Understanding Polymeric Amylose Retrogradation in Presence of Additives

Deeptangshu S. Chaudhary,[1] Benu P. Adhikari[2]

[1]Department of Chemical Engineering, Curtin University of Technology, Perth, Western Australia 6845, Australia
[2]School of Science and Engineering, University of Ballarat, Mt Helen, Ballarat, Victoria 3353, Australia

ABSTRACT: Polymeric aging in starch is an important equilibrating process leading to long-chain amylose recrystallization and anisotropic properties of material made from starch. Investigations into a number of plasticizers and hydrocolloids showed that their water retention/binding capability influence the starch polymer's crystallinity. Addition of up to 8 wt % glycerol and 3 wt % xylitol acted as an antiplasticizer and apparently increased the total crystalline phase, which reduced the degree of elongation of amylose matrix by 15%. Maltodextrin and xanthan gum also reduced matrix elongation capability but X-ray diffraction (XRD) analysis showed samples with varying crystallinity, and the extent of crystallinity did not correlate with respective tensile properties. Additives such as maltodextrin, with similar molecular structure as amylose, were ineffective in increasing degree of elongation even at 15 wt % addition rate, because of formation of pockets of crystalline region, as observed by XRD analysis. Both xylitol and xanthan gum samples showed similar tensile strength and elongation properties, but the water retention capability of xylitol-filled starch samples was about 27% lower than xanthan gum samples, at their respective higher concentrations. A dynamic structural unit is proposed to satisfy the isotropic increase in tensile strength and degree of elongation in oriented starch matrix. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 2703–2709, 2010

Key words: water-soluble polymers; structure-property relations; crystallization; aging; biopolymers

INTRODUCTION

Starch-based packaging is a class of flexible packaging materials from renewable resources that are biodegradable and are extremely environmentally friendly. However, an important limitation of this biodegradable packaging material is the slow recrystallization of the amylose chains in starch matrix leading to increased brittleness and loss of mechanical strength, called "aging".[1] Water is a known plasticizer for starch polymer,[1,2] and the uncontrolled loss of water over a long period of time occurs concurrently with the slow association of amylose chains. This fundamental behavior of the amylose polymer to recrystallize and reassociate is closely linked with its interaction with its surrounding water, as seen in Figure 1. Previous studies have shown that amylose reassociation releases some of the bound water[1] and it is believed to be a complex process[2] and requires further investigation to understand the process of starch polymeric retrogradation.[1–3]

Overview of the complex retrogradation process

The fundamental difference between high-amylose and low-amylose starch is the long-chain amylose content in the starch matrix. The other component being amylopectin, a highly branched structure, prone to short-range association (recrystallization) causing local brittleness and reduced mechanical properties.[4,5] One key effect of such "aging" is the uncontrolled loss of water. Initially, the higher wt % water is to impart effective plasticization, but the resulting amylose mobility generates greater chain association. Larger size plasticizers, along with water, are commonly included to restrict this amylose recrystallization,[6] but this inherently reduces the tensile properties of samples, in general.[7]

The long amylose chains reorganize to either cocrystallize with amylopectin or self-associate to increase the brittle character of the starch matrix.[5,7] Interestingly, with high amylose content, there is a significant reduction in the fractional association of amylopectin and amylose, allowing only amylose-amylose interaction in the presence of water.[8,9] Further, excess water increases the matrix free volume[10,11] and allows a greater degree of chain freedom/mobility and increased flexibility (greater amorphous nature); which, however, also causes water loss and "aging" of starch compounds.[12,13] Other plasticizers such as glycerol and xylitol are added to the starch matrix to
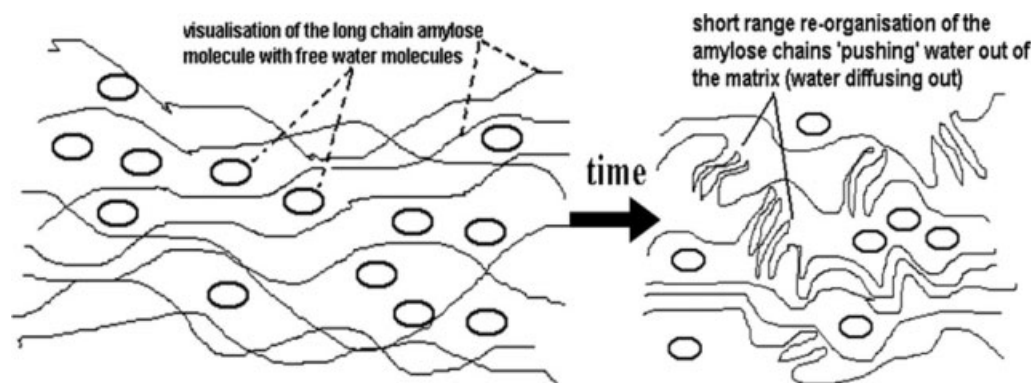
Figure 1 Presence of water provides the mobility to amylose chain to reorganize over long period, which results in water being "pushed out" of the matrix and creating pockets of crystalline amylose.

