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# MODIFIED METHODS FOR THE ANALYSIS OF THE LIGNIN-LIKE PHENOLIC POLYMER CONTENTS OF COTTON FIBERS

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## ABSTRACT

The lignin-like phenolic polymer contents of cotton fibers are too little to be directly measured using existing methods. In this study, the thioglycolic acid (TGA), acetyl bromide (AB) and Klason methods were modified in order to determine the lignin-like phenolic polymer contents of cotton fibers. Varying sample weight, thioglycolicacidtoHCl ratios, extraction times, and degrees of acidificationto precipitate the lignin-likephenolicpolymers were used to study their effects on the TGA method. In addition, the effects of the digestive temperature, time, volume of the digestive solution, and ratio of NaOH to hydroxylamine hydrochlorideon the elimination of background absorbance in the AB method were tested. Furthermore, the effects of the drying temperature and sample weights on the measurement of lignin-like phenolic polymer contents using the Klason method were analyzed. The modified TGA, AB, and Klason methods were established based on their corresponding parameter modifications. These modified methods could be directly used to determine the contents of lignin-like phenolicpolymers in cotton fibers in different ways.

**Keywords:**lignin-like phenolic polymers, cotton fiber, thioglycolic acid (TGA), acetyl bromide (AB),Klason method

## INTRODUCTION

Plant cell wall phenolicsare comprised of two compounds: (i) lignin, a polymer consisting of monolignol units linked by oxidative coupling, and (ii) low-molecular-weight hydroxycinnamic acids, which bind to various cell wall components and are involved in cross-linkages (Wallace and Fry, 1994; Fan *et al*., 2009).

Lignin-like phenolic polymersare the second most abundant terrestrial biopolymer, accounting for approximately 30% of the organic carbon in the biosphere (Boerjan*et al*., 2003; Liu *et al*., 2011).Lignin-like phenolic polymersare complex aromatic polymers formed from phenolic precursors, which are products of the plant phenylpropanoid pathway (Boerjan *et al*., 2003). The formation of lignin-like phenolic polymers predominantly occurs in the mature metaxylem and secondary tracheary elements of roots and stems, followed by very young tissue (Müsel *et al*.,1997; Fan*et al*.,2006)and cell walls (Day *et al*.,2005; Fan *et al*., 2009). This formation process can influence the mechanical strength, growth, morphogenesis and response to biotic and abiotic stress of cell walls (Lewis and Yamamoto, 1990; Wallace and Fry, 1994;Boerjan *et al*., 2003; Bhuiyan *et al*.,2009).

In order to accurately evaluate the effects of lignin-like phenolic polymers on the properties of plant cell walls, analytical methods that are suitable for different plant materials must be used.

Numerous methods have been developed and modified in order to quantitatively determine the amounts of lignin-like phenolic polymers in plant tissues (Hatfield and Fukushima, 2005).These methods include: (i) non-gravimetric methods, such as the thioglycolic acid (TGA)(Bruce and West, 1989; Müsel *et al*.,1997; Hatfield and Fukushima, 2005) and acetyl bromide (AB) procedures (Iiyama and Wallis,1988; Fukushima and Dehority,2000; Hatfield and Fukushima, 2005),which depend on the sufficient derivatization of lignin-like phenolic polymers to be soluble in a suitable solvent and measure absorbance at 280 nm; and (ii) gravimetric procedures, such as the Klason method, which rely on the removal of non-lignin materials since any remaining materials are considered to be lignin-like phenolic polymers (Iiyama and Wallis,1988; Hatfield *et al*.,1994; Hatfield and Fukushima, 2005). While the TGA method can be used to accurately reflect lignin-like phenolic polymers (Hatfield and Fukushima, 2005), the AB method can be used to reveal both ligninandhydroxycinnamic acids, and the Klason method can be used to determine the presence of the insoluble residue of lignin-like phenolic polymers. Thus, these methods are often chosen to analyze the contents of lignin-like phenolic polymers in different ways. However, the TGA, AB, and Klason methods are mainly

used to measure high contents of lignin-like phenolic polymers in woody and herbaceous plants (Iiyama and Wallis,1988; Bruce and West, 1989;Guerra*et al*.,2006).

