efficiency and decreasing agrochemical use; thus, providing a greener alternative. The addition of urea, a common fertiliser, could not only functionalise but also plasticise the film matrix being this later released to the soil.

In general, plasticizers are included in material formulations for two main purposes: as processing aid agents and as final product properties modifiers [6]. In the first case, plasticizers lower the processing temperature, reduce sticking in moulds and enhance wetting. In the second one, they increase the temperature range of usage; increase flexibility and toughness; and lower the glass transition temperature. There is a consensus in the scientific community that plasticizers reduce intermolecular forces along the polymer chains, thus increasing the free volume and chain movements. However, the plasticizer selection depends on its compatibility, efficiency and permanence in the polymer matrix [6]. Moreover, plasticisation is particularly important on biopolymer films, since the dehydration of these structures produces strong cohesive films with poor mechanical and barrier properties [7]. Since most plasticizers contain hydrophilic groups, these compounds can interact by means of hydrogen bonds not only with polymer matrix but also with water molecules, increasing therefore films moisture absorption [8]. As regards starch-based materials, many studies have been carried out on different plasticizers to evaluate their performance, being polyols -especially glycerol- the most commonly used [9-23].

In comparison to polyols, urea exhibits a strong hydrophilicity due to its chemical structure -containing two amino groups and one carbonyl group- and a tendency to crystallise. It has been used for plasticisation of starch [17, 24-26], as well as cellulose [27], poly(vinyl alcohol) [28] and soy protein [29].

http://dx.doi.org/10.22161/ijeab/3.5.38 *ISSN: 2456-1878*

In respect of external plasticization, hydrocolloid-based films admit a maximum amount of plasticizer that is limited by their migration towards the film surface. As regards glycerol plasticised-films migration is evidenced by the oily appearance of films surface [30, 31], whereas when urea is used superficial crystallisation can occur [26]. Therefore, several studies on plasticizer content effect have been reported, being 30%w/w of dry basis the maximum concentrations reported for both glycerol and urea in starch-based materials [20, 26, 30, 32]. In addition, references on plasticizers-mixtures to extend migrations limits can been found, for instance urea/formaldehyde and urea/ethanolamine [24, 25]. The use of glycerol-urea, in particular, blends has been reported in thermoplastic starch (TPS) films [2, 33]. Nonetheless, no research addressing urea and glycerol mixture effect on cassava starch films obtained by casting has been published hitherto.

In this paper, we are therefore attempting to reveal the structure and behaviour of urea, glycerol and their mixture as plasticizers for cassava starch films, regarding the effects on the water susceptibility, barrier and mechanical properties of the films considering potential agronomic applications.

II. MATERIALS AND METHODS

2.1 Materials

Native cassava (*Manihot esculenta*) starch was purchased from Cooperativa de Productores de Jardín América Ltda. (Misiones, Argentina). Reagent grade glycerol (CAS# 56-81-5, Anedra, Argentina) and urea (CAS# 57-13-6, Biopack, Argentina) were used as plasticizers.

2.2 Film preparation

Native cassava starch films were prepared by casting and plasticised with glycerol, urea or its half-and-half mixture (w/w). Aqueous suspensions of 3 %w/w starch were gelatinised at 90 ºC during 20 min. Plasticizers were added after gelatinisation in a ratio of 25:100 of plasticizers to starch (w/w). A control film without any plasticizer (C) was also prepared as matrix reference.

Approximately 20 g of the film-forming suspensions were cast onto Petri dishes (diameter 8.7cm) and later dried in a ventilated oven (GMX 9203A PEET LAB, USA) at 50 ºC for 4 h; films were removed from the plates and stored at 20ºC and 65 % relative humidity (RH) for at least 48 h.

2.3 Film properties

2.3.1 Wettability and water content

2.3.1.1 Water content

Films moisture content was determined gravimetrically by measuring the weight loss of films upon drying in an oven at 105 ºC until constant weight. Reported values correspond to the mean value of three determinations.

2.3.1.2 Water sorption

Water sorption was measured gravimetrically on 2 cm × 2 cm films exposed to 100 % constant relative humidity at 20 ºC. Films were previously dried to constant weight in an anhydrous $CaCl_2$ atmosphere with an accuracy of ± 0.0001 g. Water uptake curves were fitted to the experimental model of Elizaldey col. [34]:

$$q = (Q\ t)/(B + t) \qquad (1)$$

Where q and Q are water taken up at time (t) and at equilibrium respectively and B is the time needed for samples to gain half of equilibrium value. Water content are given on dry basis; therefore, the samples dry matter was determined gravimetrically by oven drying at 105 ºC. At least three replicates were measured for each sample.

By differentiation of Eq. (1) a specific rate of water uptake constant (K) was determined as follows:

$$K = 1/(Q\ B) \qquad (2)$$

2.3.1.3 Wettability

Films wetting was evaluated through static contact angle measurements by the sessile drop method, using a Ramé-Hart Model 250 Standard Goniometer (USA). A 2 - 3 µL doubly distilled and deionized water droplet was released on the film surface, then the contact angle was calculated from a digital picture taken as soon as the droplet had reached the sample to avoid the anomalous behaviour of swelling. The contact angle (θ) was determined from the angle made between the baseline representing the film surface (liquid-solid interface) and the tangent to the droplet surface curvature (liquid-air interface). The mean value of ten replicates were taken on each film sample.

