Université de Lorraine
Faculté des Sciences et Technologies
Ecole Doctorale (Sciences et Ingénierie) Ressources, Procédés, Produits et Environnement (RP2E)

THESE

Pour obtenir le grade de Doctorat d‟Université
Discipline : Sciences du bois et des fibres
Soutenue à Nanjing le 19 octobre 2012

Présentée par

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Condensed tannins extraction from grape pomace: characterization and utilization as wood adhesives for wood particleboard

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Acknowlegement

This dissertation was finished under Professor Nicolas Brosse and Professor Zhou Dingguo direction. They have put a great deal of time and effort in thesis topic selection, research scheme customization, thesis modified and finalized. On the occasion of the accomplishment of this dissertation, I express my sincere gratitude to my supervisors. Their rigorous thinking capacity in academic and wisdom generosity style in taking on things, which give great inspiration to me. It is a great wealth worthy of keeping lifelong.

Sincerely thank Associate Professor Laurent Chrusciel from Lermab (UHP Nancy 1 university, France) for meticulous guiding my experiments, as well as helping me apply for scholarship, visa and registration; I would like to thank Professor Christine Gerardin for his help in sample freeze drying ; thank Engineer Mehdi Yemloul and Lyne Desharnais for their help in sample analysis; thank Doctor Roland El Hage for his great help in my experiment, as well as his wife Maria El Hage caring of my life; thank doctor Shi Yuting for giving warmly help and to me. I also sincerely thank Professor Antonio Pizzi from enstib lab (UHP Nancy 1 university, France) for providing valuable advice in research programmes and experimental ideas; Doctor Paola Navarrette helped me in wood particleboard pressing and performance testing. I would prefer to thank Madam Christine Fivet from RP2E doctor school helped me in doctor registration and thesis defense.

During my Ph.D study period, I have gotten kindly advice and guidance from Professor Xu Yonglan, Professor Zhang Yang, Professor Jin Juwan, Professor Zhou Xiaoyan, Professor Mei Changtong, senior engineer Wu Yufei and Professor Lian Hailan from college of wood science and technology, Nanjing Forestry University, and great help from Associate Professor Pan Mingzhu, Yang Rui, Li Xiancheng, Wu Jinsheng and Zhao Qingwu from fast-growing tree and agriculture fiber material engineering center. Thanks for all the teachers and colleagues who support and help me!

Finally, sincerely thank my family who give great spiritual and material support to me. Special thanks to my father Lan Shiqiu and mother Lei Jinjiao give me neticulous care, my mother-in-law Lu Aiying takes care of my life and looks after my young son Chen Kaiye, my husband Chen Feng takes care of my life and supports my study. Thank them for their silence understanding and support. This dissertation is dedicated to them!

Lan Ping
October, 2012 in Nanjing

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LIST OF ABBREVIATIONS

^{13}C-NMR: Carbon nuclear magnetic resonance spectroscopy

DSC: Differential scanning calorimetry

DTG: differential thermo gravimetric curve

FT-IR: Fourier transform-infrared spectroscopy

FAO: Food and Agriculture Organization

HPAEC-PAD: high-performance anion-exchange chromatography with pulsed amperometric detection

IB: Internal bond strength

MALDI-TOF: Matrix assisted laser desorption ionization time of flight mass spectrometry

MOE: Modulus of elasticity

OIV: International Vine and Wine Organization

TMA: Thermo mechanical analysis

TGA: Thermogravimetric analysis

UV: Ultraviolet

DMSO-d_6: Dimethylsulfoxide-d_6

DHB: 2, 5-Dihydroxy benzoic acid

p-MDI: Polymeric 4, 4’-diphenyl methane diisocyanate

m_1: Weight of oven-dry crucible and lid

m_2: Weight of the crucible with oven-dry sample

m_3: Weight crucible with ash sample

T: Temperature

V_O: Volume of original filtrate

V_D: Volume of diluted filtrate

Abstract

The extraction of condensed tannins from grape pomace was examined using water medium in the presence of different bases as catalyst (NaOH, Na_2CO_3, $NaHCO_3$ and Na_2SO_3). Two different extraction processes and the influence of 4 parameters (i.e., temperature, reaction time, chemical reagents and concentration of the chemical reagents) on the tannin extracts yields and properties were studied. The tannin fractions were characterized by Fourier transform-infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), carbon nuclear magnetic resonance spectroscopy (^{13}C-NMR) and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF).

It was demonstrated that it is possible to extract reactive tannins from grape pomace in basic medium. The tannin extracts obtained by precipitation in acidic conditions display a high phenolic content (Stiasny number higher 95%) but low water solubility and low reactivity toward formaldehyde because of the formation of catechinic acid phlobatannins during the acidification step. The tannins extracts obtained by lyophilization of the liquid, despite of their lower phenolic contents, displayed promising properties for adhesive applications. The optimum temperature of the extraction process was 100 °C, reaction time was 120 min; the best concentration of reagent was 10% (*w/w*). It was also shown that addition of sulphite ions during the extraction step improved the process :y the introduction of a sulfonic group through sulfitation increased both tannin solubility and reactivity as a result from the opening of the heterocyclic ring during extraction

The structure of grape pomace sulfited tannin extracts did not present noticeable discrepancy exception of the ratio of the opening pyran ring which was different as a function of the catalyst used. The opening of pyran ring during the tannins extraction seem to be more important by using Na_2CO_3 and $NaHCO_3$ than when using NaOH. The FT-IR bands assigned to aromatic ring vibration and carbonyl groups were stronger and no bands attributed to sulfited groups were tested detected ?? in acidified tannins compared to lyophilized tannins. The TG results showed that the weight lost of these tannin extracts mainly composed of two steps. The first step starts from room temperature to 200 °C. It is attributed to the mass lost of water and some easy-degraded small low molecules. The secondly steps which is the mainly degradation step of tannin extract samples from 200 to 400 °C. The results from ^{13}C-NMR showed that condensed tannin extracts from grape pomace were consistent with dominant procyanidin units with a minor amount of prodelphinidin units that are linked together by a C4-C8 bond. Relatively low carbonhydrate and high catechinc acid content was obeserved in acidified tannins compared to lyophilized tannins. It was shown by MADI-TOF experiments that grape pomace tannin extracts are mainly composed of flavoinoid oligomers up to 6 repeating units in lyophilized tannins and 10 repeating units in acidified tannins respectively, with dominant procyanidin units. A small proportion of substitution with glucose and gallic acid was detected in procyanidin units of polyflavonoid oligomers. The degree of polymerization of acidified tannins is higher than lyophilized tannins.

Two different formulations (nonfortified tannin adhesive and fortified with addition of 20% of polymeric 4, 4’-diphenyl methane diisocyanate (*p*-MDI)) were used to press one layer wood particle board. It was shown that the nature of the extraction reagent (NaOH, Na_2CO_3 or $NaHCO_3$) greatly impacts the properties of the resins and the mechanical properties of the panel internal bonding strengths. The particleboards bonded by the tannins extracted using Na_2CO_3 as catalyst give the best performance and were good enough to pass relevant international standard specifications for interior grade panels. Formaldehyde emission of these panels was below the European Norm requirements (≤ 6.5 mg/ 100g panel).

Keywords: grape pomace; condensed tannins; tannin-based adhesives; wood particleboard; formaldehyde emission

Chapter 1. Introduction

1.1 Bibliography study

1.1.1 World grape and wine industry

The grapes are the largest planted fruit in the world, accounting for more than 10 % for the global fruit cultivation area; the grape industry represent the highest proportion, the longest industrial chain and the most important production in the world. Around 75 % the world's grape production is used to make wine, dried food and juice processing. The grapes cultivation area increased greatly and reached its peak in the 1980's, then appeared relatively shrink in 1991-1995; after that time the development stabilized without big fluctuation.

According to the United Nations Food and Agriculture Organization (FAO) and International Vine and Wine Organization (OIV) statistics in 2008, the world grapes cultivation area were basically stable about 7 900 000 hm^2 in 2002-2004. It declined after 2005, about 7 872 000 hm^2 in 2005; the area is 7 408 000 hm^2, accounting for 12.3 % of the total area of the world fruit in 2008, which is the largest planting fruit, the followed fruits are plantain, apple banana and mango. The world grape production zone is relatively concentrated; the distribution of the grape cultivation area in every continent is inequality. Fig.1.1 is all continents grape planting area statistics in 2008, Europe grape cultivation area closed to 54.9 %, Asia closed to 23.8 %, the remaining part were distributed in the other 3 continents, of which nearly 13 % in the American (Tian et al., 2011). Table 1.1 gives the main countries for grape planting area in all continents (Tu et al., 2009). There are 6 countries in the world (Spain, France, Italy, Turkey, China and United states) for which grape planting area is more than 300 000 hm^2. Europe is the world's largest grape production zone, the planting area occupies more than half of the total area, in which Spain is the biggest grape planting country and France is the traditional power grape planting country. In recent years, the ratio of Europe cultivation area gradually decreases, Asia and American ratio gradually increases, the growth of Asia is more remarkable due to the quickly rise of grape industry in China, Iran. China grape cultivation area grew significantly, ranked from the tenth spot in 1995 to the fifth spot in 2007 in the world.

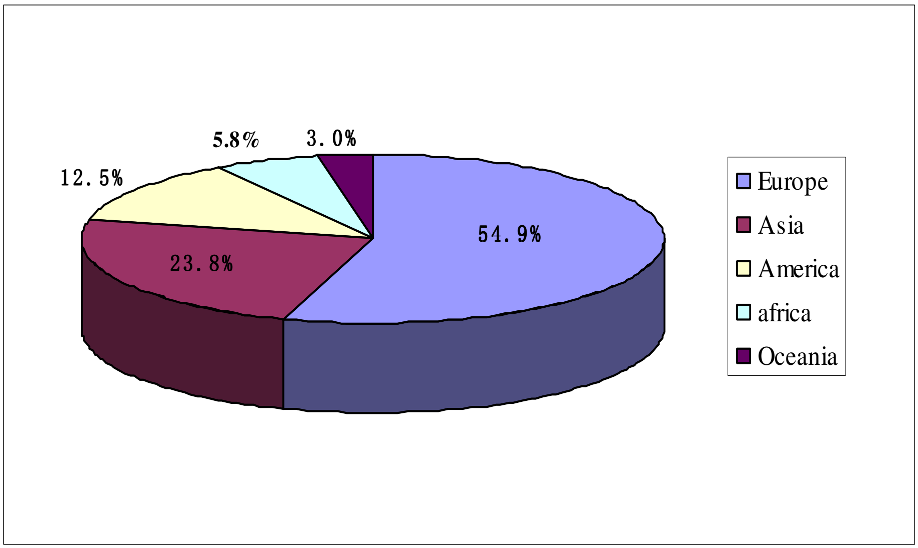

Fig.1. 1 The distribution of continent grape cultivation area (2008)

Table 1. 1 The main world countries of grape cultivation area (thousand hm^2)

conutries	2000	2001	2002	2003	2004	2005	2006	2007
Spain	122.9	121.1	120.1	120.7	120.0	118.0	117.4	116.9
France	**90.7**	**90.0**	**89.8**	**88.8**	**88.9**	**89.4**	**88.7**	**86.7**
Italy	90.8	79.8	79.7	79.1	78.7	80.2	80.2	80.0
United States	41.2	42.6	41.5	41.5	39.8	39.9	40.6	40.9
China	**30.4**	**35.9**	**42.1**	**45.5**	**46.0**	**48.5**	**49.0**	**49.0**
Iran	29.2	30.1	30.2	30.0	32.9	33.8	33.8	-
Turkey	57.5	56.4	57.0	57.0	55.9	55.5	52.7	52.5
Portugal	24.6	24.8	24.9	24.9	24.7	24.8	24.9	24.8
Romania	24.8	24.7	24.3	24.0	22.2	21.7	21.3	20.5
Argentina	20.1	20.4	20.8	21.1	21.3	21.9	22.3	23.0
Chile	17.4	18.1	18.4	18.5	18.9	19.3	19.5	19.7
South Africa	12.4	12.6	12.9	13.2	13.3	13.4	13.4	13.5
Australia	14.0	14.8	15.9	15.7	16.4	16.7	16.9	17.4
Moldavia	14.7	15.5	15.3	14.9	14.6	14.7	14.7	14.7

The grape production is correlated to the grape planting area, but it is also affected by the varieties, cultivation, pest and disease and other factors. Overall, the annual yield of grape fluctuations is more obvious than the area, however, the change are relatively stable in recent years. Global grape crop is 67.71 million tons in 2008; slightly riser than 2007, which was benefited from breeding, cultivation techniques and pest control technology. The average per unit area yield obtained in 2008 higher than before, which was 9 140 Kg/hm^2, grew more than 20% compared to 1995. The distribution of grape yield in all continents is not balanced also, European production accounted for the absolute superiority. It was accounted for above 60 % in 1990, but now has been reduced to 42%; Asia is the most rapid rise area, from 15% in 1990 to nearly 1/3 of world output now, American, Africa and Oceania appear slow growth trend (Tian et al., 2011).

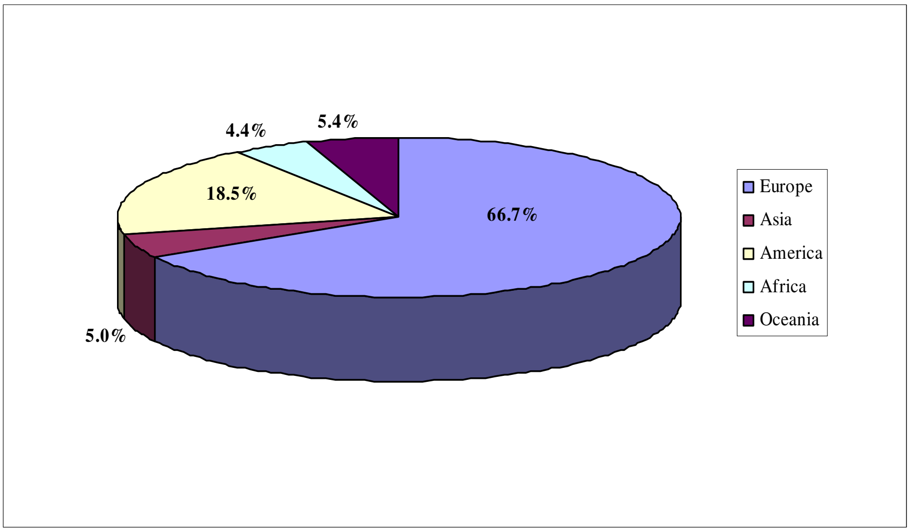

Fig.1. 2 The distribution of world wine output (2008)

Wine industry is the main part of the grape international trade, the trade volume is far more than fresh grapes, raisins and grapes juice. The world wine production reflects the world grape yield condition from a profile. It can be seen from Fig. 1.2 that Europe is the 1st wine producer, accounting for 66.7 %, followed by the America, accounting for 18.5%, the other continents are accounted for 4-5 %. Fig.1.3 and Fig.1.4 is the top ten world wine production and consumption countries in the world. France occupied the second and China ranked the seventh in the manufacture countries, France occupied the first and China ranked the fifth in the consumer countries.

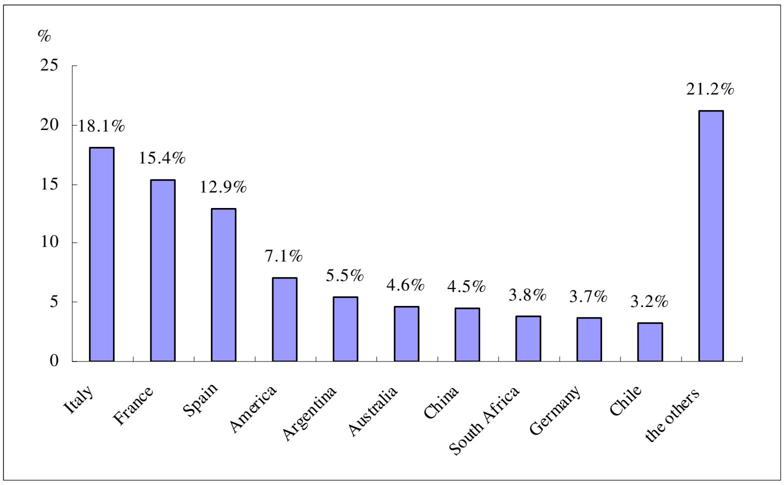

Fig.1. 3 The top ten world wine produced countries (2008)

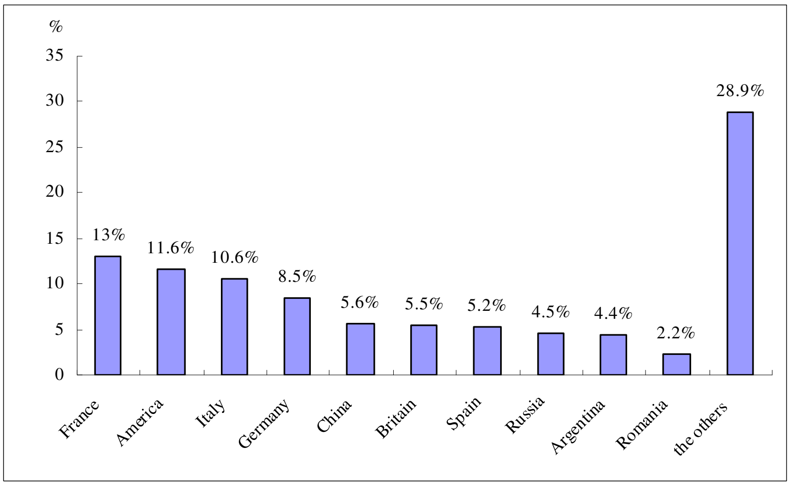

Fig.1. 4 The top ten world wine consumed countries (2008)

According to the latest data from OIV, the world wine total output was 26 billion liters in 2010, although compared to 2009 France wine production fell about 2%, still with 4.475 billion liters back to the world's first location. Italy was in second location with 4.258 billion liters, Spain kept the balance to the last year with 3.514 billion liters ranked the third location. China is a new wine production country compared to European major wine producing and consuming counties like France. There are more than 500 wine production enterprises in China now. Since few years, we observe an unprecedented extraordinary performance about China wine market regardless of the production, import, marketing, distribution, investment as well as the understanding of the wine and the level of consumption. Wine production was 0.636 billion liters from January to August, up about 15 % year-on-year; wine import was 0.202 billion liters from January to September, up about 67 % in 2010.

1.1.2 The comprehensive utilization of grape pomace

Grape is one of the world's largest fruit crops and grape pomace is one of wastes generated by grape juice and wine-making processes. The pomace consists of skins, seeds, and in certain case of some stems, with the skins and seeds making up the major part. It represents approximately 25-30% (*w/w*) of the harvested grapes after pressing (Laufenberg et al., 2003; Zhang et al., 2008). Only in France, about 700 000 tons of natural dry grape by-product were produced in 2009; in China, a large number of grape residue is also produced with the rapid development of wine industry in recent decades. So far, only small amounts of these by-products are up-graded or recycled, most of them still used for low-valued animal feed or soil fertilizer (Peng et al., 2010).

