Biochemical studies on some cotton by products Part I- Chemical constituents and cellulose extraction of Egyptian cotton stalks

*Fouad A. Ahmed; *Nadia M. Abdel-Moein ; **Amal S. Mohamed and **Shaimaa E. Ahmed

*Agric. Biochemistry Dep., Fac. Agric., Cairo University

** Cotton Research Institute, Agricultural Research Center, Giza, Egypt

shrose22@yahoo.com

ABSTRACT: The main objectives of the current investigation are to compare some chemical constituents, mainly cell wall components (cellulose and lignin), of stalks of five Egyptian cotton cultivars, as a step to convert a low valued bio-wastes of cotton plant stalks into highly value product as pure cellulose, which will, also, contributed in solving major environmental and health problem in Egypt. Lignocellulosic raw material cultivars; Giza 80, Giza 85, Giza 89, Giza 86, and Giza 90 were used in this study. They were obtained from Cotton Research Institute experimental fields. As first stage, chemical analysis comparison among aforementioned cultivars was conducted. The results showed that, there were significant differences among the five studied cultivars in moisture, Lipids, wax, crude fibers and β cellulose contents. As coincides, ash, protein, holocellulose, hemicellulose, α cellulose and lignin percentages exhibits no significant differences among cultivars. The highest percentages of moisture estimated in Giza 89 (7.74%), also in ash and lignin (3.39% and 25.75%, respectively), but it was the lowest cultivar in wax percentage (2.43%). Giza 86 showed the highest percentage in lipids and crude fibers (1.96% and 46.92, respectively), also in protein and holocellulose percentages (5.12 and 77.26 %, respectively), but it was the lowest cultivar in β cellulose (1.11%) as well as ash (2.95 %). The highest percentage in wax and β cellulose estimate (3.67% and 2.72%, respectively) was in Giza 90, but it was the lowest cultivar in Lipids (0.96%) and hemicellulose (40.04%). The highest percentages in α cellulose (49.21%) was in Giza 80 which reflected the lowest percentage in the crude fibers (38.75%). The second stage was the preparation of cellulose by removing the waxes, lignin, and hemicellulose, since cotton stalk consists of 75±2% holocellulose percentage and 44±5% α cellulose %. The third stage was conducting physical test by analyzing the sample that was prepared by X-ray, then comparison with standard cellulose sample chart to confirm its structure as cellulose.

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INTRODUCTION

In Egypt, crop residues are by-products of common crops such as cotton, wheat, maize and rice, with total amount of about 16 million tons of dry matter per year (El Saeidy, 2004). The Egyptian cotton is a unique descender of cotton that is characterized by high quality, it gained worldwide reputation for more than century and half as being of the highest lint quality among the world cottons for textile industry, beside seeds use in oil production (Bailey, 1958 and Simpson and Ogorzaly, 2001). Cotton crop area accounts for about 5% of the cultivated area in Egypt (El Saeidy, 2004). Cotton stalks produced annually as agricultural residues reached 1.9 million tons (Mona, *et al.*, 2001). These post-harvest by-product cause many severe problems; fires causing significant environmental and health disorders, in addition, eggs and larvae of harmful pink boll worm lays inside it (Amal *et al*., 2010). The cotton stalks are rich in cellulose and close to the fibrous structure of hard wood (Metcalfe and Chalk, 1950). Commercially, this material is being used as fuel in rural areas and for raising edible mushroom crops (Balasubramanya, 1981 and Pandey and Shaikh, 1987).