2.3.2 Optical and barrier properties

2.3.2.1 Water vapour permeability (WVP)

Water vapour permeability (WVP) tests were conducted using ASTM Standard Method E96 with several modifications according to [30]. After steady-state condition was reached, the acrylic permeation cells were weighed (0.0001 g) at initial time and at 1h interval over 8hs. The WVP (g/m s Pa) was calculated considering the thickness of each tested film, as well as the cell area and the water vapour partial pressure difference across the film at 20 ºC. Samples were analysed at least in triplicate.

Additionally, a digital coating thickness gauge for non-conductive materials CM-8822 (SolTec, Argentina), was used to evaluate the films thickness. Ten measurements were randomly taken at different locations for each specimen and the mean value was reported.

2.3.2.2 Optical properties

To evaluate the films light barrier capacity the absorbance spectrum (200 – 700 nm) was recorded using a HITACHI U-1900 Spectrophotometer (Japan). Films were cut into rectangles (3 cm × 1 cm) and placed on the internal side of a quartz spectrophotometer cell. Film opacity and UV-barrier capacity (AU ×nm) were defined as the area under the recorded curve between 400 – 700 nm and 200 – 400

nm, respectively, as described by Castilloy col. [35] and the standard test method for haze and luminous transmittance of transparent plastics recommendations ASTMD1003-00 Standard.

2.3.3 Film microstructure and mechanical properties

2.3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of plasticised films were measured in a FTIR Nicolet-iS10 Thermo Scientific Spectrometer (USA) with Attenuated Total Reflection (ATR) accessory. Spectra were taken in the wavenumber range: 4000 – 500 cm^{-1} by accumulation of 64 scans at 4 cm^{-1} resolution. Data was analysed by using the Software Omnic 9 (Thermo Scientific, USA). The spectral deconvolution of the data was performed using curve fitting algorithms within the following regions: 3700 – 2800 cm^{-1}, 1700 – 1500 cm^{-1} and 1200 – 900 cm^{-1}, as described in a previous work [8].

2.3.3.2 Differential Scanning Calorimetry (DSC)

Thermal properties of plasticised films were analysed by DSC employing a Q100 TA Instruments DSC equipment (USA) controlled by a TA 5000 module, with a quench cooling accessory, under a N_2 atmosphere (20ml/min). Film samples (5 - 6 mg) were weighed in aluminium pans and hermetically sealed, using an empty pan as a reference. Samples were analysed between −80 and 12 ºC, at a 10 ºC/min heating rate. Glass transition temperature (Tg, ºC) was determined using the Universal Analysis V1.7 F software (TA Instruments, USA). All measurements were performed at least by duplicate.

2.3.3.3 Mechanical testing

Mechanical performance of the studied films was evaluated by tensile tests using a texturometerTA.XT2i-Stable Micro Systems (UK) with a tension grip system A/TG. Ten probes of 7 mm × 60 mm were assayed for each sample and stress-strain curves were recorded. Maximum tensile strength (R_{max}), elongation at break (E_{max}), elastic modulus (Ec) and tenacity (Eg) were calculated according to the ASTM D882 - 00 Standard. At least ten replicates were measured, and the mean value was reported. Additionally, films thickness was determined as described previously.

2.3.3.4 Scanning Electron Microscopy (SEM)

Besides, some tested probes were mounted on bronze stubs and coated with a gold layer (40 – 50 nm) to be studied by SEM with a FEI QUANTA 200 SEM (Japan) with Apollo 40 electron detector. All samples were analysed using an accelerating voltage of 10 kV, under high vacuum mode.

2.3.4 Statistical analysis

Multifactor analyses of variance were performed using InfoStat Software [36]. Differences in the properties of the films were determined by Fisher's Least Significant Difference (LSD) mean discrimination test, using a significance level of $\alpha = 0.05$. In addition, in order to analyse the interdependence and variability of the results obtained a Principal Components Analysis (PCA) was carried out. The software (InfoStat) was used for the analysis considering two main components. From the analysis performed, the Biplot graph and the cophenetic correlation coefficient were reported, the latter being indicative of the efficiency of the variable clustering.

III. RESULTS AND DISCUSSION

I.1. Wettability and water content

Since both plasticizers evaluated present a hydrophilic nature, films water susceptibility was studied by different simple tests: water sorption, moisture content and contact angle.

Films water uptake behaviour was similar for all plasticised films. A relatively good fit ($r^2 > 0.920$) of the experimental data to the model of Elizaldey col. [34] was observed (Fig.1), from which parameters Q, B and K were obtained (Table 1).

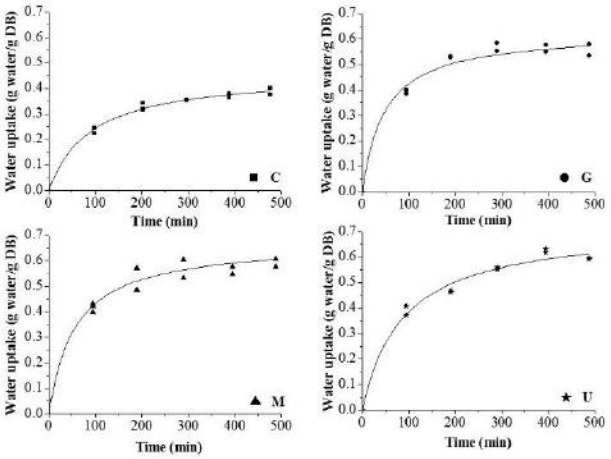

Fig. 1: Predicted (solid lines) and experimental (single points) sorption curves at 100 %RH of cassava starch-based films: unplasticized (C) and plasticised with glycerol (G), urea (U) and the mixture of both (M). (DB = dry basis)