increase amylose-amylose interaction, without the long-term "aging".[14] Hydrocolloids such as maltodextrin molecules have molecular similarity to amylose and their smaller size results in greater mobility compared with amylose chains,[15] and xanthan gum is a known water-binder and may reduce the "aging" by gel formation with water[16,17] and allowing limited amylose chain folding. Generally, the increase in polymeric crystallinity increases Young's modulus and reduces degree of elongation properties. Further, addition of plasticizer or hydrocolloids increases degree of elongation but reduces the Young's modulus.

A key area lacking a fundamental understanding is how the addition of various small molecules influences the microstructural crystallinity development of starch and the rate of water loss from the starch matrix. This study highlights the complex interplay of amylose chain reorganization and water migration in the presence of different plasticizers.

MATERIALS AND TESTING

High-amylose maize starch ($\sim$ 80% amylose) was obtained from Timstock (Victoria, Australia). Commercial grade plasticizers were received from Consolidated Chemicals (Consolidated Chemicals Pty, Vic, Australia). All starch samples were prepared using extrusion on a DSE 25 counter-rotating extruder (Brabender, Duisburg, Germany) to make pellets, which were compressed into thin sheet samples. The extrusion was performed with additional water added during extrusion, and the amount of added water is shown in Table I as water content. Extrusion torque was maintained at 30% by varying the water content. This prevented starch degradation during extrusion. The extrusion processing conditions are listed in Table I.

Table II outlines the different samples prepared for this investigation. The sheet samples were collected and cut into 10 cm $\times$ 10 cm square pieces. Normal polyolefinic bags called poly-seal bags were used to store samples in a controlled humidity and temperature cabinet (20°C and 55% relative humidity). Moisture loss kinetics for each sample was determined by weighing individual samples (10 replicates) at fixed time intervals. For determination of absolute moisture content, samples were removed from their equilibrating conditions and heated in a KERN standard moisture analyzer equipped with infra-red heaters at 120°C.

Tensile properties of the starch samples were tested according to ASTM for flexible films using an INSTRON Universal Testing Machine (5560 Series, Instron dual column model, Vic, Australia). X-ray diffraction (XRD) investigations, useful in determining ordered structures, were carried out on a Philips X-ray generator (PW 1830 XRD, Philips, The Netherlands) with 30 kV accelerating voltage and 30 mA current. Intensities from $2\theta = 1.2°$ to 30° were recorded using Ni filtered Cu-K radiation $\lambda = 0.154$ nm). The wide-angle component was used to determine the formation of long-range crystalline regions.