Cotton fibers are elongated seed epidermal cells with thickened secondary cell walls comprised almost entirely of the purest form of naturally occurring cellulose; this cellulose accounts for more than 90% of the dry weight of cotton fibers (Timpa and Triplett, 1993; Singh *et al*.,2009;Ruan, 2013). Lignin-like phenolic polymers have been shown to be linked to the cell walls of white cotton (*Gossypiumhirsutum* L.) fibers (Fan *et al*., 2009). Since the lignin-like phenolic polymer contents of cotton fibers are very low, and mature cotton fibers have dead, hollow, soft, dried cell walls that are difficult to triturate with conventional grinding techniques, the existing methods used to directly analyze the contents of lignin-like phenolic polymers are unsuitable for use in cotton fibers.

In this paper, the conventional TGA, AB and Klason methods were modified in order to make them applicable to the direct measurement of the lignin-like phenolic polymer contents of cotton fibers. The modified TGA, AB, and Klason methods were established based on their corresponding parameter modifications. These modified methods could be used to determine the lignin-like phenolic polymer contents of cotton fibers in different ways.

## EXPERIMENT

**Materials:** *Gossypiumhirsutum* L. cv.Xinluzao 19 samples were selected for the purposes of this study. The cotton fibers were washed twice with a homogenization buffer (50 mMTris-HCl, 10 g/L Triton X-100, 1 MNaCl;pH8.3), twice with 80% (V/V) acetone, and once with pure acetone (Niklas *et al*., 2000). After each wash, the samples were squeezed with a metal clamping apparatus, and the cleaning solutions were discarded. The washed cotton fibers were dried and used for measurements.

**Special Reagents:** The thioglycolic acid, acetyl bromide, and alkaline kraft lignin used in this study were purchased from Sigma-Aldrich.

### Modified methods

(1) The TGA method was modified based on the conventional method proposed by Bruce and Müsel *et al*. (Bruce and West, 1989; Müsel *et al*.,1997; Hatfield and Fukushima, 2005).

**Cotton fibers weights:** In order to determine the appropriate cotton fiber weight, 0.050, 0.100, 0.150, 0.200, and 0.250 g (±0.001 g, respectively) of cotton fibers were tested.

**Proportions of thioglycolic acid to different HCl concentrations:** Once the appropriate cotton fibers weight was ascertained, twenty cotton fiber samples were placed into twenty glass screw-cap bottles. Then, 5 mL of 1, 2 , 3, or 4 MHCl were added to the samples, and the thioglycolic acid volumes were mixed according to the following proportions of $V_{HCl}$:$V_{TGA}$: 5:0.5, 5:0.625, 5:0.75, 5:0.875, or 5:1.

**Extraction times:** In order to determine the extraction times, once the proportion of thioglycolic acid toHCl was determined, five samples of theappropriatecotton fiber weight were treated with the selected proportion of thioglycolic acid toHCl.These steps were repeated for the sample handling and lignin-like phenolic polymers extraction processes. The sample residues were extracted twice with 5 mL of 0.5 MNaOH, then re-extracted with 0.5, 1, 2, 3 or4 MNaOH. The solutions extracted three times were mixed, and different volumes of concentrated HCl(37% by weight) were added to each tube based on whether the ratio of $[H^+]/[OH^-]$ was equal to 5/24.

**Volume of the concentrated HCl:** In order to determine the effects of varying volumes of concentrated HCl on the lignin-like phenolic polymer contents, eleven cotton fiber samples were treated according to the above parameters and extraction steps. The supernatants were mixed and divided into eleven aliquots. Then, various volumes of concentrated HCl, 0.375, 0.5, 0.75, 1.5, 3, 4.5, 6, 7.5, 9, 10.5,and 12 mL, were added correspondingly.

**Extraction time:**After these parameters were established, NaOH extraction time ranging from 10 hours to 20 hours was compared in order to study the effects of the extraction time on the extraction process.

(2) The modified AB method was tested based on the method proposed byIiyama *et al*. (Iiyama and Wallis,1988; Fukushima and Dehority,2000; Hatfield and Fukushima, 2005).

**Temperature during digestion:** The 0.100-g cotton fibers samples were placed into 30-mL glass screw-cap bottles with 4 mL of freshly prepared AcBr/HAc (1:3 V/V, HAc:glacial acetic acid), and sealed immediately with Teflon caps. The bottles were heated in a water-bath at 50ºC, 60ºC, or 70ºC. The water-bath was gently shaken to promote the dissolution of the samples. Heating time ranged from 30 minutes to 4 hours.

