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ScienceDirect
Carbohydrate Polymers 72 (2008) 652–656
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Component analysis and microfiber arrangement of *Apocynum venetum* fibers: The MS and AFM study

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Received 25 August 2007; accepted 4 October 2007
Available online 12 October 2007

Abstract

In this paper, electrospray ionization/mass spectrometry (ESI-MS) was used to investigate the changes of flavonoids in the extract of the bast of AV and AV fibers. It is suggested that the quercetin structure maybe exist in the extract of the bast of AV, and then high resolution time-of-flight (TOF) MS was used to further characterize the possible ions. The identification of quercetin in the extract of the bast of AV was confirmed, while it was disappeared or tailed off in that of AV fibers. This indicated that such kind of compounds maybe destroyed during the degumming process. The microstructures of AV fibers and ramie fibers have been studied by atomic force microscopy (AFM) and it can be seen that the arrangements of microfiber of ramie were more compact than that of the AV fibers. These results may contribute to further clarify the functions of health-care and antibacterial functions of the AV fabrics.

Keywords: *Apocynum venetum* fiber; ESI-MS; TOF-MS; AFM

1. Introduction

Apocynum venetum (AV) is one type of wild shrubs, which is widely spread throughout mid and northwestern China. The AV leaves have been used widely as a kind of Chinese traditional medicine and a healthy beverage. In recent years, the AV fibers have been used in textile industry due to its excellent physical and mechanical properties, and some antibacterial textiles made from AV fibers are very popular at home and abroad (Mohanty, Misra, & Drzal, 2005; Netravali & Chabba, 2003). So, to cater for the development of clothing industry with the function of comfortable and health protection, AV fabrics have been attracted increasing interest. And the research about AV fiber was mainly focused on exploration of the healthy functions and degumming methods in the textile industry (Zhang & Han, 2006; Wu, Jiang, Li, Zhang, & Xu, 2004). It was reported that the AV fabrics have good effect on the hypertension and coronary heart disease, etc. (Lei et al., 1995; Murakami, Hishi, & Matsuda, 2001; Nishibe, Takenaka, & Kodama, 2001; Yokzawa & Kashiwada, 2004; Yokozawa, Kashiwada, & Hatori, 2002). Consequently, many degumming methods, such as chemical degumming method and bacterial degumming method were investigated to improve efficiency and obtain high quality AV fibers. In our laboratory, the fast chemical degumming method and the bacterial degumming method were explored (Han, Zhang, & Su, 2006; Wang, Han, & Zhang, 2007). It was found that the AV fibers obtained by both chemical and bacterial degumming methods have the typical cellulose I structure and can be suitable for the textile industry due to the well-defined fiber structure and the successful remove of the non-cellulose substances. Furthermore, the bacterial degumming method has superior features for industrial applications than those of chemical degumming methods due to the high efficiency, low cost and, especially, the environmental-benign nature.

Although the AV fibers in textile industry have been studied for a long time, to the best of our knowledge, the research did not have a definite explanation about the reason of health

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0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.10.002

care and antibacterial functions of the AV fabrics, and some reports presumed that such functions of AV fabrics were mainly attributed to flavonoids, which was a medicine ingredient (Lei et al., 1995; Murakami et al., 2001; Nishibe et al., 2001; Yokzawa & Kashiwada, 2004; Yokozawa et al., 2002). In this paper, the presence of flavonoids in both bast of AV and AV fibers was detected by electrospray ionization/mass spectrometry (ESI-MS) and time-of-flight mass spectrometry (TOF-MS). The microfiber arrangements of AV fibers as well as those of ramie fibers were investigated by atomic force microscopy (AFM). It was found that the flavonoids were existed in the extract of the bast of AV, but disappeared or tailed off in those of AV fibers, and the arrangement of ramie microfibers were more compact than that of the AV fibers. The small opening between the microstructure in AV fibers improved breathability and keep the AV fabrics dryness, which can destroy the growth environment of bacteria.