Films containing glycerol (G, M) reached similar equilibrium water uptake (Q) and time needed to gain half of equilibrium value (B), while films plasticised only with urea (U) presented significantly higher values ($p<0.05$) in both parameters though showing lower water uptake rate (K). Control films (C), on the other hand, gave intermediate K values compared to those of plasticised films however showing significantly lower Q values

http://dx.doi.org/10.22161/ijeab/3.5.38 *ISSN: 2456-1878*

Table 1. Water uptake kinetic parameters, water content and wettability of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	Water uptake			Water content (%)	Contact angle (Θ)
	Q (g water/g DB*)	B (hr)	K (g DB/g water hr)		
C	0.45 ± 0.02 ^{a}	1.5 ± 0.02 ^{b}	1.5 ± 0.07 ^{ab}	13.4 ± 0.8 ^{a}	38.2 ± 4.3 ^{a}
G	0.64 ± 0.01 ^{b}	0.8 ± 0.01 ^{a}	2.0 ± 0.01 ^{b}	21.1 ± 0.4 ^{d}	48.9 ± 2.9 ^{b}
M	0.67 ± 0.01 ^{bc}	0.9 ± 0.05 ^{a}	1.7 ± 0.10 ^{b}	17.7 ± 0.9 ^{c}	46.6 ± 3.6 ^{b}
U	0.71 ± 0.02 ^{c}	1.4 ± 0.11 ^{b}	1.05 ± 0.10 ^{a}	15.8 ± 0.9 ^{b}	46.5 ± 3.3 ^{b}

Reported values correspond to the mean ± standard deviation.
*DB = dry basis

($p<0.05$). As outlined by Elizaldey col. [34] at RH > 90, water uptake represent mostly multilayer water, water held
in voids, crevices and capillaries. In the absence of plasticizer films present a more compact structure [6], therefore holding less water at the same equilibrium moisture than plasticised films.

The water content of the plasticised polymer samples ranged between 15.8 and 21.1 %, with significant differences ($p<0.05$) among the three samples tested, and significantly higher than unplasticized films (C) water content (Table 1).Even though all samples contained the same amount of plasticizer (25 %w/w), the addition of urea resulted in lower moisture content, indicating differential interaction among polymer-plasticizer-water depending on the plasticizer type.

The contact angle measurement is a useful tool to determine the hydrophobic or hydrophilic character of a film surface: low contact angle values ($\Theta<90°$) correspond to surfaces that are more wettable, on the contrary, hydrophobic surfaces show high values ($\Theta>90°$) of this parameter [37]. All films presented low contact angle values (Table 1). Nevertheless, a slight but significant ($p<0.05$) increase with respect to unplasticized films (C) was observed with plasticizers inclusion independently of their nature or concentration (G, M and U). Correspondingly, reported values of corn starch based films confirm the hydrophilic nature of these materials, though the comparison of contact angle values results difficult, since it strongly depends on additives and film [38, 39].

I.2. Barrier properties

Even though G films plasticised presented higher mean WVP values than those plasticised with M and U there were no significant differences ($p>0.05$) among plasticised films (Table 2), yet these resulted significantly ($p<0.05$) lower than C films WVP. These results are in accordance with other published works, considering that all samples contained the same total amount of plasticizer. Plasticizers interfere with polymeric chain association decreasing the rigidity of the network, producing a less ordered film structure, such an effect has great impact on films WVP [6]. In comparison to unplasticized cassava starch films, WVP decreases significantly ($p<0.05$) with 25 % of plasticizer addition, since more homogeneous and compact films are obtained without pores or cracks evidenced by SEM [30, 32].

Table 2. Light barrier capacity and WVP of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	WVP (g/m s Pa × 10^{10})	Opacity (AU × nm)	UV-barrier (AU × nm)
C	1.53 ± 0.3 ^{b}	32.69 ± 2.1 ^{c}	40.84 ± 1.3 ^{a}
G	1.03 ± 0.1 ^{a}	28.83 ± 2.9 ^{bc}	39.43 ± 3.1 ^{a}
M	0.99 ± 0.1 ^{a}	25.03 ± 3.0 ^{ab}	46.00 ± 3.2 ^{b}
U	0.93 ± 0.1 ^{a}	23.45 ± 2.5 ^{a}	46.90 ± 0.94 ^{b}

Reported values correspond to the mean ± standard deviation.

With regard to films light barrier capacity, the UV barrier capacity (200 – 400 nm) was higher for films plasticised with urea, due to its characteristic absorption peak. On the contrary, unplasticized films showed higher absorption in the visible region (400 – 700 nm) attributed to a more compact structure, hence the addition of plasticizer significantly reduced ($p<0.05$) films opacity being glycerol influence lower than that of urea.