**Volume of the digestive solution:** Based on the above temperatures, 3, 4, or 5 mL of freshly prepared AcBr/HAc were used to determine a suitable volume for the digestive solution at a fixed temperature.

**Digestive time:** Digestive time ranging from 30 minutes to 210 minutes was used to test the cotton fibers.

**Elimination background absorbance:** Twenty-one 0.100-gsamples were digested using the suitable AcBr/HAc volume. Then, the samples were treated and

the extraction steps were repeated. When the digestion process was complete ,the solutions were mixed, and the mixtures were separated into twenty-one 5-mL samples, which were placed in flasks. Next, twenty-one varying4 MNaOH (2.0, 2.5, 3.0, 3.5, 4.0, 4.5 or 5 mL)to5 M hydroxylamine hydrochloride (1.0, 1.5, or 2.0 mL)ratio solutions were added to the flasks. HAc was used to fill each flask to a total volume of 25 mL.

(3) The conventional Klason method used in this study was based on the method proposed byIiyama *et al*. (Iiyama and Wallis,1988; Hatfield *et al*.,1994; Hatfield and Fukushima, 2005).

**Drying temperature:** The filter papers were dried at 60°C, 80°C, or 100°C until a constant weight (±0.3 mg upon reheating and reweighing) was achieved.

**Cotton fiber weights:**Various amounts of cotton fibers, 0.500, 1.000, 1.500, 2.000 or 2.500 g (±0.001 g, respectively),were treated with15.0 mL of 72% (V/V) $H_2SO_4$in order to determine the appropriate cotton fibers weight.

All of the experiments were analyzed in triplicate with duplicates for each treatment.

**Statistical Analysis:** A statistical analysis was conducted using Microsoft Excel 2003, and the figures were constructed with GraphPad Prism 5. The data was denoted in terms of the means of the measurements (±SE: standard error). Error bars were used to indicate the 95% significance confidence intervals.

## RESULTS AND DISCUSSION

Cotton fibers have high cellulose contents and unique characteristics. Conventional methods cannot be used to directly analyze the lignin-like phenolic polymer contents of cotton fibers. Based on the effects of various factors, the conventional TGA, AcBr and Klason methods were modified in order to measure the lignin-like phenolic polymer contents of cotton fibers. Several procedural parameters were investigated in order to determine their effects on the methods (Table 1).

**The TGA method:** As shown in Figure 1A, the amount of extracted lignin-like phenolic polymers was directly affected by the amount of cotton fibers (Figure 1A). Different amounts of cotton fibers were used in order to determine an appropriate sample size for the analysis. The absorbance spectrums of the lignin-like phenolic polymers extracted from the 0.100-g cotton fiber samples within the ultraviolet wavelength range corresponded with the Lambert-Beer law. The percentage lignin-like phenolic polymers decreased when the amountof cotton fibers was greater than 0.100 g. This indicated that the lignin-like phenolic polymer contents of cotton fibers less than or equal to0.100 gin weight can be extracted more thoroughly than that of cotton fibers greater than 0.100gin weight. Fan *et al*. (2009) reported that the thioglycolate phenolic contents of cotton fibers are low (0.13-0.35%).Since the 280-nm absorbance of the small amounts of lignin-like phenolic polymers in the cotton fibers was too low to affect the standard curve, the experimental error increased. Thus, 0.100-g of cotton fiber samples was chosen for the purposes of this study.

The $V_{TGA}$: $V_{HCl}$ concentrations significantly impacted the total absorbance in that the lignin-like phenolic polymers were displaced from their normal covalent attachments to the cell wall during TGA derivatization. In order to obtain a suitable ratio of thioglycolic acid to concentrated HCl, the effects of various ratios on the degradation of cotton fibers were tested (Figure 1B). Combinations of 5 mL of 2 MHCl with different amounts of TGA yielded high absorbance values, especially when 0.75 mL of TGA was used. Thus, the 0.100-g cotton fiber samples were treated with 5 mL of 2 MHCl and 0.75 mL of TGA. Some of the samples, which were treated twice with HCl+TGA, yielded lower absorbance values after the second treatment, indicating that repeated treatment did not increase the extracted amounts of lignin-like phenolic polymers.

