

Modification and Chemical Characterization of Barley Starch

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Abstract

Acetylation is a chemical modification for esterification, in which hydroxyl groups are replaced with acetyl groups that offers him major stability, changing the physicochemical and functional properties of the starches. Acetylated starches presents 6-10 ° C less at the gelatinization temperature and the maximum peak viscosity is greater than that of a native starch, so are more readily dispersed. The acetylation also increases the clarity and stability of the gels and reduces the retrogradation. Isolate the barley starch and modify his propierties by means of chemical agents: anhydride acetic (AA) and vinyl acetate (VA) and characterize them, have been the aim of this work. With regard to the content of present starch in the grain (60%) the obtained yield was 50 %. The percentage of acetyls reached was between 3 and 6%; with the use of the AA, was 1.6 times times higher than VA and were obtained major incorporation of acetyl groups; the degree of substitution was of 0.143 and 0.240, acording VA or AA was used, respectively. A difference was observed in the average diameter of particle, for the native starch (NS) was of 19µm, while for the starches treated with VA and AA was 22µm and 104µm respectively, due to the introduction of acetyl groups. The morphology of the granules is not affected by the acetylation process. FTIR analysis confirmed the degree of acetylation of starch.

Key words: *acetylation, barley starch, anhydride acetic, native starch*

1.0 Introduction

The acetylated starches have different characteristics to the natives, presents 6-10 °C less in the gelatinization temperature (Tg) and the peak of maximum viscosity is major with regard to the natives, which indicates that the acetylated starches be disperse easier than the natives. The acetylation also increases the clarity and stability of the gels and reduces the retrogradation. The reactions that happen during the acetylation are illustrated in the figure 1; in the principal reaction, the starch is acetylated with AA and with an alcali as catalyst.

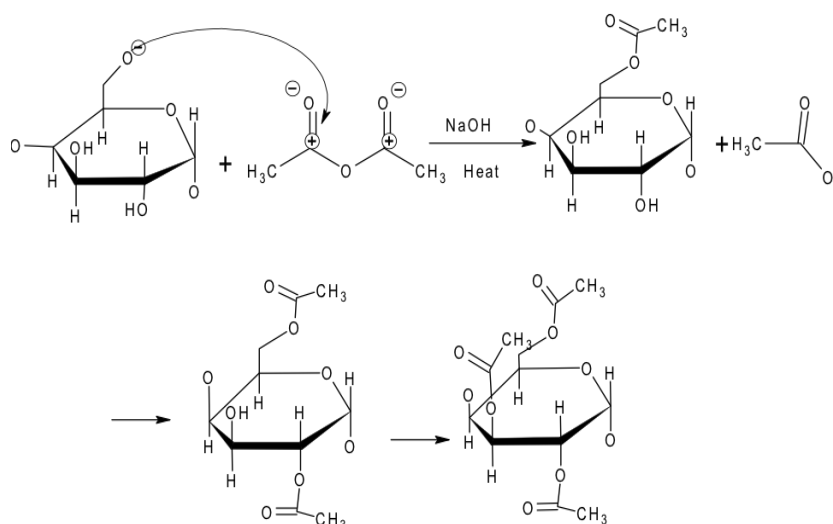


Figure 1. Chemical reaction during the acetylation (Della, 2007)

This reaction is an example of a nucleophilic substitution in an unsaturated carbon of the AA (Della, 2007). In addition three different hydroxyl groups have different reactivity. The first -OH in the carbon 6 is more reagent and the acetylation is carried out easier than in the carbons 2 and 3. Of the secondary -OH groups, the group -OH in the C2 is more reagent than that of the C3 (Xu *et al.*, 2004); nevertheless, Heins and collaborators (Heins *et al.*, 1998) demonstrated by means of nuclear magnetic resonance (NMR) that on having done the analysis of the starches acetylated the position is found mainly acetylated C3.

The acetylation of the starches depends on certain factors, as the source of obtaining of the starch, concentration of the reagent, time of reaction and pH. Several researchers mention that the acetylation reaction is reversible, proving to be a deacetylation in the starch (de Graaf *et al.*, 1995; Xu *et al.*, 2004).

2.0 Materials and methods

The method described by Huang and collaborators (2005), was used to modify by acetylation the barley starch. The starch was dispersed in distilled water (45mL) and waved during an hour to 25°C. NaOH solution to 3 % was used to fit the pH to 8.0. The AA (11.6mL) was added drop to drop to the mixture, while the pH was kept in an interval of 8.0-8.3 with the NaOH to 3 %. After adding the reagent the reaction was kept for 10min. The mixture was exact to pH 4.5 with HCl 0.5M, later it was washed two times by distilled water and once by ethylic alcohol to 96 %. It dried off for 24hrs to 40°C. The percentage of acetylation and the substitution degree (SD) were calculated.

The acetylated starch (1g) was placed in a 250mL flask, they added 50mL of ethanol 75 %, was stirred and homogenized and was warmed in a water bath to 50°C for 30min., after this time it was left to cool to temperature environment and they added 40mL of KOH (0.5M). Was titrated by HCl 0.5M using phenolphthalein as indicator. The same procedure was realized in NS to use it as reference. Was analyzed by laser diffraction size and particle distribution. It was held in Mastersizer 2000 (Malvern Instrument, USA) considering a refractive index of 1.494 and accuracy of $\pm 1\%$, according to rules of Malvern, the air was used as a means of transport.