RESULTS AND DISCUSSION

Mechanical properties

Figure 2 shows the variation in Young's modulus for all the starch samples. The addition of small amount of plasticizers or hydrocolloids tends to increase the Young's modulus; however, as the plasticizer concentration is increased, there is a notable reversal in

TABLE I
Processing Conditions for Preparing Various Starch Samples

Parameters	Processing conditions
Screw speed (rpm)	120–135
Highest temperature (°C)	110
Water content (%)	22–28
Residence time (s)	240–275

TABLE II
Starch Samples Nomenclature for this Investigation

Sample	Sample code	Sample	Sample code
High-amylose starch	HAS		
3 wt % Glycerol	G3	5 wt % maltodextrin	M5
8 wt % Glycerol	G8	10 wt % maltodextrin	M10
12 wt % Glycerol	G12	15 wt % maltodextrin	M15
3 wt % Xylitol	X3	1 wt % xanthan gum	XG1
8 wt % Xylitol	X8	2 wt % xanthan gum	XG2
12 wt % Xylitol	X12	4 wt % xanthan gum	XG4

modulus. In Figure 3, the initial effect of reduced elongation at low concentration of additives is widely recognized as antiplasticization. Such behavior is determined by the solubility extent of the additives in the polymer matrix; glycerol has excellent solubility in starch and is a typical example of an antiplasticizer compound.[14] As expected, Figure 3 shows once there is excess of plasticizers in the matrix, it generally increased the degree of elongation.

Interestingly, antiplasticization effect is shown by all additives in starch matrix. In plasticizers, glycerol and xylitol have smaller molecular size and greater degree of solubility in the starch matrix, which tends to the increased amylose-amylose interaction and antiplasticization behavior. Further, with lower quantities of the hydrocolloids in starch, as can be seen from Figures 2 and 3, maltodextrin and xanthan gum reduced the degree of elongation significantly at lower quantities but unlike maltodextrin, xanthan gum increases the degree of elongation at higher hydrocolloids concentration. The antiplasticization effect, because of these hydrocolloids, is based on their nature. For example, the short-chain maltodextrin, in the presence of water, have greater mobility compared with amylose chains[15] and because of the inherent molecular similarity between the amylose

and maltodextrin, the extent of cocrystallization of maltodextrin and amylose chains is increased, resulting in water loss. Earlier studies showed that by increasing the water activity of gelatinized starch samples, the net matrix crystallinity was increased,[18] which is similar to antiplasticization behavior. This is due to the increased free volume by smaller plasticizers that lead to greater chain association to coalesce and "squeeze-out" water. This mechanism is similar to protein folding and the subsequent expulsion of water.[12] On the other hand, as the concentration of xanthan gum is increased, it first interacts only with water to form a gel[16,17] and subsequently interacts with amylose chains and water resulting in reduced chain association.[19] This would initially prevent chain movement and reduce the degree of elongation, but as its concentration is increased, there is greater interaction between the gel structure and the amylose chains, and moderate increase in degree of elongation.

Moreover, it is well known that extrusion processing is heavily biased toward the axial (in the direction of extrusion) direction[20,21] - meaning that invariably, the tensile strength or the elongation properties in the axial direction will be significantly higher than those in the transverse direction. To understand the
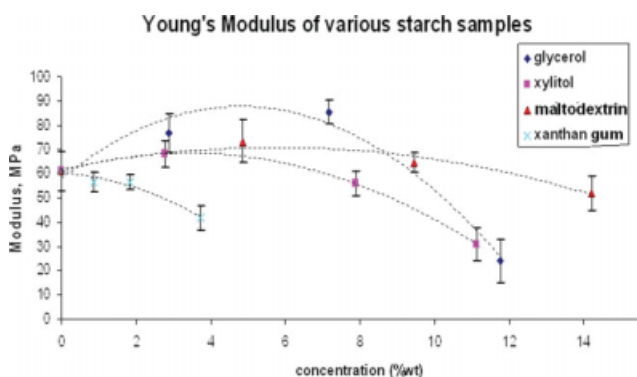

Figure 2 Young's modulus of starch samples as function of plasticizer type and concentration. The data points are connected by a dotted line for simple visual impact with no scientific intention. [Color figure can be viewed in the online issue, which is now available at www.interscience.wiley.com.]

