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PROPERTIES AND MACROMOLECULAR STRUCTURE OF UNACETYLATED AND ACETYLATED *NATA DE COCO*

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This study aims to gain more insight into the supramolecular structure of the cellulose in *nata de coco*, and to compare its ability to be modified with the known types of cellulose. The investigations were done using IR spectroscopy and thermal analysis.

The IR spectra of microcrystalline cellulose were identical to cellulose spectra. However, those of dried *nata de coco* cellulose showed additional peaks that indicated a loss of inter- and intra-sheet hydrogen bonding, usually extensive in other cellulose forms where sheets are the prevalent secondary structures. This kind of structure would be consistent with the necessary framework for gel formation. Details in the fingerprint region further supported a different macromolecular structure from the microcrystalline cellulose. Acetylation of the fresh and pressed-and-dried *nata de coco* yielded only partial acetylation, as indicated by the C=O stretching as well as the remaining broad OH-stretch. Acetylated samples showed reduced water retention capacities, and were more prone to variable water retention characteristics during pH changes.

The DSC and TGA behavior of microcrystalline cellulose, acetylated and unacetylated forms of *nata de coco* showed differences in the macromolecular structures, as well as in stability of the material.

Keywords: *Nata de coco*, microbial cellulose, acetylated *nata de coco*, macromolecular structure

INTRODUCTION

Nata de coco is also known as microbial cellulose gel, having been formed by *Acetobacter aceti* subspecies *xylinum* in a growth medium under proper temperature, pH, and aerobic conditions. Its chemical composition is reported to be 89-93% water, 4-10% cellulose and 1-3% ash [1,2].

L. Dimaguila's thesis indicates that it is cellulosic in nature through solubility tests, cellulose test, hydrolysis by cellulase, and similarity of IR spectra [2]. *Nata de coco* cellulose, however, appears in gel form with the percentage of water several times over the usual absorption of other cellulosic material. Other types of microbial cellulose similar to our *nata* have also been reported [3].

The interest of this study is to gain more insight into the macromolecular structure of the cellulose in *nata*, and to compare its ability to be modified with the known cellulose materials.

Cellulose is a carbohydrate made of glucose units in β-1,4-linkage. It occurs in long chains which periodically alternate between crystalline and amorphous segments (Fig. 1) [4].

It usually assumes the crystalline structure of cellulose I, which is made up of parallel chains with strong intra- and intermolecular hydrogen bonding. These chains are then arranged in sheets which are staggered relative to each other, with strong intra-sheet hydrogen bonding [4-6]. It can be converted to microcrystalline cellulose through acid hydrolysis, in which the amorphous bridges are removed, leaving only the crystalline portions [7,8]. Strong acid hydrolysis is fast and leaves the microcrystalline parts undissolved [7].

EXPERIMENTAL

Materials. Raw *nata de coco* samples were bought from the Marikina market. About one-inch cubes were cut, pressed with a rolling pin, air-dried for five days, and finally stored in a vacuum dessicator for another three days.

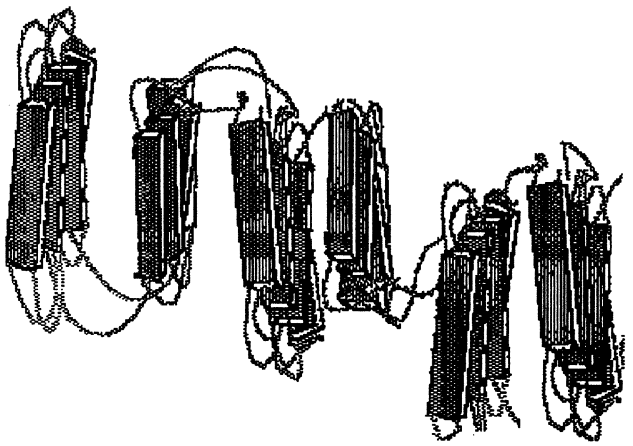

Fig. 1. Schematic picture of crystalline and amorphous segments of cellulose (adapted from O.A. Battista [4])

Instrumental Measurements. Samples of *nata de coco* were pressed and air-dried, then analyzed on the PE-283 IR spectrophotometer and on a Shimadzu FTIR spectrophotometer. Similar samples, as well as the gel forms, were run on Shimadzu DSC-50 and TGA-50, using nitrogen atmosphere (industrial grade, CIGI) on the DSC of gel samples.

