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Research Article

Effects of Enzymatic Hydrolysis on the Physicochemical and Structural Properties of Cassava Bagasse (*Manihot esculenta Cranz*).

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Abstract: This study evaluated the effect of enzymatic hydrolysis on the physicochemical and structural properties of cassava bagasse (*Manihot esculenta Cranz*). Cassava bagasse is a byproduct of the cassava starch process with limited applications in the industry. Were applied enzymatic treatments to three ratios a substrate (cassava bagasse): Buffer volume (1:10, 1:15, 1:20). Were used two commercial enzymes, cellulase (CelluSEB TL) and α-amylase (Licquozyme supra 2.2X). The physicochemical and structural analysis was performed after each treatment, including a control. The physicochemical analysis showed that the cassava bagasse had a high content of carbohydrates (61,19%) and fiber (22,63%); additionally, there were significant differences ($p<0,05$) between the bagasse control and the three enzymatic treatments. The FT-IR spectroscopies of the enzymatic treatments showed the absence of the absorption signal 1374 cm^{-1} corresponding to the cellulose chemical bond CH, as well as the decrease in the intensity of the band 2927 cm^{-1} corresponding to the CH bonds and CH_2, which may be related to a decrease of the crystallinity in the enzymatically treated bagasse. It was found that, due to its physicochemical composition, cassava bagasse is a material that could be used for biotechnology or food purposes; moreover, enzymatic hydrolysis produces the decrystallization of cellulose and significant changes in its physicochemical properties.

Keywords: α-amylase, cellulose, cellulose, crystallinity, FT-IR spectroscopy, lignocellulosic

INTRODUCTION

Cassava (*Manihot esculenta Cranz*) is one of the main energy sources in many tropical countries; it appears to have originated in Venezuela during 2700 BC (Pandey *et al*., 2000). According to Aristizábal and Sánchez (2007), among the main characteristics of cassava are: the high potential to produce starch, tolerance to drought and worn soils and large adaptability to different growth conditions. Worldwide, more than 600 million people depend on cassava in the Africa, Asia and Latin America (Tonukari, 2004). According to FAO, world cassava production during 2014 was 268.277.743 Ton., the African continent was the largest cassava producer with 145.770.528 Ton (53,9% of world production), followed by Asia, which reported a production of 89.833.397 Ton (30,3%). America had a production of 32.421.670 Ton (15,7%) and Oceania reported a production of 252.148 Ton. (0,1%). Also during 2014, cassava production in Latin America was 30.641.834 Ton., where Colombia is the third largest producer of cassava. The departments of the Colombian Caribbean Region have an important place in the production of cassava in this country. In 2015, the departments of Córdoba, Sucre, Bolívar, Atlántico, Magdalena and Cesar was among the top ten cassava producers in Colombia, contributing a total production of 183.731 Ton (DANE, 2016). More than two-thirds of total cassava production is used for human consumption and the other fraction is used in the animal feed industry (John, 2009; Tonukari, 2004). A considerable part of cassava production is processed and marketed as starch (Alarcón and Dufour, 1998); therefore, cassava starch is the most important industrial product obtained from this raw material (Ceballos and De la Cruz, 2002; Suárez and Mederos, 2011).

Cassava bagasse is a fibrous by-product resulting from the industrial processing of cassava during the extraction of starch, which is generated in large

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quantities and treated as a solid residue (Lu *et al*., 2012). It is the most abundant by-product of cassava, approximately 90% of its wet weight (Chen *et al*., 2015). Since the bagasse has a high humidity (about 85%) after the starch extraction process, it is difficult to store and transport due to its high perishable nature (Farias *et al*., 2014). The dry bran of cassava bagasse has a dry matter content of 80% to 85%; 60% -70% of which corresponds to starch and 12%-14% to fiber (Alarcón and Dufour, 1998). Cassava bagasse (dry matter) contains starch and fibrous compounds in equivalent proportions, with low levels of minerals, proteins and lipids, which together represent less than 5% a dry basis (Farias *et al*., 2014). This residue contains high contents of starch (40-64%), cellulose, hemicellulose and lignin (15-50%) (Chen *et al*., 2015).