I.3. Film microstructure and mechanical properties

I.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for pure components: starch; glycerol and urea, and the 50:50 mixture of the later are shown in Fig.2. Urea exhibits characteristic absorption bands in two main regions: 3700 – 3000 cm^{-1} (N-H amide stretching) and 1700 – 1300 cm^{-1} (N-H amide bending and carbonyl group stretching), presenting three characteristic peaks located at 1675, 1618 and 1585 cm^{-1}. Glycerol, on the contrary, has a broad band between 3700 – 3000 cm^{-1} (O-H stretching and bending) and the characteristic peaks of C-H bonds in the region of 3000 –

http://dx.doi.org/10.22161/ijeab/3.5.38 *ISSN: 2456-1878*

2800 cm^{-1} and 1500 – 1200 cm^{-1}. Urea and glycerol mixture showed characteristics absorption bands of both pure components, though maximums shift towards higher wavenumbers were observed on the 3700 – 3000 cm^{-1} and 1700-1300 cm^{-1} regions, representative of H-bridges interactions between both compounds. Moreover, native cassava starch spectrum presents a wide band between 3700 – 3000 cm^{-1} corresponding to the O-H stretching and bending, another peak at 1643 cm^{-1} associated to the O-H stretching in water molecules clusters with moderately strong H-bonded, and the characteristic bands of the C–O-C stretching vibrations and C-O-H bending vibrations in glycoside and pyranose rings in amylose and amylopectin at 1200 – 900 cm^{-1} [40, 41].

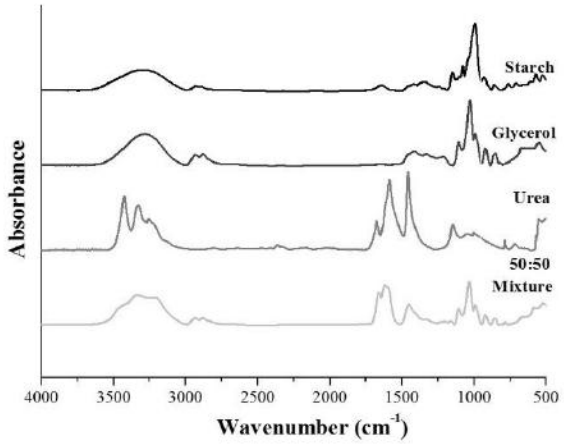

Fig.2: ATR-FTIR spectra of pure film components: starch, glycerol, urea and its 50:50 mixture.

Likewise, FTIR spectra of plasticised films with glycerol, urea and the mixture of both revealed the characteristic bands of the pure components. Nonetheless, variations in intensity and maximums shifts were observed indicating distinctive interactions among components. Main differences were displayed in 3700 – 3000 cm^{-1}, 3000 – 2800 cm^{-1}, 1700 – 1500 cm^{-1} and 1200 – 900 cm^{-1} regions (Fig.3). Table 3 shows the most important absorption peaks in each spectral window.

On the one hand, C and G films revealed a broad and intense absorption band in 3700 – 3000 cm^{-1}region centred at 3281 cm^{-1}, which is assigned to O–H stretching and bending vibrations. Films containing urea on the other hand, presented three important contributions in this region: about 3200, 3345 and 3452 cm^{-1} (Fig.3a). The latter are attributed to the characteristic amide N-H stretching peaks of urea (3254, 3327 and 3427 cm^{-1}), although the significant shifts observed indicate -as expected- that these N-H groups are involved in the H-bridge interactions within the matrix. The individual contributions of each of these peaks to the band depend on the urea content,resulting relative areas of 3200 and 3345 cm^{-1} peaks the most affected (Table 3).

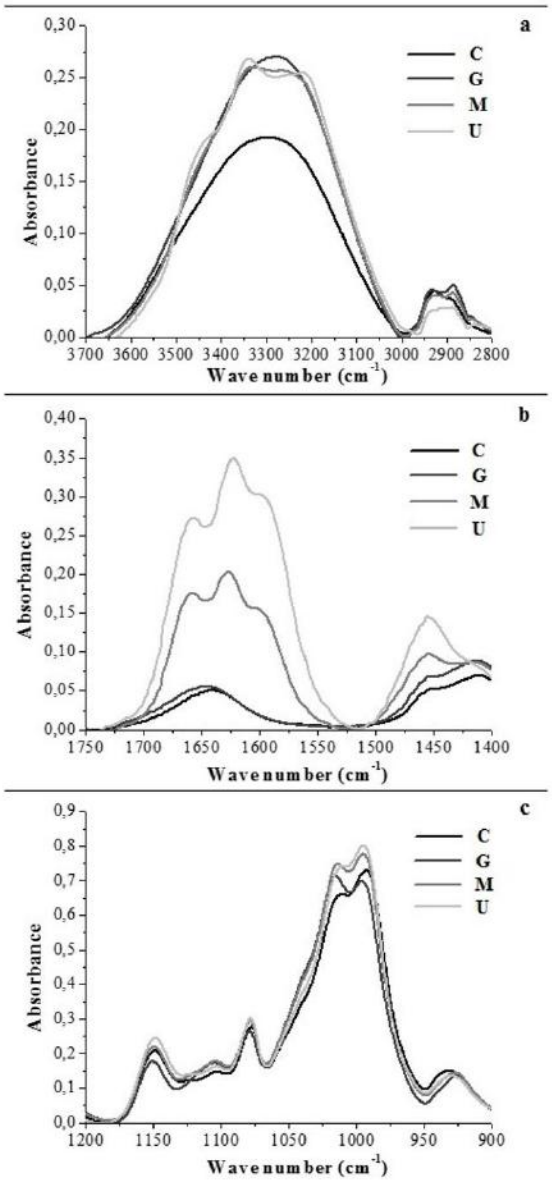

Fig.3: ATR-FTIR spectra of cassava starch-based films: unplasticized control (C), plasticised with 25% glycerol (G), 12.5% glycerol and 12.5% urea (M) and 25% urea (U). Three main regions are shown: a) 3700-2800cm^{-1}; b) 1750-1400cm^{-1}; and c) 1200-900cm^{-1}.