Figure 3 The degree (%) of elongation of starch samples as a function of plasticizer type and concentration. The data points are connected by a dotted line for simple visual impact with no scientific intention. [Color figure can be viewed in the online issue, which is now available at www.interscience.wiley.com.]
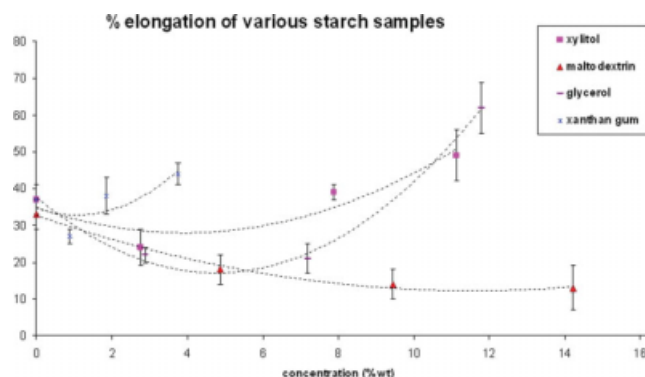
TABLE III
Stretch-Ratio of Various Starch Samples along with Their Mean Moisture Content (10 Specimen of Each Sample Were Tested at 55% RH and 23°C)

Sample type	Moisture (wt %)		Stretch ratio	
	Mean	SD	Mean	SD
G3	10.148	(±0.211)	1.873	(±0.134)
G8	11.263	(±0.124)	1.641	(±0.104)
G12	11.412	(±0.051)	1.117	(±0.051)
X3	9.802	(±0.171)	1.282	(±0.116)
X8	10.041	(±0.082)	1.225	(±0.22)
X12	10.772	(±0.092)	1.116	(±0.122)
M5	9.145	(±0.311)	1.776	(±0.233)
M10	8.457	(±0.213)	1.533	(±0.123)
M15	8.325	(±0.193)	1.384	(±0.041)
XG1	10.851	(±0.432)	1.344	(±0.054)
XG2	11.425	(±0.367)	1.311	(±.073)
XG4	14.324	(±0.336)	1.217	(±0.103)

mechanism of interaction of the amylose-plasticizers compared with the amylose-hydrocolloids, this study proposes a factor called the "stretch ratio" to represent the microstructural response from a tensile strength perspective. The stretch ratio is the ratio of tensile strength in axial to transverse direction, and Table III shows the values of stretch ratio for all samples against the moisture retention of the matrix. The lower the value of the "stretch ratio", the greater is the matrix isotropy and therefore, greater is the ability to deform in different directions. From this table, it is difficult to correlate large stretch ratio values with the fractional water retention. For example, at highest concentrations, xanthan gum and xylitol have lower stretch ratio values but significantly different moisture retention; meaning that they have greater ability to prevent matrix rupture but this ability arises from their different nature. Xylitol has five hydroxyl

groups to link with amylose and water. Its effectiveness as a low-molecular-weight plasticizer to generate greater reorganization capacity for the amylose chains has been previously discussed.[22,23] Xanthan gum, however, can interlink and adsorb large quantities of water molecules,[24] which may increase the free volume and create larger amorphous phase. This is different from plasticization (such as by xylitol) as the amorphous fraction influences the mechanical properties of composites mainly by development of an organized crystalline structure.[25,26]

A final observation of maltodextrin samples indicates that it failed to shown any significant improvement in degree of elongation even at 15% addition. As seen from Table III, maltodextrin samples equilibrated with significantly lower amount of water. Also, Figures 2 and 3 show that the increasing concentration had insignificant change in modulus but significant change in % elongation of starch samples. As discussed earlier, it is suggested here that presence of maltodextrin chains increases two complimentary behavior—(a) increased linking between maltodextrin and amylose chains causing increased crystalline "rigid" structure and (b) increased miscibility of amylose with amylopectin in matrix to "squeeze-out" free water. A combined effect of such behavior will be a net reduction in water retention and a simultaneous reduction in degree of elongation.