The infrared spectra (FTIR) of the NS and acetylated were obtained by method of Pushpamalar's and collaborators (2006). The dry sample was mixed with KBr in relation starch/KBr of 4:1. The mixture was pressed to obtain a tablet and was analyzed in a spectrometer Nicolet Magna-IRTM 550; the spectrum was analyzed in an interval of resolution of 400-4000 cm^{-1} and 16 sweeps were realized.

The starch samples were dried in an oven at a temperature of 35 °C \pm 3 °C for two days, is placed on a conductive strip of double adhesion, which is fixed on an aluminum support; were covered with a thin film gold, on a computer for electrodeposition (Toothy marks, model Vaccum Desk II). The samples were observed in an scanning electronic microscope (SEM), JOEL, model JSM-G-300 to a voltage of 20 KV, 18 mm. The micrographics were taken to extents of 2000x, 1500x, 1000x, 500x and 100x. There was placed a minimum quantity of sample of starch in the microscope slide, there was added a drop of distilled water and put on a coverslip.

The samples were observed with a polarized light microscope Nikon EFD-3 connected to a video camera mark Dage, model MTI DC - 330 using a program About Meta Morph (AMM) version 6.1r0, with a lens of 10x. The starch granules were random selected and the birefringence phenomenon was observed by means of the formation of the malt cross in the center of the starch granule. Using the same microscope the sample was observed in clear field by an lens of 10x. The obtained images were analyzed using the program Sigma Scan Pro, version 5.0.0.

To evaluate the gelatinization temperature (T_g) of starches were taken 2.2 mg dry weight of sample in an aluminum crucible and added deionized water 7.5 μ L. The crucible was sealed hermetically and was left to balance for approximately 30 minutes before realizing the analysis.

Since reference was used an empties aluminum crucible . Passed the time of wetting (1 hour) the sample was submitted to a program of warming in an interval of temperature of 10 to 120°C and one speed of 10°C/min. The temperature of beginning (T_b), gelatinization temperature or of peak (T_g or T_p), final temperature (T_f) and the enthalpy (ΔH) were obtained from the thermograms directly of the analysis of the software TA Instruments OS/2 version 2.1. For the determination of retrogradation, the aluminum crucibles were stored in refrigeration for seven days.

3.0 Results and discussion

The percentage of acetyls that was found for barley starch, in dependence of the agent used (VA or AA) are shown in the table 1.

Table 1. Percentage of acetyls of the starches modified with different agents and degree of substitution (DS)

	Percentage of acetyls	
	VA	AA
Barley Starch	3.66 \pm 0.07	6.00 \pm 0.11
	Degree of Substitution (DS)	
	0.143 \pm 0.008	0.240 \pm 0.008
	VA (Vinil Acetate)	AA (Acetic Anhydride)

It is possible to observe how it is reached between 3 and 6 % of acetylation. With the use of the AA were obtained 1.6 times major incorporation of acetyl groups. These results guard direct relation with the degree of substitution (DS) that is equivalent to the average number of -OH groups that were replaced by $\text{CH}_3\text{-C=O}$ groups in the molecule of barley starch in this case. The level of substitution of the hydroxyl groups along the chain of starch, are expressed as DS. In table 1 show themselves the results obtained during the process acetylation of the NS of barley. Is possible to observe that the DS of the starch obtained after the treatment with 11.4mL of VA and 11.6mL of AA, in equal conditions, is greater for the starch treated with AA, this owes to AA that is a very reagent agent.

The substitution was carried out in the carbons, 2, 3 and 6, because sterically are more unprotected along the chain of amylase, with what the being a linear chain these groups -OH they can be replaced easier. The DS also is limited by the availability of the -OH groups inside the chain of starch, provided that the reaction always is going to begin for the carbon that is freer to react. In studies realized by Huang and collaborators (2005), in starches extracted of leguminous, the DS was higher for the starches treated with VA.

The degree of substitution in the starches of peas with AA was of 0.066, for those that one used VA it was of 0.070, for the starches of chick-pea the DS it was of 0.059 with AA and 0.064 with VA and in the starches extracted from the bean it was of 0.057 with AA and 0.068 with VA.

Lawal (2004) obtained lower values of DS, in acetylated starches with AA at different concentrations, (6% and 8%) for starch from oats. Was obtained a DS of 0.05 and 0.11, respectively. Treating the oat starch with 10 g and 15 g of AA, the DS obtained was 0.03 and 0.04 respectively.

Likewise Mormoghtadaide and collaborators (2009), with concentrations used of AA in 27.77mL and 37.88mL for the oats starch, they determined a DS of 0.05 and 0.11 respectively. The variations in the DS can owe to the utilization of different sources of starch, different contents of amylase and amilopectyn, size and morphology of the granule and the manner in which the acetylation reaction is carried out, as well as in the variation of different parameters such as the time of reaction and temperature.

Low DS obtained in these starches are them suitable for his utilization in the food industry, since the Food and Agriculture Organization (FAO) allows the utilization of acetylated starches with DS in low values. Likewise the Food and Drugs Administration of the United States (FDA) allows in food, starches with SD low. These must be values less than 2.5% of acetyl groups in starch for use in industry (Riçón *et al.*, 2007).