The lignocellulosic materials comprise three polymers: cellulose, hemicellulose and lignin (Meng and Ragauskas, 2014; Hendriks and Zeeman, 2009). Due to the large amounts of lignocellulosic biomass generated in the agroindustry, forestry and agriculture, there is an accumulation of this one, which can cause environmental problems (Maitan-Alfenas *et al*., 2015). Lignocellulosic biomass can be degraded to simple sugars through enzymatic or chemical treatments; however enzymatic hydrolysis proves to be more suitable because it has less energetic requirements, it is environmentally friendly and generate few inhibitory products, which is an advantage for the following fermentation (Zhang *et al*., 2012; Brummer *et al*., 2014). There are used effective methods of multi-enzymatic hydrolysis for lignocellulosic materials containing starch, these methods involve the amylolytic activity combined with the action of other hydrolytic enzymes; there are studies where the combination of different enzymes have been used for the hydrolysis of complex materials such as cassava pulp and other materials (Chaikaew *et al*., 2012; Wang *et al*., 2006). The enzymatic hydrolysis of lignocellulosic materials may be limited by the action of several factors such as cellulose crystallinity, the degree of polymerization, moisture content, available contact surface, lignin content and the degree of milling (Hendriks and Zeeman, 2009; Nair *et al*., 2011).

The objective of this study was to evaluate the effect of enzymatic hydrolysis on the physicochemical and structural properties of cassava bagasse (*Manihot esculenta Cranz*).

MATERIALS AND METHODS

Collection of the raw material: Samples of cassava bagasse were collected from the company Almidones de Sucre S.A.S located at Km 4.5 via Sincelejo-Corozal (Colombia).

Physico-chemical characterization: Was performed the analysis of moisture, crude protein, crude fiber, lipids and ash according to the official methods described by AOAC (1990).

The content of cellulose, hemicellulose and lignin in cassava bagasse were determined by the NREL/TP-510-42618 method (Sluiter *et al*., 2012).

Starch content: Was determined the starch content by enzymatic hydrolysis (Belitz and Grosch, 1997), were added 200 mg of the sample to 42 mL of distilled water. Subsequently, 20 µL of α-amylase solution was added and heated into a water bath at 80-90°C during 15 min under constant agitation. The suspension was then cooled down and was added 2,5 mL of 0,1M sodium-acetic acid buffer solution pH 4,8. Then, 300 µL of amyloglucosidase solution was added and heated into a water bath at a temperature of 60°C during 30 min with constant agitation. Then the bioreaction was cooled down at room temperature and two drops of NaOH 2N solution were added to neutralize. It was transferred to a 125-mL Erlenmeyer flask by filtering the solution with gauze; the sample was made up to a volume of 125 mL by adding distilled water. Was determined the concentration of reducing sugars (RA) by using the DNS method proposed by Miller (1959). The starch content was calculated using Eq. (1) and (2):

$$\%Sugars = RA \times 0.125 \times 1000 \quad (1)$$

$$\%Starch = \frac{\%Sugars \times 0.9 \times 100}{Sample\ weight} \quad (2)$$

Amylose content: Was determined the amylose content with the method ISO 6647-1 (2007); was placed 100 mg of lipid-free-cassava bagasse into a 100-mL flask with one mL of ethanol (96%) and 9 mL of NaOH (1M). Was placed the flask in a water bath at 90-100°C during 10 min. Then, the solution was cooled down up to room temperature.

Was subjected the sample to a colorimetric reaction; 200 µL of acetic acid (1 M) and 400 µL of Lugol solution were added to 1 mL of the solution transferred in a test tube, then made up to 20 mL with distilled water. The solution was stored in the dark for 20 min to develop the color. Then, was measured absorbance at 620 nm. Was made the quantification of amylose by using a calibration curve (Concentration vs. absorbance) between 0 and 1 mg of amylose. Was estimated the amount of amylose in each sample by applying the straight line regression formula of the calibration curve Eq. (3):

$$\%Amylose = \frac{(Y-B)}{A} \times \frac{100}{P} \quad (3)$$

where,

Y = The absorbance of the simple
A = The slope of the line
B = The intersection with the Y axis
P = The weight of the sample in mg

Infrared spectroscopy with Fourier transform (FT-IR): Were obtained cassava bagasse infrared spectra by using a Fourier transform infrared spectrometer (Thermo scientific reference Nicolet iS5 Transmission iD1) in the region of 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1}. Was made the formation of the crystals by mixing 20 mg of the cassava bagasse with KBr in a ratio of 1:5 (Cassava bagasse: KBr).