In the spectral region between 3000 – 2800 cm^{-1} urea does not reveal any band, whereas glycerol has a band with two clear peaks at 2937 and 2875 cm^{-1}. Cassava starch C and G films exhibit an analogous band though relative intensities shift due to the presence of glycerol's methylene groups (Table 3). Moreover, in films containing urea this band unfolds into three contributions with similar relative intensities (Table 3), indicating that the environment of C-

http://dx.doi.org/10.22161/ijeab/3.5.38 *ISSN: 2456-1878*

Table 3. FTIR band deconvolution peaks in the analysed spectral windows of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	C	G	M	U
3700-3000 cm^{-1}	3297.0 (1)	3281.9 (1)	3203.4(0.41)	3199.6 (0.48)
	--	--	3346.2(0.41)	3343.2 (0.34)
	--	--	3467.1(0.18)	3458.8(0.18)
3000-2800 cm^{-1}	2886.0 (0.36)	2884.4 (0.58)	2881.3 (0.43)	2884.1 (0.40)
	--	--	2914.6 (0.26)	2914.0 (0.30)
	2931.1 (0.64)	2936.3 (0.42)	2938.1 (0.31)	2940.2 (0.30)
1700-1500 cm^{-1}	--	--	1593.8 (0.25)	1587.6 (0.32)
	1643.7 (1)	1651.5 (1)	1628.5 (0.45)	1623.8 (0.40)
	--	--	1664.8 (0.30)	1663.9 (0.28)
1200-900 cm^{-1}	928.7 (0.07)	923.8 (0.06)	925.6 (0.06)	927.8 (0.06)
	989.7 (0.34)	993.9 (0.35)	992.9 (0.36)	992.4 (0.36)
	1016.9 (0.23)	1017.3 (0.17)	1016.5 (0.19)	1016.1 (0.22)
	1045.9 (0.12)	1040.1 (0.20)	1041.2 (0.17)	1043.1 (0.13)
	1078.8 (0.06)	1079.8 (0.05)	1079.3 (0.05)	1079.0 (0.06)
	1101.8 (0.06)	1103.6 (0.07)	1102.9 (0.06)	1102.6 (0.05)
	1124.6 (0.03)	1122.5 (0.03)	1123.4 (0.03)	1123.8 (0.03)
	1152.0 (0.09)	1151.1 (0.07)	1150.3 (0.08)	1149.7 (0.09)

In the case of deconvoluted bands each informed value corresponds to the peak position and in between brackets its relative contribution to the total band area.

H groups' change in the presence of urea affecting its vibrational transition.

In the FTIR spectra, films containing urea present three similar signals in terms of peak position and relative contribution in the region comprised among 1700 – 1500 cm^{-1} yet with higher absorbance in the U samples (Table 3). The observed shifts with respect to pure urea peaks indicate that both plasticizer-matrix and plasticizer-water interactions occur in the material. Similar results were published by Wangy col. [26] in oxidized corn-starch films plasticised with urea, being the peak at 1659 cm^{-1} attributed to C=O stretching (amide-I region) and the one at 1626 cm^{-1} assigned to N-H bending (amide-II region). In both C and G films, a single contribution could be seen in this region, associated with the O-H bonds of the water molecules that interact with the matrix (Table 3). Shifts observed in C and G films compared to those of pure components (Fig.2 and 3b) proved to be less important than that seen in films containing urea (M and U). From these results, it is clear that urea-matrix interactions are stronger than glycerol-matrix ones. MayYu [17] have exhaustively analysed the effect of plasticizers containing amide groups on the properties of thermoplastic starch, revealing that the hydrogen bond-forming abilities with starch was higher for urea than for polyols. Thus, in films containing urea the reaming hydrophilic groups available for interaction with free water are reduced, leading to lower film water content as shown in Table 1.

An increase in urea content led to an increase in the intensity of the peak located at 1455 cm^{-1}, which correlates with the C-N bond stretching in urea structure. Wangy col. [26] associated the absorbance ratio of this peak to that of 2930 cm^{-1} (A_{1455}/A_{2930}) to the superficial urea content of the material. The authors had observed a significant increase in A_{1455}/A_{2930} ratio when its concentrations exceeded 30 %w/w at the same time migration of this plasticizer to the surface of potato starch films had been detected by SEM. Despite the fact that A_{1455}/A_{2930} doubled from M (3,5 ± 0,6) samples to U (8 ± 0,6), urea superficial crystallisation was not observed in either samples probably because urea contents assayed were below those reported by Wangy col. [26] for urea migration to occur. Nonetheless, such differences can only be attributed to the greater concentration of urea molecules, and therefore C-N bonds, in films with 25 % of urea.

As shown in Fig.3c, in the fingerprint region of the spectrum all films components absorb, presenting distinctive peaks and intensities. Since in this region the major contributions are related to the starch matrix no major spectral variations were observed. Besides, the bands in this region of the infrared spectrum result mainly from C-O and C-C vibrational modes that are highly coupled, therefore the assignment of individual bands results difficult [42]. The main contributions found are shown in Table 3.

http://dx.doi.org/10.22161/ijeab/3.5.38 *ISSN: 2456-1878*

Table 4. Glass transition temperature, thickness and tensile resistance properties of control cassava starch films (C) and films plasticized with: glycerol (G), urea (U) and their mixture (M).