The largest share in cotton plant dry weight is for stem which comprises 23.15 % (Mcbryde, 1891). Cellulose comprises the major part of all plant biomass, and the source of all cellulose is the structural tissues of plants. Cellulose often occurs in close association with hemicellulose and lignin (Farone *et al*., 1998)

Brown (1927) found that cotton stalk consist of ash 3.09%, protein 4.00%, fibers 45.31% and fats

1.11% (percentage of constituents in water-free material). It was found that, as average chemical compositions of pulping woods, waxes, holocellulose, alphacellulose and lignin percentages are 1%-5%, 70-73%, 42-44% and 27%-31%, respectively (Grant, 1958). Rizk (1970) mentioned that the cotton stem fibers contained cellulose 65.43%-69.28%, lignin ranged from 21.19% to 22.93 % , wax from 1.91% to 2.06%., and ash content percentage was ranged from 1.89% to 2.53%. Kebeasy (1988) found that, the value of lipids in methanol benzene solution is (4.24%) which, mostly, belong to the waxes, whereas petroleum ether extract reflect lower values (2.93%). The estimations on cotton stalk of ash, crude protein, crude fibers, lignin, and cellulose were 2.94%, 3.48%, 47.35%, 23.6% and 35.6%, respectively. Paralikar and Bhatawdekar (1987) found that moisture content of raw cotton stalk was 9.5%. Silverstein, *et al*. (2007) reported that, the estimated percentage of acid-insoluble lignin was (27.9%), acid-soluble lignin was (2.2%) and ash was (6.0%).

Lignin is an integral part of plant cell walls and makes up one-quarter to one-third of the dry mass of wood, along with hemicellulose, is nature's cement which exploits the strength of cellulose and confers flexibility (Parajuli, 2006). Wassel (1985) found that, for sowing date, mean values of fiber cellulose and lignin were significantly increased as the time of sowing was delaying (between 67.45%-71.63% and 24.19-28%, respectively), on the contrary wax and ash percentages were (between 1.64%-1.015%, and 2.67-1.98%, respectively). The effect of retting methods was significant on the mean values of lignin and ash percentages scoring, in stagnant water retting, 23.36% and 1.79%, respectively. In 1.2% ammonium oxalate solution at 100 ºC for 1-2 hours, the highest percentage values were scored in cellulose percent, wax and ash (75.75%, 1.998% and 2.71%, respectively). On the other hand, the lowest percentages of cellulose (67.29%), lignin (21.22%) and wax (1.015%) were found in 1.2% sodium carbonate at 100 ºC for 1-2 hrs.

Acidified sodium chlorite is an effective method to remove lignin; however, during the delignification process the hydroxyl groups and reducing end groups of cellulose can also be oxidized (Fengel and Wegener, 1984).

MATERIALS AND METHODS

1. **Plant materials:** The current investigation was carried out on the dry stalks for five Egyptian cotton (*Gossypium barbadense* L.) cultivars; Giza85, Giza86, Giza89, Giza80 and Giza90.
2. **Work procedures:** The work was conducted in the Cotton Seed Technology and Natural Products Laboratory, Cotton Chemistry Department, Cotton Research Institute (CRI), Agricultural Research Center (ARC), Giza, Egypt. Cotton stalks of all studied cultivars were collected from ARC experimental fields, after complete harvesting of the economic crop, in August 2006, then cut into splinters about 2.5-4 cm in length by hand, then samples milled in a mill, using 0.4 mm screen, and finally, the milled samples were subjected to compositional analysis to compare among the five studied cotton cultivars. Mixed sample of cultivars stalks was subjected to cellulose extraction method then preparing derivatives from it. The chemical comparison among five studied cultivars was statistically analyzed as Randomized Complete Block Design (RCBD) with one factor (cultivars) in three replicates to obtain L.S.D. (at alpha 0.05) among averages for each studied character.
3. **Chemical analysis** (was determined on dry weight basis).

The chemical composition of the cotton stalks *i.e.* moisture, ash, Lipids, protein, Lignin, crude fibers, wax, (holocellulose and hemicellulose) and (α and β cellulose) were determined according to (AOAC, 1984), (AOAC, 2000), (AOAC, 1990), (AOAC, 1955), (Tanaka *et al*., 1985), (A.O.A.C., 1970), (Dorée , 1947), (whistler *et al.*, 1948) , and (Whister and Wolform, 1963), respectively.