Acetylation. The samples were pre-treated with glacial acetic acid (Merck, AR). For every 0.5 g dry *nata de coco*, 50 mL toluene (Univar, AR), and 50 mL acetic anhydride (Riedel de Haen, AR) and 0.015 mL perchloric acid (Merck, AR) was used as acetylating solution. For fresh (undried) samples, it was simply assumed that the cellulosic material was 10% of the total weight, so that the proportional amounts of acetylating reagents were used.

After stirring for about 60 hours or reacting in an ultrasonic bath (L&R Solid State/Ultrasonic T-14B) for about an hour, the mixture was decanted and washed repeatedly with ethanol (Merck, AR) until it was free of toluene and acetic acid.

Water Retention. The dried samples were initially weighed, then soaked in distilled water or aqueous buffer solutions with different pH. The samples were then removed from solution, drained of excess water, then weighed again. The process was repeated until the weight of the soaked samples level off.

Percent water retention was calculated according to the following equation: % water retention = $(W_{wet} - W_{dry})/W_{wet}$ x 100 where W_{wet} is the weight of the wet sample and W_{dry} is the weight of the dry sample.

The results are compared for the different pH solutions.

RESULTS

***IR Spectra of Unacetylated and Acetylated* Nata de Coco.** The IR spectrum of microcrystalline cellulose (Avicel) is identical to the spectrum of cellulose reported in literature. As expected, relatively broad peaks are characteristic of polymers with extensive hydrogen bonding which come not only from intramolecular and inter-chain H-bonds but also from intra- and inter-sheet H-bonds, arising from its secondary structure [4-6]. The extensive hydrogen bonding is evident in the increased band widths, shifts, and splitting of some frequencies (Figs. 2*a*, 2*b*) [10, 11].

In contrast, the IR spectrum of dried *nata de coco* (Figs. 2*a*, 3*a*, and 3*b*) shows a shift of the OH-stretching to lower frequencies, improved resolution between 3000 and 2700 cm^{-1} and between 1435 and 1300 cm^{-1}, additional peaks at 2856 cm^{-1} and between 1750 and 1550 cm^{-1}, and more intensive peaks in the fingerprint region relative to the OH stretching.

The peaks between 1750 and 1550 cm^{-1} vary their position, depending on batch or drying time (Fig. 3*b*). The peak at 1743 cm^{-1} eventually disappears, the peak at 1693 cm^{-1} shifts

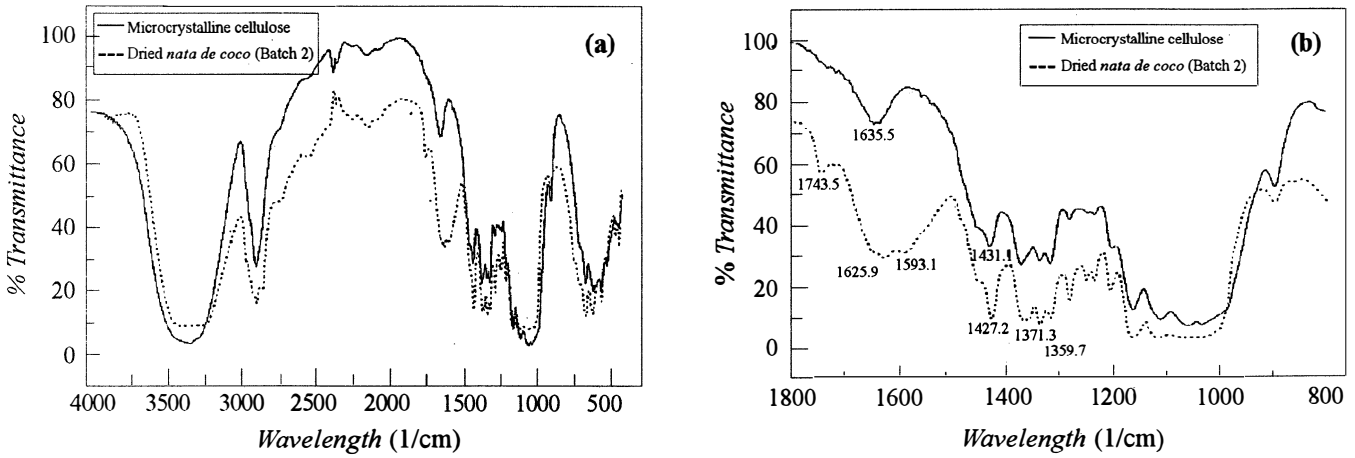

Fig. 2. IR of Microcrystalline cellulose and dried nata de coco: (a) *4000-400 cm^{-1}; and* (b) *1800-800 cm^{-1}*