2. Materials and methods

2.1. Materials

The pith tissue cannot be used to obtain fibers and was, therefore, mechanically separated from the bast parts. The bast of the AV was used as raw material. These were all chosen from the same group of one year's white AV living in Xinjiang province, PR China. The gum content was 54.26%.

2.2. Fiber production

The fiber pretreatment conditions mentioned below were the optimized conditions. Many trials of fiber pretreatment were conducted by varying the concentration, time and temperature of treatment and the liquor-to-bast ratio (Zhang & Han, 2005). The optimized conditions were decided based on the quality of the fibers produced.

Process: pre-impregnation → washing → first cooking → washing → second cooking → washing → acid rinsing → washing → dewatering → shaking → drying.

Pre-impregnation: H_2O_2 solution (6 g/L), normal temperature and pressure, liquor ratio 1:15, pH value 6.5, time 20 min.

First cooking: NaOH solution (10 g/L), $NaSiO_3$ solution (2%), sodium tripolyphosphate (2%), normal pressure, temperature 100 °C, liquor ratio 1:15, time 1 h.

Second cooking: similar to the first cooking step except for the treated time elongated to 2.5 h.

Acid rinsing: sulfuric acid (1 g/L), normal temperature and pressure, liquor ratio 1:15, time 2 min.

Washing: washing with the hot water above 70 °C, then hand washing and spray rinsing with tap water.

2.3. Electrospray ionization (ESI) and time-of-flight (TOF) mass spectrometry

The bast of AV and the AV fibers were dried in an oven with forced air circulation at 40 °C for 24 h. The dry samples were then extracted by anhydrous ethanol at 80 °C for 6 h. The 500 ml of anhydrous ethanol was used to extract 8 g of samples, respectively. After extraction, anhydrous ethanol was evaporated under low pressure to 10 ml. The two samples were tested for the presence of flavonoids.

The mass spectra measurements were carried out using an APEX IIFT-ICR mass spectrometer.

2.4. AFM measurement

Samples of the AV fibers and the ramie fibers were put into the mixed solution of 5 g/L H_2O_2 and 9 g/L urea at room temperature for 20 min. They were then boiled in 10 g/L ammonium oxalate monohydrate for 3 h, rinsed throughly with excess water. The samples were purified by boiling in distilled water, and then put them into the mixed solution of 30% H_2O_2 and acetic acid (1:1, v/v), rinsed in water, and allowed to air dry. The samples were prepared for AFM observation after the solution was dried. AFM measurement was performed on SPI 3800.

3. Results and discussion

3.1. MS analysis

The extract of the bast of AV and the AV fiber samples were analyzed by mass spectroscopy to detect the changes of flavonoids in AV fibers before and after the degumming process. Mass spectra of the two samples were obtained using electrospray ionization in the negative ionization (NI).

Fig. 1 shows the ESI-MS spectra of the extract of the bast of AV and the AV fibers. It can be seen that these two MS spectra were obviously different. The ESI-MS profiles of the extract of the bast of AV showed three main peaks, which were identified at m/z 377.1 $[M-H]^-$, m/z 463.1 $[M-H]^-$ and m/z 625.1 $[M-H]^-$. However, these fragments cannot be detected from those of the AV fibers with the same ESI-MS measurement. Due to the nature of the natural fibers, it is suggested that flavonoids may be existed in the natural AV. And the quercetin was supposed to be one of the components of AV, whose structure is shown in Fig. 2. The peak at m/z 377.1 $[M-H]^-$ was contributed to the disaccharide, in which two monosaccharide were connected to form stable disaccharide structure. Due to the nature of the AV fiber, the fragment at m/z 463.1 $[M-H]^-$ was ascribed to the combination of one quercetin molecule and one saccharide molecule, which released one H_2O molecule during the process. The appearance of the peak at m/z 625.1 $[M-H]^-$ was suggested to be due to the chemical combination process of quercetin with two monosaccharide molecules.