In figure 2 it is possible to estimate the average of the size of particles of the NS and modified by acetylation. It is possible to observe a difference in the average diameter of particle between the three starches, the size for the NS was of 19 μ m, whereas for the starches treated with VA and AA was of 22 μ m and 104 μ m respectively. The increase in the size is due to the introduction of the acetyl groups to the chains of starch.

Long and branched molecules of amilopectyn in a regular orientation between amorphous and crystalline regions give rise to the birefringence characteristics of the granules as some authors report (Fox and Cameron, 2002). These amorphous regions are contained in the molecules of amylase fundamentally. This makes possible that when the starch granule absorbs water, the amorphous areas are remain more available and therefore these can move freer inside the granule.

The conditions of gelatinization that were determined to different temperatures and in correspondence with the size and distribution of NS starch barley granules, (variety Emerald) with rind (SER) or pearly (SEP), show themselves in the Table 3. To the initial temperatures of 20°C and up to 35°C, no was observed variation either in the average size of the granules of the NS nor barley either significant difference between samples of SEP and SER. This indicates that at least even 35°C it does not produced swelling in the granules.

Starches are semicrystalline materials, and his crystallinity depends on the structural characteristics of the amilopectyn and the reassociation of the amylase (Della, 2007). X-rays diffraction (DRX), is a technology that is in use for the study of the crystalline nature of the starches. In figure 3 it proves to be the boss of DRX corresponding to the NS and acetylated.

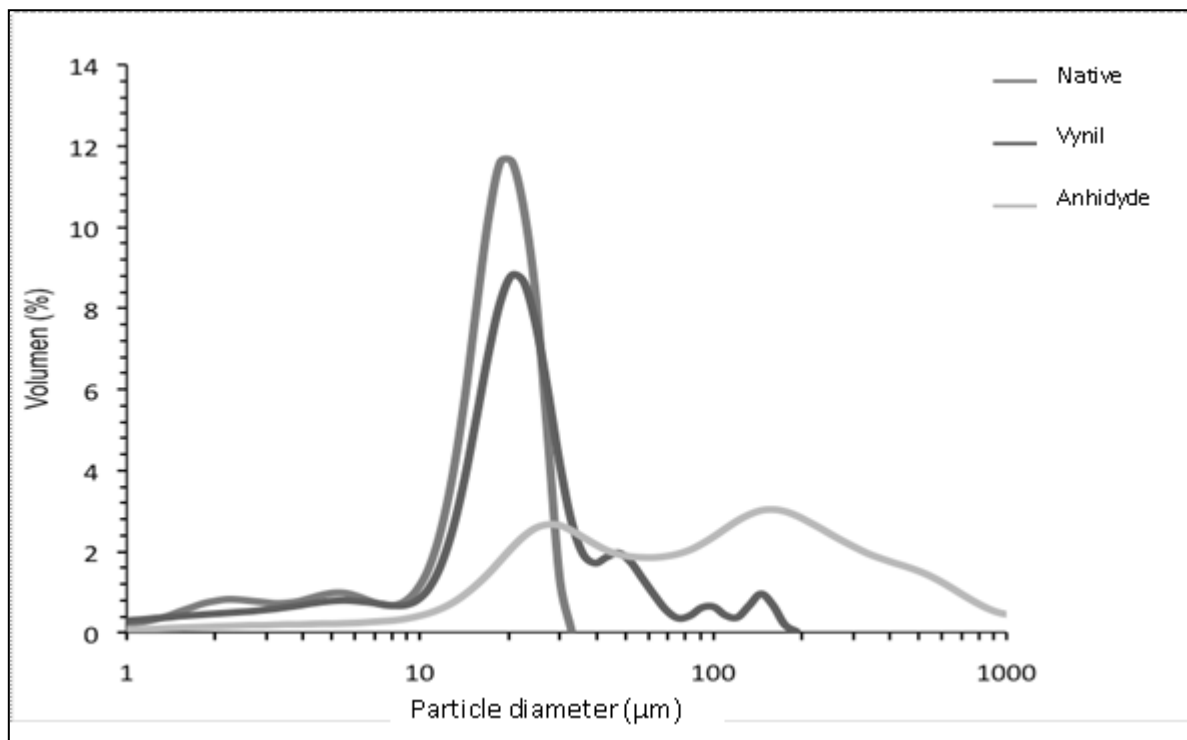
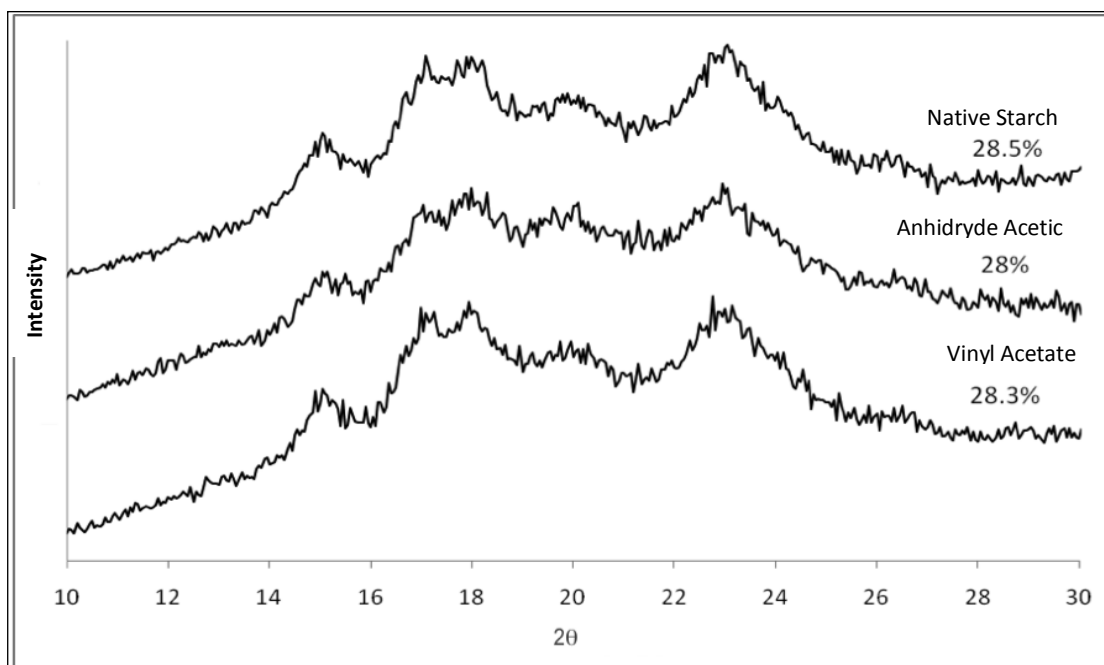