The lignin-like phenolic polymers extractions were affected by multiple extraction conditions. In this study, various NaOH concentration and extraction times were tested (Figure1C). High percentages oflignin-like phenolic polymer contents were extracted with2 MNaOH. However, when the concentration of NaOH was greater than 2 M, a large amount of gelatiniform precipitation was produced, and the measured lignin-like phenolic polymer contents decreased. The precipitation could have been a result of the high concentrations of NaOH (>2M), which caused the esterification of polysaccharides extracted from the cotton fibers.

The results also indicated that the extraction time, ranging from 10 to 12 hours, did not significantly affect the extracted amount of lignin-like phenolic polymers; thus,10-12 hours was selected as the processing time in order to conserve time and ensure thorough extraction.

Lignin-like phenolic polymers dissolved in NaOH can be precipitated through the addition of concentrated HCl. The amount of recovered lignin-like phenolic polymers was dependent on the volume of HCl (Figure 1D**)**, which corresponded with the results of Fukushima and Hatfield (Fukushima, 1991; Hatfield *et al*., 1999, 2005). The highest absorbance value was obtained when 1.5 mL of concentrated HCl was added; thus, 1.5 mL of concentrated HCl was added to the NaOH solutions in order to precipitate the lignin-like phenolic polymers.

In summary, the conventional TGA method was modified as follows:

Cotton fiber samples (0.100 g) were placed in a glass screw-cap tube and treated by 5 mL of 2 MHCl and 0.75 mL of TGA for 4 hours at 98ºC. The insoluble residue was obtained using a centrifugation and washed three times with water. Then, theresidue was extracted twice with 5 mL of 0.5 MNaOH, then once with 5 mL of 2 MNaOH. The supernatant of the extraction solutions were mixed, acidified with 1.5 mL of concentrated HCl for 4 h at 4°C,and centrifuged in order to obtain the lignin-like phenolic polymers. Next, the lignin-like phenolic polymers were dried and dissolved in 5 mL of 0.5 MNaOH. Their absorbance values were measured at 280 nm. A 0.5-M NaOH solution was used as a blank solution, and alkalinekraft lignin was used as the calibration standard.

**The AB method:** In the AB method, the extracted lignin-like phenolic polymer contents were affected by several experimental parameters, including the heating temperature, heating time, AcBr/HAc (1:3, V/V) volume, and ratio of NaOH to hydroxylamine hydrochloride solution. Therefore, these parameters were tested in order to determine their effects on the lignin-like phenolic polymers obtained from the cotton fibers (Figure 2A). The cotton fibers were not thoroughly digested at 50°C, even after lengthy digestive time. The amount of digestion increasedat60°C and was highest at 70°C. The cotton fibers were completely digested after 1.5 to 2 hours, and the background colors were removed with4 MNaOH and 5 Mhydroxylaminehydrochlorideat70°C. Deep red-colored materials, similar to carbonization, appeared in the reaction solution after 3 hours at 60°C and after 2.5 hours at 70°C, resulting in measurement errors.

As shown in Figure 2B, 5 mL of AcBr/HAc (1:3, V/V) yielded a high absorbance at 280 nm.Thus, 5 mL of the AcBr/HAc (1:3, V/V) was selected for the purposes of this study.

Measurements conducted every 10 minutes after 1.5 to 2 hours indicated that 1 hourand45 minutes was the optimal digestive time at 70°C (Figure2C).

Bromine, a contaminant in AcBr, reacts with sodium bromide to form polybromide ions, which has very high absorbance at 280 nm (Fukushima *et al*.,1991). Bromine and polybromide were removed through the addition of the hydroxylamine hydrochloride solution (Fukushima *et al*.,1991).High NaOH and hydroxylamine hydrochloride solution concentrations were chosen, which introduced minimal amounts of water to the mixture and removed the strong absorption peak. In this experiment, the NaOH and hydroxylamine hydrochloride concentration significantly affected the results, which corresponded with the results of obtained by Iiyama (1988) and Fukushima (2000). The use of 4.5 mL of 4 MNaOH and 1.5 mL of 5 Mhydroxylamine hydrochloride eliminated the absorbance of the background solutions and promoted the absorbance of the alkali lignin (Figure2D).

Thus, the following modifications were made to the conventional AB method.