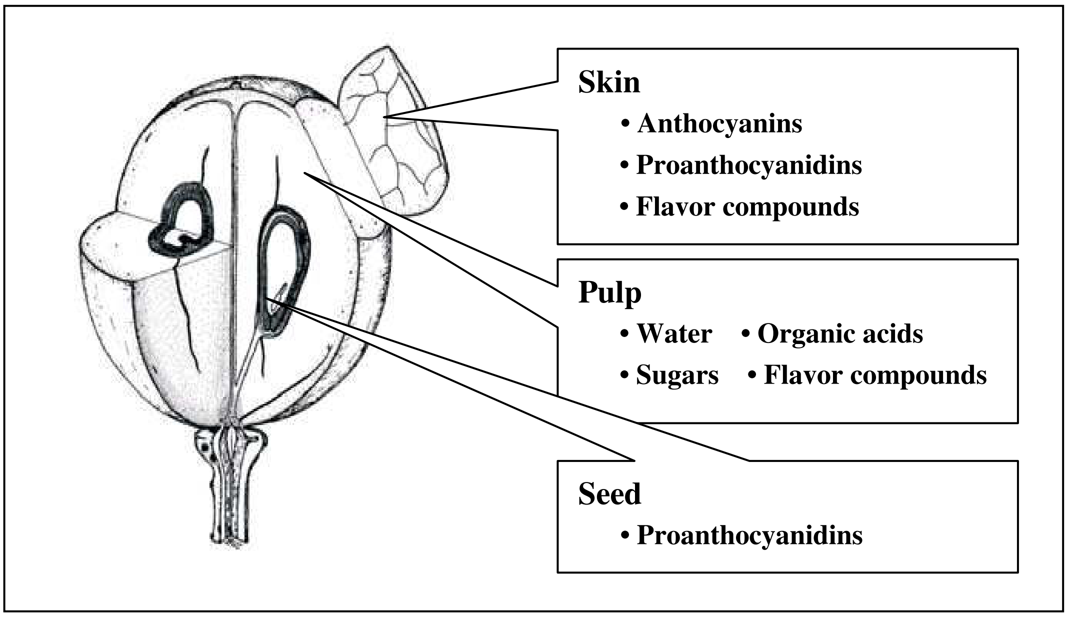

Fig.1. 5 The main chemical compounds distribution within the grape berry

The recovery of by-products from agriculture industries to be converted into value-added products has attracted great interest in the past several years. The main chemical compounds distribution within the grape berry can be seem in Fig.1.5 (Kennedy, 2008). Grape berry is mainly composed of anthocyanins, proanthocyanidins, water, organic acids, sugars and flavor compounds. The anthocyanins and proanthocyanidins are mainly in grape skin and grape seed; the other chemical compounds are in grape pulp. The wine-making processes are mainly press water, organic acids, sugars and flavor compounds for grape pulp, leave grape skin and grape seed which still retains high levels of proanthocyanidins and other polyphenols which will pose potentially pollution problems when it is used as soil fertilizer in a short period (Northup et al., 1998).

Since the first observation of the “French paradox” (Renaud and De Lorgeril, 1992), many recent studies have highlighted the beneficial effects of grape or wine polyphenols for human health. As a consequence, considerable efforts have been devoted to optimizing the extraction of high purity phenolic compounds from winery by-products for food additive applications but also for cosmetic and pharmaceutical industries (Monagas et al., 2006; Ozkan et al., 2004; Torres et al., 2002; Bonilla et al., 1999). In recent years, researcheres in China have gradually developed the value-added productions from grape pomace. Mainly extracts pigment, flavour compounds, proanthocyanidins, tartaric acid and pectin from grape skin and produces oil, oligomeric proanthocyanidins and tannins from seed (Kang et al., 2003; Liu et al., 2006; Li et al., 2006; Wei et al., 2001; Ju et al, 1990). In addition, researchers have extracted *resveratrol* from grape pomace using ultrasonic and macroporous absorption resin method, which is a kind of stilbene structure non-flavonoid polyphenols compounds (molecular formula $C_{14}H_{12}O_{3,}$) (Kang et al ., 2006, 2007 ; Li et al., 2007 ; Yao and Qi ., 1999 ; Du et al., 2007). *Resveratrol* as natural products in grapes, with many physiological and pharmacological activities, especially plays an important role in the prevention of cardiovascular disease and cancer.

Some researches have been done on the development of high value-added products from grape pomace, but the techniques generally employed used organic solvents (Guerrero et al., 2008; Negro et al., 2003; Ruberto et al., 2007; Spigno and De Faveri, 2007; Pinelo et al., 2005;

Makris et al., 2007; Monrad et al., 2010) or supercritical CO_2 (Pinelo et al., 2007; Vatai et al., 2008) for the isolation of low yield/high purity extract fractions which required high technology in equipment and processing. Until now, the high amounts of grape pomace produce by wine industry mainly used for fertilizer and animal feed.

1.1.3 The structure of tannins

Tannins also called vegetable tannins, are secondary plant metabolites, which are traditionnaly used for tanning leather. Tannins can be divided into hydrolysable tannins and condensed tannins based on the chemical structural characteristics by Freudenberg in 1920. Hydrolysable tannins are complex compounds composed of polyphenolic acid and sugar (mainly is *D*-glucose) or other material (such as polylols), bonding with ester or glucoside linkage. These compounds are easy to hydrolysis into poly alcohols and polyphenolic acid (gallic acid and ellagic acid) under the action of enzyme, acid or in alkali conditions (Fig. 1.6). Hydrolysable tannins can be divided into gallotannins and ellagitannins according to the hydrolysis products. Condensed tannins are composed of flavan-3-ol units (such as catechin or its derivatives of galloctechin) linked together by C-C linkage, which are not easy to decomposed in aqueous solution (Sun., 1988). Tannins widely exist in the plant stem, bark, roots, leaves and fruits.

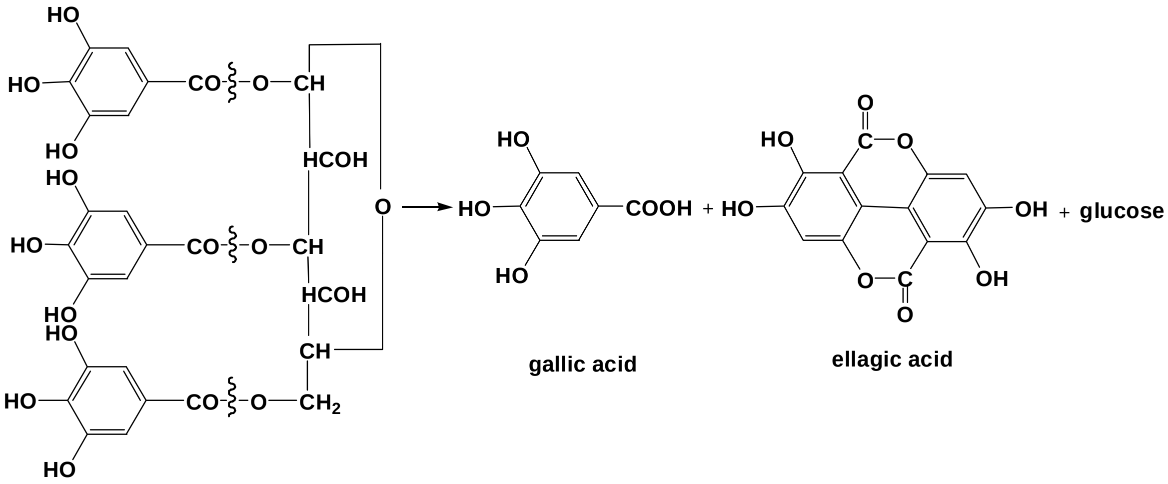

Fig.1. 6 The hydrolysis of hydrolysable tannins

Proanthocyanidins (condensed tannins) are natural polymers which contain polyflavonoids and can be found in the bark, stem, phloem, seeds, fruits, wood leaves and needles of dicotyledon plants (Zuker., 1983; Hemingway and Karchesy., 1989). condensed tannins are polyphenolic compounds with a molecular weight (*Mw*) ranging from 500 to 3000 Da (Hemingway and Karchesy., 1989) and they are used in several industrial applications such as leather tanning and wood adhesive (Pizzi., 1983). Condensed tannins extracts are mostly composed of flavan-3-ols repeating unit (Fig. 1.7), smaller fractions of polysaccharides and simple sugars.

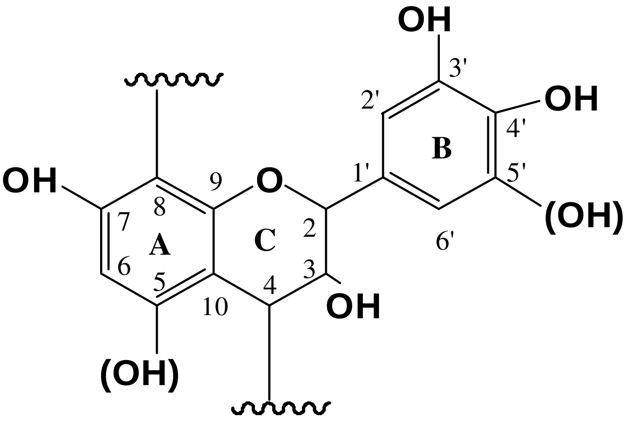

Fig.1. 7 The chemical structure of condensed tannins

There are some fundamental differences between different polyflavonoid tannins that can be used for thermosetting wood adhesive. Flavonoid units in such tannins present phloroglucinol or resorcinol A-rings and catechol or pyrogallol B-rings. The classification of the polymers composed by different repeating units is shown in Fig. 1.8. Different tannins are composed by different repeating unit. Some researches display that minosa and quebracho tannins are resorcinol-like tannins and pine extracts phloroglucinol-like tannins. The electron cloud density of in the aromatic ring of phloroglucinol A-ring is increased by the influence of two electron-donating group (phenolic hydroxyl,-OH), especially in C6 and C8 position. Thus, an electrophonic substitution reaction is facilitated due to stronger nucleophilicity of the aromatic ring. This is why pine tannins, mainly composed by phloroglucinol A-ring, exhibite higher reactivity and faster curing speed(Pizzi., 1994).

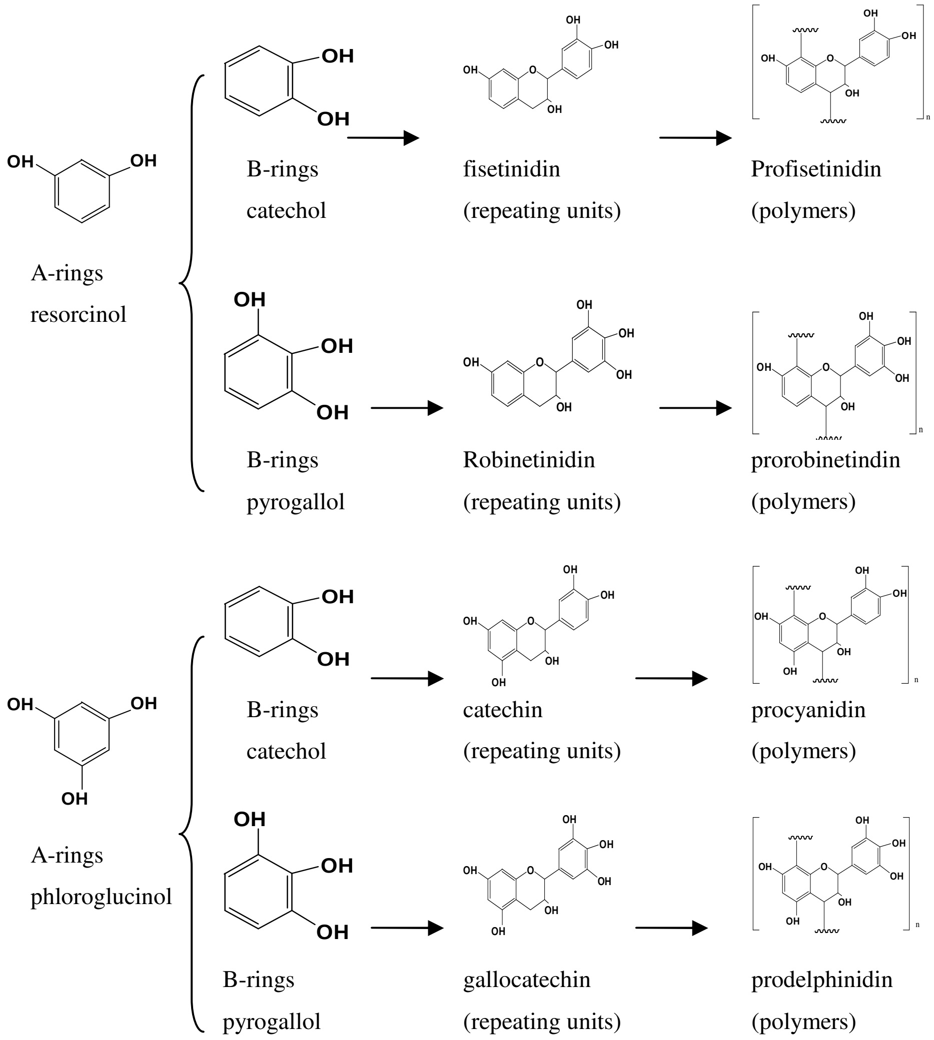

Fig.1. 8 The classification of the condensed tannins polymers

The flavonoid repeating units are mainly linked to each other at C4-C6 in profisetindins and prorobinetinidins. On the other hand, the repeating units in procyanidins and prodelphinidins are linked at C4-C8. The linkage of the repeating units is also different in different tannin extracts. The possible linkage in the mimosa and quebracho tannins is: C4-C6 between resorcinol A-ring unit and phloroglucinol A-ring unit < C4-C8 between resorcinol A-ring units < C4-C6 between resorcinol A-ring units. It can be seen that the main linkage is C4-C6 link. The pine tannins mainly linked by C4-C8 (Hoong et al., 2010; Lei et al., 2008; Pizzi., 1982). If the monomers are linked only through C4-C6 or C4-C8 linkages, the resulting tannins molecule exhibits a linear structure; on the other hand, if monomers are linked through both C4-C6 and C4-C8 linkages, the structure presents angular unit. The reactivity will be very low even no reactivity towards to formaldehyde in branched tannins. According to Pash et al (2001) that mimosa tannin is heavily branched due to the presence of considerable proportion of angular units in its structure while quebracho tannin is almost completely linear. These structural differences also contribute to the considerable differences in viscosity of water solutions of the two tannins.

To the best of our knowledge, polyflavonoid tannins extracted from grape pomace, the structure of their main oligomer repeating units and their degree of polymerisation distribution have never been studied. It is necessary to define their characteristics to verify of these tannin fractions used for industrial exploitation.

1.1.4 The research progress of tannin-based wood adhesive

The adhesive used in the early wood industry mainly was natural polymer material, but it was gradually replaced by petrochemical products from the beginning of 19th century (such as PF, UF). Now, natural adhesives (such as tannin-based adhesive, soybean protein adhesive and lignin adhesive have attracted attention again with the rising of petrochemical product prices and strengthen of peoples environmental protection awareness. Tannin-based adhesive is the most successful natural adhesive on study and application, which has been used in industrial production in many countries.

Tannin-based adhesives are similar to the phenolic resin adhesives and are produced from tannins as raw material by addition of appropriate curing agents. Its main characteristic is high chemical reactivity and fast curing speed. Due to its similar chemical structure compared to phenol, tannin can replace a part or totally the phenol material in phenolic resin adhesive preparation,. Condensed tannins accounted for 90% of the tannins and have a high economic value as raw chemical materials for the production of green adhesives and resins. Condensed tannins and the flavonoid monomers are widely distributed in nature, especially in the bark of minosa, quebraocho, chestnut, valonea and pine. At present, the commercial tannins include pine, mimosa and quebraocho tannins (Pizzi., 1982; Gu., 1999; Vazquez et al., 2000; Panamgama., 2007; Fradinho et al., 2002).

The preparation of tannin-based adhesive has a long history over 30 years; a depth research had been studied and developed some kinds of industrial tannin adhesive with good performance meet the industrial requirement in South America, Australia and South Africa. Industrial tannins not only can be directly used for wood adhesive, but also could replace part of phenol for phenolic resin production and as filler or additives on phenolic resin, urea-formaldehyde resin modification. The research of tannin-based adhesive started in the 70's and caused widespread concern in the early 90's of last century in China.

Tannin-based adhesive are obtained by hardering of polymeric flavonoids of natural origin, or condensed tannins, by polycondensation with a cross linker agent. The common cross linker agent used in tannins-based adhesive are formaldehyde, normally used for formalin solution or paraformaldehyde which can quickly release formaldehyde in alkaline conditions. Tannins reactivity toward formaldehyde can be adjusted by dilution, pH value and addition of alcohols. The most commonly used alcohol is methanol, which can be combined with formaldehyde to form $CH_2(OH)(OCH_3)$. When the resin is heated, formaldehyde is gradually released in response to tannins.

Based on it higher reactivity, action of formaldehyde is between 10 and 50 times faster than the reaction of phenol with formaldehyde. On the other hand, it is not neceasry to prebuild the polymer as in the reaction of phenols with formaldehyde, this leads to low amounts of formaldehyde being required. Nevertheless, it still exists formaldehyde emission problem. Because of its toxicity, a extensive research is carried out to reduce formaldehyde using new alternative to formaldehyde. Instead of formaldehyde, non volatile cross linker agents can be used (such as: trishydroxymethylnitromethane (TN), Hexamethylenetetramine and glyoxal) with no aldehydes production or through generation of aldehydes in situ (Kim et al., 2003;

Heinrich et al.,1996; Ballerini et al., 2005).

On the other hand, self-condensation can be used to reduce formaldehyde emission. The principle of self-condensation is breaking the flavonoid monomer's heterocyclic ether bond O1-C2 to react with other tannin's C6 or C8. This condensation reaction just increases the viscosity but not gelatin. Pizzi (1995) showed that the reaction of auto condensation four commercial tannins was carried out when catalyzed by a lignocellulosic substrate or by a weak Lewis acid as alkali dissolved silica. Pecan nut tannin and pine tannin need only lignocellulosic induced auto condensation to give excellent interior grade particleboard. Slower reacting tannins such as mimosa need instead both the catalytic effect of the substrate as well as that of a weak Lewis acid such as silica. Only pecan nut tannin appears at this stage to be able to give boards of acceptable dry internal bond strength at industrially significant pressing time.

Based on structure characteristics, there are still some problems in tannins-based adhesive application. High molecular weight, high viscosity, high reaction activity toward formaldehyde, short application period, low bonding strength, poor water resistance, formaldehyde emission by using formaldehyde as harder are (Lei et al., 2008) drawbacks which restrict utilization of condensed tannins in wood adhesive industry. Some solutions have been proposed to overcome these drawbacks. Utilization of acid or alkali treatments not only can damage gums structure to reduce the viscosity, but also decrease tannin's molecular weight, increasing resin flow and improving tannins reaction toward to formaldehyde. At the same time, this treatment can increase resin flexibility and extend water residence time by opening flavonoid structure of heterocyclic ring (Pizzi., 2003; Hoong et al., 2009. Sowunmi et al (1996) treated tannins extract with acetic anhydride and then sodium hydroxide followed by modification with 20 % resole-type PF resin. This treatment significantlty affected the chemical structure and reactivity of tannins: heat of reaction of tannin with formaldehyde was increased while the activation energy was drastically reduced. Premature cure was also reduced. It was demonstrated that these fortified formulations had good plywood adhesive properties. Chen (2007) illustrated the reaction of larch bark tannin extracts with sulfite and sodium hydroxide and tested its solution viscosity. It was shown that the solution viscosity was dropped and the relative molecular mass was reduced with narrow distribution. The high sulfited larch bark tannins extracts were more available to decrease the solution viscosity than the alkali treated extracts. The modified larch bark tannins extract was used for the production of bakelite bond.