To further confirm if the flavonoid components were existed in the AV sample, the secondary MS technique was used to analyze the corresponding three fragments and the measurement of high resolution time-of-flight (TOF) mass spectroscopy was performed. Fig. 3 shows

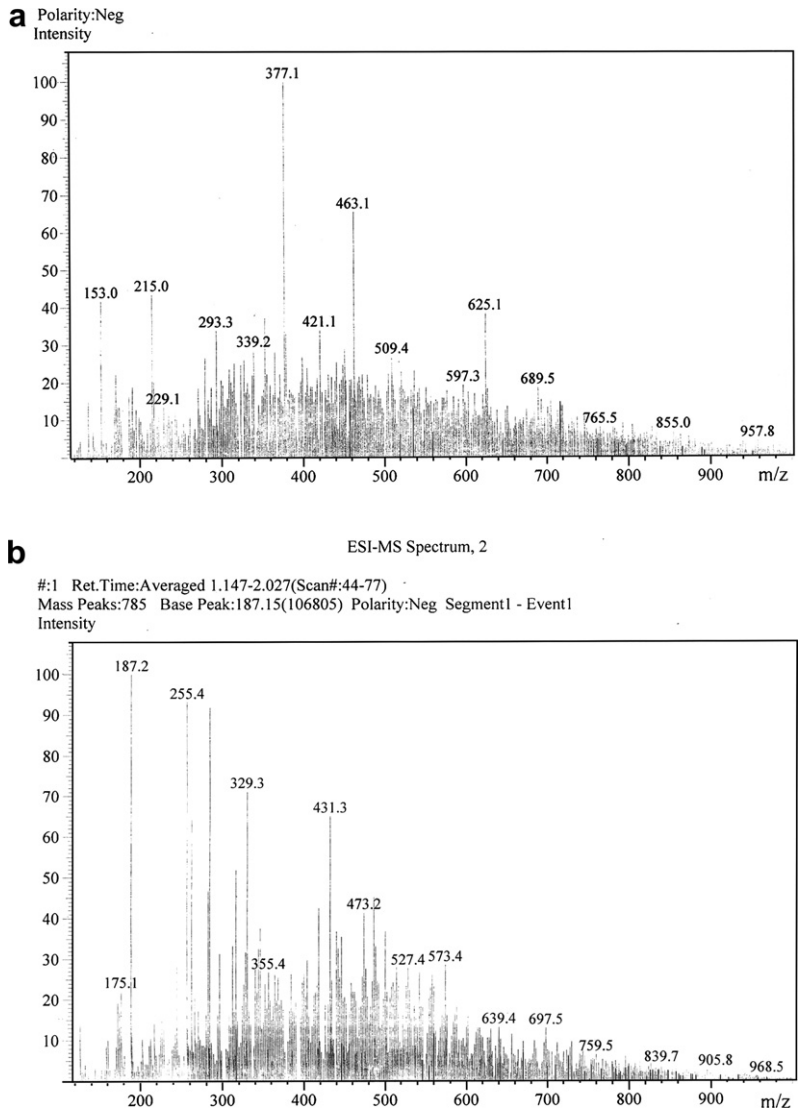

Fig. 1. ESI-MS profiles of the extract of the bast of AV (a) and that of the AV fibers (b).

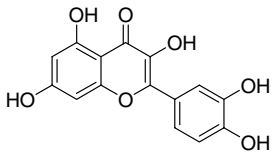

Fig. 2. The chemical structure of quercetin.

the secondary TOF-MS results of these three fragments at *m/z* 625.1 $[M-H]^-$, *m/z* 463.1 $[M-H]^-$ and *m/z* 377.1 $[M-H]^-$. From the TOF-MS spectra, it can be seen that both the pseudomolecular ions at *m/z* 625.1 $[M-H]^-$ and *m/z* 463.1 $[M-H]^-$ showed fragment ions at *m/z* 300.6 $[M-H]^-$, which further indicated the presence of quercetin in the natural AV. The peak at 341.6 $[M-H]^-$ from the results of the pseudomolecular ion at *m/z* 377.1 $[M-H]^-$ was ascribed to disaccharide. From these TOF-MS results, it can be concluded that the flavonoids in the bast of AV disappeared or tailed off after pretreatment methods. It is suggested that flavonoids consisted in the natural AV maybe reacted or degraded due to the strong base or acid conditions during the nature of the chemical degumming process. These MS results can give some evidence that health care and antibacterial functions of the AV fabrics maybe not mainly attributed to flavonoids.