Cotton fiber samples (0.100 g) were placed in a 30 mL glass screw-cap bottle. Then, 5 mL of freshly prepared AcBr/HAc (1:3 V/V) was added to the bottle, which was sealed immediately with a Teflon-lined screw-cap. The bottle was heated in a water bath at 70°C for 1 hour and 45 minutes and shaken gently at 200 rpm to promote dissolution. All of the bottle's contents were completely transferred into a 25-mL volumetric flask that contained 4.5 mL of 4 MNaOH.The bottle was rinsed with 10 mL of HAc, then put the HAc to the flask. After mixing, 1.5 mL of 5 Mhydroxylamine hydrochloridewas added to the flask for a total volume of 25 mL including with HAc. After 30 minutes, the lignin-like phenolic polymer contents were obtained through an absorbance measurement of the resulting solution at 280 nm using a GBC Cintra 20 UV/Visible spectrophotometer calibrated with alkaline kraft lignin. The 280-nm absorbance measurement was compared to that of a blank solution.

**The Klason method:** The drying temperature, drying time and the amount of cotton fibers affected the extracted amounts of acid-insoluble lignin-like phenolic polymers. In the modified Klason method, the treated filter papers were dried at 60°C, 80°C, or 100°C (Figure3A). A constant weight was achieved over 3 hours at 60°C while maintaining the integrity of the filter papers. The constant weight was also achieved over 2 hours at 80°C and in 1 hour at 100ºC. However, because the filter papers were treated by 3% (V/V) $H_2SO_4$, they became brittle and less effective when the drying temperature was greater than or equal to 80°C. Thus, although 60°Cis a low temperature, since the results obtained at this temperature were consistent before and after filtration, this temperature was selected for the purposes of this study.

Most existing studies have used<1.000 g of other samples (Kajita *et al*.,1997; Abdullah *et al*.,2006); however, since the lignin-like phenolic polymer contents of cotton fibers are very low, they were determined via the weight difference method, in which the weighing error in the experiment was increased if the amount of cotton fibers was <1.000 g. The lignin-like phenolic polymer contents measurements were consistent for cotton fiber weights ranging from 1.000 to 2.000 g (Figure3B). The measurement error decreased as the amount of cotton fibers increased, but the measured lignin-like phenolic polymers values decreased for sample weights greater than 2.000 g. Thus, 2.000-g cotton fibers weights were used for the Klason method analysis. In this experiment, the autoclaved samples were kept in sealed bottles for 1 hour at 121±3°C instead of being refluxed for 4 hours ± 5 minutes on the reflux

condenser during the digestive process in order to reduce environmental pollution and experimental danger as well as reduce the reaction time.

Therefore, the following modifications were made to the conventional Klason method. After treatment with 3% $H_2SO_4$, the filter papers were cleaned with distilled water until the cleaning water from the papers was neutral. The filter papers were dried at 60°C until a constant weight was achieved (±0.300 mg upon reheating, recorded as $W_1$). Next, 2.000-g cotton fibers (recorded as $W_2$) were weighted, then suspended in 15 mL of 72% $H_2SO_4$and stirred with a glass rod at 20ºC for 2 hours. The contents were transferred to an Erlenmeyer flask and diluted with distilled water to a total volume of 560 mL. The sealed bottle was autoclaved for 1 hour at 121±3°C. After cooling, the lignin-like phenolic polymers were collected with the pre-weighed filter paper and washed with distilled water to remove any residual acid. Then, the contents were dried to a constant weight (recorded as $W_3$). The dried filter paper and corresponding lignin-like phenolic polymers were placed in crucibles then heated at 575±25°C in a muffle furnace for a minimum of 3 hours until all of the residues were equal in weight ($W_4$). The acid-insoluble lignin-like phenolic polymers (%) were calculated based on their "as received" dry weights according to

Acid-insoluble lignin-like phenolic polymers= $(W_3–W_1–W_4)/W_2 \times 100\%$

where, $W_1$ represents the initial weight of the filter paper, $W_2$ represents the weight of the sample, $W_3$represents the weight of the filter paper and contents, and $W_4$represents the weight of the ash contents.