Film	Tg (ºC)	Thickness (µm)	R_{max} (MPa)	E_{max} (%)	Ec (MPa)	Eg (kJ/m^3)
C	68.3 ± 1.8 ^{c}	75.8 ± 2.4 ^{a}	61.8 ± 2.9 ^{b}	6.1 ± 0.8 ^{a}	1936 ± 376 ^{b}	1995 ± 344 ^{c}
G	37.8 ± 0.5 ^{b}	75.9 ± 5.3 ^{a}	2.2 ± 0.6 ^{a}	47.3 ± 9.6 ^{b}	14 ± 3 ^{a}	1091 ± 73 ^{a}
M	35.0 ± 1.2 ^{b}	74.5 ± 5.8 ^{a}	2.9 ± 0.3 ^{a}	68.9 ± 4.5 ^{b}	7 ± 2 ^{a}	977 ± 122 ^{a}
U	13.6 ± 1.0 ^{a}	74.9 ± 3.1 ^{a}	3.0 ± 0.4 ^{a}	81.8 ± 7.2 ^{c}	29 ± 7 ^{a}	1740 ± 99 ^{b}

Reported values correspond to the mean ± standard deviation.

The peaks between 990 and 1030 cm^{-1} were attributed to the anhydroglucose ring C–C, C–O, C–H bonds stretching and C-O-H bending modes [25, 43],while those at around 1150 and 1080 cm^{-1} were assigned to C–O–H stretching in starch [44]. The slight deviations detected in peaks maximums were attributed to plasticizers-starch interaction.

In addition, many authors have emphasised that both the absorbance ratios of the peaks at 994 and 1047 cm^{-1} relative to that of 1022 cm^{-1} could be indicative of the degree of crystallinity of starch [42, 43]. In line with these studies, C films presented A_{990}/A_{1022} and A_{1047}/A_{1022} ratios 15% lower than native starch, since in C films starch had been gelatinised and therefore present a rather amorphous gel structure [45]. Likewise, films plasticised with U showed similar results than C films. Films containing glycerol in their formulation (M and G) presented significantly higher A_{990}/A_{1022} and A_{1047}/A_{1022} ratios ($p<0.05$), that should be indicative of more crystalline regions in the films structure. Nonetheless, these unusual results could be attributed to the band at 995 cm^{-1} that correspond to the vibration of the skeleton C–C and the peak at 1045 cm^{-1} associated to the stretching of the C–O linkage in C1 and C3 in glycerol [46]. Consequently, this criterion would not be adequate to estimate the crystallinity of plasticised starch films.

I.3.2. Differential Scanning Calorimetry (DSC)

The DSC measurements served to determine the relaxation transitions of starch films (Table 4). The registered glass transition temperature (Tg) value for C films proved to be noticeably higher than that reported for films including plasticizer. Such results are in agreement with transition temperature registered for unplasticized starch films by other authors in literature [47, 48]. Moreover, a significant decrease ($p<0.05$) in the Tg of cassava starch films was observed in films plasticised with urea (U), being this effect less important in the co-plasticisation with glycerol (M). Lowering of Tg is regarded as an indicative of plasticisation efficiency [6], therefore these results imply that urea is a more efficient a plasticizer than glycerol and their mixture.

I.3.3. Mechanical testing and SEM characterisation

Furthermore, the mechanical behaviour of starch-based films depends heavily on their composition and thickness, yet all studied films had an average thickness circa 75 µm with no significant differences ($p>0.05$) among samples (Table 4).

Mechanical tensile resistance parameters of cassava starch-based films are shown in Table 4. From the results, it is clear that unplasticized films (C) present a brittle behaviour due to the strong cohesive forces among amylose and amylopectin chains [6]. The addition of 25 %w/w of plasticizer had a substantial impact on films maximum resistance (R_{max}) and elongation (E_{max}) as well as their elastic modulus (Ec), regardless of their nature (Table 4). Plasticised films mechanical profiles are shown in Fig.4.

As expected, the films mechanical behaviour was markedly affected by the type of plasticizer. Films plasticised with urea (U) resulted more flexible and resistant than that plasticised with the same content of glycerol (G). A significant increase ($p<0.05$) was observed in the elongation at break and tenacity of the material, although no significant differences were observed in the maximum tensile strength at rupture (Table 4). The materials elastic modulus follow a similar tendency (Table 4), thus it could be concluded that in terms of mechanical performance urea is a more efficient plasticizer than glycerol, most probably due to molecular size difference between both plasticizers [6]. In this regard, the higher mechanical resistance of U films is attributed to the stronger interactions between urea and the starch matrix, which was evidenced by FTIR analysis. With regards to film flexibility, Ivaničy col. [33] had revealed an opposite behaviour studying native corn starch films plasticised with urea and glycerol, reporting higher Tg values for urea plasticised starch films; therefore, in their study films were in a vitreous-amorphous state at ambient temperature which would explain their brittleness. Differences are attributed to processing conditions since in this work plasticizer was incorporated before starch gelatinisation.

When both plasticizers were added to the matrix, the resultant mechanical profile is in between that of a flexible and a flexible-tenacious material, exhibiting

http://dx.doi.org/10.22161/ijeab/3.5.38 *ISSN: 2456-1878*

though poorer characteristics than that of films with a single plasticizer. In spite the fact that there are no significant differences ($p>0.05$) in matrix elongation at break between U and M samples, both the maximum strength and the tenacity of the latter are significantly lower ($p<0.05$) than those of films plasticised either with urea or glycerol (U and G, respectively). Presumably, H-bridges interactions could be favoured among urea and glycerol molecules rather than interactions with the polymer matrix, negatively affecting the films mechanical properties.