Enzymatic hydrolysis: Was made the enzymatic hydrolysis by using the commercial enzymes Licquozyme Supra 2.2X from Novozymes (α-amylase, obtained from *Bacillus licheniformis*, 300 KNU/g) and CelluSEB from Enzyme Innovation (Cellulase, 8000 CMC/g). Initially, citrate buffer 0,1M (pH 5,0) and the enzyme cellulase were added, to the cassava bagasse sample, heated in a water bath at 70°C during 30 min with constant agitation. Then, was added the α-amylase enzyme and was raised the temperature to 80-90°C under constant agitation (120 rpm). After the hydrolysis process finished, was determined the concentration of reducing sugars (RA) by using the DNS method (Miller, 1959). The percentage of saccharification was calculated by Eq. (4); this value is an indicator of hydrolysis conversion (Salcedo *et al*., 2011).

$$\%\text{Saccharification} = \frac{RA\ (\frac{g}{l})\times 0{,}9\times 100}{Conc.substratum\ (\frac{g}{l})} \qquad (4)$$

Experimental design: To evaluate the effect of enzymatic hydrolysis on cassava bagasse was applied a Completely Randomized Design (CRD) with a single categorical factor. The Solid: Liquid ratio (Bagasse: Buffer) was the factor evaluated and a control treatment corresponding to cassava bagasse without enzymatic treatment. In Table 1 is shown the factorial arrangement used.

Statistic analysis: Data collected were analyzed by Statgraphics® Centurion XVI statistical program, an Analysis of Variance (ANOVA) was applied, considering the factorial arrangement of the treatments and a Tukey Test was performed to compare the means of the treatments.

RESULTS AND DISCUSSION

Table 2 shows the physicochemical properties of cassava bagasse. The protein content (1,78%) and fiber (22,63%) are between the range reported by other authors, 0,13-3,5% and 10-58,19%, respectively; the ash value (2,27%) was below to those obtained by other authors who reported values between 7,0 and 11,9%; the lipids value (1,69%) is higher than the estimated in other studies, between 0,54% and 0,12% (Paternina *et al*., 2016; Ray *et al*., 2008; Vandenberghe *et al*., 1998; Sriroth *et al*., 2000; Chaikaew *et al*., 2012; Tumwesigye *et al*., 2016; Zhang *et al*., 2016). The carbohydrate value was 61,19%, which is similar to that reported by Vandenberghe *et al*. (1998). The values of hemicellulose and lignin, 5,36% and 4,53%, respectively, are similar to those reported by Chaikaew *et al*. (2012), who obtained values of 5,1% and 4,6% for these components, respectively. The percentage of cellulose found (35,57%) is higher than the obtained in other studies, between 4.11 and 17.4% (Chaikaew *et al*., 2012; Rattanachomsri *et al*., 2009; Virunanon *et al*., 2013). Differences in the values of physicochemical properties may be due to the fact that the nutritional value of the root can be affected by the use of different cassava varieties, soil conditions and climate, fertilization (Gil and Buitrago, 2002; Pandey *et al*., 2000); on the other hand, the chemical composition of the cassava bagasse could also be affected by the starch extraction technology applied in these researchers (Rattanachomsri *et al*., 2009; Paternina *et al*., 2016).

Table 1: Factor arrangement employed in the enzymatic hydrolysis on cassava bagasse

Treatment	Solid: Liquid ratio
T1	1:10
T2	1:15
T3	1:20

Solid: Cassava bagasse; Liquid: Citrate buffer 0.1M (pH 5.0)

Table 2: Physicochemical properties of cassava bagasse

Composition	Content (%)
Moisture	10.44±0.02
Protein	1.78±0.05
Fiber	22.63±0.34
Ash	2.27±0.04
Lipids	1.69±0.08
Carbohydrates (*)	61.19
Cellulose	35.57
Hemicellulose	5.36
Lignin	4.53