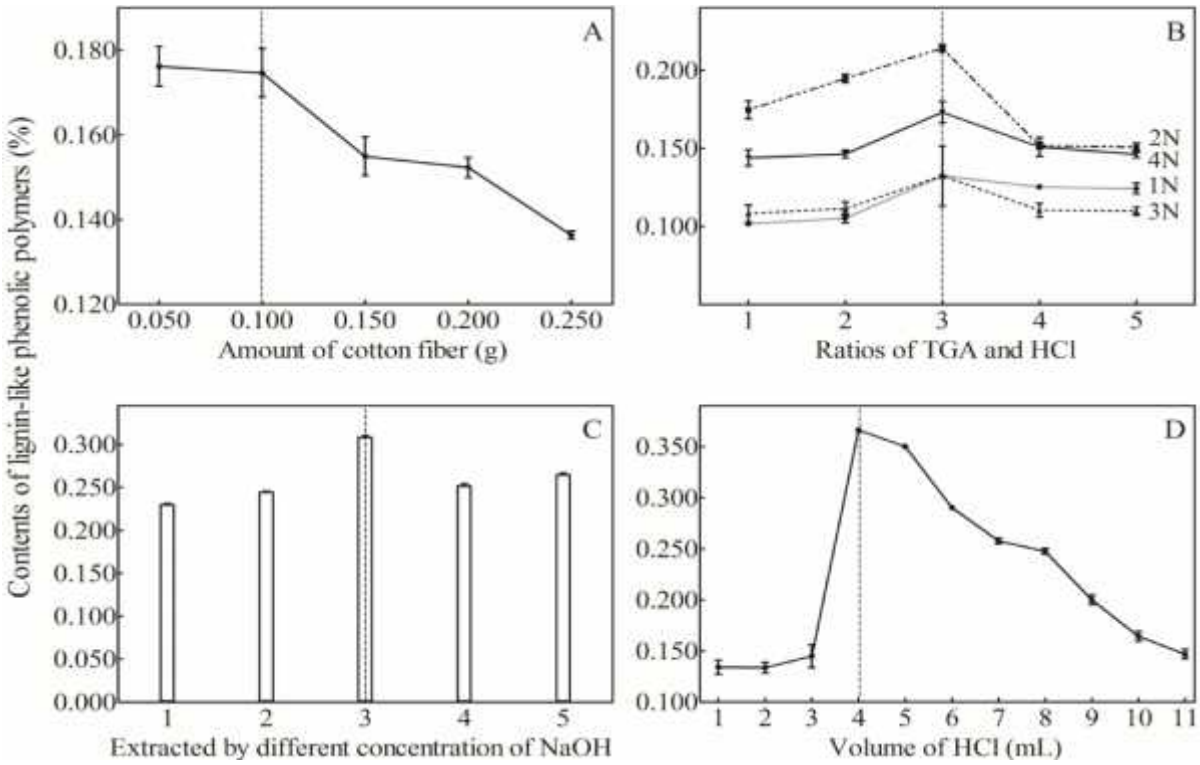


**Figure 1TGA method modifications**

(A) Different amounts of cotton fibers

(B) Different TGA/ HCl (V/V) ratios. On the horizontal axis: (1) 0.5 mL of TGA and 5 mL of1 MHCl, (2) 0.625 mL of TGA and 5 mL of1 MHCl, (3) 0.75 mL of TGA and 5 mL of1 MHCl, (4) 0.875 mL of TGA and 5 mL of1 MHCl, and(5) 1 mL of TGA and 5 mL of1 MHCl

(C) Different NaOH concentrations and numbers of extractions. On the horizontal axis: (1) 0.5 MNaOH extracted three times, (2) 0.5 MNaOH extracted two times and 1 MNaOH extracted once, (3) 0.5 MNaOH extracted two times and 2 MNaOHextracted once, (4) 0.5 MNaOH extracted two and 3 MNaOH extracted once, and (5) 0.5 MNaOH extracted two times and 4 MNaOH extracted once

(D) Different concentrated HClvolumes during the acidification of the alkaline solutions. On the horizontal axis: (1) 0.375 mL of concentrated HCl, (2) 0.5 mL of concentrated HCl, (3) 0.75 mL of concentrated HCl, (4) 1.5 mL of concentrated HCl, (5) 3 mL of concentrated HCl, (6) 4.5 mL of concentrated HCl, (7) 6 mL of concentrated HCl, (8) 7.5 mL of concentratedHCl, (9) 9 mL of concentratedHCl, (10) 10.5 mL of concentratedHCl, and (11) 12 mL of concentratedHCl