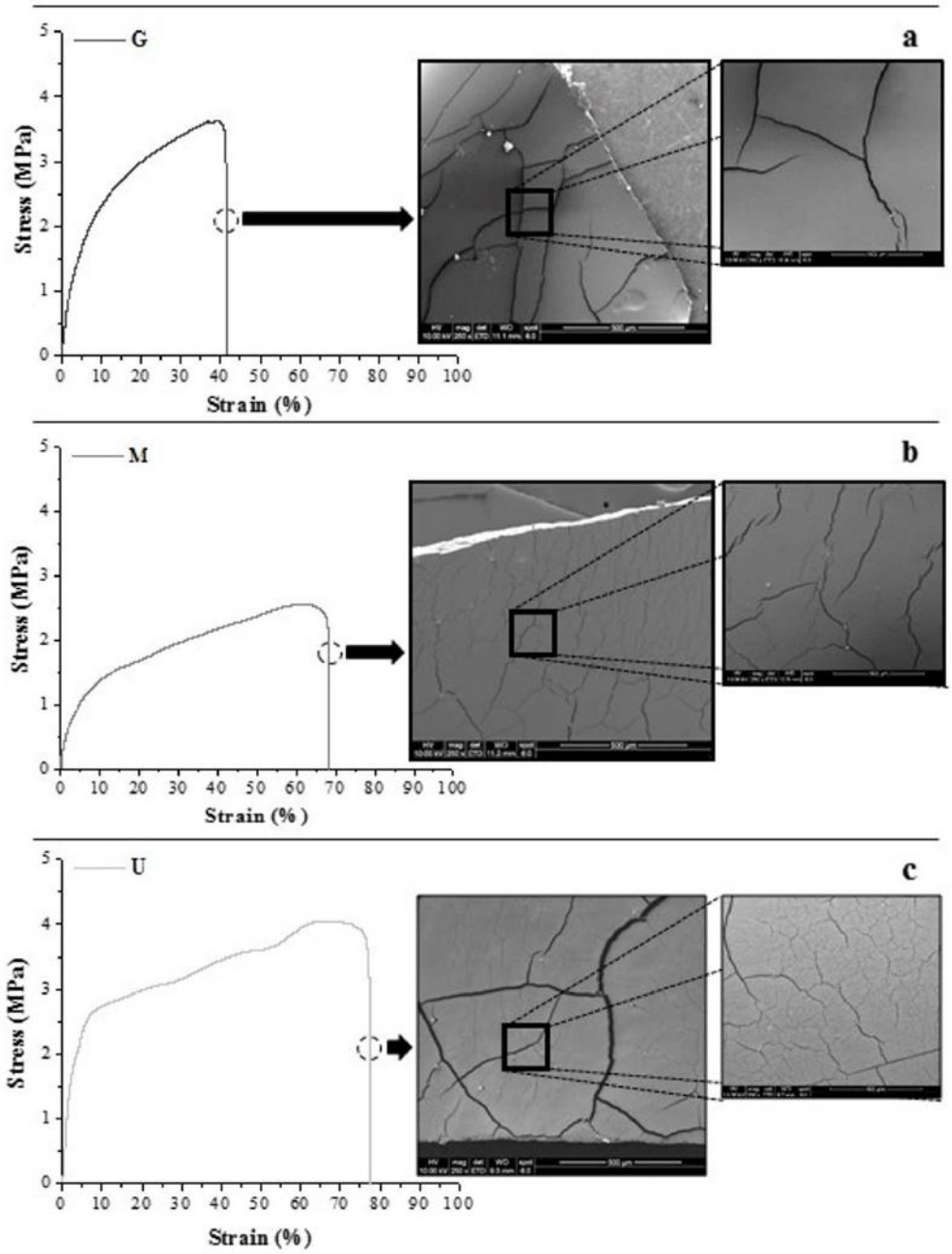

Fig.4: Tensile stress-strain curves and SEM micrograph of fractured plasticised cassava starch films with: a) 25% glycerol (G); b) 12.5% glycerol and 12.5% urea (M); and c) 25% urea (U). The SEM micrographs correspond to the cross section and a close-up image of the films surface.

Moreover, the topography of the surface and cross sections of the plasticised films subjected to tensile rupture test were studied using the SEM technique. Fig.4 shows the films probe rupture cross section and surface close-up of cassava starch films containing different types of plasticizer. Cracks or micro-cracks were observed on the surface of the materials in the direction in which the fracture of the specimen was propagated. However, films

with the addition of urea presented smaller cracks in every other direction probably because of the amorphous structure of the plasticised matrix. Besides, it should be remarked that no superficial urea migration, nor crystallisation, was detected. The micro-cracks observed result from the non-elastic elongations of films containing urea (detail box in Fig.4b and 4c). These results proved again the more flexible and resistant structure developed in U films, due to urea-starch interactions development as was confirmed by FTIR.

I.4. Principal Components Analysis (PCA)

In order to illustrate the aforementioned effects of the plasticizers on the polymer matrixes a PCA was carried out (Fig.5). Two separate analysis were done: one comparing plasticised and unplasticized samples all together (Fig.5a), and another one comparing only plasticised films to evaluate the effect of plasticizer type (Fig.5b). Both analyses gave a *cophenetic correlation coefficient* value circa 1, showing that such data grouping is representative of the experimental variables studied.