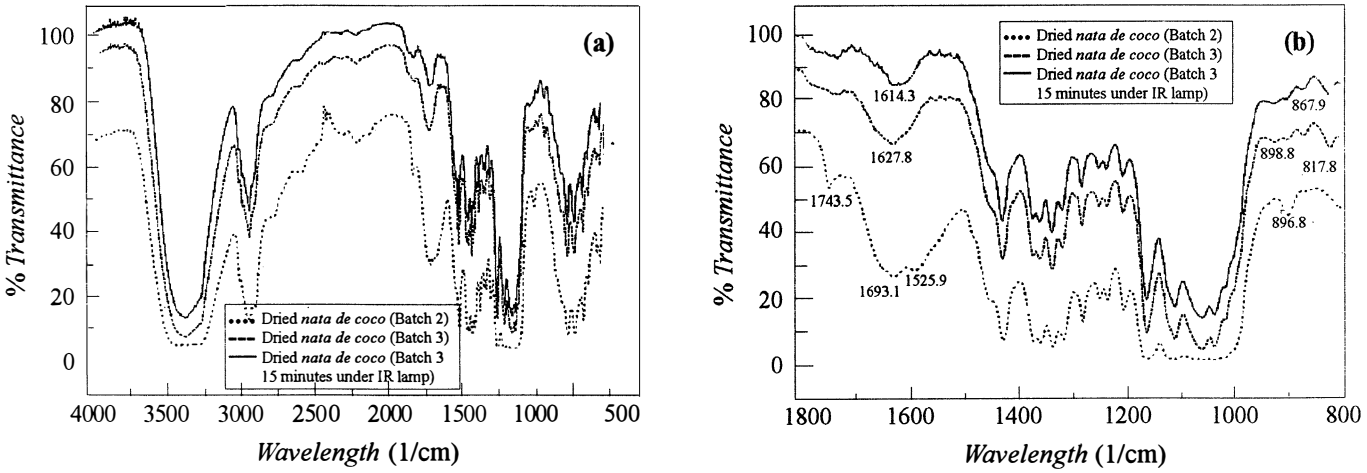

Fig. 3. IR of dried nata de coco (different batches): (a) *4000-400 cm^{-1}; and* (b) *1800-800 cm^{-1}*

to lower frequencies, and the peak at 1593 cm^{-1} shifts to higher frequencies until there seems to be just one peak at about 1614 cm^{-1}.

In addition, batch and drying time affects the 900 to 800 cm^{-1} region, with additional peaks appearing at 922, 868 and 818 cm^{-1}.

Acetylation of *nata* is possible, as indicated by the presence of the C=O stretching, but not all the way to the triacetylation, as shown by the big broad bands of OH (Fig. 4). When it is acetylated as fresh *nata*, the peak at 1652 cm^{-1} is more pronounced than the 1634 cm^{-1} peak when it is acetylated after pressing and drying the material (Figs. 5*a* and 5*b*).

Water Retention Characteristics. Water is better retained in the case of unacetylated *nata*, as Table 1 and Figure 6 show. Interestingly, the pH-variations of the acetylated form seem to be more pronounced.

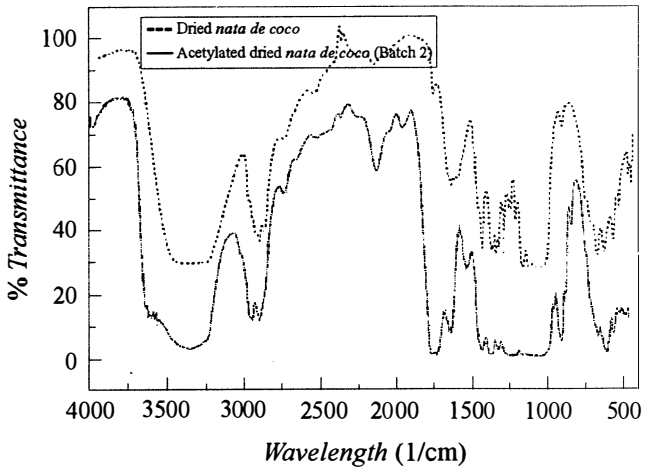

Fig. 4. IR of dried and acetylated nata de coco (4000-400 cm^{-1})