4. **Preparation of cellulose:** The method described by Chahal *et al.*, (1979) was used for preparation of cellulose as follows:

Cotton stalks (50 g) were separately extracted with ethanol– benzene (1:2 v/v) for 6 hours in soxhlet apparatus then dried at 45 ºC. The dried samples were heated with sodium chlorite solution [Blend 23.5 g of sodium chlorite ($NaClO_2$) in 1000 ml of water containing 5 g of glacial acetic acid] at 75-80 ºC for 5 hrs. to remove the lignin. This treatment was repeated twice and the solution filtered. After filteration the residual material was washed with distilled water until free from chloride ions, then treat with ethyl alcohol to give holocellulose (hemicellulose and cellulose). To obtain the hemicellulose and cellulose fractions the

holocellulose was treated with 500 ml. NaOH 10% (w/v) for 4 hr. at 25 ºC with occasional stirring then filtered. The residue was again treated with 250 ml. NaOH 10% (w/v) for 3 hr. at 80 – 90 ºC, filtered and washed with water, ethanol, acetone and ether then air dried. This fraction contained the cellulose. The obtained cellulose was used in the preparation of hydrocellulose, cellulose acetate, cellulose nitrate

5. X-ray diffraction: The prepared cellulose sample was exposure to x-ray treatment in Central Laboratories Sector, The Egyptian Mineral Resources Authoroty (EMRA), The Ministry of Petroleum. A Philips X-Ray Diffraction equipment model PW/1710 with monochromator, Cu-radiation (λ=1.542 Å) at 40 K.V., 35 m.A. and scanning speed 0.02º/sec. were used. The reflection peaks between 2Θ = 2º and 60º, corresponding spacing (d, Å) and relative intensities (I/Iº) were obtained. The diffraction charts and relative intensities are obtained and compared with ICDD files. Obtained diffraction chart of prepared cellulose was checked against chart of standard cellulose sample (Kennedy *et al*., 1992) treated by 25% aqueous ammonia at 10 ºC for 15 and 240 min.

RESULTS AND DISCUSSION

In a trial to solve major environmental and health problem in Egypt, chemical constituents, mainly cell wall components, of five Egyptian cotton cultivars were investigated as presented in Table (a,b) and illustrated by Fig. (1a, 1b).

I- Chemical analysis:

1. Moisture %:

Data in Table (a) illustrated by Fig. (1a) indicated that there were significant differences among studied cultivars. Estimated stalk moisture ranged between 7.21 to 7.74% corresponding to studied samples. Giza 89 showed the highest value for this trait, significantly differed than Giza 90 and Giza 85 by 5.59 and 7.35%, respectively.

Table (a): Chemical composition of the cotton stalks.

Characters / Cultivars	Moisture%	Ash%	Lipids%	Wax%	Protein%
Giza 85	7.21	3.25	1.13	3.26	2.55
Giza 86	7.62	2.95	1.96	3.38	5.13
Giza 89	7.74	3.39	1.24	2.43	3.09
Giza 80	7.61	3.04	1.14	2.57	3.10
Giza 90	7.33	2.98	0.96	3.67	4.05
L.S.D at 0.05%	0.332	N.S	0.502	0.601	N.S