Figure 2. Distribution of the size of particle of the native and modified starches

Table 3. Size of starch granules (μm) for types (%SRD)

Temperature ($^{\circ}\text{C}$)	SER	SEP
20	19.76 (0.77)	19.54 (0.53)
35	19.76 (0.77)	19.54 (0.31)
45	30.45 (1.05)	25.12 (0.79)
55	49.56 (2.04)	30.15 (1.68)
65	88.60 (2.76)	36.65 (2.07)
75	96.35 (3.21)	40.25 (2.16)
85	94.60 (5.12)	34.56 (1.88)
95	42.60 (2.22)	28.70 (0.93)
100	39.20 (1.85)	27.00 (0.68)
105	37.60 (1.66)	26.50 (0.64)

SER, starch barley granules, (variety Emerald) with rind; SEP, starch barley granules, (variety Emerald) pearly



Figures 3. Patron of X-rays diffraction of praise starches of barley native and modified for acetylation.

It is possible to observe that the peaks of crystallinity of the native starch are more great and are better defined in comparison with the starch treated with VA, presenting a crystallinity of 28.5%. On the other hand the starch modified with AA diminishes his peaks of crystallinity and these are of 28%, this loss of the crystallinity is owed to the modification that was carried out in the crystalline region of the starch. Due to the fact that the modification is carried out with major facility in the amorphous region (composed by the amylase), and that does have a high degree of substitution, his crystalline region is not damaged, as it is observed in the boss of diffraction of the acetylated starch with VA.

This also can be observed in figure 4 of microscopy with normal light (a) and polarized light (b), where is observed that the starch loses neither his cross of Malta nor his birefringence, opposite of what happened with the starch treated with AA, since it increased his DS, the substitution being carried out in the crystalline region of the granule. In figure 5 it appears as the granule loses his crystallinity and the malt cross not is observed.

Della (2007), observed that the crystallinity in the starches of banana acetylated is diminishing as the time of the reaction is increasing. Other studies realized in acetylated maize starches, show that these, with DS low, have crystallinity profiles, similar that native starches (NS). Nevertheless when it increases the DS the crystalline structure of the starch is destroyed (Xu *et al.*, 20004). The thermal properties of the native and modified starches, were evaluated by means of DSC. Peak temperature (T_p) or of gelatinization and enthalpy (ΔH) of the NS and acetylated, is presented in table 4.

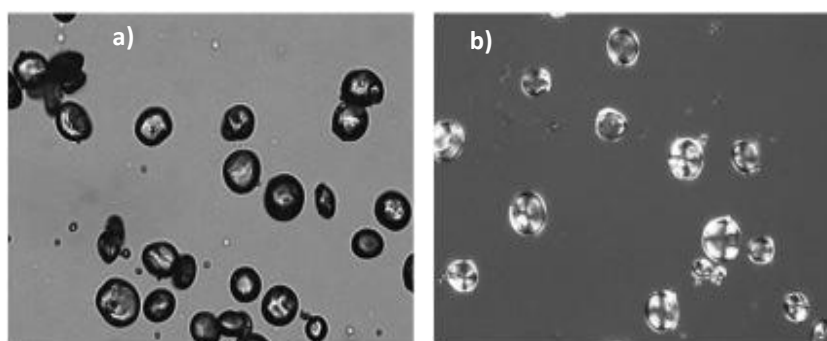


Figure 4. Micrography of the starch modified with VA a) Normal Light, b) Polarized Light