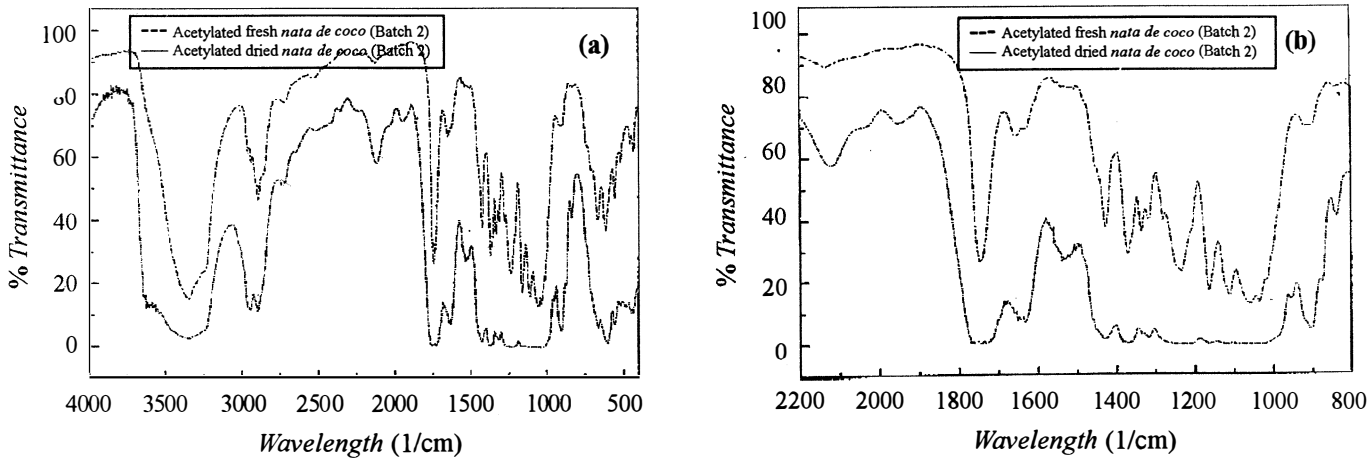

Fig. 5. IR of acetylated from fresh and pressed-and-dried nata de coco: (a) *4000-400 cm^{-1}; and* (b) *1800-800 cm^{-1}*

Table 1. Percent Water Retention of Unacetylated and Acetylated *Nata de coco.*

pH	*Nata de coco*	Acetylated *nata de coco*
2	85.7%	50.0%
5	85.7%	40.0%
7	91.3%	65.5%
9	85.7%	60.0%