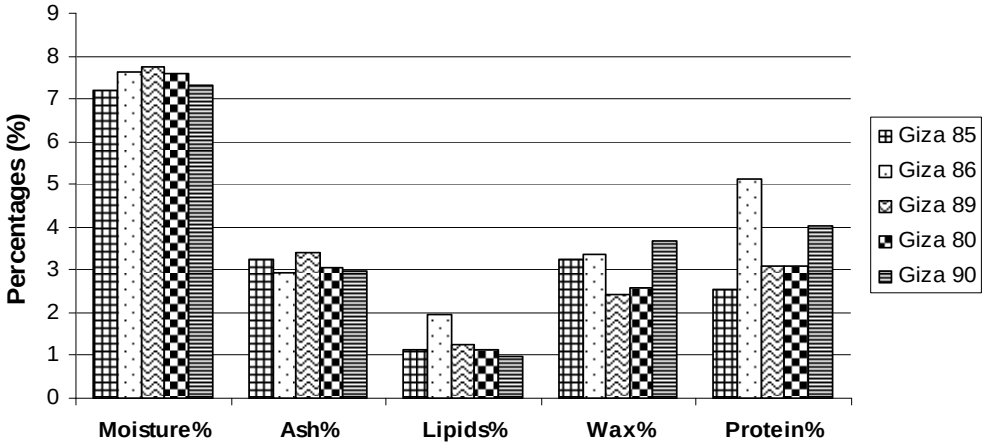

Fig. (1a): Chemical composition of cotton

2. Ash %:

No significant differences among cultivars understudied were observed, as indicated in Table (a) and Fig. (1a). Ash percentages ranged from 2.95% to 3.39%. The highest cultivar (Giza 89) increased by 14.92% than lowest one (Giza 86).

3. Lipids %:

As shown in Table (a) and Fig. (1a), there were significant differences among studied cultivars. Percentages of lipid % enclosed between 0.96% and 1.96% according to cultivar. Giza 86 showed the highest value significantly excelled Giza 89, Giza 80, Giza 85 and Giza 90 by 58.06%, 71.93%, 73.45% and 104.17%, respectively.

4. Wax %:

It was obvious of Table (a) and Fig. (1a) that, significant differences were statistically estimated among studied cultivars. Wax percentages % ranged from 2.43% to 3.67% due to cultivar. *Gossypium barbadense* cv. Giza 90 significantly exceeded Giza 80 and Giza 89 by 42.8% and 51.03%, respectively.

5. Protein %:

According to data in Table (a) and Fig. (1a), no significant differences were estimated among studied cultivars. Protein % percentages were elevates from 2.55% to 5.13% as to genotypic variations among cultivars. Giza 86 was the superior than other cultivars and exceeded Giza 85 (the lowest one) by 101.18%.

6. Crude fibers %:

Data in Table (b) and Fig. (1b.), confirmed the existence of significant differences among studied cultivars. Crude fibers % values were between 38.75% and 46.92% as to cultivar. Highest value was achieved in Giza 86 which score significant elevation by 21.08% over the lowest valued cultivar; Giza 80.

7. Lignin %:

As shown in Table (b) and Fig. (1 b), there were no significant differences among studied cultivars. Percentages of lignin % enclosed between 21.84% and 25.76% according to cultivar. Giza 89 showed the highest value exceeded Giza 85 (the lowest value) by 17.95%.

8. Holocellulose %:

It was obtained from data in Table (b) and Fig. (1b) that, no significant differences among cultivars understudied were observed. Percentages of holocellulose% ranged between 73.44% and 77.26% according to cultivar. Giza 86 showed the highest value exceeded the lowest value; Giza 85 by 5.20%.

9. Hemicellulose %:

Data in Table (b) and Fig. (1b) indicated that, there were no significant differences among studied cultivars. Estimated hemicellulose%, related to dry sample weight, ranged between 40.04% to 49.89% corresponding to studied cultivar. Giza 85 showed highest value for this trait, differed than Giza 90 which showed the least value by 24.60%.

Table (b): Chemical composition of the cotton stalks

Characters / Cultivars	Crude fibers%	Lignin%	Holo-cellulose%	Hemi-cellulose%	α Cellulose%	β Cellulose%
Giza 85	39.50	21.84	73.44	49.89	39.74	1.74
Giza 86	46.92	23.98	77.26	45.83	41.08	1.11
Giza 89	44.98	25.76	75.62	47.66	41.25	2.51
Giza 80	38.75	25.48	75.69	44.60	49.21	2.051
Giza 90	43.43	22.27	76.52	40.04	45.73	2.72
L.S.D at 0.05%	7.993	N.S	N.S	N.S	N.S	1.035