(*) It was calculated by difference

Table 3: Starch and amylose content of cassava bagasse

Composition	Content (%)
Starch	50.55±0.32
Amylose	7.70±0.06

Table 3 is shown the starch and amylose content in cassava bagasse. The residual starch content of 50,55% is similar to that reported by Chaikaew *et al*. (2012), but is lower than the obtained in other studies, which range was between 60,1 and 75,1% (Rattanachomsri *et al*., 2009; Sriroth *et al*., 2000; Virunanon *et al*., 2013). Because of the high starch content, cassava bagasse can be used in biotransformation processes with edible fungus cultures (Pandey *et al*., 2000). The amylose content (7,70%) is lower than the reported in cassava starch which may be between 20,93 and 22,61%; this value depends on the age and variety of the plant (Tan *et al*., 2017).

The values of fiber, ash, lipids, amylose and starch, shown in Table 4, had significant differences ($p<0.05$) between treatments, also between the three enzymatic treatments and the cassava bagasse (control). The

Table 4: Effect of the enzymatic treatments on the chemical composition of the cassava bagasse (means±SD)

	Composition (%)					
Treatments	Protein	Fiber	Ash	Lipids	Amylose	Starch
Cassava bagasse (control)	1.78±0.05 a	22.63±0.34 a	2.27±0.04 a	1.69±0.08 a	7.70±0.06 a	50.55±0.32 a
T1	1.31±0.12 ab	18.50±0.55 b	31.21±0.40 b	0.94±0.21 b	1.38±0.04 b	32.7±1.71 b
T2	1.26±0.18 b	17.36±0.02 b	31.4±0.15 bc	0.33±0.14 bc	1.385±0.04 b	35.33±0.55 bc
T3	1.00±0.05 b	14.73±1.00 c	32.57±0.50 c	0.44±0.01 c	1.32±0.00 b	38.54±1.81 c

Values followed by a different letter, within a column, are significantly different ($p<0.05$) according to the Tukey test.

Table 5: Percentages of saccharification in each treatment

Treatment	Saccharification (%)±SD
T1	21.50±0.44 a
T2	29.98±0.95 b
T3	21.79±0.24 a

Values followed by a different letter, within a column, are significantly different ($p<0.05$) according to the Tukey test.

augment of ash after the three enzymatic treatments could have been due to the addition of the citrate buffer, which was prepared with sodium citrate. Protein value had significant differences ($p<0.05$) between control and treatments 2 and 3. Furthermore, the means of the three treatments are lower than the control; these results are not similar to that obtained by Jasko *et al*. (2011) who reported increments in the percentage of protein after performing the enzymatic hydrolysis of cassava bagasse. The means of fiber and lipids for the control are higher than the means values obtained for the three treatment evaluated, which differs with the results obtained by Jasko *et al*. (2011), where the fiber increased after enzymatic hydrolysis. The percentage of starch decreased in the enzymatic treatments due to the possible action of α-amylase. Since amylose is part of the crystalline fragment in the starch molecule (Lourdin *et al*., 2015), the decrease in its value may indicate a reduction in the crystallinity of the residual starch present in the bagasse (Van Soest and Essers, 1997).

Table 5 shows the percentages of saccharification after each treatment evaluated. The ANOVA indicates that there are significant differences ($p<0.05$) between treatments and the Tukey test showed that there are significant differences between treatment 2 and treatments 1 and 3. The mean of treatment 2 (29,98%) is greater than in the treatments 1 and 3, which may indicate that when using a solid: liquid ratio of 1:15, there is a high conversion to reducing sugars. Chaikaew *et al*. (2012) obtained a 20% higher saccharification percentage when using an enzymatic cocktail (cellulases and α-amylase) than when using individual enzymes in the hydrolysis of cassava pulp. In their study, Jasko *et al*. (2011) reported soluble reducing sugars percentages of 14,5% and 7,7%, obtained with an enzymatic hydrolysis of cassava bagasse using cocktails of α-amylase plus amyloglucosidase and α-amylase, amyloglucosidase plus cellulase. The enzymatic hydrolysis of cassava bagasse is different from that of pure starch, since there are non-starch polysaccharides such as cellulose, hemicellulose and lignin, also the starch granules are trapped in this structure; therefore, to achieve a good degradation of this material is needed the action of both cellulolytic and amylolytic enzymes (Chaikaew *et al*., 2012). Alvira *et al*. (2010) mentioned in his study that not only the effectiveness of the enzymes affect the development of the enzymatic hydrolysis, but also factors that influence the enzymatic hydrolysis such as the physical, chemical and morphological characteristics of the lignocellulosic materials; likewise the access of the enzyme to the cellulose can be affected by the cellulose crystallinity, the content and distribution of lignin and hemicellulose and the available surface area.