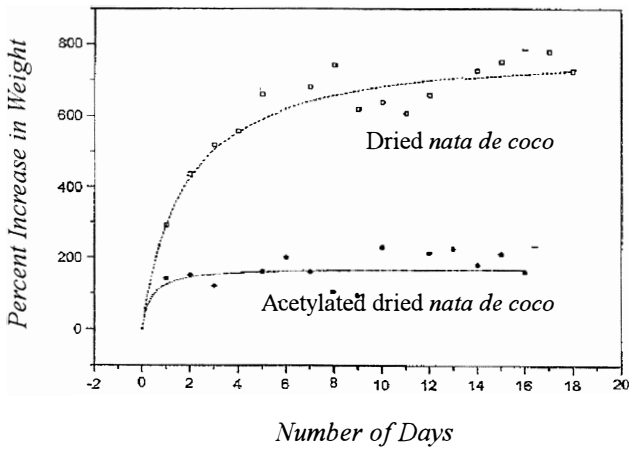

Fig. 6. Swelling rate of acetylated and unacetylated nata de coco *at pH 7*

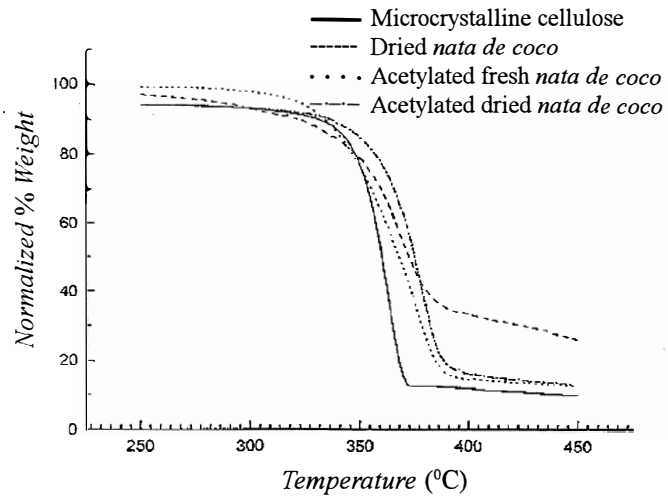

Fig. 7. TGA of microcrystalline cellulose, dried nata de coco, *acetylated fresh and acetylated pressed-and-dried* nata de coco

Thermal Analysis. TGA results show that microcrystalline cellulose degraded at a lower temperature than the rest of the samples with the midpoint falling at 360°C (Fig. 7). The *nata de coco* degraded at slightly higher temperatures (365°C) and with more residual material. The two acetylated forms are similar to each other, in their overall form, but the degradation temperature of acetylated fresh *nata* (364°C) is closer to the unacetylated one. The acetylated pressed-and-dried *nata* degrades at a slightly higher temperature of 374° C. Both do not show much residual material after degradation.

DSC analysis indicates that microcrystalline cellulose shows a transition at 67.3°C while the pressed-and-dried *nata* peaked at 62.6°C. The peaks exhibited by acetylated materials are at lower temperatures, and are less intense for approximately the same sample size (Fig. 8). Acetylated fresh *nata de coco* showes peaks at 51.2°C and at 97.2°C. The latter peak is better resolved for the acetylated fresh *nata.*

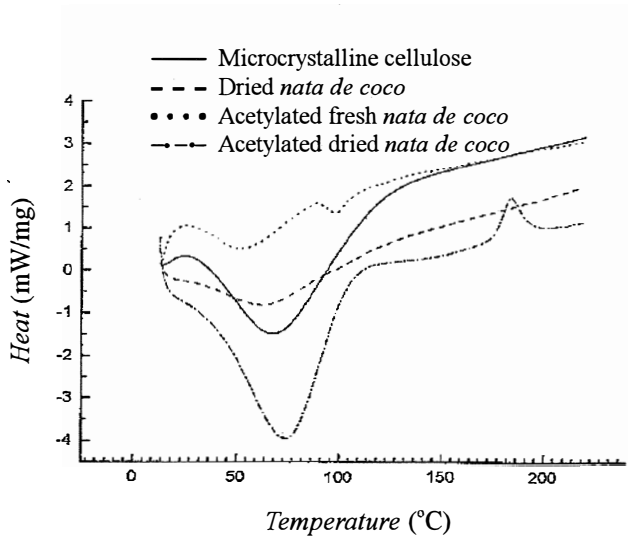

Fig. 8. DSC of microcrystalline cellulose, dried nata de coco, *acetylated fresh and acetylated pressed-and-dried* nata de coco

DISCUSSION

IR Spectra. While the overall appearance of the IR spectra of *nata de coco* looks similar to cellulose, there are variations in certain details which may be due to differences in macromolecular structure. Changes due to hydrogen bonding may be reflected in the IR spectra in several ways: H-bonding can lower the -O-H frequency (3400-3200 cm^{-1}), increase O-H deformation frequency if present (1455-1441, 1340-1310, ca 1200, 750-650 cm^{-1}), increase band widths for these modes, and give rise to shifts and splittings due to coupling between similar vibrational modes within the molecular or with other molecules [10,11]. In the spectrum for ordinary or microcrystalline cellulose, only the OH stretch is clear compared to the other broad and unresolved peaks. This is shifted to lower frequencies in the *nata de coco* spectrum, but this is also ambiguous because it may just be due to absorbed water.

A better gauge for changes in hydrogen bonding due to macromolecular structure would be in the O-C-O acetal stretching, the ring oxygen of which directly participates in the intra-molecular hydrogen bond of the sheet forms of cellulose.