There are lots of reports about tannin-based adhesive applied in exterior and interior plywood and particleboard since 70's of last century. Now, people focus again on the natural adhesive due to the shortage of energy and improvement of human's environmental awareness. Industrial tannins not only can be directly used for wood adhesive, but also could replace part of phenol for phenolic resin production and as filler or additives on phenolic resin, urea-formaldehyde resin modification(Lei et al., 2008 ; Tondi and Pizzi., 2009). However, not all the tannin extracts can be used as wood adhesive based on its structural characteristics and composition. Yazaki and Collins (1994) examined the suitability of barks from four mature pine species, one young pine species and one spruce species. Only the bark extracts of *pinus caribaea* and *pinus pinaster* gave high quality wood adhesives. At present, the variety of industrial tannins suitable to wood adhesive is less and mainly is condensed tannins, which is mainly derived from bark of some wood species rich in tannin (mimosa tannins, qeobracho tannins and larch tannins).

Wine industry produces a large amount of grape pomace every year, although in recent year, people gradually realized the grape pomace is rich in anthocyanins, proanthnocyanins and other polyphenol compounds, and start to purified for pharmaceutical and cosmetic industries, but the utilization is few. Until now, most of the grape pomace only used for soil fertilizer or animal feed. Therefore, the author considers that we can extract condensed tannin from grape pomace and use as wood adhesive. However, the research and application still blanks in this field in the world.

1.2 The aim and innovation point of thesis

The main objective of this study is to extract condensed tannin from grape pomace for the production of wood particleboard. The goal is to explore a kind of environmentally

friendly natural adhesive, from its raw material, extraction technique and application, in order to provide technical support and theoretical basis for the future industrial application.

The innovation points of this study are:

1. The feedstock: condensed tannins are extracted from grape pomace, a low cost and abundant raw material. This work will allow an improved the use of an industrial by-product, currently underexploited.

2. The process: the extraction process developed in this study uses water medium and simple equipments, making possible a future large scale extraction and industriallisation.

3. The application: The tannin-based adhesives produced in this study were employed for the elaboration of green particule boards. This study proposes an economically and environmentally valuable utilization of grape pomaces.

1.3 The structure of thesis

Industrial tannin is a kind of polymer mixture polymerized by flavonoids composition, which also contains non-tannin components such as carbonhydrate and simple sugars. Therefore, the distribution of molecular weight and chemical composition are inhomogeneous. Not all the tannin extracts are suitable for wood adhesive due to its tannin content and structure characteristics varies by raw material and extraction technique. In this way, although there are a lot of reports on tannin extraction process and characterization, the industrial tannins used as wood adhesive are few. The goal of this study is extraction of condensed tannins from grape tannin use as wood adhesive. A system research was done on extraction process, structure characterization and application, the specific thesis structure as follow:

Chapter 1. Introduction

Introduce the development of world grape and wine industry, the comprehensive utilization of grape pomace and the research progress of tannin-based wood adhesive.

Chapter 2. Materials and Methods

Introduce the materials and methods used in this thesis.

Chapter 3. Extraction of condensed tannins from grape pomace

Comparison of two different alkali extraction processes, the influence of different extraction reagent (NaOH, Na_2CO_3, $NaHCO_3$ and Na_2SO_3), dosage of extraction reagent, extraction time, temperature on the yield and properties of tannin extracts was investigated.

Chapter 4. Characterization of condensed tannins from grape pomace

Characterization of tannin composition, molecular weight distribution and structure of tannin extracts by different extraction processes using Fourier transform-infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), carbon nuclear magnetic resonance spectroscopy (^{13}C-NMR) and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). FT-IR spectrometer was used to determine the presence of functional groups and structures of tannin extracts; TGA was applied to determine the thermal stability and degradation of tannin extracts; ^{13}C-NMR was used to investigate tannin extracts composition and structures, determine the oligomer type of condensed tannins; MALDI-TOF-MS was used to examine polyflavonoid tannin oligomers molecular weight. Then combined with the result of tannins' yield, effective condensed tannins content and the basic performance of tannins-based adhesive to optimal the tannin extracts suitable as wood adhesive.

Chapter 5. Utilization of condensed tannins from grape pomace in wood particleboard

production

The properties of tannins adhesive were compared in different extraction methods and different formulations. Physical properties and formaldehyde emission were determined in wood particle board production pressed by tannin-based adhesive. The purpose is to investigate the influence of different tannin extracts and different formulations on the properties of production.

Chapter 6. General conclusions and perspectives

Introduce the general conclusions and perspectives of this thesis.

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Chapter 2. Materials and Methods

The samples correspond to mixtures of by-products of the winemaking process of red grape variety (Vitis vinifera), growing in south-east of France and were provided by a French distillery (Franck Le Net, UDM). The air-dried grape pomace was composed by grape skin, seeds and stalks manually separated. It was stored at room temperature during the course of this study. In this study, all chemicals are marketed by Sigma-Aldrich and VWR International.

2.1 Condensed tannins extraction treatment

Twenty grams (oven-dry matter) of grape pomace were treated with an aqueous solution of sodium chemicals with a solid-to-liquid ratio of 1:8. The reaction mixture was heated at different extraction parameters. Concerning experiments conducted at atmospheric pressure (T = 70°C and 100°C), the treatments were carried out in a round bottle flask connected by water condenser. For reactions performed at 120°C, the treatments were carried out in a 4560 Mini Beach Top reactors with a Parr 4836 temperature controller (Fig. 2.1, Parr Instrument Company, Moline, IL) and the reaction mixture was heated at a rate of ~5 °C/min with continuous stirring. After heating, the pomace was cooled, washed and filtered through filter paper. Two different extraction paths were adopted in this study (Fig. 2.2). In path 1, 3 N HCl was added to adjust the pH at a 1.5 value in order to precipitate the acidified tannins, which were collected by centrifugation and then air-dried. In path 2, the washed liquid was directly evaporated to a moderate concentration (rotary evaporator, temperature: 60°C), then lyophilised to yield lyophilised tannins. A sample of each solid residue, liquid phase and tannin extracts was separated and stored in a freezer at -5 °C before analysis.

The kinetics of tannins extraction from grape pomace was studied by the measurement of tannin concentrations in the hydrolysates from the absorbance at 330 nm (UV-2500 PC series spectrophotometer). Results were given according to a calibration plot for a solution of procyanidins prepared by the method of hydroalcoholic maceration of grape seeds extracted with ethyl acetate in the laboratory.

Fig.2. 1 4560 Mini Beach Pressure Reactor

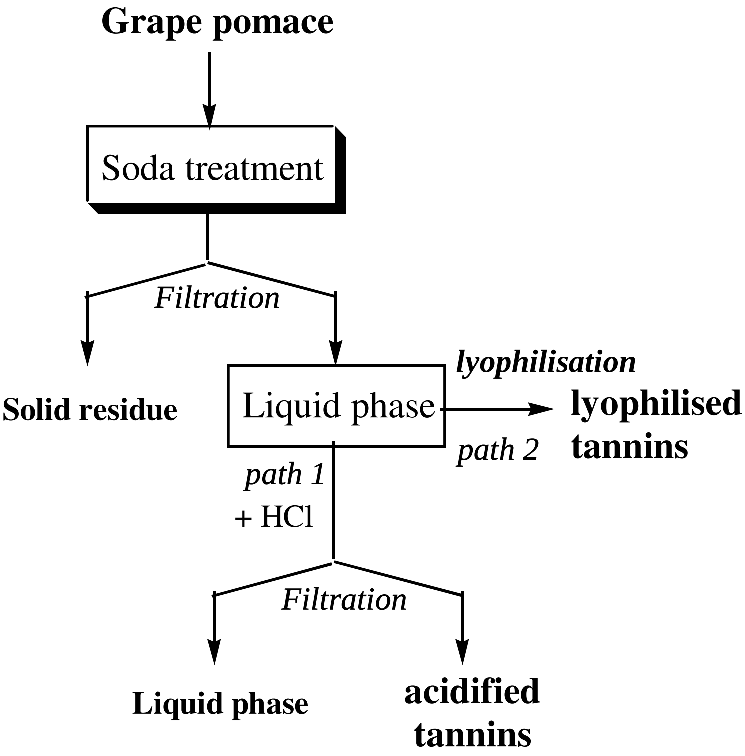

Fig.2. 2 Schematic of the treatment

2.2 Analysis of the raw material, solid residue and liquid phase

2.2.1 Moisture content

Moisture content of the raw materials and solid residue after treatment was determined using KERN MRS 120-3 infra-red moisture analyser.

2.2.2 Ash content

Ash content of the raw materials was determined by Muffle furnace method. Firstly, took the weight of oven-dry crucible and lid (m_1), added at least 100 mg room temperature weight of sample and place then in the 105 °C oven 6 h, weighted the crucible with oven-dry sample (m_2). Then placed crucible with oven-dry sample in to 525 °C Muffle furnace 2 h, after temperature returned to 105 °C, placed in desiccators to cool and weight crucible with ash sample (m_3). So, calculated ash content as follow:

$$Ash(\%) = \frac{m_3 - m_1}{m_2 - m_1} \times 100\%$$

2.2.3 Element analysis

Carbon, hydrogen, oxygen, sulphur and nitrogen elemental analysis were obtained on a Perkin Elmer 240C microanalyser. The protein was calculated from the formula: protein (%) = Nitrogen (%) × 6.25.

2.2.4 Total soluble polyphenols

Total soluble polyphenols were spectrophotometrically determined using gallic acid as standard. 1ml of dilute sample solution, 1ml of 10 % Na_2CO_3 (*W: V*) were added to a glass tube (10 ml) sealed with a PTFE screw cap. The tube was placed in a water bath at 38 °C for 10 min. Afterward, 1ml of Folin reagent (1:2, *V:V*) was added, shaken, rested 15 min at room temperature in darkness and finally measured at 660 nm (UV-2500 PC series spectrophotometer).

2.2.5 Carbohydrate and lignin contents

Carbohydrate and lignin contents were measured on extractive-free material (soxhlet extracted with dichloromethane overnight), ground to pass a 40-mesh screen, according to the laboratory analytical procedure (LAP). Samples were hydrolyzed with 72 % sulfuric acid for 1 h and then autoclaved after being diluted to 3 % sulfuric acid through the addition of distilled water. The autoclaved samples were filtered and the dried residue was weighed to give the Klason lignin content. Monosaccharide contents in the filtrate were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Pronto., 1998).

Separation and quantification of neutral sugars were performed using a Dionex ICS-3000 (Fig. 2.3) system consisting of a SP gradient pump, an AS autosampler, an ED electrochemical detector with a gold working electrode, an Ag/AgCl reference electrode and Chromeleon version 6.8 (Dionex Corp., USA). A Carbopac PA1 (4 -250 mm, Dionex) column with a guard column (4 -50 mm, Dionex) was used as a stationary phase using isocratic conditions with 1 mM sodium hydroxide as the eluent. Eluents were prepared by dilution of a 46–48% NaOH solution (PA S/4930/05 Fisher Scientific) in ultrapure water. All eluents were degassed before use by flushing helium through for 20 min; subsequently they were kept under a constant helium pressure (eluent degassing module, Dionex). After each run, the column was washed for 10 min with 200 mM NaOH solution and reequilibrated for 15 min with the starting conditions. Samples were injected through a 25 μL full loop and separations were performed 25 °C at a rate of 1 mL/min. The pulse sequence for pulsed amperometric detection consisted of a potential of

+100 mV (0-200 ms), +100 mV integration (200-400 ms), -2000 mV (410-420 ms), +600 mV (430 ms), and -100 mV (440–500 ms).

Fig.2. 3 High-performance anion-exchange chromatography

The acid soluble lignin content was determined from absorbance at 205 nm (UV-2500 PC series spectrophotometer) using 3% H_2SO_4 as a blank according to Lin and Dence (1992). If the absorbance is higher than 0.7, the sample is diluted in a volumetric flask with 3% H_2SO_4 to obtain an absorbance reading in the range 0.2 to 0.7. The lignin content was calculated from the following expression of Beer's law:

$$Lignin(g/l) = \frac{Absorbance}{110}$$, in undiluted filtrate

$$Lignin(g/l) = \frac{Absorbance}{110} D$$, in diluted filtrate

Where $D = \frac{V_D}{V_O}$; V_D= Volume of diluted filtrate and V_O= Volume of original filtrate taken

2.3 Characterization of tannin extracts

2.3.1 The reactive tannin content in tannin extracts

The Stiasny number reaction was used to determine the reactivity of tannins towards formaldehyde. According to Yazaki and Collins (1994) about 0.2 gm (oven-dry mass) of tannins sample, 5 ml of 37 % aqueous formaldehyde and 5 ml of 10 M hydrochloric acid solution (HCl) were mixed together and heated under reflux for 30 min. At the end of this reaction, the mixture was filtered through a sintered glass filter (filter no.3) while it was still hot. The precipitate was washed with hot distill water (5x10 ml) and dried to a constant weight in an oven at 105 °C. The Stiasny number was determined as a percentage of the weight of the precipitate to the weight of the starting sample. The test was duplicated and the average value was reported.

2.3.2 The condensed tannins content in tannin extracts

Condensed tannins was calculated from the absorbance at 550 nm of polyphenolic solutions obtained after 5% HCl-BuOH treatment (100°C, 3h) of sample (Reed et al., 1982). Results were given according to the same calibration standard in the kinetics testing of tannins extraction.

2.3.3 Thermo gravimetric analysis (TGA)

Thermo gravimetric analysis (TGA) was used to observe the function of sample's mass corresponding to temperature or time. It was applied to determine the thermal stability and degradation of tannin extracts. The TGA was done using a Pyris 1 TGA (PerkinElmer Corporation).The temperature range of 25 °C -700 °C at a heating rate of 20 °C/ min.

2.3.4 Fourier transform-infrared spectrometer (FT-IR)

FT-IR spectrometer was used to determine the presence of functional groups and structures of sample molecules. The infrared spectrum is produced by the transition of molecular vibration energy accompanied with rotational energy. The absorption peaks is a certain width band. When a certain frequency infrared irradiated one molecule, if a group vibration frequency is consistent with it, and associated with a change in dipole moment at the same time, this molecule would produce infrared absorption. The experiment was determined using Nexus 870 FT-IR instrument (Nicolet Corporation). Resolution is 0.09 cm^{-1}, wave number precision is 0.01 cm^{-1}, transmittance precision is 0.07 % T. Tannins (0.2 mg) were added into KBr powder (30 mg), mixed and grinded to powder which diameter reached 2 μm， then pressed to a small piece sample in a press machine.

2.3.5 High resolution nuclear magnetic resonance spectrometer (NMR)

The principle of NMR is based on measuring the absorption of radiation of radio frequency (RF) by an atomic nucleus in a strong magnetic field. The absorption of radiation caused the nuclear spin return in the direction of the highest energy. After absorbing energy, the atomic nuclei re-transmit RF radiation and return to their initial state of lower energy level.

All NMR experiments were performed on a Bruker Avance-400 spectrometer operating at a frequency of 100.59 MHz. ^{13}C-NMR spectra were accomplished by using a pulse sequence (inverse-gated) that eliminated the nuclear over Hauser effect (NOE) and had a sufficiently long pulse delay, allowing for all nuclei to be fully relaxed before the next pulse. The acquisition parameters used during the recording of the spectra included 9-15 K numbers of acquisitions, 90°C pulse width and a 12 second pulse delay. The total acquisition time for recording each spectrum was ranged from 36 to 48 h (Robert., 1992). All samples were extracted with pentane (Soxhlet) to remove impurities before testing. Acidified tannins (100 mg) were dissolved in DMSO-d_6 (0.5 ml) and lyophilised tannins (100 mg) were dissolved in D_2O (0.5 ml) with slight heating. Samples were placed in 5 mm NMR tube (sample volume 0.5 ml) and heated to 50 °C in order to reduce viscosity. Data were processed offline using XWinNMR processing software (Acorn NMR Inc).

2.3.6 Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF)

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is a novel soft ionization mass spectrometry developed in recent years. Instrument is mainly composed of two parts: Matrix assisted laser desorption ionization source (MALDI) and time of flight mass analyzer (TOF). The principle of MALDI is used laser irradiated the eutectic crystalline film formed by samples and matrix. The substrate absorbed energy form the laser and delivered to the sample molecular, which made the sample molecular ionization with the proton transfer to or from sample molecular. The principle of TOF is ions were accelerated by electric field across the flight line, according to the different flight time arriving the detector and detected the ion mass-to-charge ratio(m/z) corresponding to flight time.

MALDI-TOF-MS is a suitable method for examining polyflavonoid tannin oligomers. The spectra were recorded on a KRATOS Kompact MALDI 4 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The experimental conditions used were: mass- high (20 kV acceleration voltages), polarity-positive, flight path-linear 100-150 pulse per spectrum and 200-800 ns time of delayed extraction technique. The calibration has been conducted with a calibration standard with an average molar mass of 3000 g/mol (Mw) and a polydispersity of 1.05.

Acidified grape pomace tannin powder (4 mg) was dissolved in 1 ml acetone. Lyophilised grape pomace tannin powder (4 mg) was dissolved in 1 ml distill water. 2, 5-Dihydroxy benzoic acid (DHB) was used as the matrix and 10 mg NaCl were added to the matrix solution to enhance the ion formation. According to Pasch et al (1996) the best results for tannin analysis were obtained by using the combination of matrix DHB/NaCl. The sample of the solution and the matrix were mixed in equal amounts and 0.5–1μl of the resulting solution was placed on the MALDI target. After the solvent has evaporated, the MALDI target was deposited into the spectrometer (Pasch et al., 2001)

2.4 Error analyses

The composition of the raw material and the fraction after extraction treatments were established from two independent experiments performed using the same conditions. For all the samples, Klason lignin, acid soluble lignin and Stiasny number were calculated from analysis of duplicate samples. Error values <0.6% (standard deviation), <0.1% (standard deviation) and <2.5% (standard deviation) were, respectively, determined for the Klason lignin, acid soluble lignin and Stiasny number.

2.5 Particleboard manufacture and testing

2.5.1 Preparation of resin formulations

Two different adhesive formulations were investigated in this study, the first formulation is nonfortified tannin-formaldehyde adhesive, a certain concentration of tannin solution was prepared, 5 % of powdered *Para* formaldehyde, by weight of dry tannin extract, was added. The second Formulation is fortified with a synthetic cross linker: 5 % of powdered *para*formaldehyde, 20% of polymeric 4, 4'-diphenyl methane diisocyanate (*p*-MDI), by weight of dry tannin extract, was added to a certain concentration of tannin solution respectively.

2.5.2 Gelation time (T-gel)

The gelation time (T-gel) of tannin was referred to the working time of tannin towards formaldehyde from a colloidal solution to become a solid or semi-solid jelly or gel. About 10 g tannin adhesive were added to a test tube and placed in a water bath, which was maintained at boiling temperature (just below 100 °C) at normal atmospheric pressure. The time taken to reach the gel point was recorded during constant stirring with the aid of a wire spring and a stopwatch. The test was duplicated and the average value was reported. The pH of the mixture was measured using a microprocessor pH meter (Hanna pH 211). 33 % of sodium hydroxide solution (NaOH) and hydrochloric acid solution (HCl 4M) were used to adjust different pH of the mixture.

2.5.3 Thermal mechanical analysis (TMA)

Thermal mechanical analysis (TMA) is to study the rigidity of the wood-adhesive joint as a function of temperature. Modulus of elasticity (MOE) of the tannins formulations were studied using thermal mechanical analyzer with TA controller (Mettler TMA 40), STARe date processing software. The experiments were conducted under the conditions at a heat rate of 10 °C/ min from 25 °C to 250 °C, 30 mg of resin, three points bending TMA mode. The maximum of MOE and its increase corresponding to temperature or time for wood-adhesive systems indicated the performance of the adhesive and the strength of the final application.