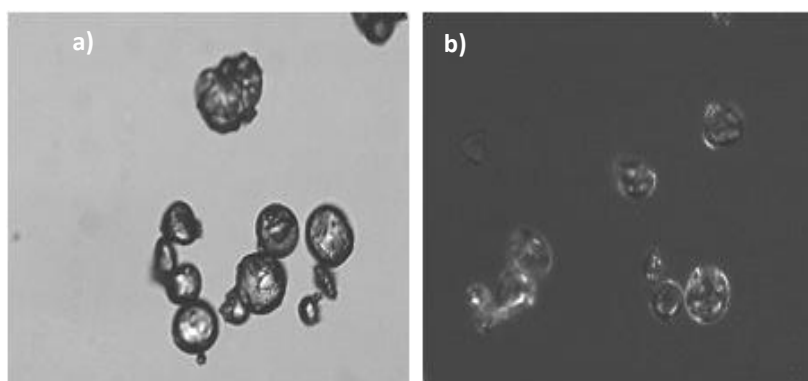


Figure 5. Micrography of the modified starch with AA a) Normal Light, b) Polarized Light

Table 4. Thermal analysis of the modified starch.

	Gelatinization			Retrogradation (7 days)			Retrogradation (14 days)		
	NS	VA	AA	NS	VA	AA	NS	VA	AA
Starch T_i	55.52±0.35	49.96±0.21	48.05±0.19	55.07±0.14	49.96±0.17	44.18±0.48	55.89±0.06	46.56±1.38	46.65±1.40
Starch T_p	59.48±0.10	54.17±0.02	52.58±0.20	59.38±0.14	54.17±0.01	49.65±1.40	59.13±0.13	51.28±1.12	51.63±0.96
Barley T_f	77.20±0.94	66.54±0.34	61.90±0.51	74.77±1.89	66.54±0.28	59.63±0.96	76.47±0.83	63.58±0.19	61.51±0.49
ΔH	7.65±0.14	4.84±0.11	2.36±0.14	8.26±0.68	4.84±0.09	2.76±0.32	7.87±0.31	5.39±0.16	2.47±0.14

NS (Native starch)

VA (Vinyl Acetate)

AA (Acetic Anhydride)

It is possible to observe that there is a decrease as for the temperatures of the starches modified between 6.4-12.9 °C with regard to the temperatures of the NS. Mirmoghtadaie and collaborators (2009) obtained a reduction in the acetylated starches of rice and waxen rice. Also it observed a decrease in T_i and T_c in starches modified of oats, this decrease owes to the weakening of the intragranulars forces and intergranular of the molecules of starch.

To seven days is observed that there is no great variation with regard to the temperatures initial and finals of gelatinization with regard to the NS and acetylated, since they are kept in the same range; this itself happens to fourteen days of retrogradation, they does not show significant variations. As for the enthalpy of gelatinization it is possible to observe a decrease in the starches derivatized, being 4.84J/g for the starch acetylated with VA and minor in the starch treated with AA of 2.36J/g.

This assumes to during the modification the granule, this suffers damage and is losing his organization, which provokes that it diminishes the quantity of energy necessary for there be realized the phase transition (Della, 2007). Lawal (2004) observed a reduction in the enthalpy of gelatinization after the second and seventh day, between the samples of starch derivatized the highest level of reduction was observed in the acetylated starch after the second and seventh day.

The crystallization of the molecules of starch took was produced during the frozen storage and the overheating of the gel in a DSC, produced an endothermic transition that was absent in the first samples.

The profiles of viscosity of the NS and acetylated, appears in figure 6, is observed that the peak of viscosity of the NS is below that of the starches treated with acetates. The starch modified with AA shows a peak of viscosity of 386 Pa.s to a temperature of 90 °C, whereas the peak of viscosity of the starch treated with VA he is in 234 Pa.s to a temperature of 82.5 °C.

This owes to itself the acetylation damages in the granules of starch. Ricón and collaborators (2007) have obtained in acetylated starches of fruit of Bread an increase in the values of viscosity to an initial temperature of gelatinization of 69.3 °C. Also they observed an increase in the peaks of viscosity of starches waxen of rice modified by acetylation to minor temperatures that the NS.

The NS showed bands for FTIR that correspond to the elongation (stretching) of the principal groups typical of the molecules of starch (figure 7) what coincides with what indicate some authors (Xu *et al.*, 2007; Mano *et al.*, 2003; Guerra *et al.*, 2008). Of equal way, the acetylated samples, they presented also bands in the interval of 900-1280 cm^{-1} , that corresponds to the stretching of the link C-O (figures 8 and 9).