Microstructure and effect of additives

Figure 4 shows the raw XRD traces of five representative samples (starch, gycerol 8 wt %, xylitol 8 wt %, maltodextrin 10 wt %, and xanthan gum 2 wt %). The traces indicate that addition of the different plasticizers and hydrocolloids influence the growth of
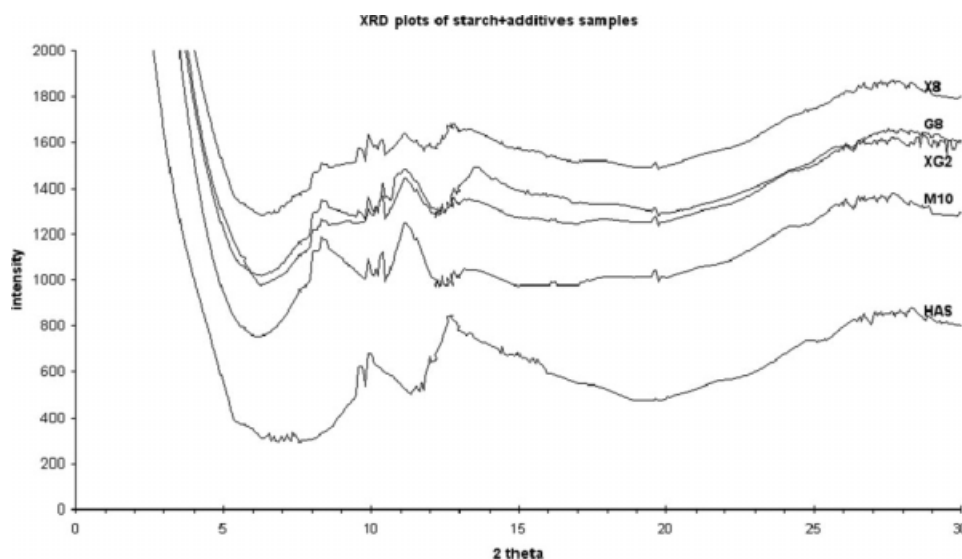

Figure 4 XRD traces of powdered samples of high amylose starch (HAS), G8, X8, M10, and XG2.

TABLE IV
Total Crystallinity Values and Fractional Rigid Amorphous Values
of Various Starch Samples

Sample	Moisture (wt %)		Crystalline region		Rigid amorphous' region	
	Mean	SD	Mean	SD	Mean	SD
G3	10.148	(±0.211)	28.548	(±0.213)	2.681	(±0.367)
G8	11.263	(±0.124)	33.486	(±0.311)	3.301	(±0.22)
G12	11.412	(±0.051)	15.114	(±0.311)	9.547	(±0.124)
X3	9.802	(±0.171)	26.471	(±0.193)	1.584	(±0.211)
X8	10.041	(±0.082)	18.586	(±0.367)	5.872	(±0.432)
X12	10.772	(±0.092)	14.15	(±0.122)	15.886	(±0.193)
M5	9.145	(±0.311)	30.581	(±0.124)	5.309	(±0.082)
M10	8.457	(±0.213)	33.18	(±.073)	4.667	(±0.051)
M15	8.325	(±0.193)	29.465	(±0.054)	2.115	(±0.123)
XG1	10.851	(±0.432)	26.88	(±0.193)	2.185	(±0.213)
XG2	11.425	(±0.367)	31.475	(±0.432)	7.683	(±0.336)
XG4	14.324	(±0.336)	22.011	(±0.116)	11.541	(±0.193)

crystalline regions as seen by the broadening of shoulder and diffusion of some peaks. Further, with 8 wt % xylitol, the peaks appear to be significantly diffused when compared with the rest of the samples.

It is imperative to understand that the XRD traces only reflect an overall "ordered" structure in the matrix and the broadness of the peak reflect the size of the "ordered" structure. It has been previously shown that such orderness could exist with significantly less molecular crytallinity (because of chain association or chain folding).[27] Because of the high amylose proportion in the starch samples used in this study, most ordered structures resulted from a continuous amylose network.[28] Previous XRD investigations reported that high amylose structures have a low crystalline matrix fraction of around 23%.[29] Interestingly, the XRD traces of the present starch samples showed about 15% greater "orderness" in matrix, when compared with the literature,[29] with broader peak or peaks with large shoulder (Fig. 4), similar to previously mentioned structures.[20] This is not a true molecular crystallinity and should be interpreted as development of "net orderness," which is a combined effect of real chain folding (molecular crystallinity) and long-range chain-additive organization[21] (refer to Table IV for total crystallinity values). Clearly, factors like "stretch ratio", mentioned earlier, will be sensitive to such morphology development.