2.5.4 Elaboration and testing of particleboards

Single layer laboratory particleboards of 340 mm × 300 mm × 14 mm dimension were prepared using particles of beech (*Fagus sylvatica*) and Norway spruce *(Picea abies*) at 35.7 kg/cm^2 maximum pressure, 195 °C press temperature and 7.5 min press time. 10 % (w/w based on dry particles) of adhesive resin solid was loaded. Fig.2.4 is the press system of particleboards. Fig.2.5 is the pressing process curve.

The dry internal bond (IB) strength of the particleboards was tested according to a relevant international standard. European Norm EN 312, wood particleboard-specifications. The distribution vertical density profile of particleboards was determined by DAX 5000 density instrument.

Fig.2. 4 The press system of particleboards

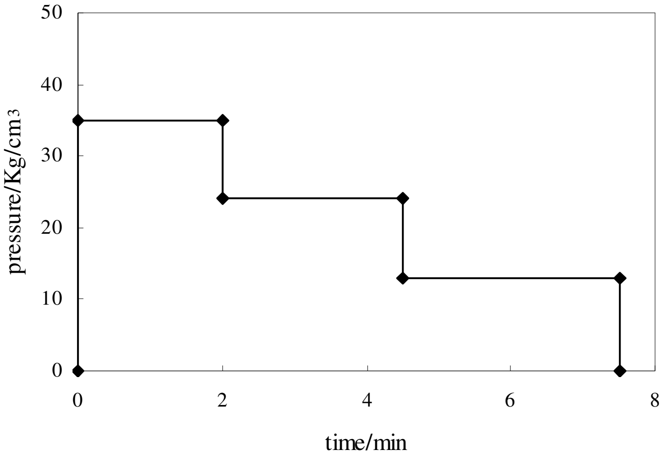

Fig.2. 5 Hot pressing process curve

The formaldehyde emissions from the particleboards were determined according to the European Norm (EN-717-3) using a 500 ml glass jar. Three test pieces, with dimensions of 25 mm×25 mm×14 mm, which were cut from Single layer laboratory particleboards, are positioned in the jar. The formaldehyde released from the test pieces at 40±1°C, during 180 min is absorbed in 50 ml distilled water and determined from absorbance at 412 nm with spectrophotometer. The specification curve was drafted by the standard formaldehyde solution in different concentrations (Fig.2.6).

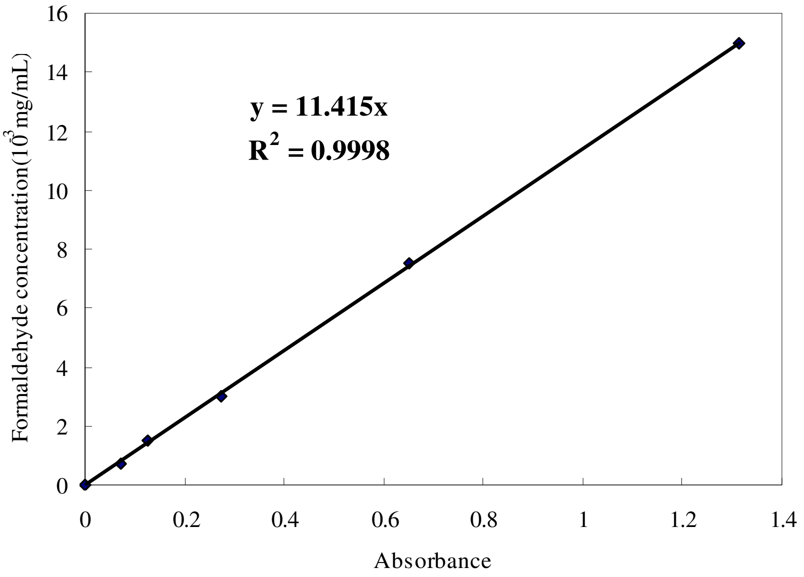

Fig.2. 6 The specification curve of formaldehyde emission drafted by standard formaldehyde solution

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Chapter 3. Extraction of condensed tannins from grape pomace

3.1 Introduction

Many different extraction methods for the extraction of condensed tannins from tree bark have been described using methanol, acetone and other organic solvent (Chavana et al., 2001; Zhao et al., 2010). Organic solvents extraction is expensive and unacceptable taking into account the simple technological level of extraction factories. So the industrial extraction of tannins is generally a fairly simple procedure in a water medium. However, not only are different tannins often extracted with slightly different procedures, but some important conditions must be kept in mind to obtain an extract that is at least reasonably suitable for wood adhesives application.

Extraction is generally conducted by placing the raw vegetable material in a series of enclosed or covered vats called autoclaves. Concerning mimosa bark, only water is used; in quebracho, water containing 5 to 10 % sodium sulfite or metabisulfite; in pine, water containing 2 to 3 % sodium sulfite or metabisulfite; in pecan nut, 5 % sodium sulfite with 3 to 4 % sodium carbonate or bicarbonate. The initial temperature of the extraction water used also varies: for mimosa, temperatures of 94 to 100 °C are used; in quebracho, pine and pecan nut tannins, temperatures of not more than 70 °C are used. the use of higher temperatures does not improve the industrial yields of the phenolic materials; it often differentially favours extraction of nonphenolic materials (mainly sugars). The extraction yield in a water medium is different as a function of the raw materials. The extraction yields were around 13 to 15% for pine barks and 30 to 33 % for mimosa barks, (Pizzi., 1994).

The industrial tannins extracts are generally a mixture of poly and monoflavonoids, with a noticeable or even considerable proportion of nonphenolic materials, mainly simple sugars and polymeric carbohydrates and hydrophilic gum. The tannin content is 70-80 % for mimosa and quebracho industrial tannin extracts and 50-60 % for pine tannin extracts. The non-phenolic materials can not react with formaldehyde in resin synthesis and sugar has a dilution function in resin solid content. Even if the content of hydrophilic gum is very low in tannin extracts, (usually containing 3 to 6 %), it has a great influence of viscosity of tannin resin, and it is the primary reason of the high viscosity of tannin-based adhesive. Better wetting and diffusion of resin in wood surface is an important factor to obtain good glue joint ability. The wettability has great effects on wood products bonding properties if the resin viscosity is too large (Lei et al., 2008; Gu., 1999). The industrial extraction yield could be improved by increasing the temperature and dosage of alkali, but this mainly improves the yield of non-phenolic material in extracts. Furthermore, higher temperature often induces structural modifications in the tannin such as auto-condensed and tannin-derived products.

As presented in section 1, grape pomace retains high level of condensed tannins which could be extracted and valorized through the production of adhesives. Because the composition of this raw material is very different to the tree barks, the extraction conditions developed for grape pomace tannins have to be adapted. In this Chapter, the extraction of condensed tannins from grape pomace was examined using water medium in the present of NaOH, Na_2CO_3, $NaHCO_3$ and Na_2SO_3. Two different extraction processes and the influence of 4 parameters (i.e., temperature, reaction time, chemical reagents and concentration of the chemical reagents) on the extracts yield, properties and the composition of the solid residue were studied.

3.2 Results and discussions

3.2.1 Composition of the raw material

The air-dried grape pomace was provided by a French distillery (Franck le Net, UDM). It was composed by grape skin, seed and stalk, with the skin and seed making up the major part and a small quantity of stalk. It was sealed in plastic sack and stored at room temperature during the course of this study. Before the analysis the composition of the grape pomace, the grape skin, seed and stalk were manually separated, the grape skin which used in this study was milled to powder to pass a 40 mesh screen. The grape pomace which used in this study was the same batch. The composition of the grape pomace is presented in Table 3.1.

Table 3. 1 Composition of untreated grape pomace[a,]

Components	**%**
Condensed tannins	24.9±0.9
Klason lignin	48.7±0.2
Soluble lignin	4.3±0.3
Glucanes	14.6±0.6
Other sugars [b]	6.2±0.5
Ash	7.0±0.2
Proteins	13.4±0.04
Ether extract	5.0±0.38
DCM extracts [c]	4.8±0.37

[a] All data are yield of components (g) per 100g of oven-dried pomace.
[b] Arabinanes, xylanes, mannanes, galactanes.
[c] methylene dichloride (CH_2Cl_2) extracts

The content of condensed tannins of grape pomace raw material was determined as the same method described for tannin extracts in section 2.3.2. The condensed tannins present a value of 24.9 ± 0.9 % of dry matter, near than that described by Llobera and Canellas (2007) for red grape pomace (22.3 %) by Bravo and Saura-Calixto (1998) for skins of red grapes (27 %). Total polyphenol content of grape pomace was gravimetrically determined as Klason lignin (48.7 ± 0.2 %) and as soluble lignin (4.3 ± 0.3 %) by UV quantification. According to Rebole (1994) components such as tannins (condensed tannins) and a considerable amount of proteins (as protein-tannins complexes or as Maillard products) and ash are in the Klason lignin fraction, which results in a total composition exceeding 100 %. The carbohydrate of raw material was measured using a Dionex ICS-3000 high performace anion exchange chromatography with pulsed amperometric detection. The ether extracts and DCM extracts were performed by soxhlet extraction in order to extract oil and organic compouds from grape pomace raw materials respectively.

3.2.2 Comparison of two different extraction processes

The conditions used for the tannins extraction from bark previously exposed (section 3.1, table), applied to grape pomace were unsuccessful No tannins extract were obtained from grape pomace in this condition and the results were not reported in this study. In order to check the possibility to extract polyphenols from grape pomace, we choose to use harsher conditions with hydroxide (NaOH) in high concentration and higher temperature using pressure reactor in this section.

The tannins extracted from grape pomace were examined using a mixture water-sodium hydroxide (NaOH) at 120 °C, 120 min according to Fig.2.2. Two different processes were used after the extraction step for tannins recovery:

Path 1: addition of dilute HCl to adjust the pH of the liquid phase reach 1.5 to precipitate the tannins which are collected by centrifugation and then air-dried;

Path 2: lyophilization of the liquid phase to yield a crude residue.

The influence of soda concentration (5-15%) on the yield, properties of extracted tannins and on the composition of the solid residue and liquid phase was examined (Table 3.2 and Table 3.3). Table 3.2 gives the experimental conditions used for all the experiments and the results obtained including the yield of crude tannins and the Stiasny number recovered following paths 1 and 2.

As shown in Table 3.2, the yield of crude extracts increased with the higher concentration of soda medium. For path 1, the yield ranged from 5 to 30 %; for path 2, the yield ranged from 24 to 101 %. The yield of crude lyophilized tannins is higher than crude acidified tannins in the same experimental condition. High yields of crude lyophilized tannins (higher than 100 % for test 6) were obtained according path 2 because of the high sodium hydroxide content recovered after lyophilisation.

Table 3. 2 Experimental conditions for the treatment of grape pomace in water medium. Yields and Stiasny number of path 1 and path 2 tannins

Treatment condition		Acidified tannins (path 1)		Lyophilised tannins (path 2)		
test	NaOH $\%^a$	Yield $\%^b$	Stiasny number	Yield $\%^b$	Stiasny number	T-Gel (pH)
1	5	4.8	95	23.8	46	174 (6.7)
2	10	21.7	89	45.2	55	53 (8.7)
3	15	31.4	97	58.8	50	100 (9.2)
4	20	29.1	98	71.1	49	138 (9.6)
5	30	31.8	97	88.4	39	1111 (10.7)
6	40	25.8	98	101.5	32	∞ (12.9)

[a] % based on weight of dry pomace ;

[b] yield calculated from the raw material (oven dry mass) ;

[c] modulus of elasticity

The Stiasny number is an indication of the purity of the polyphenol extracts (Stiasny, 1906). The determination of The Stiasny number is studied according to method reported by Yazaki and Collins (1994).

Regarding path 1, extracts bearing high Stiasny number values (95-98 %) were recovered indicating a high phenolic content. For lyophilized tannins (path 2) Stiasny number between 32 and 55 % were observed. The low polyphenol content of these extracts is rationalized by (1) the high sodium hydroxide content in the crude tannin (isolation of the sodium salts) and (2) the important quantity of non-tannin fractions extracted from pomace and recovered. For high sodium hydroxide concentration (test 5 and 6) large non-tannin fractions were extracted which did not react with formaldehyde (Pizzi., 1994).

It also shows that using 5 % (*w/w*) of NaOH low amounts of polyphenols were isolated. When the concentration of soda was increased to 15 %, the tannin yield markedly increased to about 30 % (*w/w*) of pure polyphenols.

Table 3. 3 Experimental conditions for the treatment of grape pomace in water medium, Composition of solid residue and liquid phase of extraction

Treatment condition		**Solid residue**				**Liquid phase**	
test	**NaOH %**	**Klason lignin**	**Soluble lignin**	**Glucanes**	**Other sugars**[a]	**Total sugars**[b]	**Polyphenol**[c]
Raw material	-	48.7	4.34	14.61	6.19	-	-
1	5	41.9	1.65	13.29	4.95	0.12	4.7
2	10	28.8	1.29	5.05	1.95	0.06	5.8
3	15	24.2	1.21	7.15	2.49	0.05	10.7
4	20	16.9	0.62	6.87	2.91	0.05	12.6
5	30	7.6	0.36	8.99	2.49	0.05	21.8
6	40	7.7	0.65	7.32	1.75	0.05	23.2

[a] Arabinanes, xylanes, mannanes, galactanes.

[b] total sugars in liquid phase including glucanes, arbinanes, xylanes, mannanes and glactanes.

[c] total soluble polyphenol content in the liquid phase

Table 3.3 gathered the chemical compostion of the solid residues and liquid phases after extraction. The methods for sugars and lignin contents determination and of total soluble polyphenol content are given respectively in section 2.2.5 and the 2.2.4.

The Klason lignin content and soluble lignin content in solid residue deceased with the increase of the soda concentration used in this study compared to grape pomace raw material. It indicates the higher extraction of tannins along higher soda concentration. The same variation trend of sugars content (including glucanes and other sugars) coukld be explained by a higher extraction of sugars from grape pomace with higher soda concentration. Normally sugars didn't precipitate in acid conditions, and stay in the solution. The very low monosaccharides contents measured in the liquid phase suggesting an extensive degradation of sugars during the extraction process.

Concerning the content of polyphenol in the liquid phase (Table 3.3), the gradual increase in NaOH concentration of the medium caused a gradual increase in the soluble phenol concentration in the liquid phase. This is in accordance with the Klason lignin and soluble lignin data established from solid residues analysed after extraction showing a gradual decrease of phenolics with higher soda concentration. (see section 3.2.1).

Gel time is an indication of the reactivity of the sites of tannins molecules toward formaldehyde. Gel time is highly dependent on the pH of the tannin solutions; the gel time generally decreases when the pH of the tannin solution is increased.

Concerning acidified tannins (path 1), because of the low solubility in water of the fractions and the low reactivity of these tannins toward paraformaldehyde, it was not possible to establish gel times. This behaviour could be both explained by the well know catechinic acid rearrangement (Fig.3.1) (Sears et al., 1974; Kiatgrajai et al., 1982) and rearrangement of flavonoid to phlobatannins (Fig.3.2) (Sealy-Fisher and Pizzi., 1992) which would occur during the acidification step. The formation of phlobatannins is in accordance with the redish colour of the extracts isolated through acidification.

The catechinic acid rearrangement should affect the more reactive A-aromatic ring the catechnic moiety. Phlobatannins are reddish coloured, water insoluble phenolic substances which are related to tannins, phlobatannins can easily be precipitated from a water solution of polyflavonoid tannins by acid-induced condensation reactions. Consequently, these two rearrangements should decrease the water solubility of acidified tannins extract and the reactivity toward prarformaldehyde.

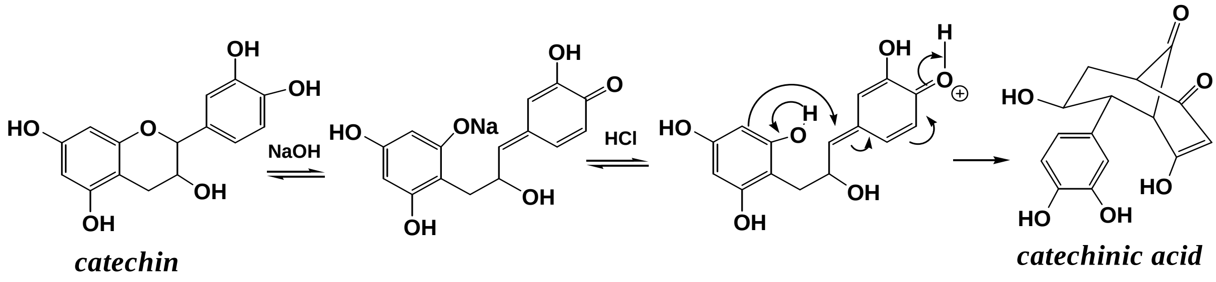

Fig.3. 1 Mechanism of formation of catechinic acid

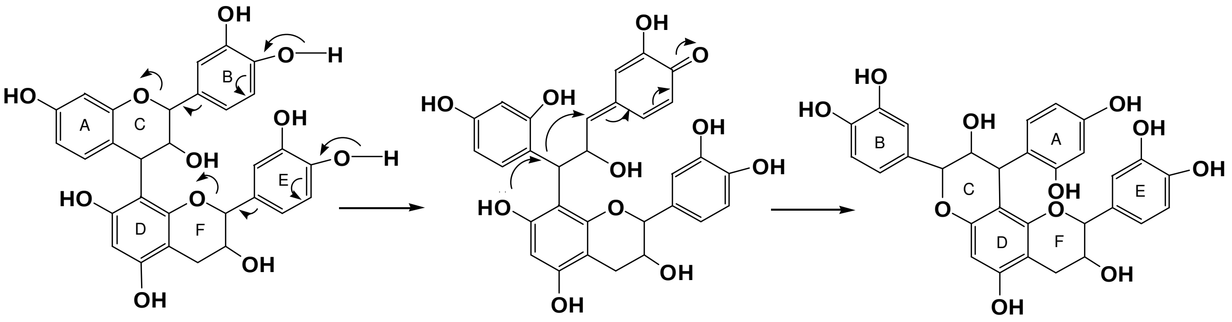

Fig.3. 2 Mechanism of rearrangement of flavonoid dimmer to phlobatannins

Concerning lyophilized tannins (path 2) the gel time results of tannins formaldehyde-based glues are given in Table 3.1 accompanied with the pH of the tannins solutions in water (30%, *w/w*, tannin/water). It appears that gel times are directly correlated with the Stiasny number of the tannins fractions and a dramatic increase in the gel time was observed when high sodium hydroxide concentrations were employed. Fig.3.3 gives the gel times of 3 lyophilized tannins extracts (test 1, 2 and 3) as a function of pH of the tannins solution. The best result was observed for test using a concentration of NaOH of 10 % (*w/w*) during the extraction process: in these conditions extracts bearing short gel times were isolated (72 s at pH 8 and 32 s at pH 10). Compared to previously described results, this tannins fraction can not reach the gel times yet obtained at pH 8 for pine tannins and pecan nut tannins, which are equal to 26 and 22 s, respectively (Pizzi., 1994). However, despite their lower purity (about 50 % of active phenolic ingredients), this grape pomace extract displays gel time values lower than the ones of mimosa and quebracho tannins, demonstrating the good reactivity of these crude tannins toward paraformaldehyde. Compared to acidified tannins, the higher reactivity of the lyophilized extract is probably due to the low catechinic acid and phlobatannins content.