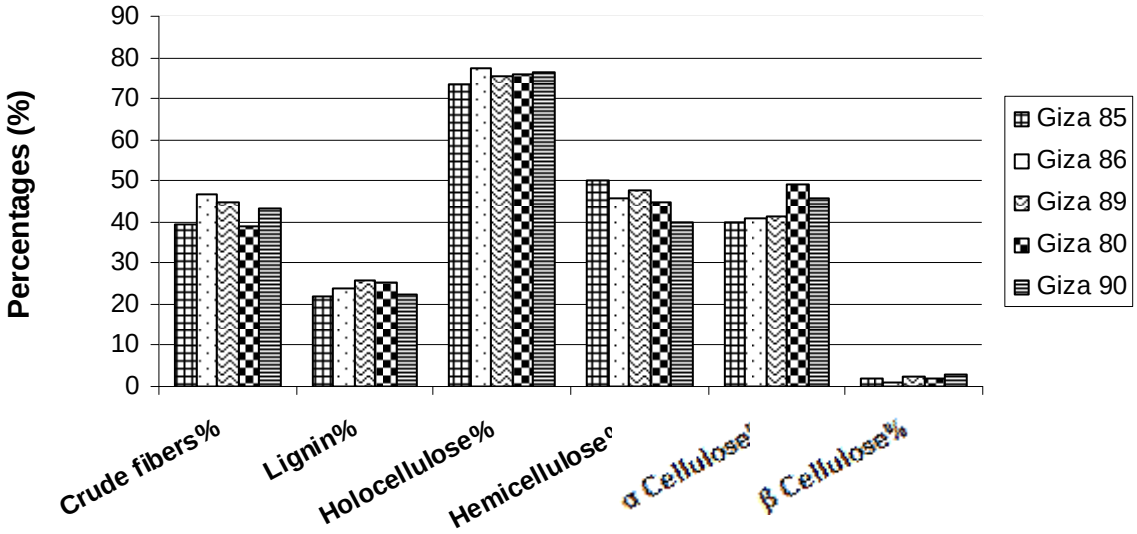

Fig. (1b): Chemical composition of cotton stalks

10. α Cellulose%

Differences among cultivars understudied were insignificant as observed in Table (b) and Fig. (1 b). Percentages of α cellulose%, related to cellulose sample weight, ranged from 39.74% to 49.21% as to studied cultivar. Highest cultivar (Giza 80) showed increment by 23.83% than lowest one (Giza 85).

11. β Cellulose%

It was obvious from Table (1 cont.) and Fig. (1 cont.) that, significant differences were statistically estimated among studied cultivars. Percentages of β Cellulose%, related to cellulose, enclosed from 1.11% to 2.72% due to cultivar. Cultivar Giza 90 significantly exceeded Giza 86 by 145.05%.

Aforementioned results are in agreement with those found by Brown, 1927, Grant, 1958 and Rizk, 1970, and partially in accordance with data estimated by Wassel, 1985 and Kebeasy, 1988. Previous results are coincides those found by Paralikar and Bhatawdekar, 1987 and Silverstein, *et al*., 2007.

II- X-ray diffraction:

Extracted cellulose sample was applied to X-Ray Diffraction equipment. Obtained diffraction chart of prepared cellulose (Fig. 3) was checked against chart of standard cellulose sample treated by 25% aqueous ammonia at 10 ºC for 15 and 240 min. (Fig 2), and this comparison showed that, the cellulose sample at D.p. (degree of polymerization) was treated with 25% aqueous ammonia for 15 and 240 min., and the tested sample, which was extracted from cotton stalks, had the same trend and enclosed in the same range. This aforementioned result confirmed that the tested extracted sample is cellulose.