It is likely that a rigid amorphous phase is formed because of the interaction between free water, additives, and amylose chains with the addition of plasticizers and hydrocolloids. From the XRD crystallinity data, the quantity of rigid amorphous is calculated by separating the microcrystalline peaks from the shoulder and from the base using a deconvolution method.[30] Different quantities of microstructure are thus obtained, some having short-range ordered structure (because of the free volume generated by the additives and "locked" water) and appear as the broader

shoulder in XRD. Further, when such "ordered" structures are arranged close to each other, we obtain the typical diffused peak in XRD. Table IV shows the rigid amorphous values for the different starch samples. In Table IV, samples with large rigid amorphous fractions also have greater water retention; for example, X12 and XG4 samples showed the largest fraction of rigid amorphous with more than 10 wt % water in the matrix. On the other hand, with increasing maltodextrin content, both the amorphous content and the moisture content decreased, which further supported the earlier discussion on stretch ratio data in Table III.

Furthermore, the data in Table IV highlight the strong antiplasticization behavior of glycerol, which was highlighted by the increase in modulus and reduction in degree of elongation (Figs. 2 and 3). It can be seen from Table IV data that the rigid
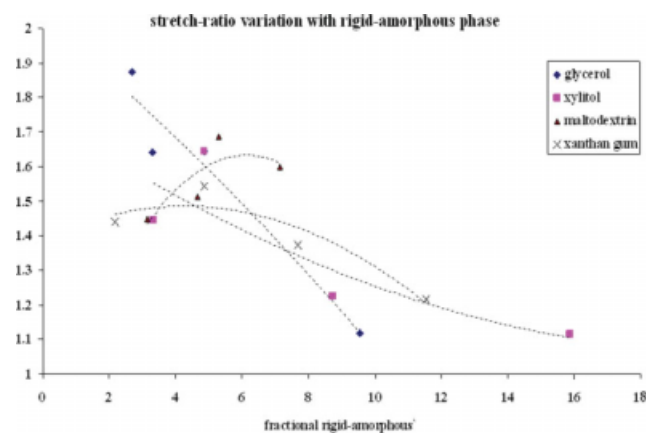

Figure 5 Peer variation in stretch ratio and fractional rigid amorphous content of starch samples as a function of plasticizer type and concentration. The data points are connected by a dotted line for simple visual impact with no scientific intention. [Color figure can be viewed in the online issue, which is now available at www.interscience. wiley.com.]
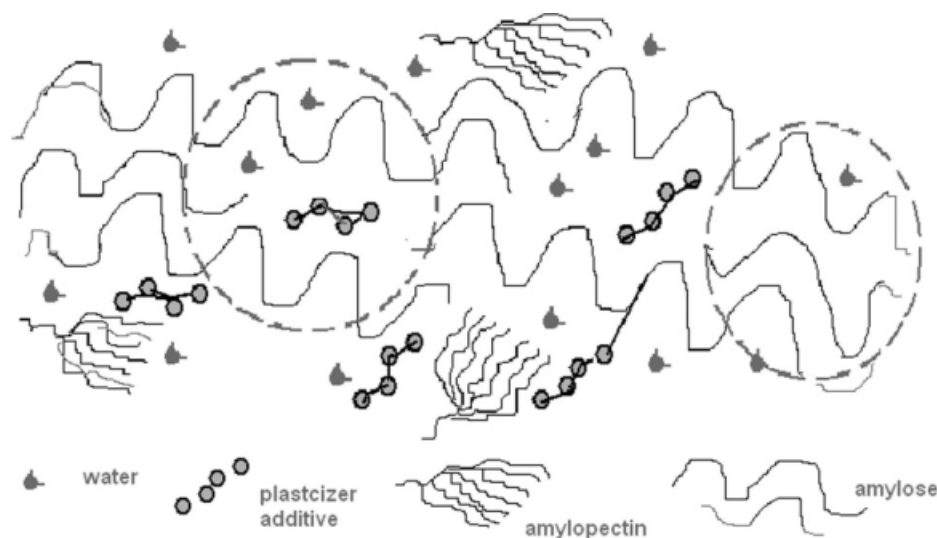
Figure 6 Schematic diagram of starch matrix with quasi-units, shown by the dotted ovals. Some units have greater amylose fractions (ordered structures) and some units have greater plasticizer fractions (nonordered), and these units can interact under external stresses because of larger free volume.