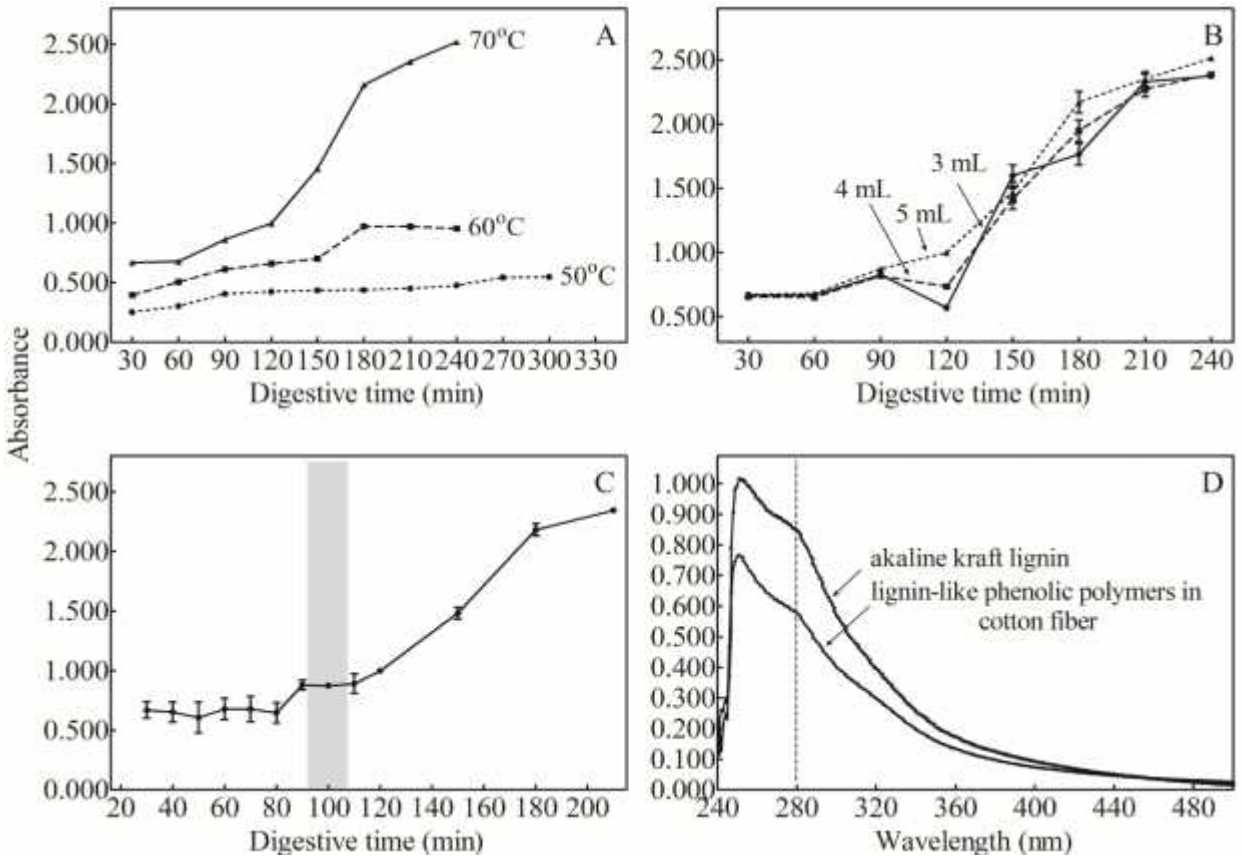


**Figure 2AB method modifications**

(A) Time lapsethe reactions between theAcBr and cotton fibers at different temperatures

(B) Different digestive volumes at different lengths of time at 70°C

(C) Digestion over different lengths of time at 70°C

(D) UV absorbance spectra of the indicated standards and the AcBr extracts of the cotton fibers