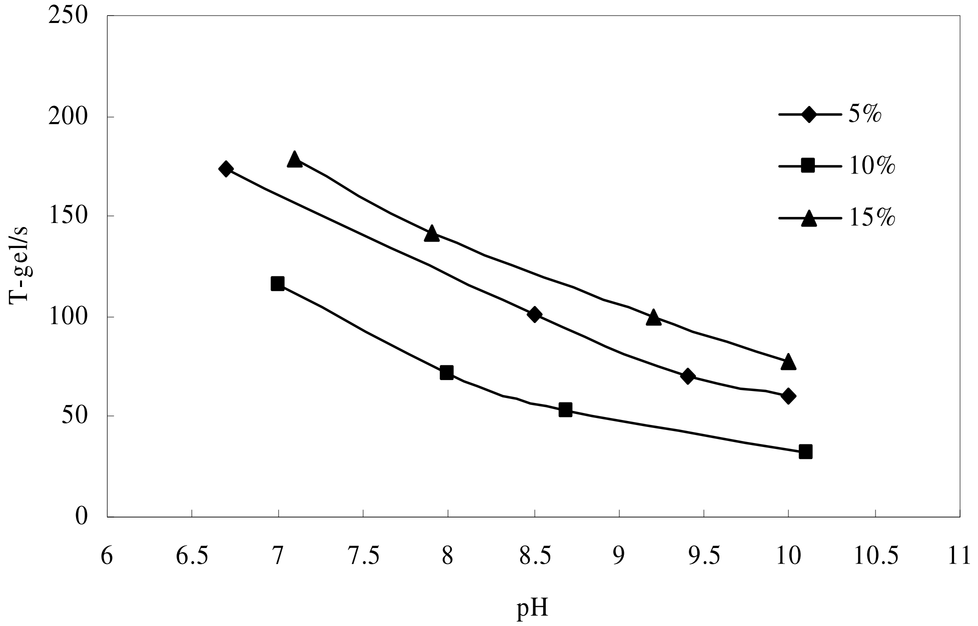

Fig.3. 3 Gel time of lyophilized grape pomace tannin solutions (30%) at 120 °C

To conclude, it was demonstrated in this section that it is possible to extraction of reactive tannins from grape pomace in basic medium used harsher extraction conditions with higher soda concentration and higher reaction temperature. Comparison the result of two different extraction processes, including the preliminary tannin adhesive properties by gel time, the recovery of the crude tannin extracts by lyophilization is better than acidification in application as wood adhesive for wood based panels. The optimization of the extraction parameters is then described in the following section.

3.2.3 Optimization of the tannins extraction using sodium hydroxide

The influence of reaction temperature, reaction time and concentration of sodium hydroxide (NaOH) on the extract yield and the composition of the solid residue were studies on this section. Three treatment temperatures were experimented: 70 °C, 100 °C (both at atmospheric pressure) and 120 °C (using a pressure reactor). Three different reaction times (60 min, 120 min and 180 min) were also experimented.

The results of tannins extraction using sodium hydroxide are presented in Annexe 1 from test 1 to test 14. Fig.3.4 and Fig. 3.5 give the yield of crude tannin extracts from grape pomace as a function of different reaction temperature and reaction time, respectively.

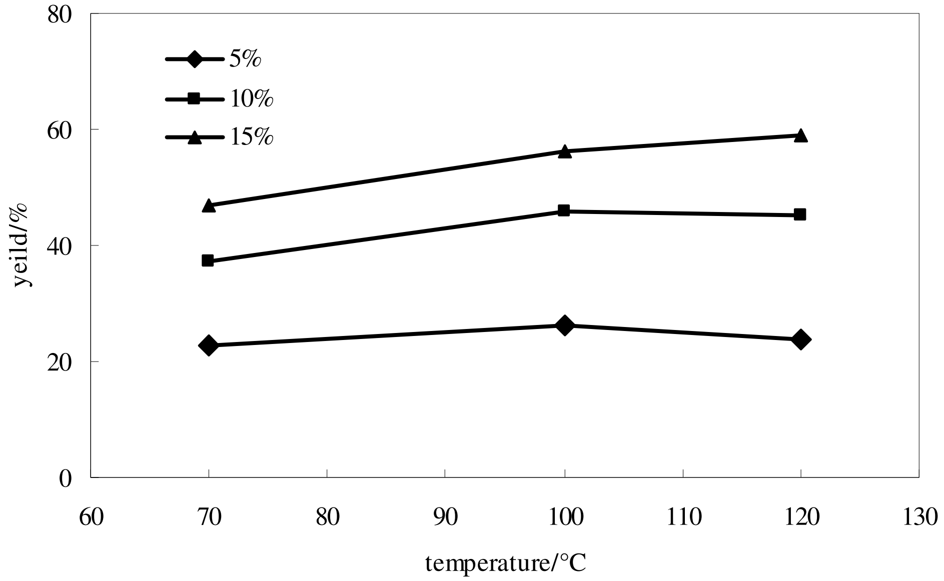

Fig.3. 4 The yield of crude tannin extracts as a function of different reaction temperature from 70 °C to 120 °C with reaction time is 120 min

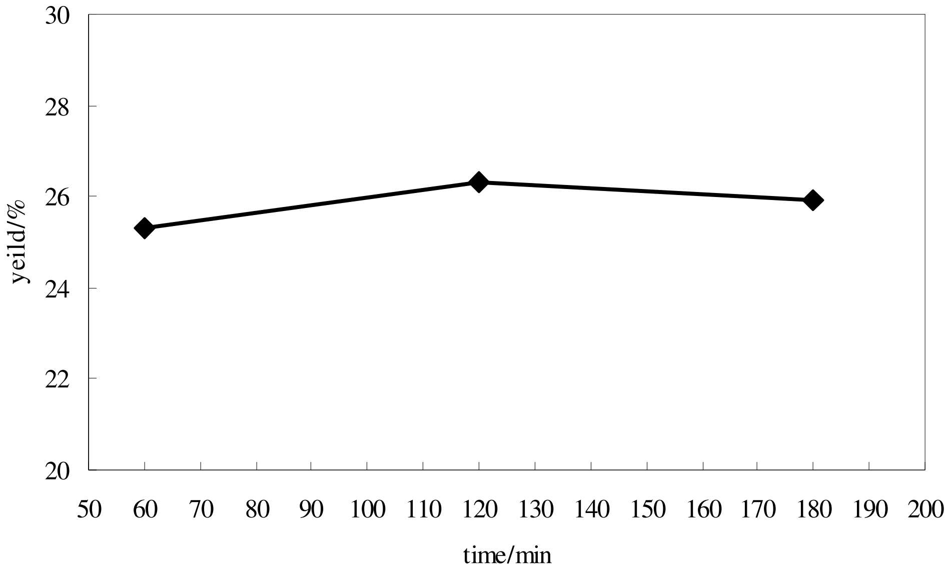

Fig.3. 5 The yield of crude tannin extracts as a function of different reaction time from 60 min to 180 min with reaction temperature is 100 °C

As shown in Fig.3.4, the concentration of NaOH affected the yield of tannin extracts, the higher yields were obtained with higher concentrations of NaOH. The yield of crude tannin extracts reaches more than 50 % when a NaOH concentration of 15 % was used (test 3, test 9). The second influence parameter is reaction temperature, the yield slightly improves along with the increase temperature, but it is not the higher temperature used, the higher yield was obtained. When the temperature increases from 100 to 120 °C, the yield has a little bit fell down (test 1-3, test 7-9, test 10-11). Comparison of the results for NaOH=5 % indicates that the highest amount of tannin extracts (26.3 %) was reached for test 7 when the temperature is 100 °C. it appears in Fig.3.5 that no significant increase in yield was observed for longer reaction time (test 7, 13,14).

Fig.3.6 and Fig. 3.7 give the Stiasny numbers of crude tannin extracts from grape pomace as a function of different reaction temperature and reaction time, respectively.

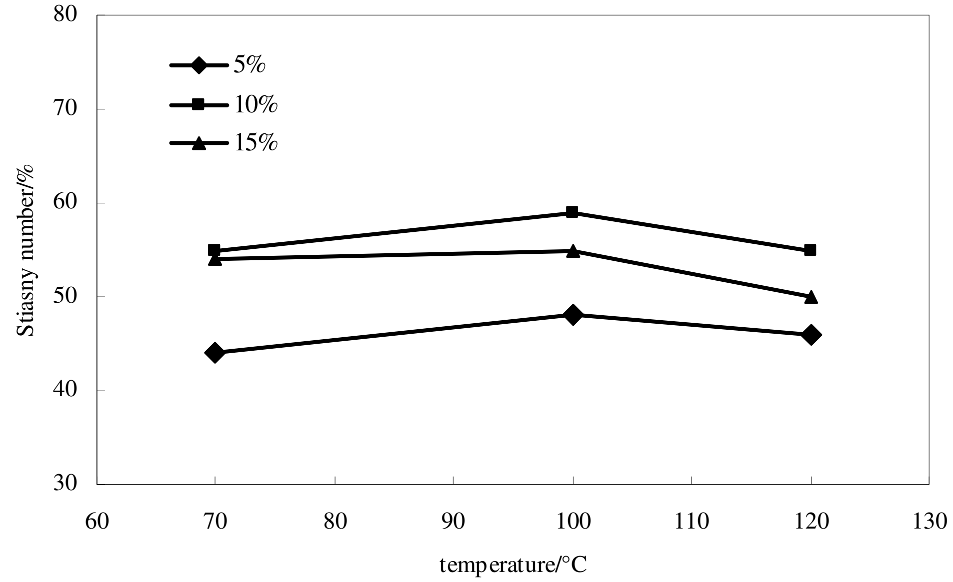

Fig.3. 6 The Stiasny numbers of crude tannin extracts as a function of different reaction temperature from 70 °C to 120 °C with reaction time is 120 min

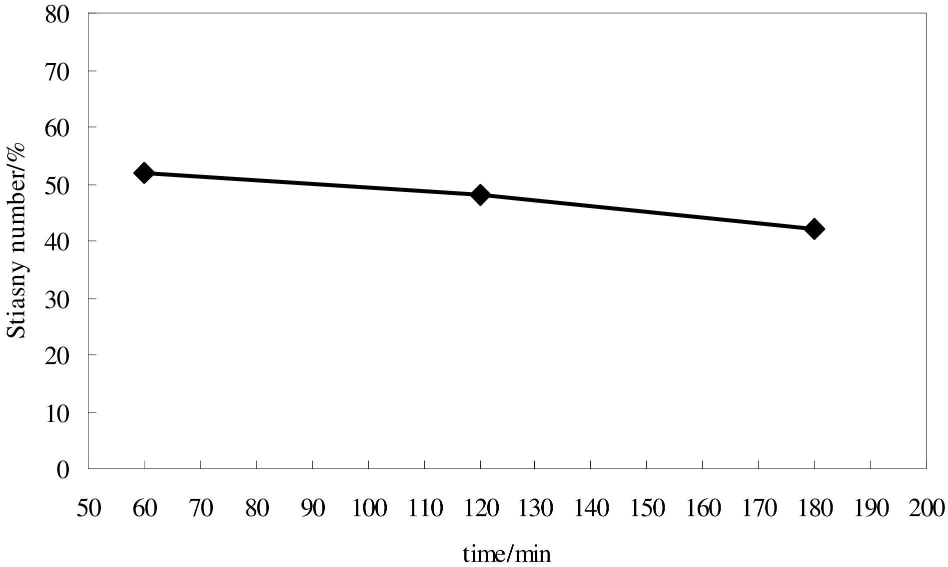

Fig.3. 7 The Stiasny numbers of crude tannin extracts as a function of different reaction time from 60 min to 180 min with reaction temperature is 100 °C

As shown in Fig.3.6, Stiasny numbers seem no markedly variation in different temperature (test 1,7,10; test 2,8,11; test 3, 9,12), but Stiasny number are also largely affected by the concentration of NaOH (test 1-3; test 7-9; test 10-12), higher Stiasny number values were observed for the soda concentration range from 5% to 10%, then decreases from 10% to 15%. The highest Stiasny number is 59 % for test 8 (NaOH=10 %). It can be explained by the reason that large non-tannin fractions were extracted when higher concentration of soda was used.

Fig.3.7 presents that Stiasny numbers slightly decrease as a function of the duration of the treatment (test 7, 13, 14). It could be rationalized that by the fact that a part of polyphenols could be degraded for longer reaction time and or by the extraction of non phenoluic compounds.

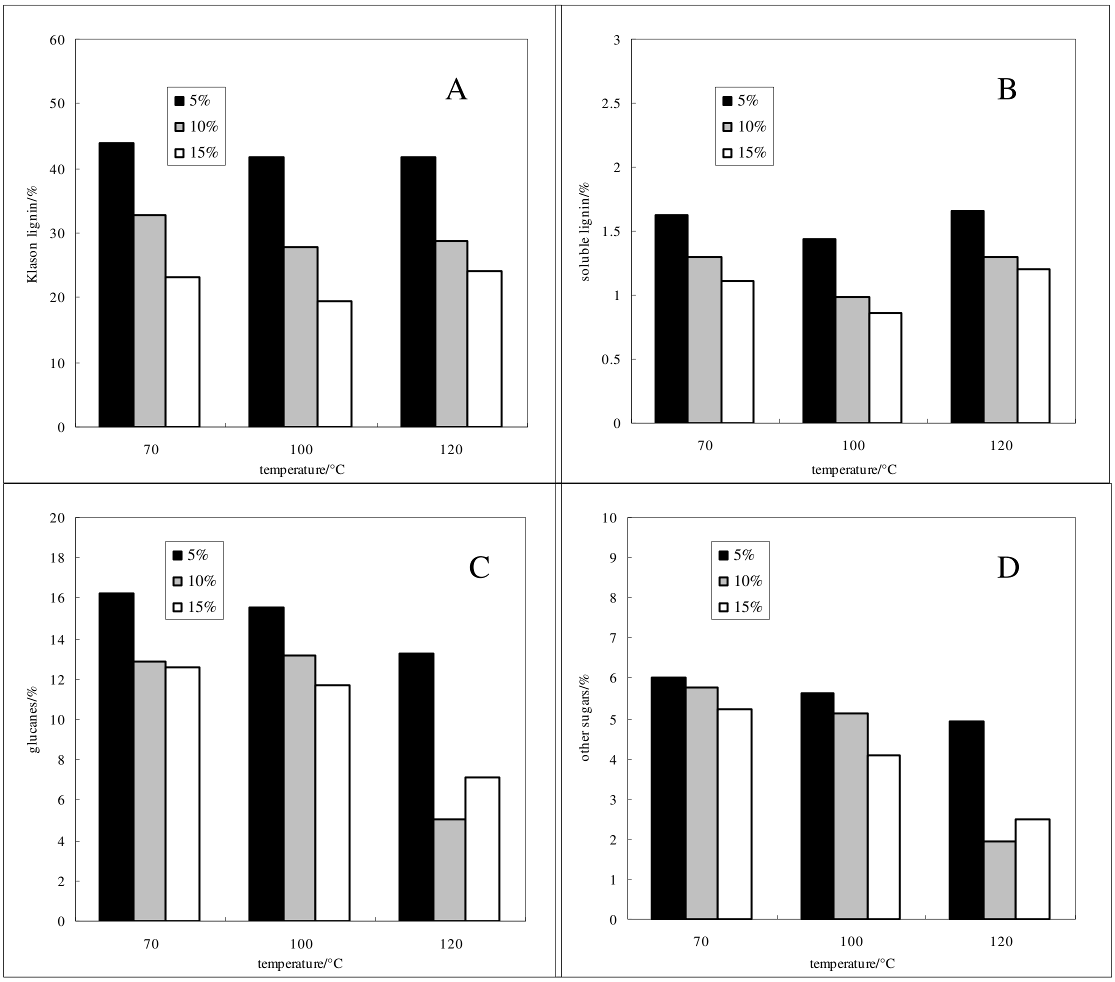

Fig.3. 8 The chemical composition of the solid residue as a function of different reaction temperature from 70 °C to 120 °C with reaction time is 120 min

Fig.3.8 presents the influence of extraction parameters on the composition of the solid residue. Fig.3.8 A and B show a decrease in the phenolic content (Klason lignin and soluble lignin) corresponding to different temperature and reagent concentration, rationalized by the removal of a part of the tannins content from the grape pomace. This decrease trend is obviously affected by soda concentration, which is in accordance with crude tannin extracts yield results. The data concerning the amount of glucans and other sugars (Fig.3.8 C and D) shows that an important fraction of the sugar content is removed and recovered in the extracts. This observation is in agreement with the low Stiasny number obtained from the extracts.

Fig.3.9 gives the solid residue chemical compostions plotted against reaction time. Interestingly, an increase in the temperature of the process or in the reagent concentration resulted in a reduction of the sugar content of the residue (see fig.3.8.) whereas a longer reaction time seems to have a little effect.

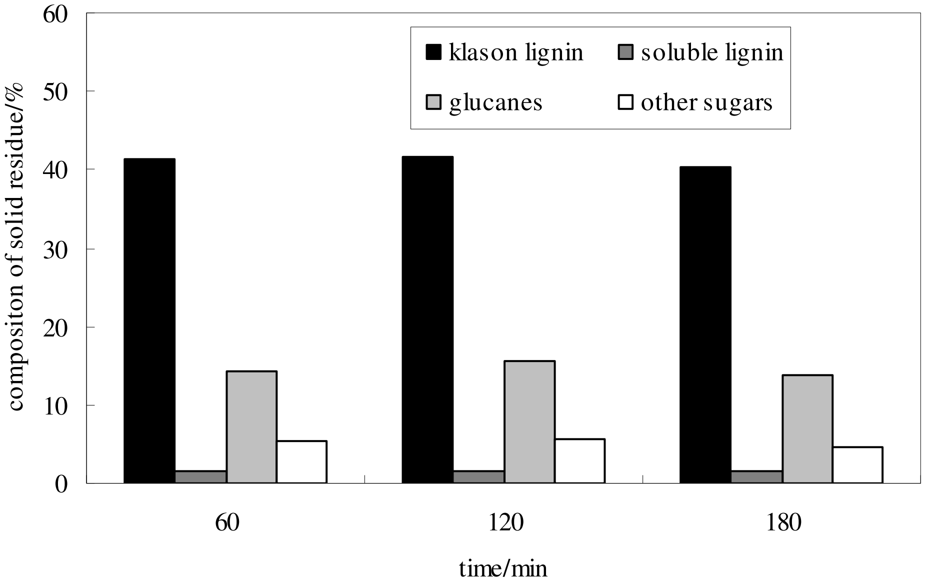

Fig.3. 9 The chemical compostion of the solid residue as a function of different reaction time from 60 min to 180 min with reaction temperature is 100 °C

It is noteworthy to mention that tannins extracted at 70 °C (test 10-12) exhibited a very low solubility in water, consequently it was not possible to determine a gel time for these extract. Fig.3.10 gives the gel times of tannins (extracted at 100 °C) as a function of pH of the tannin solution. Compared to Fig.3.3 (extracted at 120 °C), it appears that the temperature of the process largely impacts the reactivity of the extracts, tannins extracted at 120 °C show lower reactivity towards formaldehyde. The gel time and the pH of tannin solution seem to exhibit no significant change in different reaction time.