amorphous region has a small increase between G3 and G8, when compared with X3 and X8, even though the total moisture retention increased in both cases. It is well known that the solubility of smaller plasticizer, such as glycerol, in starch is higher than that of xylitol, a much larger molecule. So, glycerol has a greater tendency to create a three-way amylose-glycerol-water interaction, leading to the well-known antiplasticization. Because of the excellent mixing achieved in the extrusion, antiplasticization effect is observed with all the additives in this investigation, albeit at different concentration, which is expected because different additives have different solubility levels in the amylose matrix. However, with excess plasticizer, there is significant decrease in the amylose-amylose association and decrease in the stretch ratio values with increase in the rigid amorphous content, as seen in Tables III and IV.

Figure 5 attempts to highlight the significance of the stretch ratio factor from a structural point of view. Moisture retention data in Table III could not explain the tensile stretch ratio behavior of all the samples by itself; however, when combined with the rigid amorphous phase, a decrease in the "stretch ratio" value is observed as the "rigid amorphous" content is increased. This is shown in Figure 5. Based on the experimental evidence, i.e., greater complexity and larger size of the plasticizer leads to greater water retention (Table III) and further increases the "rigid amorphous base", which has shown to improve the stretching ability of the polymer samples (Fig. 5), a structural unit is suggested for the rigid amorphous phase (see Fig. 6). When compared against the smaller plasticizers interaction, it is found that presence of smaller molecules such as glycerol or maltodextrin

support faster reorganization of the amylose chains by "displacing" water with other plasticizer molecules, and this is directly measured against a quicker water loss rate (lower equilibrium water content under identical conditions in Table IV). The structure suggested in Figure 6 is formed by simultaneous multiple interaction of additives, water, and amylose chains to "lock" water molecules. Also, the rigid amorphous network interactions maintain sufficient orderness and chain flexibility by chain sliding and rearrangements as found in other structures.[31,32] Such structures are then able to absorb external stresses and achieve greater deformation.[33] This microstructure can be visualized not as a fixed unit but as a quasi-unit, continuously changing the amount of starch-water-additive within its boundaries as it reacts to external stresses.

CONCLUSIONS

The addition of plasticizer or hydrocolloids increases the degree of elongation but reduces the young's modulus, once there is excess of additives in the starch matrix. Maltodextrin and xanthan gum reduce the degree of elongation significantly at lower quantities but unlike maltodextrin, xanthan gum increases the degree of elongation at higher hydrocolloids concentration. Maltodextrin's inability to act as a plasticizer lies in its identical molecular structure to amylose, which increases the overall association of amylose and maltodextrin. The stretch ratio values for xanthan gum and xylitol samples showed increased isotropic behavior, even though the increase in tensile strength and matrix flexibility may come through different mechanisms. Xanthan gum retained the

greatest fraction of moisture amongst all the tested samples, whereas the maltodextrin samples showed the poorest water retention. Xylitol and glycerol samples retained similar mositure in the starch matrix, but gave rise to different crystalline fractions. This was attributed to glycerols' smaller size and its ability to increase amylose chain association by increasing free volume in the starch. We propose here that glycerol, xylitol, maltodextrin, and xanthan gum compete with water to interact with hydrophilic sites on the amylose chains to form a unique molecular structure that "locks" greater fraction of the water molecules. Analysis of the amylose crystalline region indicates that this new structure enhances the isotropic tensile properties using both the crystalline regions and the extra water, which is corroborated by the moisture loss kinetics. We believe that it is of great significance in controlling the moisture transport from the starch structure and reducing the recrystallization of starch components. Further work is being carried out in the important area of glass transition temperature of amylose chains and linking it with investigations into the diffusion mechanism of water molecules from plasticized starch matrix.

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