In the first case, it is clear that the first main component (CP1) -that explains the 70.2% of the total variance- represents the plasticisation effect on starch films. In comparison, considering the plasticised films (left side of Fig.5a) it can be seen that U films generate more important changes in films properties, since it is further away from the centre axis. Considering that C and M samples were not considerably affected by the second main component (CP2), in this case representing the 25.7% of the total variance, it was therefore attributed to the presence of urea or glycerol as single plasticizers.

Similarly, in the second case (Fig.5b) this analysis showed that: the first main component (CP1) that explains 76.1% of the total variance, associated with the presence of glycerol in the film matrix; whereas the other main component (CP2) attributed to single or co-plasticisation, accounts for the remaining 23.9%. The latter effect correlates with that seen in the first case, indicating that overall co-plasticisation with 50:50 urea and glycerol mixtures have a lower impact on films end properties than single compound plasticisation.

This analysis summarizes the previously detailed results, indicating a stronger influence and efficiency of urea as plasticizer of the starch matrix.

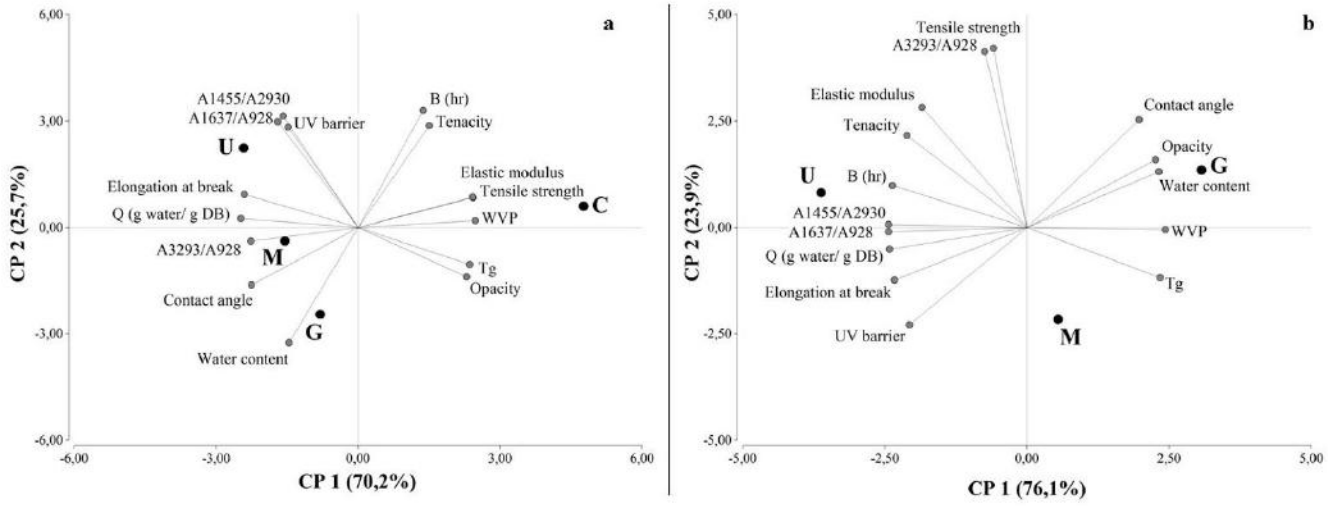

Fig.5. Principal components analysis (PCA) considering films properties with significant differences for cassava starch films, comparison of: a) plasticised (G, M and U) and unplasticized (C) films, and b) films plasticized with 25 %w/w of glycerol (G), urea (U) and their 50:50 mixture (M).

IV. CONCLUSION

In conclusion, urea, glycerol and their mixture were compatible plasticizers for cassava starch-based matrices, not detecting surface migration within the plasticizer's content tested (25 %w/w). Even though all samples contained the same amount of plasticizer, the addition of urea resulted in lower film moisture content. Films containing urea showed enhanced optical properties, especially the UV barrier capacity, since this plasticizer exhibited an electronic transition in this spectral region; though films containing a mixture of both plasticizers presented the highest UV-Vis absorption (200 - 700 nm).

Plasticizer-polymer interactions as well as those involving water molecules were evidenced by peak shifts observed in ATR-FTIR spectra: in particular, the detected shift to lower frequencies at 3300-3000 and 1700-1300 cm-1 regions, suggested stronger H-bonding interaction between starch O-H groups and N-H and C=O groups in urea than those between O-H pairs. In addition, the higher plasticizing efficiency of urea was demonstrated by both

the decrease in Tg values and the mechanical properties enhancement. On the contrary, the co-plasticization of the starch films with glycerol and urea 50:50 mixture resulted in lower mechanical resistance. As discussed, this behaviour could be ascribed to H-bridges interactions among urea and glycerol molecules -which were evidenced by FTIR of the mixture- reducing the plasticizers' interactions with the polymer matrix. Hence, the latter's use for starch- based films plasticization should be discouraged.

The obtained results provide a starting point for the study of applications of starch-based biomaterials as active compounds for controlled-release systems, particularly for agronomic purposes considering that urea is a commonly used fertilizer. In this regard, further research on co-plasticization with urea should be encouraged.

V. ACKNOWLEDGEMENTS

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Project PICT 2011–1213 and 2015-0921) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Florencia Versino wishes to thank CONCIET as well for a Doctoral Fellowship. Authors would also like to thank Ms. Marina Urriza for her technical assistance.

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