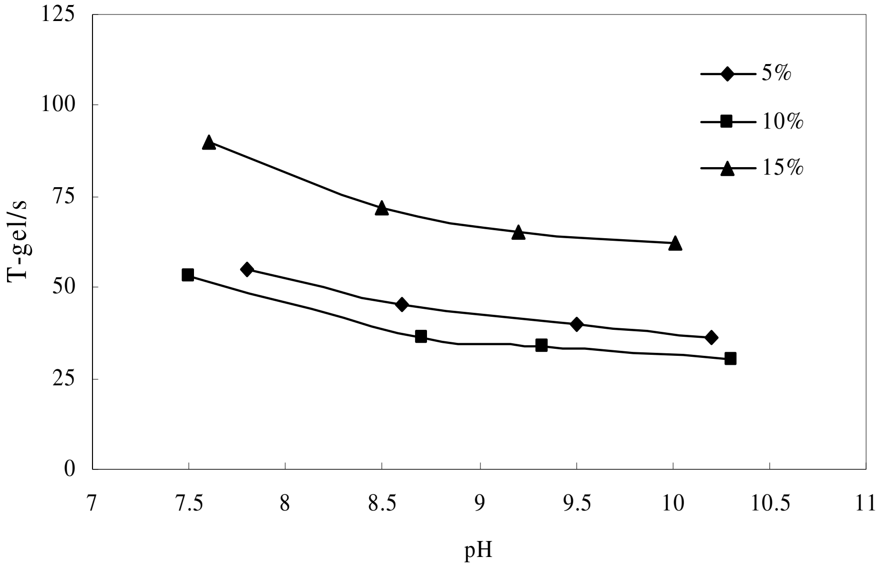

Fig.3. 10 Gel time of lyophilized grape pomace tannin solutions (30%) at 100 °C

To conclude, a comparison of three extraction parameters (reaction temperature, reaction time and concentration of sodium hydroxide (NaOH)) shows that the reagent concentration has the largest influence factor on the yield and properties of crude tannin extracts, and the reaction temperature is the second influence factor. To use higher reagent concentration and higher temperature could improve the yield of crude tannin extracts but decrease noticeably the actual percentage of phenolic material usable for wood adhesives. The optimal extraction condition in this section is concentration of NaOH =10 %, reaction temperature 100 °C and reaction time 120 min.

3.2.4 Optimization of the tannins extraction using sulfited reagents

Sulfitation of tannins is the most useful reaction in flavonoid chemistry to increase tannin extraction yield from barks. According to Pizzi (1979), the advantage of sulfitation in tannin adhesive application were lower viscosity, enhanced solubility, higher moisture retention by the tannin resin and allowing slower adhesive film dry-out. It could modify the tannin on polyflavonoid A-rings (resorcinol or phloroglucinol) during sulfitation making its more reactive towards formaldehyde due to the opening of the etherocyclic ring from the methoxyphenol to hydroxyphenol. The mechanism of the opening of pyran ring during sulfitaion of flavonoid tannins is showed in Fig. 3.11.

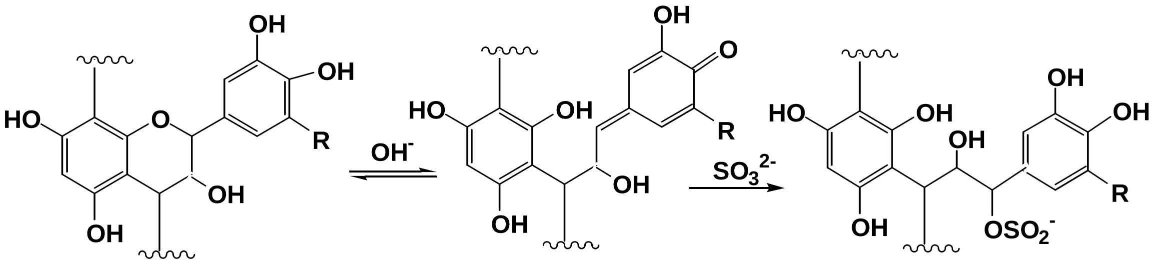

Fig.3. 11 Mechanism of the opening of pyran ring during sulfitation of flavonoid tannins

In this section, we experimented the utilization of sulfited reagents previously used to improve tannins exraction from bark, for the extraction of crude tannin from grape pomace. The sulfited extraction was examined using sodium sulfite (Na_2SO_3) in water in the presence of sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) or sodium bicarbonate ($NaHCO_3$). The influence of 3 parameters (i.e., reaction time, chemical reagents and concentration of the chemical reagents) on the extract yield and the composition of the solid residue were examined.

The total results were presents at Annex 1 from test 15 to test 32. Fig.3.12 and Fig.3.13 give the yield of crude tannin extracts as a function of different chemical reagents and different reaction time. The amount of crude tannin extracts increase obviously with the concentration of the reagents, and also with the duration timefrom 60 min to 120 min then reach a plateau with the longer time. The yield of tannins extracted by $NaHCO_3/Na_2SO_3$ is relatively lower than other two reagents. The suitable reaction is 120 min.

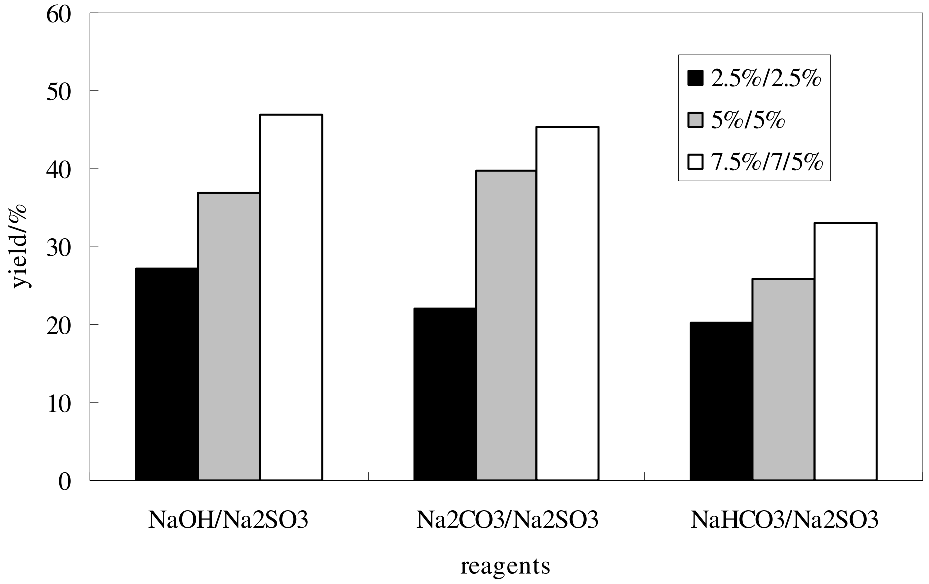

Fig.3. 12 The yield of crude tannin extracts as a function of different concentration of the chemical reagents

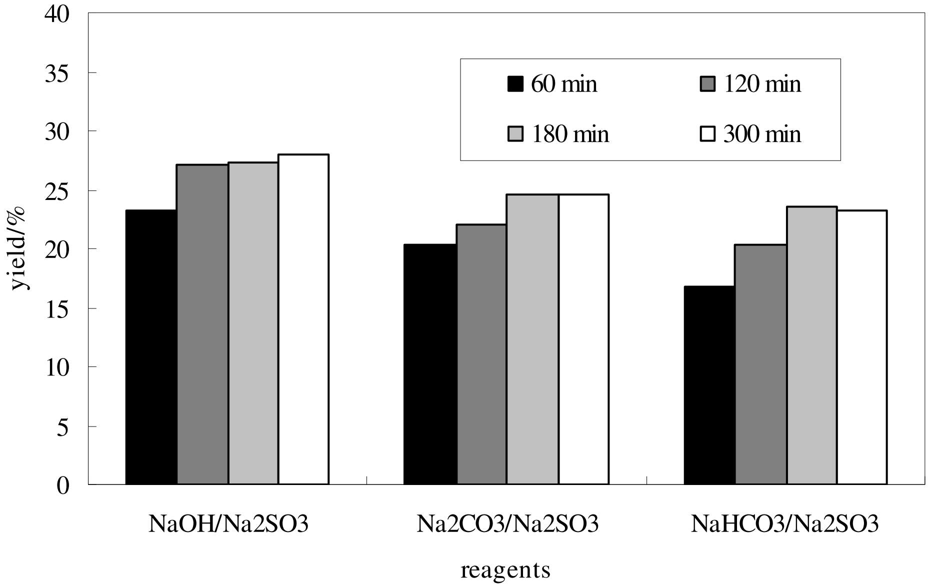

Fig.3. 13 The yield of crude tannin extracts as a function of different reaction time and different chemical reagents

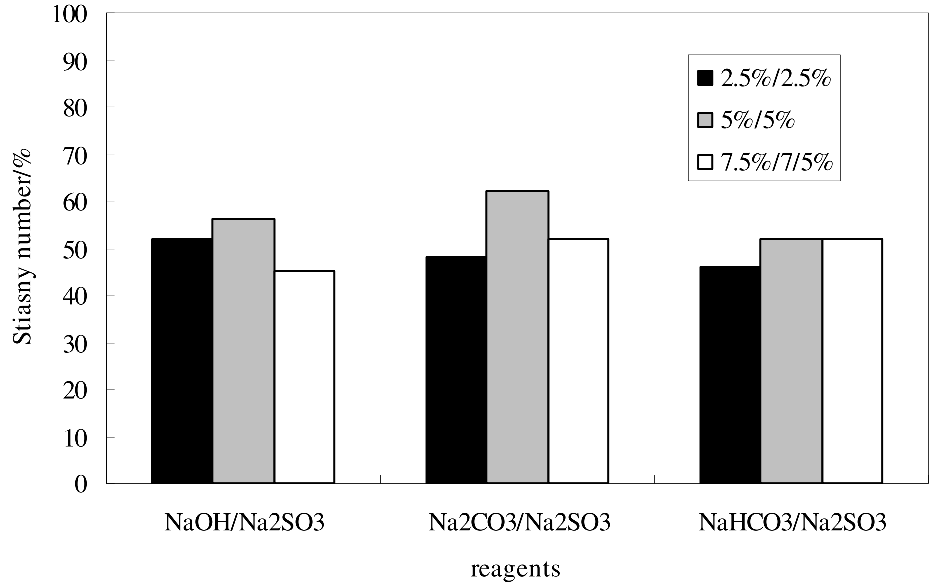

Fig.3. 14 The Stiasny numbers of crude tannin extracts as a function of different concentration of the chemical reagents

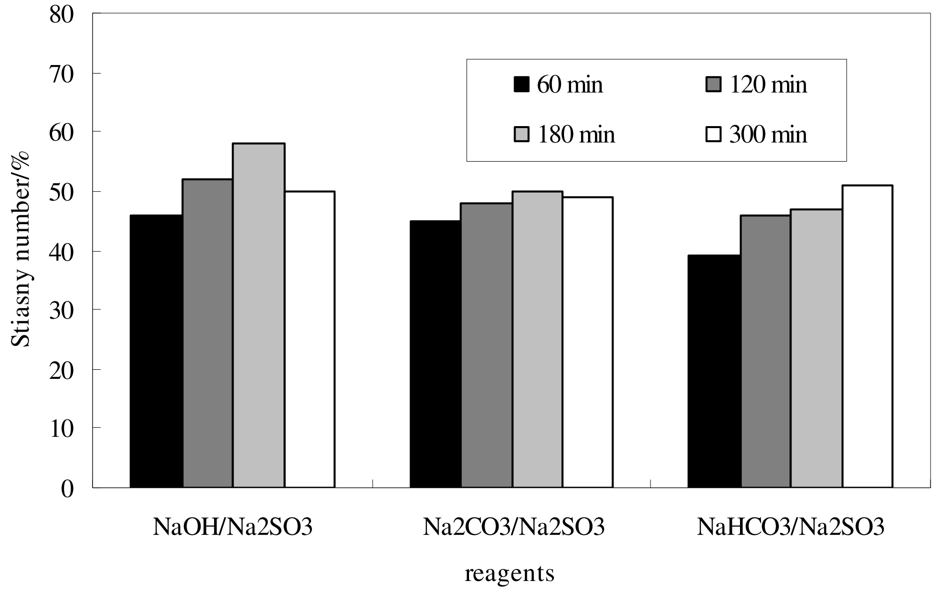

Fig.3. 15 The Stiasny numbers of crude tannin extracts as a function of different reaction time and different chemical reagents

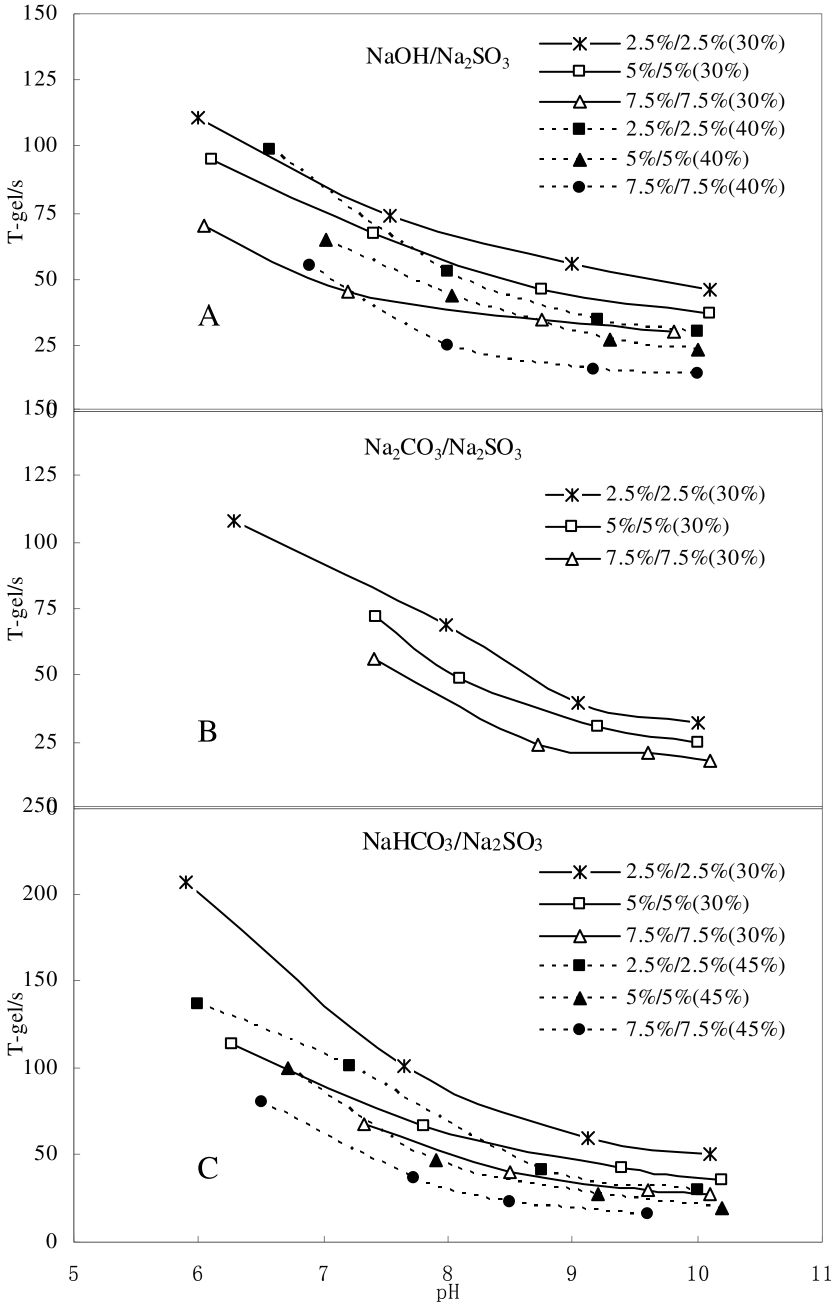

Fig.3. 16 Gel time of grape pomace sulfited tannin extracts reacted with paraformaldehyde at pH ranged from 6 to 10

Concerning the Stiasny number, it decreases for the higher concentration of reagent (Fig.3.14) and the reaction time reach to 300 (Fig.3.15), which indicated that a large amount of non-tannin fractions were extracted when using harsh extraction condition. The higher value of Stiasny number is 56 % (reagent of NaOH 5% /Na_2SO_3 5%), 62% (reagent of Na_2CO_3 5%/Na_2SO_3 5%) and 52 % (reagent of $NaHCO_3$ 5%/Na_2SO_3 5%), respectively.

The chemical compostions of solid residue by sulfitation extraction have the same variation trend as sodium hydroxide extraction. An increase in the chemical reagent concentration resulted in a reduction of the sugars and phenolic content of the residue whereas a longer reaction time seems to have a little influence. In this part, we do not analyze the solid residue chemical compositions in detail.

Gel time values for tannin extracts treated in different reagents and different concentration are given in Annex 1 accompanied with the pH of the tannins solutions in water (30 %, *w/w*, tannin/water).

Fig.3.16 gives the gel times of tannins extracts as a function of pH of the tannins solution in different concentration of sulfited reagents. As shown in Fig.3.16 A and Fig.3.16 C, the concentration of tannin solution largely impacts the reactivity of the extracts, the gel time becomes shorter as the higher concentration tannin solutions were prepared. It also appears that the gel time are directly correlated with the Stiasny number of the tannins fractions and a dramatic decrease in the gel time was observed when high reagent concentrations were employed in the presence of sulfite ions.

A comparison of the results using 5% (*w/w*) of NaOH, Na_2CO_3 and $NaHCO_3$ in the presence of 5% (*w/w*) Na_2SO_3, respectively, in these conditions crude tannin extracts bearing short gel times were isolated. The values are 67s, 72s and 66 s at the pH of 7.5, respectively.

Compared to previously described results, these values can not reach the gel times yet obtained at pH of 7.5 for faster reacting procyanidin-prodelphinidin pine and pecan nut tannins, which are equal to 25 and 32s, respectively. However, lower than the resorcinol-like mimosa and quebracho tannins, despite their lower purity (about 50-60 % of active phenolic ingredients). It demonstrates the good reactivity of these crude tannin extracts toward to paraformaldehyde. It appears that the tannins extracted by reagent of Na_2CO_3/Na_2SO_3 show the highest reactivity towards formaldehyde compared to the other two tannins.

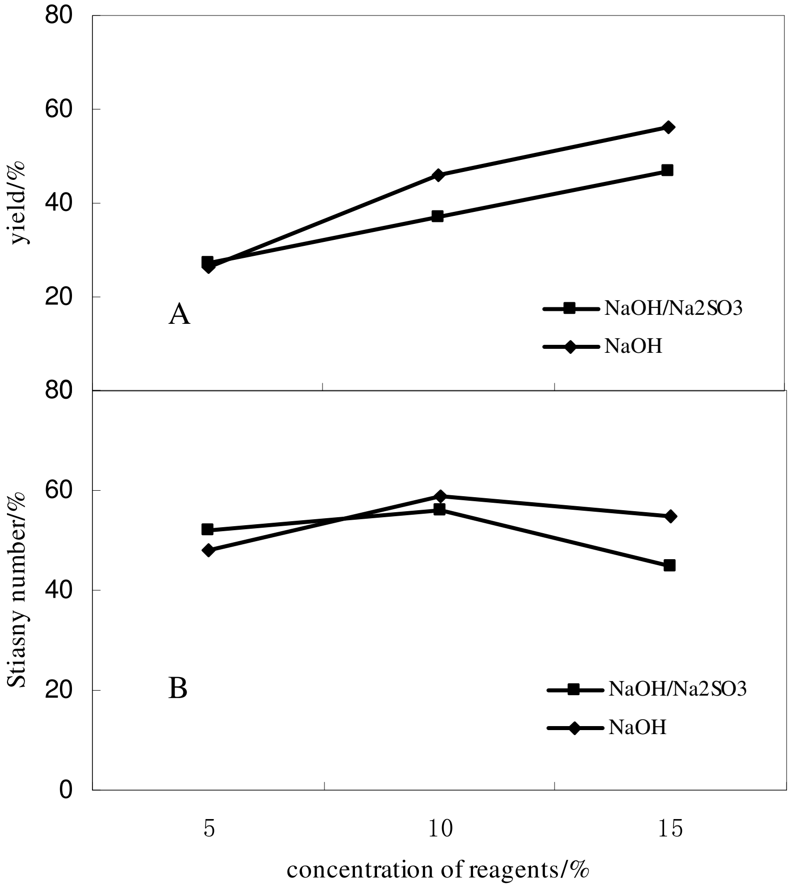

Fig.3. 17 A comparison of the yield and Stiasny numbers of grape pomace crude tannin extracts with and without sulfitation

The results of tannin extraction with and without sulfited reagent were also compared in this study. Fig.3.17 gives the yield and Stiasny number of crude tannin extracts using sodium hydroxide (NaOH) in the presence of sulfited reagents sodium sulfite (Na_2SO_3) compared to using sodium hydroxide (NaOH) only at the same reaction condition. The yield of sulfited tannins is slightly lower than soda tannins and the Stiasny number of sulfited tannins is fundamental equal to soda tannins except the concentration of reagents is 15%. It appears from these results that the extraction ability of sulfited reagents is lower than soda, but the sulfitation of tannin have the same reactive tannin content compared to soda tannin.