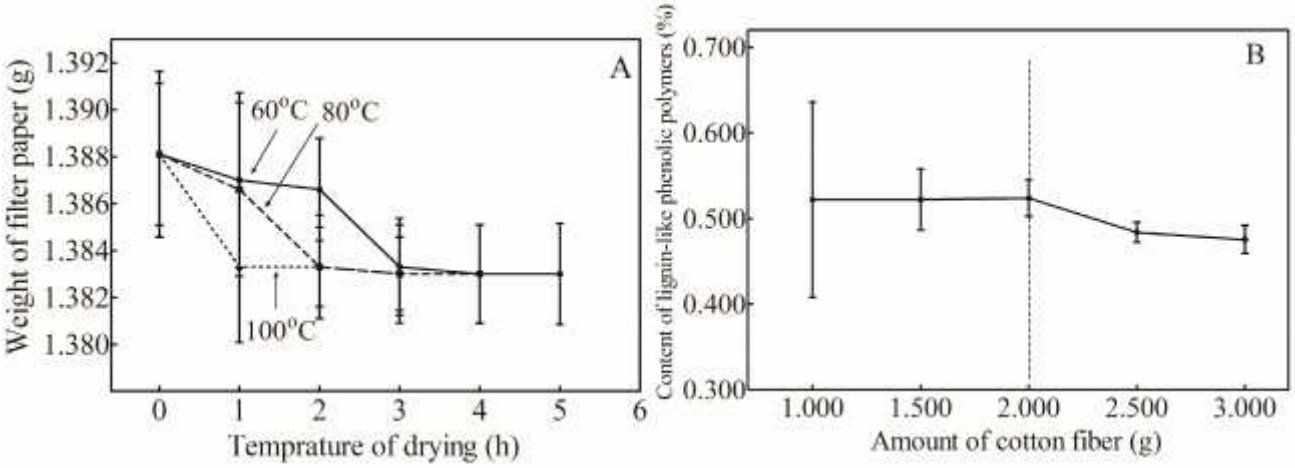


**Figure 3 Klason method modifications**

(A) Weights of the filter papers dried at different temperatures

(B) Lignin-like phenolic polymercontents of the cotton fiber samples with different weights

**Table 1 Experimental parameters**

| Method | Parameter | Reference | *In the research* | |
|---|---|---|---|---|
| | | | **Experimented** | **Determined** |
| TGA | Weight | 50 mg | 0.050, 0.100, 0.150, 0.200, 0.250 g | 0.100 g |
| | $V_{HCl}$:$V_{thioglycolic\ acid}$ | 5:0.5 (2 N HCl) | 5:0.5, 5:0.625, 5:0.75, 5:0.875, 5:1 (1, 2 , 3, or 4 NHCl) | 5:0.75 (2 N HCl) |
| | Extraction times | Twice by 5 mL of 0.5 MNaOH | Twice of 5 mL of 0.5 MNaOH, and the residues were re-extracted by 0.5, 1, 2, 3 or 4 MNaOH, respectively | Twice with 5 mL of 0.5 MNaOH and then once with 5 mL of 2 MNaOH |
| | Extraction time | 18 hours | 10-20 hours | 10-12 hours |
| | $V_{HCl}$of acidification | 1 Ml | 0.375, 0.5, 0.75, 1.5, 3, 4.5, 6, 7.5, 9, 10.5 and 12 mL | 1.5 mL |
| AB | Temperature | 60 or 70ºC | 50, 60, or 70ºC | 70°C |
| | Time | 30 minutes or 40-120 minutes | 30 minutes-4 hours 30 minutes-210 minutes | 1 hour and 45 minutes |
| | $V_{AcBr/HAc}$ | 4.0 or 5 mL | 3, 4, or 5 mL | 5 mL |
| | $V_{NaOH}$:$V_{hydroxylamine\ hydrochloride}$ | 4.5 mL(2 MNaOH):1 mL (7.5 Mhydroxylamine hydrochloride) or 4 mL (0.3 M):1mL (0.5 Mhydroxylamine hydrochloride) | 21 different ratios of 4 MNaOH (2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5 mL):5 M hydroxylamine hydrochloride (1.0, 1.5, or 2.0 mL) solutions | 4.5 mL of 4 M NaOH:1.5 mL of 5 Mhydroxylamine hydrochloride |
| Klason | Filter | Filtering crucible 100°C | Filter paper | Filter paper |
| | Drying temperature | | 60, 80 or 100°C | 60°C |
| | Weight of cotton fibers | 1.000 g | 0.500, 1.000, 1.500, 2.000 or 2.500 g | 2.000 g |

**Conclusion:** The conventional TGA, AB and Klason methods are unsuitable for analyzing the lignin-like phenolic polymer contents of cotton fibers. The modified TGA, AB, and Klason methods proposed in this paper were established based on their corresponding parameter modifications. The modified methods were used to directly measure the lignin-like phenolic polymer contents of cotton fibers (Fan *et al*., 2009; Han *et al*., 2013).The results indicated that these modified methods could be used to determine the lignin-like phenolicpolymer contents of cotton fibers in different ways.

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