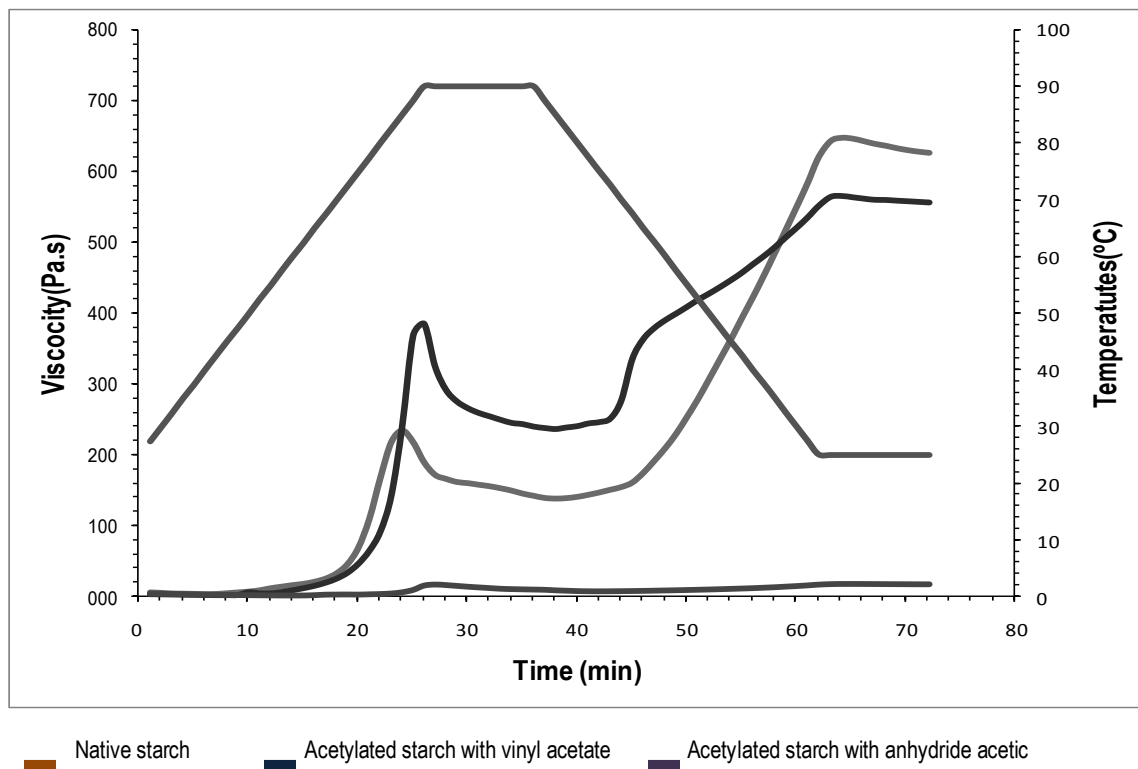


Figure 6. Profile of viscosity of the modified starches

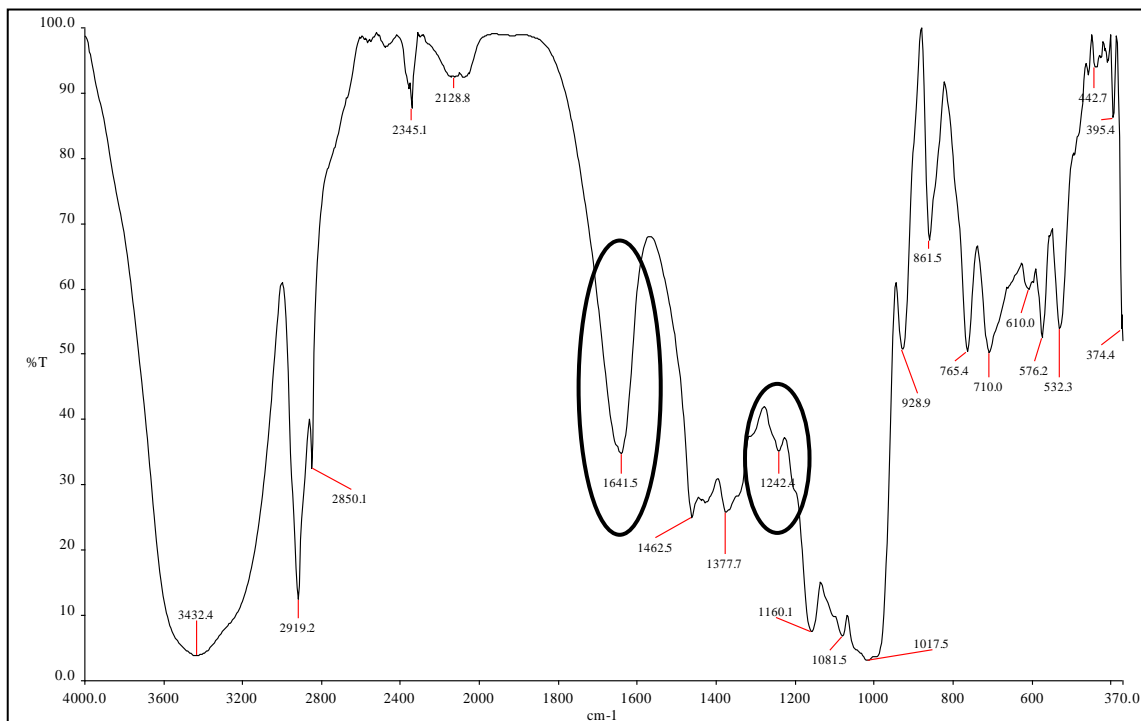


Figure 7. Analysis for FTIR of the NS of barley

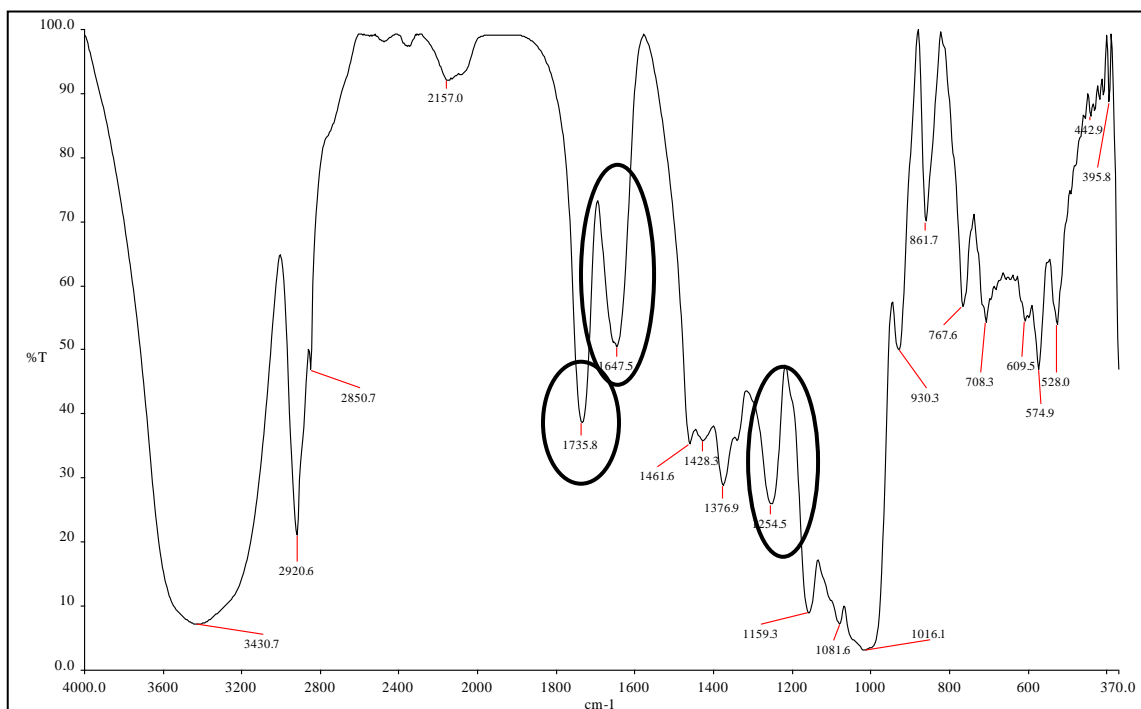


Figure 8. Analysis for FTIR of the AN of barley acetylated with VA

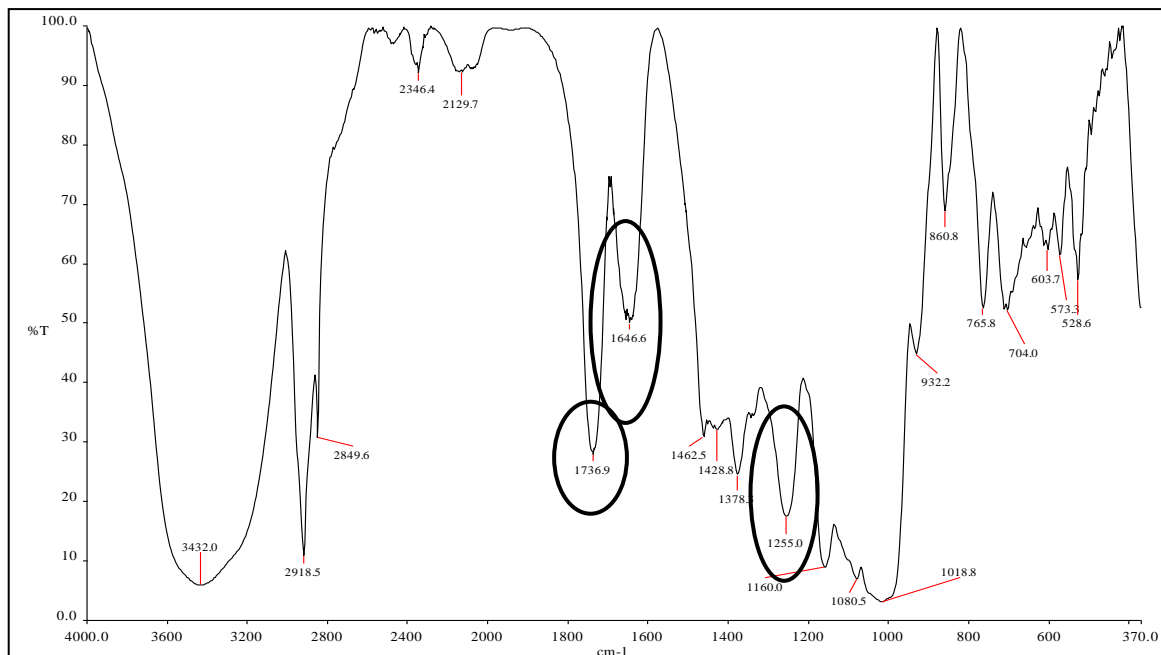


Figure 9. Analysis for FTIR of the AN of barley acetylated with AA

It is possible to observe that the sign that appears approximately to 1255 cm^{-1} corresponds specifically to the stretching of the link C-O of the acetyl group which have been confirmed and discussed by some authors (Mano *et al.*, 2003). This sign was major when the reaction was carried out by AA (figure 8) due to the increase in the DS; this allows to corroborate the major reactivity of the AA in comparison with VA. Guerra and collaborators (2008) have reported this sign in 1226 cm^{-1} for the acetylated starch of banana; in this study, for the acetylated barley starch, it is possible to report as typical this sign that appears to 1255 cm^{-1} , lightly displaced to major numbers of waves. When the reaction takes is produced by acetylation in the molecule of starch, is observes a decrease (shortening) in the signs that they correspond to the vibrations for stretching or tension (between $3000\text{--}3900\text{ cm}^{-1}$) and to the vibrations for flexion (1650 cm^{-1}) of the groups -OH (more notable in the latter vibration). This is due to the introduction of acetyl groups in the starch, which cause a steric hindrance to the vibrations for flexion, corroborating the acetylation effected in the analyzed samples.

In addition, an increase was observed in the sign to $1730\text{--}1740\text{ cm}^{-1}$ in the barley modified starch (indicated with arrows). This sign is typical for the acetylated starch and corresponds with reports of the consulted bibliography and one indicates in the figures 8 and 9 (Della, 2007; Xu *et al.