Figure 1 shows the Fourier transform infrared spectroscopy of the cassava bagasse, in which some bands of vibration characteristic of cellulose are highlighted: C-H_2 (1422 cm^{-1}), CH (1374 cm^{-1}), CO (1021 cm^{-1}), C-O-C (1158 cm^{-1}) (Contreras *et al*., 2010). The vibration band between 3500 and 3200 cm^{-1} refers to the characteristic stretching of OH in cellulose (Mandal and Chakrabarty, 2011; Široký *et al*., 2010). The band 1735 cm^{-1} is assigned to the C-O stretch of the carboxyl group in the hemicellulose (Parida *et al*., 2015). The band around 1420-1430 cm^{-1} is related to the amount of the crystal structure of the cellulose (Åkerholm *et al*., 2004). Similarly, Oh *et al*. (2005) indicated that the band 1375 cm^{-1} is especially sensitive to the state of the crystalline and amorphous regions of cellulose. Smits *et al*. (1998) indicated that the 1047 cm^{-1} vibration band is related to the crystalline (amylose) part of the starch, while the 1022 cm^{-1} vibration band has to do with amorphous (amylopectin) zones.

Figure 2 shows Fourier transform infrared spectroscopies of the cassava bagasse (control) and the three enzymatic treatments evaluated. The lack of the vibration bands 1422 and 1374 cm^{-1} in the three enzymatic treatments are related to the crystalline structure of the cellulose. The vibration bands 1374 cm^{-1} (CH bond) and 2927 cm^{-1} (deformation of the CH and CH_2 bonds) are used to determine the degree of crystallinity (Bertocchi *et al*., 1997), therefore as reported by Carreño Pineda (2011) non-detection of these bands (Fig. 2) may indicate a decrease in crystallinity in enzymatically treated bran. Crystallinity is one of the limiting factors in the enzymatic hydrolysis of cellulose because it offers resistance to the action of the enzyme; therefore, the decrease of the crystallinity indicates a better accessibility of the cellulase to the cellulose molecules in the spaces created by the decrystallization of the cellulose, thus increasing the enzymatic reaction (Choe and Shin, 2015).