3.2. AFM analysis

To get further understanding of the AV fiber microstructure, the structural characterization of AV fibers was conducted by AFM and compared with that of ramie fibers. Fig. 4 shows the AFM images of the AV fibers and the ramie fibers. It can be seen that the AV fibers and ramie fibers show interesting textures. Both the AV fibers and the ramie fibers had rippled surfaces consisting of stick which form ribbon-like structure extending along the fiber axis in twisting each other. The AV fibers surface

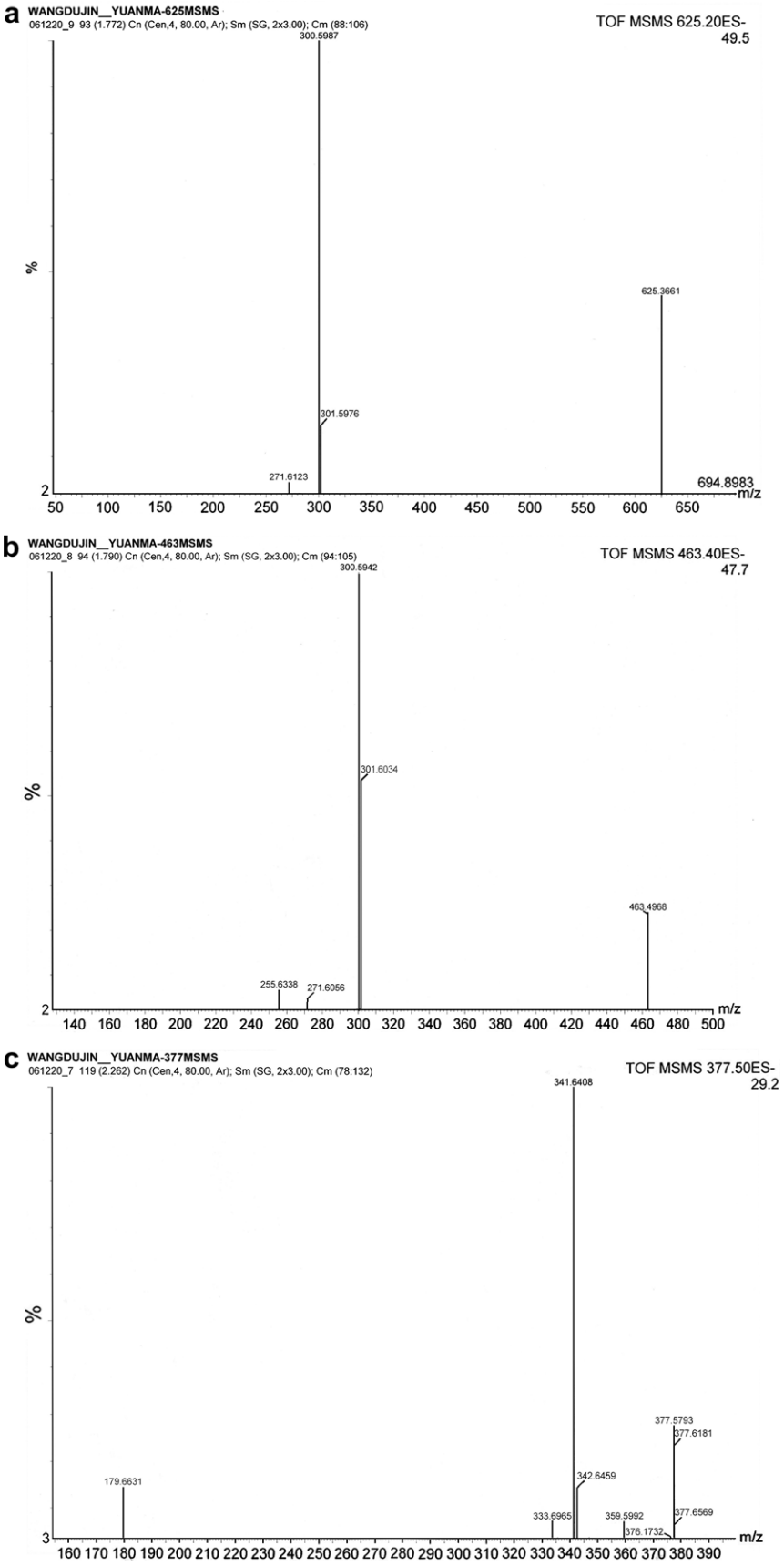

Fig. 3. The high resolution TOF-MS spectra of the pseudomolecular ion at *m/z* 625.1 $[M-H]^-$ (a), *m/z* 463.1 $[M-H]^-$ (b) and *m/z* 377.1 $[M-H]^-$(c).

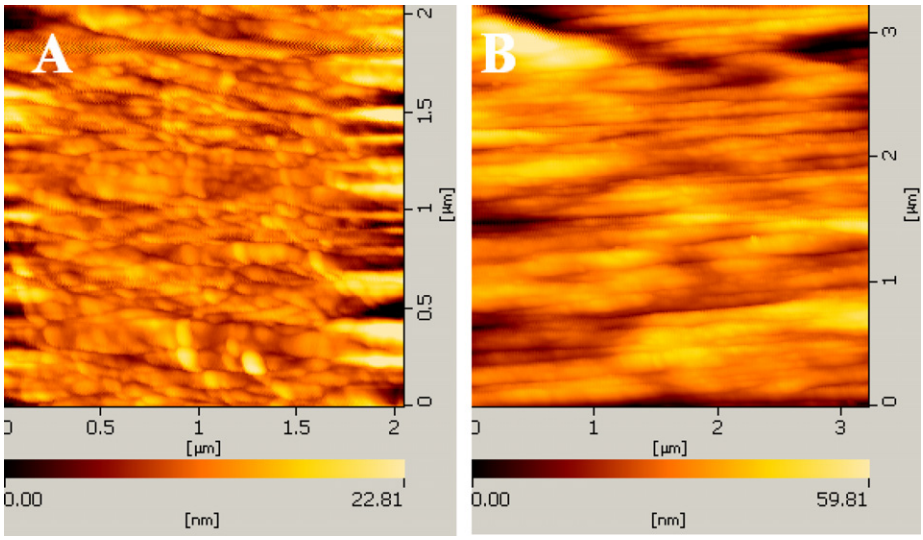

Fig. 4. AFM image of AV fibers (A) and ramie fibers (B).

structures were seen to have rougher and clearer thread structures. And as seen from the AFM images, the structure of the AV fibers had clear small opening between sticks which made the microstructure of AV fibers were looser than that of the ramie fibers. The small opening between sticks in AV fibers can improved breathability and keep the AV fabrics dryness which can destroy the growth environment of bacteria.

4. Conclusion

The component analysis of the extract of the bast of AV and the AV fibers was investigated by ESI- and TOF-MS. The experimental results showed that the flavonoids, which consisted in the extract of the bast of AV, disappeared or tailed off in that of the AV fibers. Furthermore, the microstructure of AV fibers and ramie fibers were characterized by AFM. Both fibers had rippled surfaces and the microstructure of AV fibers was looser than that of the ramie fibers. And the research results disclosed that health care and antibacterial functions of AV fabrics maybe not directly attributed to flavonoids. The small opening between sticks in AV fibers can improve breathability and keep the AV fabrics dryness. These results may help to further clarify the mechanism of the functions of health-care and antibacterial of the AV fabrics.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 50573035). The authors are very indebted to Prof. Dujin Wang and Prof. Shannong Zhu at Institute of Chemistry, the Chinese Academy of Sciences, for their kind help in structural characterization of the fibers. We also thank Dr. Peizhi Guo at Qingdao University for helpful discussions.

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