*, 2004; Guerra *et al.*, 2008). Guerra and collaborators (2008) also report this sign for the acetylated starch of banana and indicate that it becomes major, major all that is the time in which the reaction is carried out. In this work it was observed besides the fact that in the acetylated starch with AA (Figure 8) this sign is more intense, which corroborates the major reactivity of the acetic anhydride with regard to the acetate of vinyl in the process of acetylation of the starch.

4.0 Conclusions

Was possible to modify the native starches (NS) of barley by acetylation The percentage of acetyls reached was between 3 and 6%; with the use of the AA, was 1.6 times times higher than with VA and were obtained major incorporation of acetyl groups; the degree of substitution was of 0.143 and 0.240, according VA or AA was used, respectively. A difference was observed in the average diameter of particle, for the native starch (NS) was of $19\mu\text{m}$, while for the starches treated with VA and AA was $22\mu\text{m}$ and $104\mu\text{m}$ respectively, due to the introduction of acetyl groups. The X-ray diffraction allowed to observe that the percentage of crystallinity of the starch treated with VA (28.3%) is slightly lower compared that the NS (28.5%) for the modified starch AA falls to 28%; the crystallinity loss is due to the modification that was carried out in the crystalline region of the starch. The morphology of the granules is not affected by the acetylation process. FTIR analysis confirmed the degree of acetylation of starch.

References

- Della, V. D. (2007). *Efecto del nivel de acetilación en las características morfológicas y moleculares del almidón de plátano (Musa paradisiaca L)*. Tesis de maestría del Centro de Desarrollo de Productos Bióticos de Yautepec, Morelos.
- Xu Y., Miladinov V. and Hanna M. A. (2004). Synthesis and characterization of starch acetates with high substitution. *Cereal Chemistry*, 81: 735-740.