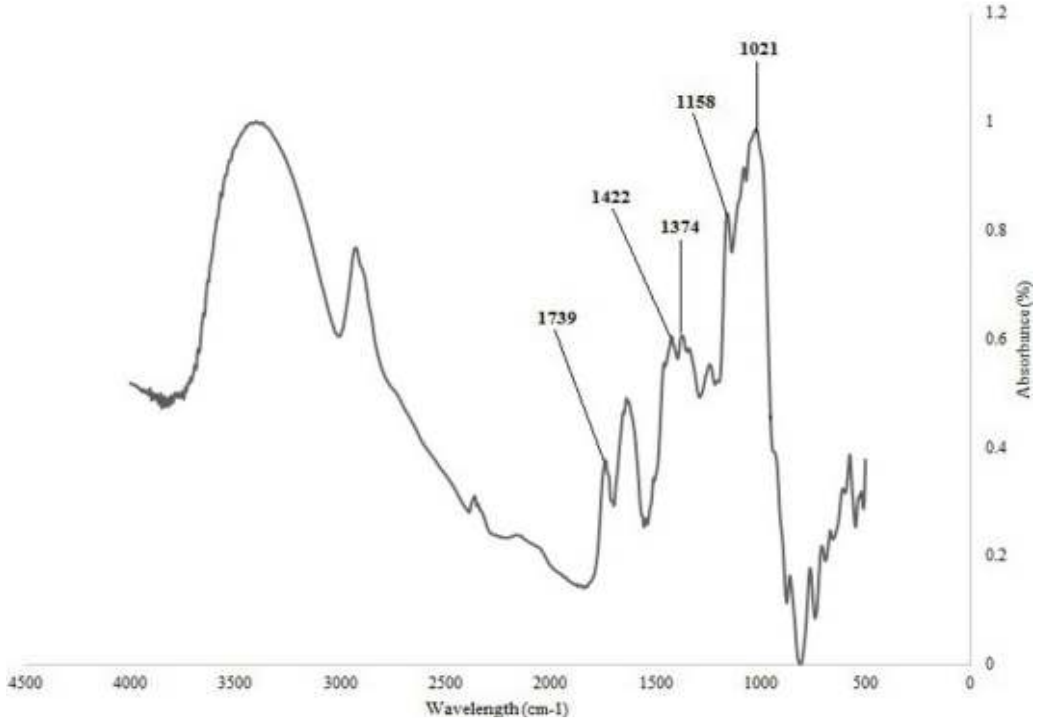

Fig. 1: FT-IR spectroscopies of cassava bagasse

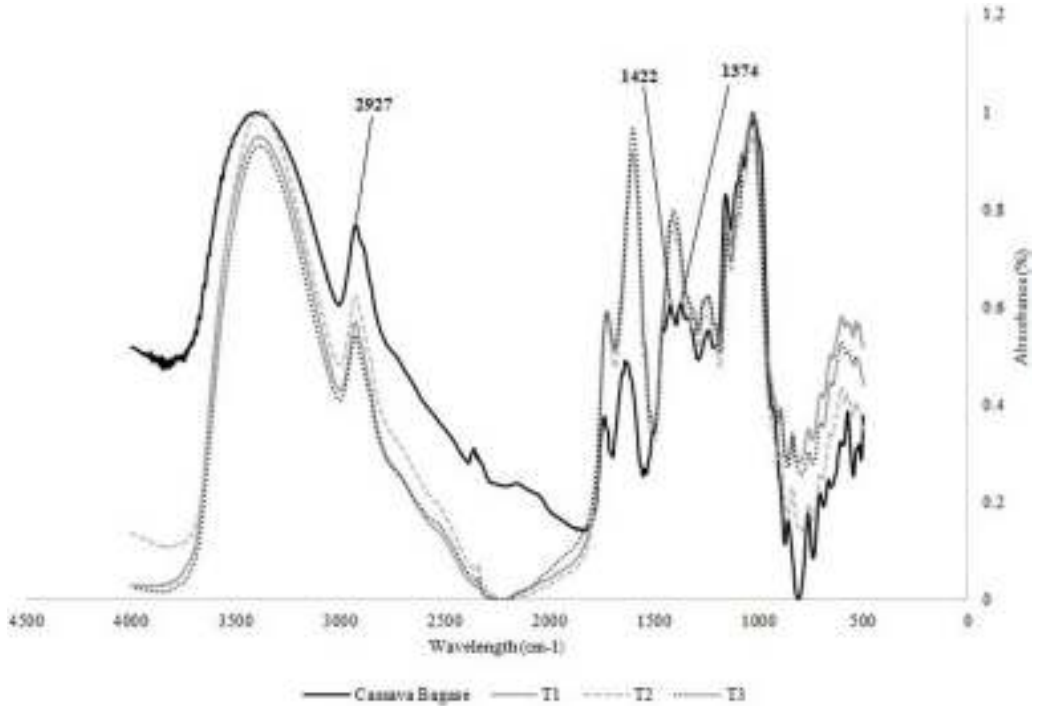

Fig. 2: FT-IR spectroscopies of cassava bagasse (control) and enzimatic tratments

CONCLUSION

Because of its high carbohydrate content, the cassava bagasse could be used is a substrate for biotech or food industry as a carbon source. Physicochemical analyses of the hydrolyzed cassava bagasse showed that enzymatic hydrolysis produces significant changes in the chemical composition of this material. The enzymatic treatment using a solid: liquid ratio of 1:15 shows the highest conversion to reducing sugars with a

saccharification percentage of 29,98%. Fourier transform infrared spectroscopy of the cassava bagasse showed cellulosic binding vibration bands and the non-detection of the 1374 cm^{-1} band together with the decrease in the intensity of the 2927 cm^{-1} band, which indicates a decrease in crystallinity associated with the applied enzymatic treatment.

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CONFLICT OF INTEREST

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