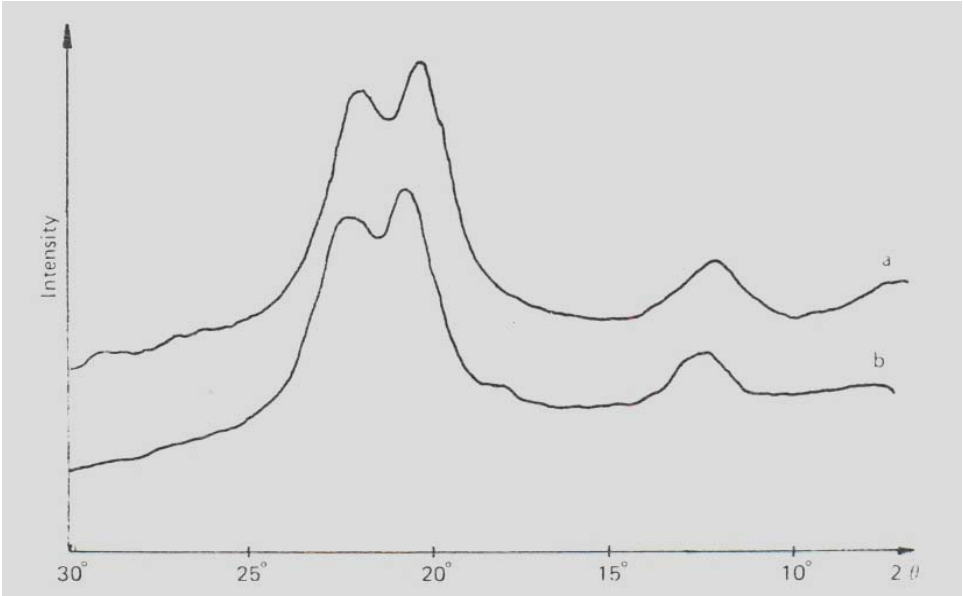

Fig. (2): X-ray diffractograms of cellulose (D.p.=425) treated by 25% aqueous ammonia at 10 ºC for :

a. 15 min.
b. 240 min.

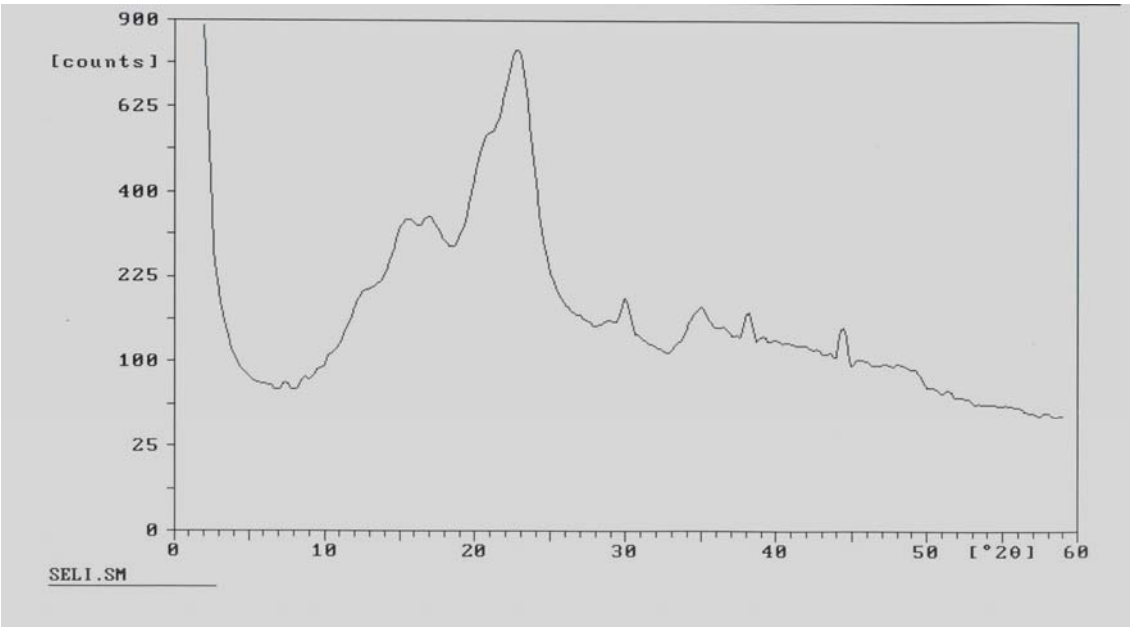

Fig (3): X-ray diffractograms of cellulose extracted from cotton stalks

CONCLUSION

Final goal of current work is achieved by extracting cellulose from cotton stalk of five cultivars understudied and use it to produce economical cellulose derivatives products. However, cultivar Giza86 has best performance for this investigation aim; it has the highest holocellulose percentage (beside some other traits) compared with other studied cultivars. Elimination of wax and lignin are prerequisite operations before cellulose extraction, thus Giza 86 has favorable (reduction in) percentage of lignin but set second (without significant difference) to Giza 90 as the highest wax percentage. Some cellulosic derivatives will be produced as future complementary investigation to current work.