- Heins D., Kulicke W. M., Käuper P. and Thielking H. (1998). Characterization of acetyl starch by means of NMR spectroscopy and SEC/MALLS in comparison with hydroxyethyl starch. *Starch/Stärke*, 50(10): 431-437.
- de Graaf R. A., Broekroelofs G. A., Janssen L. P. B. M. and Beenackers A. A. C. M. (1995). The kinetics of the acetylation of gelatinized potato starch. *Carbohydrate Polymers*, 28: 137-144.
- Huang, J., Schols, H. A., Klaver, R., Jin, Z., Sulmann, E. and Voragen, A. G. J. (2007). Characterization of differently sized granule fraction of yellow pea, cowpea and chickpea starches after modification with acetic anhydride and vinyl acetate. *Carbohydrate polymers*, 67:11-20.
- Lawal, O. S. (2004). Succinyl and acetyl Starch derivatives of a hybrid maize. Physicochemical characteristic and retrogradation properties monitored by DSC. *Carbohydrate Reserch* 339 2673-2682.
- Mirmoghtadaie, L., Kadivar, M., Shahedi, M. (2009). Effects of cross-linking and acetylation on oat starch properties. *Food Chemistry* 116, 709-713.
- Rincón, M. A., Rached, B. L., Aragoza, L. E., Padilla, F. (2007). Efecto de la acetilación y oxidación sobre algunas propiedades del almidón de semillas de Fruto de pan (*Artocarpus altilis*). *Archivos Latinoamericanos de Nutrición*. Órgano Oficial de la Sociedad Latinoamericana de Nutrición. Vol. 57, No. 3
- Fox, B. A., Cameron, A. G. (2002). *Ciencias de los Alimentos, Nutrición y Salud*. Editorial Limusa. Grupo Noriega Editores. P.150-171.
- Mano J. F., Koniarova D. y Reis R. L. (2003). Thermal properties of thermoplastic starch/synthetic polymer blends with potential biomedical applicability. *Journal of Materials Science* 14, 127-135.
- Guerra-Dellavalle, D., Bello-Pérez, L. A., González-Soto, R. A., Solorza-Feria, J., Arámbula-Villa, G. (2008). Efecto del tiempo de reacción en la acetilación de almidón de plátano. *Revista Mexicana de Ingeniería Química*. Vol. 7(3).