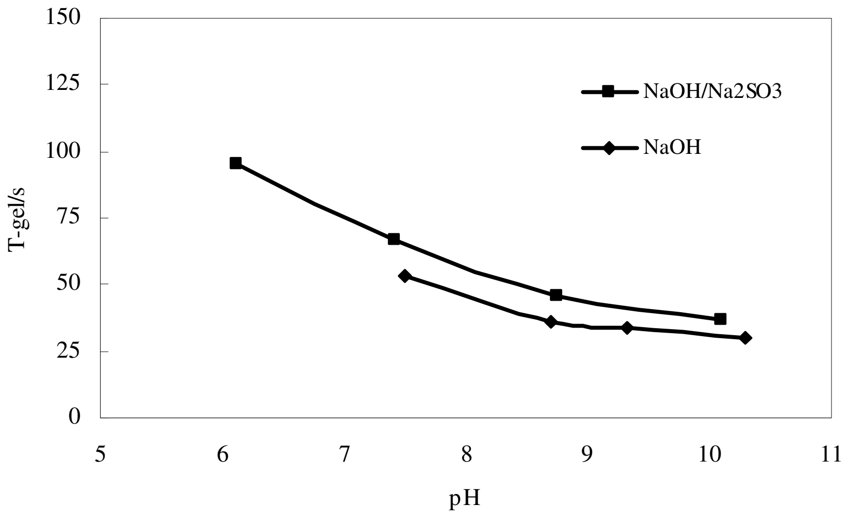

Fig.3. 18 A comparison of Gel time of grape pomace crude tannin extracted by 10 % NaOH and NaOH (5%)/Na_2SO_3 (5%)

As we known, the gel time of tannin based adhesive not only is affected by the reactive tannin content but also by the viscosity and solubility of tannin. The higher viscosity and lower solubility will shorter the tannins gel time value at the same reactive tannin content. Fig. 3.18 gives a comparison of gel time result of crud tannin extracted by 10 % NaOH and NaOH (5%)/Na2SO3 (5%), respectively. These two kind of crude tannins have nearly equal reactive tannin content (the Stiasny number of the crude soda tannins is 59 %, sulfited tannins is 56 %, respectively). The gel time of soda crude tannin extracts is shorter than sulfited crude tannin extracts. This phenomenon reflects that the sulfitation lower the viscosity of the tannin extracts using sulfited reagents from one aspect.

3.2.5 Kinetic study of tannins extraction

The kinetics of extraction of tannins from pomace in basic aqueous medium at 100 °C was carried out using the following conditions: NaOH 5 % and 10 % and NaOH/Na_2SO_3 2.5 %/2.5 % and 5%/5% (w/w based on dry mass raw material). The tannin concentrations in the hydrolysates were determined by UV quantification.

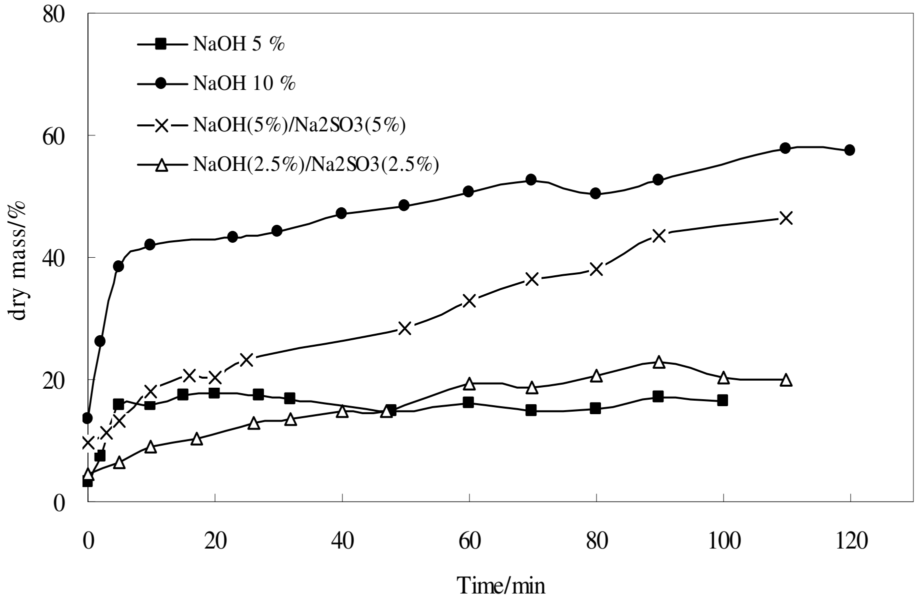

Fig.3. 19 Kinetic of tannins extraction[a]

[a] The tannin concentrations were determined by UV quantification

Fig.3.19 gives the variation of the tannin concentrations in the liquid phase (% based on the initial tannin content of the raw material) as a function of duration of the treatment. Using NaOH as reagent, the concentrations of extracted tannins increased rapidly to reach a maximum at ~ 10 minutes. No significant increase in yield was seen for longer reaction times. The addition of sulfite ions in the medium led to a different pattern. In these conditions, the tannins concentration in the liquid phase increased steadily with the duration of the treatment and no plateau effect was observed. This behaviour seems to attest to a different mechanistic pathway of extraction in presence of sodium sulfite and is in accordance with previously described mechanism (Pizzi., 1994) involving a reaction of the nucleophilic sulfite ion with a flavonoid C-ring opening (see Fig. 3.11). Moreover, it appears that the optimum of tannin extract ability is directly correlated to the base concentration: a yield of ~20% was observed using 5% (w/w) of NaOH compared to ~40% yield using 10% (w/w) of NaOH.

3.2.6 Discussions

Most of the basic reagents used in this study have been previously described in the literature to extract tannins from bark, but generally in much lower proportions, such as NaOH or Na_2CO_3 in the range of 0.5-1.0 %. Panamgama(2007) described the extraction of tannins from ***Pinus radiate*** bark using Na_2CO_3/Na_2SO_3, 0.4%/4% (*w/w*). Pizzi (2003) extracted tannins from quebracho and pecan nut using 3-5 % sodium sulfite or metabisulfite eventually in the presence of low sodium carbonate concentrations (0-3 %) at a temperature of 70 °C. the utilization of harsher conditions for the extraction of tannins from grape pomace can be rationalised by fact the easily hydrolysable fraction of condensed tannins was extracted during the vinification process. Thus, the hydrolysis and solubilisation of the residual tannin fraction of higher DP necessitates higher temperature (optimum temperature =100 °C) and a higher pH (concentration of the base in the range of 2.5-7.5 %). At higher temperature (120 °C), we have demonstrated that tannins with lower reactivity were obtained; this observation can be rationalized by the formation of higher catechinic acid content. The formation of this rearrangement product should affect the more reactive A-aromatic ring of the catechin moiety and consequently should decrease the reactivity towards formaldehyde. On the other hand, using milder condition (70°C), extracts bearing low water solubility were isolated because of the extraction of higher molecular mass tannin fractions.

The acidified tannins precipitated at pH 1.5 show a low water solubility, perhaps it is not suitable to as tannins-formaldehyde adhesive directly, but it is can be copolymerized with PF resol to afford a resin. Vázquez et al (1996) described the precipitated ***Pinus radiate*** bark at pH = 2 was copolymerized with a resol to afford a resin with a 1:1 phenol/tannins ratio, this resin was applied in plywood boards and obtained better tolerance to moisture in plies and a high levels of adhesive-repellent substances than commercial phenol-formaldehyde resins.

The lyophilization tannins obtained in this study with a gel time below 100 s at pH 7 can be classified under the same group as the faster reacting procyanidin-prodelphinidin tannins previously described such as pine bark and pecan nut pith extracts (Pizzi, 1994). These results are rationalized by the fact that the A-rings of grape pomace tannins possess the phloroglucinol type of structure (proanthocyanidins), much more reactive towards formaldehyde than a resorcinol-type structure, with important consequences in the use of these tannins for adhesives. Thus despite their low purity (about 50% of active phenolic ingredients), and the harsh experimental conditions used for their extraction (100 °C, high pH), these grape pomace extracts display a good reactivity towards formaldehyde.

3.3 conclusions

In this chapter, we examined the extraction of condensed tannins from grape pomace for the production of adhesive. Two different extraction processes and the influence of 4 parameters (i.e., temperature, reaction time, chemical reagents and concentration of the chemical reagents) on the tannin extracts yield, properties and the composition of the solid residue were studied. We demonstrated that:

1. It is possible to perform an extraction of reactive tannins from grape pomace in basic medium using harsher extraction conditions with higher alkali concentration and higher reaction temperature than for bark treatments. The tannin extracts obtained by precipitation in acidic conditions display a high phenolic content (Stiasny number higher 95%) but low water solubility and low reactivity toward formaldehyde because of the formation of catechinic acid and rearrangement of flavnoid to phlobatannins during the acidification step. The tannins

extracts obtained by lyophilisation of the liquid, despite of their lower phenolic contents, displayed promising properties for adhesive applications.

2. The optimum temperature was 100 °C, the formation of catechinic acid at high temperature (120 °C) and extraction of higher molecular mass tannin fractions at low temperature (70 °C) both impacted the reactivity of the extract towards formaldehyde. The optimum reaction time was 120 min; it appeared no significant improvement in the yield and the Stiasny number for longer reaction times.

3. The best concentration of reagent was 10% when reactive polyphenol content of tannins reaches the highest value, the increase of tannins yield but the decrease of stiasny number at higher concentration indicated the more non-tannin fractions were extracted in the tannin extracts. The higher Stiasny number reaches 58 % (NaOH 10%), 56 % (NaOH (5%)/Na_2SO_3 (5%)), 62% (Na_2CO_3 (5%)/Na_2SO_3 (5%)) and 52 % ($NaHCO_3$ (5%)/Na_2SO_3 (5%)), respectively.

4. Sulfitation of tannins is the most useful method in flavonoid chemistry and can be useful in the preparation of tannin-based adhesive. The introduction of a sulfonic group through sulfitation increased both tannin solubility and reactivity as a result from the opening of the heterocyclic ring during extraction in this study.

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Chapter 4. Characterization of condensed tannins from grape pomace

4.1 Introduction

Industrial tannin extracts is a kind of polymer mixture polymerized by flavonoids composition, which also contains non-phenolic conponents such as carbonhydrate. Therefore, the distribution and molecular weight and chemical composition are inhomogeneous, which is affected by different extraction process and the raw material. It is the first time to extract tannins from grape pomace raw material and intend to use as wood adhesive to press wood particleboard. There is not any literature about the structure characterization of grape pomace tannins before. In this way, it is necessary to identify it structure before application.

There are two methods (chemical method and physical method) to indentify the chemical structure and composition of tannins. The physical method is the main analysis method before the application of modern instrument analytical technique. For example, the chemical structure of first natural flavan-3,4-ols unit was separated by chemical method in the year of 1954. The disadvantages of classics chemical method to identify structure are big workload, long analysis time and large comsumption of samples. At present, the physical method is the main analysis method based on the development of the modern instrument analytical technique. The mainly methods such as UV-VS spectroscopy, infrared spectroscopy, mass spectroscopy and nuclear magnetic resonance spectroscopy have greatly accelerated the research of tannin structure study (Sun., 1988).

Nuclear magnetic resonance (NMR) is one of the most common method used to identify and differentiate the structure and degree of polymerization of high molecular polymers. ^{13}C-NMR analysis can indicate rapidly, simply, and directly on concentrated solution of the tannin extract, structural characteristics and properties that are of potential importance in the use of these materials as wood adhesives. The tannin extracts could be detected by ^{13}C-NMR were

(1) The relative proportions of fisetinidin-robinetidin units in relation to catechin-epicatechin units,

(2) Differences in the number average degree of polymerization,

(3) The extent of open heterocycle forms present,

(4) The extent of proper branching,

(5) The relative proportion of pyrogallol versus catechol B-rings in the flavonoid repeating units.

~~As we knowledge~~, such an approach of characteristics are fundamental for tannin extract application and formulation as adhesives. The ^{13}C-NMR band assignment for pure catechin is given in Fig. 4.1, as an reference to analyze the tannin extracts (Pizzi., 1994).

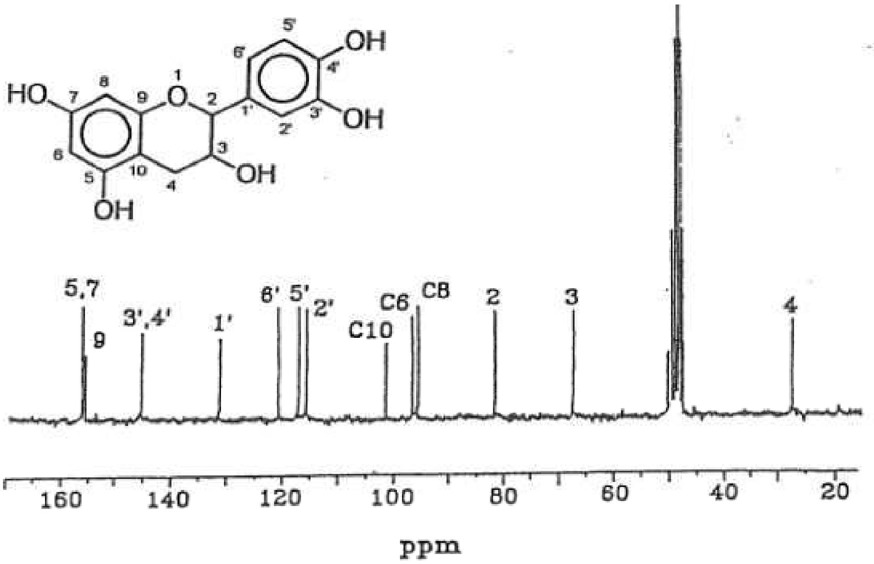


Fig.4. 1 ^{13}C-NMR spectrum of pure monomeric catechin with carbon atom assignments

Another powerful technique, matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS) has also been used recently for this purpose (Pizzi., 1994). MALDI-TOF MS was originally introduced by Karas et al (1987) which allows the characterization of macromolecules compounds and determination of the polymer chain length. It also has the ability to detect high molecular weight compounds and also the pattern of oligomers with small differences in mass. This technique has increased the upper mass limit for mass spectrometric analyses of macropolymers by more than 300,000 Da. The MALDI-TOF has been used in the analysis of condensed tannins, mainly in the field of food science (Foo et al., 2000; Ohnishi-Kameyama et al., 1997; Hedquist et al., 2000; Kruger et al., 2000; Meagher et al., 2006; Monagas et al., 2010). Pash et al (2001) had successfully introduced MALDI-TOF to the analysis structure on commercial mimosa (*Acacia mearnsii*) tannin, quebracho (*Schinopsis balansae*) tannin and pecan nut (*Crya illinoensis*) tannin. Other condensed tannin such as bark of *Pinus radiate*, bark of *Pinus pinaster*, *Pices abies* extracts, *A.auriculiformis* extracts and *Phizophora apiculata* extract have been successfully analysis using this technique (Ku and Mun., 2007; Weber et al., 2007; Clemens et al., 2004; Oo et al., 2008; Ishida et al., 2005). As so far, none has been published on the analysis of the structure of grape pomce tannin extracts.

Fourier transform-infrared spectroscopy (FT-IR) is a powerful method used to indentification the functional groups and molecular structure of chemical compounds due to each compound has its own infrared spectrum. The main chemical bonds vibration and its assignment can be concluded in Table 4.1 (Li., 2003). FT-IR can be used to indentify the functional group and molecular structure of tannin extracts. For example, the carbonyl group is the most characteristic absorption peak in tannin IR spectrum. It exists in all the hydrolysable tannin and acylation condensed tannin, but not in the condensed tannin and flavan-3-ols unit.

Table 4. 1 The main FI-IR peaks and its Assignment of chemical compounds' bonds vibration

Wavenumber cm^{-1}	Assignment
3650～2500	O-H, N-H (stretching vibration)
3300～3000	C-H (-C≡C-, -CR＝CH-, Ar-H) (stretching vibration)
3000～2700	C-H (-CH_3-, -CH_2-, R_3C-H, -CH＝O) (stretching vibration)
2275～2100	C≡C, C≡N (stretching vibration)
1870～1650	C＝O (stretching vibration)
1690～1590	C＝C, C＝N (stretching vibration)
1475～1300	Saturated C-H (in plane bending vibration)

1000～670	C-H (C= C-H, Ar-H) (out of plane bending vibration)

Thermal analysis technology is kind of analysis method based on the characteristics of thermal behavior. As we known, the material happen various physical and chemical changes in heating or cooling, these changes are usually accompanied with thermal effects and other phenomena, which became the thermal analysis testing object. Thermo gravimetric analysis (TGA) was used to test the function of sample's mass corresponding to temperature or time in control temperature condition (Li., 2003).

In this Chapter, the composition, molecular weight distribution, thermal stability and structure of grape pomace condensed tannin fractions extracted by different extraction processes and different extraction reagents were characterized using Fourier transform-infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), carbon nuclear magnetic resonance spectroscopy (^{13}C-NMR) and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS).

4.2 Results and discussions

The condensed tannin extracts from grape pomace were carried out considering previously described in chapter 3 using acidified and lyophilized processes and different extraction reagents. The reagents are sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) and sodium sulfite (Na_2SO_3) at 100 °C during 120 min in an aqueous medium.

4.2.1 Elemental analysis of tannin extracts

The elemental analysis of grape pomace crude lyophilized tannin extracted by three different basic reagents (2.5% (*w/w*) of NaOH, Na_2CO_3 and $NaHCO_3$ in the presence of 2.5% (*w/w*) Na_2SO_3, extraction conditions A, B and C respectively were studied using Perkin Elmer

240C microanalyser. The result is presented in Table 4.2. The C%, H% and N% values of tannins extracted by three different reagents are lower than grape pomace raw material, but not obviously difference among themselves. The element S is introduced by the sulfitation, the value of S% presents the sulfite group contents in the tannin extracts. It can be seen that the sulfite group in tannin fractions in condition B and C is higher than condition A (A : 1 sulfited moiety every 25 residues; B and C : ~1 sulfited flavonoid moiety for approximately every 15 residues). The protein was caculated from the value of element N, it can be dedued that the extracts contained about 10 % (*w/w*) of proteins. The value in condition B is higher than the others.