The appearance of the variable 1596 cm^{-1} peak in this area, as compared to the microcrystalline cellulose may indicate a different secondary structure affecting the acetal oxygen which is expected to absorb in the region 1560-1530 cm^{-1} from calculations [11]. The acetal oxygen is normally hydrogen-bonded with the hydrogen of the OH bonded to C-3 [4]. This means that the peak may have been shifted to higher frequencies, implying less H-bonding for the acetal oxygen. With different batches, this peak is shifted further to 1525 cm^{-1} then is finally incorporated with the peak at 1614 cm^{-1}. The shifting pattern within these comparable samples indicates that inter- or intra-molecular interactions such as the hydrogen bond are involved. If these peaks truly point to a loss of hydrogen-bonding, this would mean that the sheet structure is not the same as before.

The peak in the region of 1636 cm^{-1} may be attributed to absorbed water [11]. Its shift to 1626 cm^{-1} initially, and even to 1614 cm^{-1} in another batch may indicate the different types of (bound or unbound) water in the cellulose. The shift to lower frequencies may support a more bound water with decreased bond energy.

The peak at 1430 cm^{-1} is attributed to the CH_2 bending of C-6. The shift of this peak to 1427 cm^{-1} in the *nata* spectrum indicates greater flexibility. This would be consistent with reduced H-bonding, and could be explained by the loss of the sheet structure or of the high order since the OH-group on C-6 is involved both in inter-chain and intra-sheet H-bonding [4-6].

The rest of the changes (900-817 cm^{-1}) are more difficult to explain because they are correlated with the finer features of the molecule. They would simply confirm the differences in the overall structure of the microcrystalline and microbial celluloses.

The spectra obtained after acetylation confirm the acetylation of the celluloses but not complete acetylation, as seen from the broad OH bands. The peak at 1652 cm^{-1} in the acetylated fresh *nata* is more pronounced than the 1634 cm^{-1} peak when it is acetylated after pressing and drying the material. This difference indicates that the amount of water absorbed is greater, and since it is at higher frequency, much of it may be unbound water. In the press-and-dry process, most of the unbound water would have been removed prior to acetylation and only those that are difficult to remove are left in the polymer matrix.

Water Retention. The water retention behavior confirms the acetylation of the cellulose. This behavior is expected since acetylation reduces the hydrophilicity of the material. Interestingly, the pH-variations of the acetylated form seem to be more pronounced, which may indicate a degree of hydrolysis in different media.

Thermal Analysis. The behavior of *nata de coco* cellulose compared to microcrystalline cellulose confirms differences in their macromolecular structures. The "network" for the former, which may be in helical form, as is generally observed for gels of polysaccharides, may explain the higher temperature of degradation.

The TGA of the acetylated pressed-and-dried *nata* may support improved acetylation, leading to a more pronounced difference in their overall structures, since it degrades 10° higher than the acetylated fresh *nata.*

The transitions in the 50-60° region observed in the DSC graph may partly be related to the evaporation of water or occluded solvent since the peak is very broad and the endset temperature reaches over 100°C. This is seen in some samples which have a small weight loss from 61°C to over 100°C in the TGA diagrams. However, not all the samples contain this weight loss, so that the DSC peak may also be due to internal transitions of the polymer chains, possibly related to changes in crystalline structure [5].

In the samples shown in Figure 8, the *nata de coco* which was acetylated after it had been dried thoroughly, showed the larger and deeper trough, indicating more heat in the transition, compared to the *nata de coco* which was acetylated fresh. If we relate this to IR data which shows that more -OH had been acetylated in the dried sample, we can see that drying may lead to greater internal transitions of the chains.

CONCLUSION

The IR spectra shows the cellulosic nature of *nata de coco*, with the indications that the macromolecular structure is not sheet-like and the degree of hydrogen bonding is decreased. The microbial cellulose can be acetylated similar to the other celluloses, but the triacetylation is with greater difficulty. The water retention of acetylated and unacetylated forms are due to the reduced hydrophilicity of the acetylated cellulose. The water retention behavior of the unacetylated form is relatively independent of pH, while the acetylated form has more variations with respect to pH.

TGA indicates different structures for microcrystalline cellulose and *nata de coco*, as well as for the acetylated forms. The DSC results may indicate the evaporation of water in the cellulose superstructure or the phase transitions that affect the crystallinity of the sample.

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