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دراسات كيميائية حيوية على بعض مخلفات القطن

الجزء الاول - التركيب الكيميائى لحطب القطن المصرى

فؤاد عبد الرحيم أحمد*- نادية عبد المعين الغرباوى-*أمل صابر محمد- شيماء السيد أحمد****

***قسم الكيمياء الحيوية الزراعية - كلية الزراعة - جامعة القاهرة**

****معهد بحوث القطن- معهد البحوث الزراعية- الجيزة- مصر**

تعد الأهداف الرئيسية للبحث الحالى هى مقارنة المكونات الكيميائية وبصفة أساسية مكونات الجدار الخلوى (السليلوز و اللجنين) لحطب خمسة أصناف من الأقطان المصرية، ثم تحويل المخلفات الحيوية منخفضة القيمة إلى منتج عالى القيمة الاقتصادية كالسليلوز النقى، مما سيساهم بدوره فى حل مشكلة بيئية وصحية رئيسية فى مصر. وقد استخدم فى هذه الدراسة خمسة أصناف من أصناف القطن المصرى هى جيزة 80 وجيزة 85 وجيزة 89 وجيزة 86 وجيزة 90. ولقد تم الحصول عليهم من الحقول التجريبية لمعهد بحوث القطن. تم اجراء مقارنة للتحليل الكيميائى بين الأصناف سابقة الذكر. وقد أظهرت النتائج ما يلى

1- أن هناك اختلافات معنوية بين الأصناف المستخدمة من حيث المحتوى من الرطوبة و اللبيدات و الشمع و الألياف الخام و البيتاسليلوز

2- ليس هناك اختلافات معنوية بين الأصناف من حيث المحتوى من الرماد و البروتين والسليلوز الكلى و الهيميسليلوز و الألفا سليلوز و اللجنين

3- سجلت أعلى النسب للرطوبة فى حطب الصنف جيزة89 (7,74) أيضا فى الرماد واللجنين (3,39 و 25,75% على التوالى) ، ولكنه كان أكثر الأصناف انخفاضا فى نسبة الشمع (2,43%).

4- أظهر الصنف جيزة 86 أعلى النسب فى اللبيدات (1,96%) و أيضا فى نسب البروتين والألياف الخام والسليلوز الكلى (5,12 و46,92 و77,26 % على التوالى), بينما كان الصنف الأكثر انخفاضا فى البيتا سليلوز (1,11%) وكما فى الرماد (2,95%).

5- كانت أعلى نسبة فى الشمع (3,67%) فى جيزة 90 والذى أظهر أيضا أعلى تقدير فى البيتاسليلوز (2,72%)، ولكنه كان أكثر صنف انخفاضا فى الهيميسليلوز (40,04%) واللبيدات (0,96%).

6- كانت أعلى النسب في الألفاسليلوز (49,21%) فى جيزة 80 و الذى عكس أكثر النسب انخفاضا فى الألياف الخام (38,75%).

7- تم استخلاص سليلوز الحطب بإزالة الشموع و اللجنين والهيميسليلوز (المواد المصاحبة له) كما تم إجراء الاختبار الفيزيائى للسليلوزالمستخلص بواسطة الأشعة السينية الحيودية التى أكدت المقارنة مع الرسم البيانى لعينة سليلوزقياسية أن المستخلص هو سليلوز. يصلح لاجراء عمليات التحوير الكيميائى له لانتاج المشتقات السليلوزية وذلك فى الجزء الثانى من هذه الدراسة.

11/4/2010