Table 4. 2 Elemental analysis of grape pomace tannins extracted by different extraction reagents

extraction conditions	**C%**	**H%**	**N%**	**S%**	**Proteins %**
Raw material	46.29	5.39	2.14	-	13.4
A (NaOH 2.5%/ Na_2SO_3 2.5%)	35.92	3.84	1.43	0.01	8.94
B (Na_2CO_3 2.5%/ Na_2SO_3 2.5%)	36.64	3.81	1.48	0.03	9.25
C ($NaHCO_3$ 2.5% /Na_2SO_3 2.5%)	36.43	3.80	1.27	0.04	7.94

4.2.2 FT-IR study of tannin extracts

The functional groups of the grape pomace tannin extracts using three different basic reagents A, B and C (the same samples as elemental analysis) were analysed by FT-IR. The spectra are presented in Fig. 4.2 and the assignments are given in Table 4.3.

The band at 3318 cm^{-1} was assigned to hydroxyl both contribute to phenolic and sugar content. The bands at 2973 cm^{-1} were due to C-H stretching vibration assigned to methyl and methylene groups. A weak signal at 1878 cm^{-1} is assigned to carbonyl groups. The presence of carbonyl groups could be rationalized by the catechinic acid rearrangement occuring during the extraction process. As previously described, the formation of catechinic acid is to be minimized because it should decrease the reactivity of the extract toward cross linking agent. The bands at 1308 cm^{-1}, 1264cm^{-1} and 1212 cm^{-1} were assigned to saturated C-C stretching attribute to CR$_2$-CHR-CR(SO$_3^{2-}$) structure caused by the opening of the pyran ring during sulfitation of flavonoid tannins (Hoong et al., 2009). This reaction has a positive effect because it increases the solubility of tannins and reduces the viscosity of the extracts allowing the use of adhesive solutions of higher resin content. The mechanism of the opening of pyran ring is given in Chapter 3.

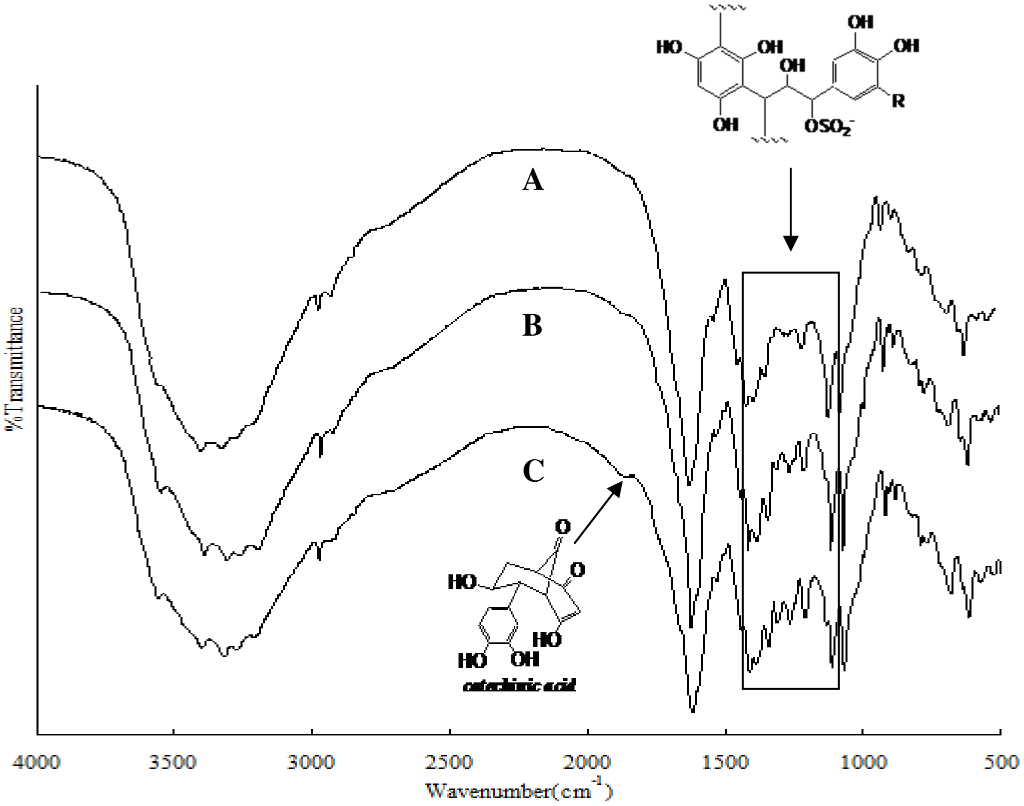


Fig.4. 2 FT-IR spectra of grape pomace tannins extracted by different extraction conditions

A comparison of the IR signals for A, B and C and of their relative intensities indicates that the ratio of the heterocyclic C-ring opening of flavonoid unit is function of the nature of the base used for the tannins extraction. It appears from Fig. 4.4 that the opening of pyran ring during the tannins extraction seems to be more important by using Na$_2$CO$_3$ and NaHCO$_3$ (respectively conditions B and C) than when using NaOH (condition A). This observation is in accordance with the sulfite group contents of the three tannin fractions established based on elemental compositions given in Table 4.2

Table 4. 3 Assignment of FT-IR spectra of grape pomace tannins extracted by different extraction conditions

Peak cm^{-1}	**assignment**
3318	-OH stretching vibration
2973,2931	$-CH_3$, $-CH_2-$ stretching vibration
1878	C=O stretching vibration
1619,1534,1501	Aromatic squel vibration
1439	CH deformation and aromatic ring vibration
1412	Aromatic vibration
1341	saturated CH in plane bending vibration
1308,1264 , 1212	C-C frame stretching(C-CHR-C)
1111	aromatic CH in plane bending vibration
1066	C-O stretching vibration
921	aromatic CH out of plane bending vibration

The FT-IR spectra of lyophilized tannins (A) (NaOH 2.5% (*w/w*)/ Na_2SO_3 2.5% (*w/w*)) and acidified tannins (D) (NaOH 5 %(*w/w*)) were given in Fig. 4.3. The acidified tannins (Fig.4.5 D) displayed a different pattern:

-No obviously band among 1308-1212 cm^{-1} was detected assigned to saturated C-C stretching attribute to CR_2-CHR-CR (SO_3^{2-}) structure caused by the opening of the pyran ring in the spectra.

-An intense signal at about 1878 cm^{-1} assigned to carbonyl groups indicates higher catechnic acid content in acidified tannins, the bands assigned to aromatic ring vibration (1600-1400 cm^{-1}) are quite stronger than lyophilized tannin extract. These results are in accordance with the catechinic acid rearrangement occoured during the acidification step and higher phenolic content with high Stiasny number values (95-98 %) in acidified tannins.

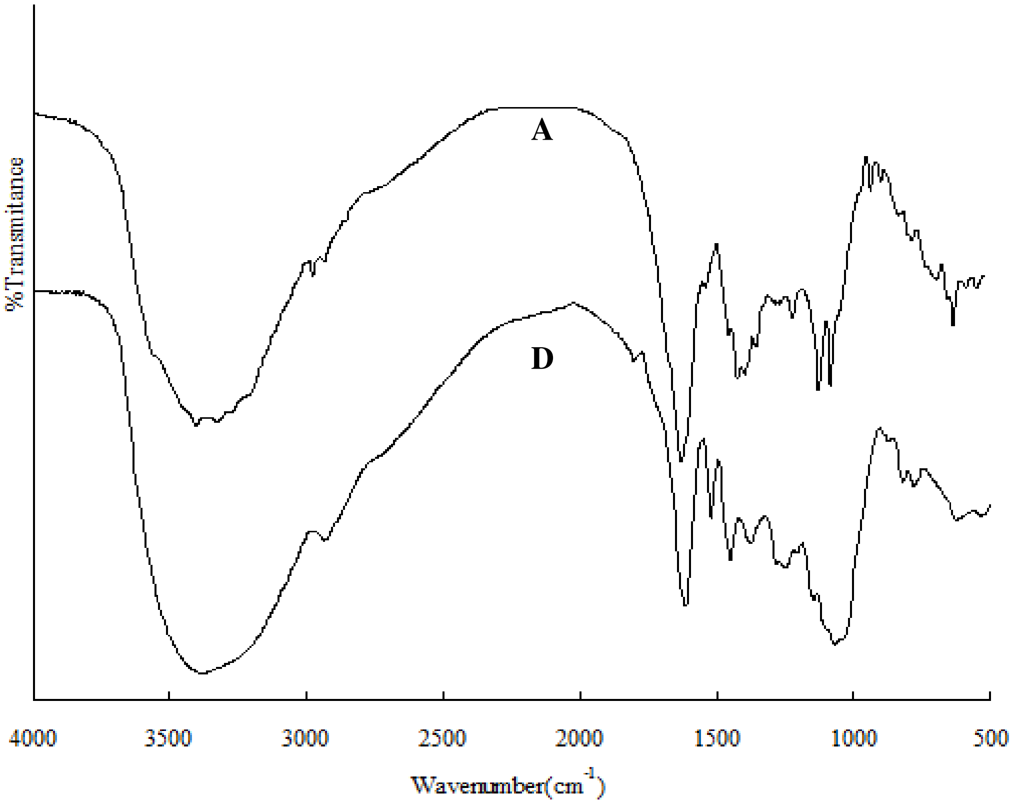


Fig.4. 3 FT-IR spectra of grape pomace tannins extracted by different extraction processes

4.2.3 TGA study of tannin extracts

Thermo gravimetric analysis (TGA) was applied to determine the thermal stability and degradation of tannin extracts from grape pomace. The TGA curve presents the weight loss percentage of the tannin extracts and the DTG cure is the differential curve, presents the mass lost rate. The thermal properties of the grape pomace tannin fractions extracted by different extraction reagents A, B and C (the same samples as elemental analysis) were studied used

TGA. The results are showed in Fig. 4.4. At the same time, the tannins extracted by different processes were also examined, the results are showed in Fig.4.5.

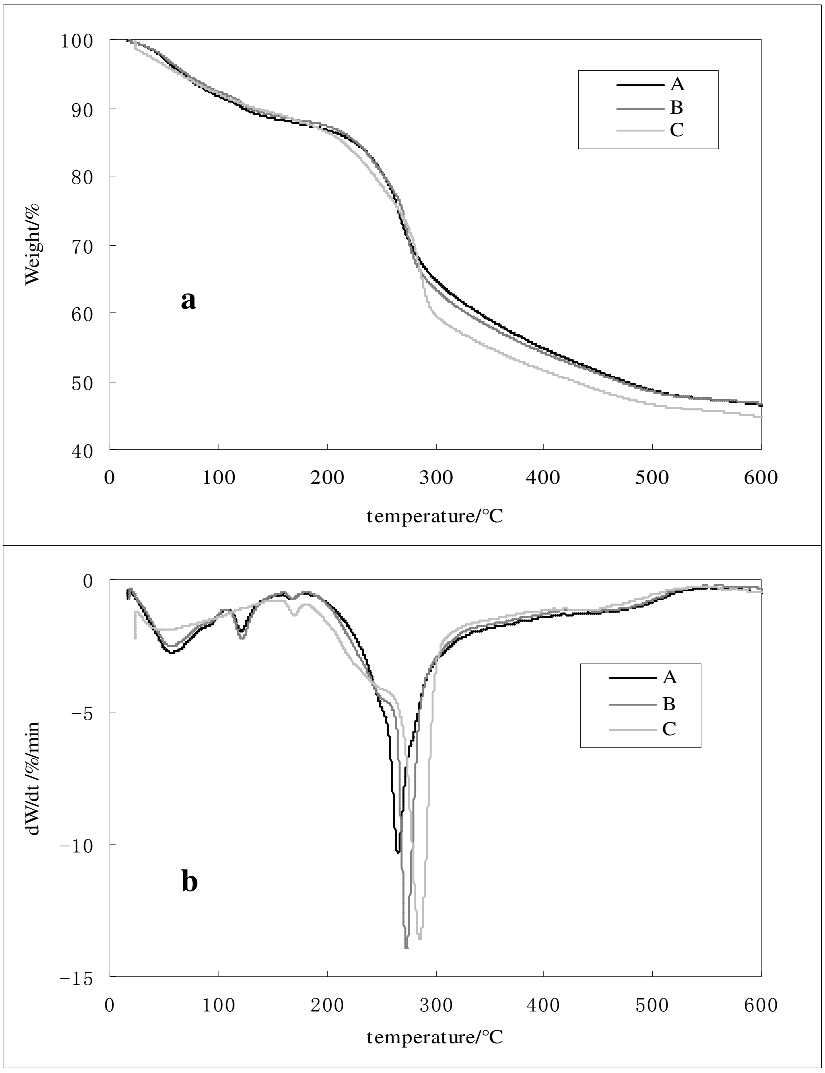


Fig.4. 4 TGA (a)and DTG(b) curves of tannins extracted by different extraction conditions

It can not seen noticeable difference among these three kinds of sulfited tannins extracts from TGA and DTG curves (Fig.4.4). The weight lost of these tannin extracts is mainly composed by two steps (see Fig.4.4a). The mass lost of first step is nearly 12 % with the temperature from room temperature to 200 °C. It is attributed to the mass lost of water and some easy-degraded small low molecular materials such as simple sugars, organic acid, phenol and pigment. The secondly steps which is the mainly degradation step of tannin extract samples from 200 to 400 °C. The mass lost is nearly 33%. It is attributed to the mass lost of polyphenol materials in the tannin extracts. It appears from Fig.4.4b that the temperature of mass loss of tannins seems to be higher by using Na_2CO_3 and $NaHCO_3$ (respectively conditions B and C) than when using NaOH (condition A). This phenomenon perhaps is caused by the different molecular weight distribution of crude grape tannins extracted by different chemical regeants.

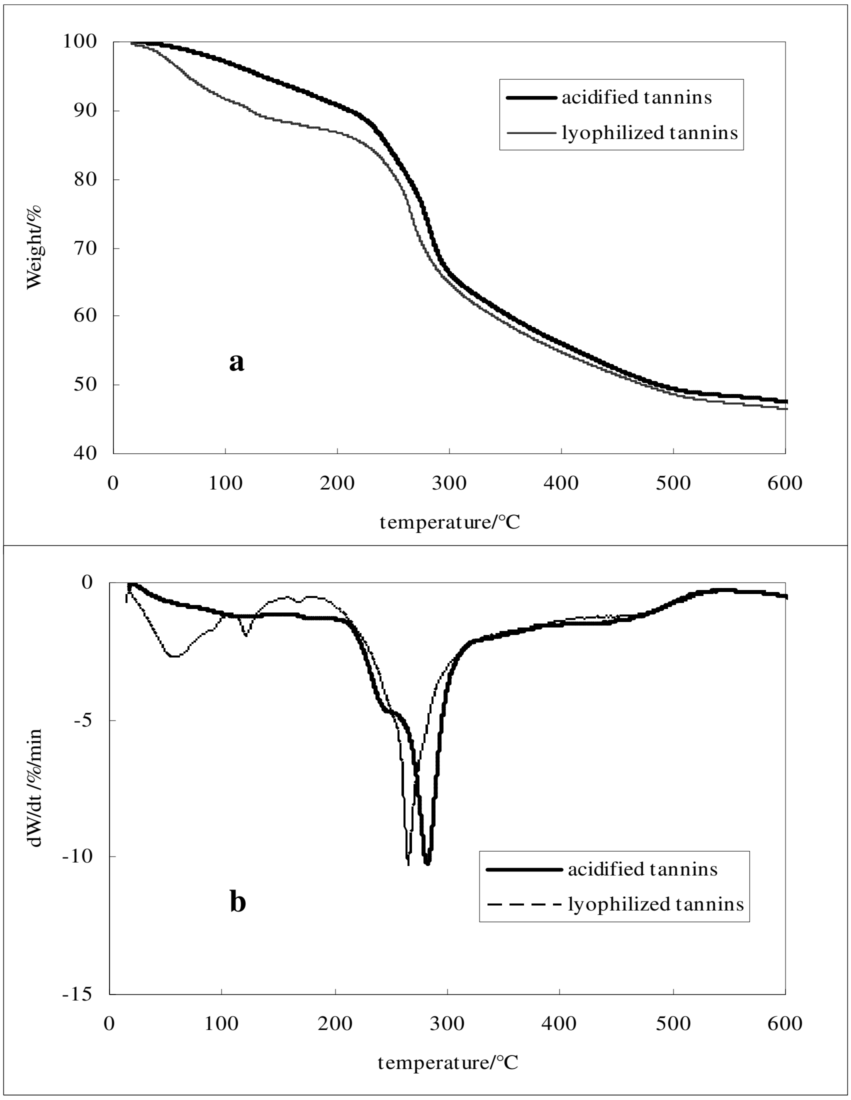


Fig.4. 5 TGA(a) and DTG(b) curves of tannins extracted by different extraction processes

A comparison of the thermal properties of acidified tannins (NaOH 5 %(*w/w*)) and lyophilized tannins (NaOH 2.5% (*w/w*)/ Na_2SO_3 2.5% (*w/w*)) is given in Fig.4.5. It appears that an obvious difference occured in the first step(room temperature to 200 °C) : concerning acidified tannins, the mass lost nearly 9%, mainly occurred by the evaporated of water content.

It never appears apparent mass lost rate point compared to lyophilized tannins. This phenomenon can be attributed to the lower non-tannin fraction (such as low molecule sugars) in the acidified tannins. This result is in agreement with high Stiasny number value of acidified tannins previously observed. We also observed that the acidified tannins mass loss temperature is higher than lyophilized tannins, this observation can be rationalized by a higher degree of polymerization of acidified tannins compared to lyophilized tannins.

4.2.4 NMR study of tannin extracts

The solution ^{13}C-NMR spectra of the acidified and of the lyophilized tannins from grape pomace were performed in d_6-DMSO and D_2O, respectively. The acidified tannins spectrum was performed in d_6-DMSO because of its low solubility in water.

The grape pomace lyophilized tannin extracts using three different basic reagents A, B and C (the same samples as elemental analysis) were analysed by solution ^{13}C-NMR. The spectra are presented in Fig. 4.6 and were interpreted according to previous studies (Fu et al., 2007; Zhang et al., 2010; Pizzi., 1994).

Tannins show characteristic ^{13}C peaks consistent with that condensed tannins with dominant procyanidin units and with a minor amount of prodelphinidin.

A single broad band at 150 to160 ppm belong to the flavonoid C5, C7 attached to phenolic –OH groups on the phloroglucinol A-ring, the band at approximately 155 ppm belong to C9 simultaneously, overlapping with the C5 and C7 band; band at 96-98 ppm are assigned to the unreacted C6 and C8 sites on A-rings. The absence of the C4-C6 interflavonoid band at 95 ppm and the presence of the C4-C8 interflavonoid band at 105 ppm seem to indicate that the units could be exclusively linked to C4-C8 (Pizzi and Stephanou, 1993; Navarrete et al., 2010), a classical pattern for a procyanidin.

The band 142-145 ppm belong to the C3′ and C4′ of procyanidin units; a small amount of prodelphinidin is also detected as its C4′ band at 129-132 ppm, overlapping with the chemical shifts of C1′ on the B-ring; the band at 120-121 ppm for C6′; the band at 115-117 ppm for C5′ and C2′, this band is an indication of the proportion of pyrogallol B-ring: the greater its intensity the lower the proportion of pyrogallol ring in the tannin.

The cluster of peaks between 170 to 182 ppm assigned to carbonyl groups. Peaks region at 60 to 90 ppm occur from carbons of polymeric and monomeric carbohydrates which are always present in industrial tannin extracts. Meanwhile, The C3 band is at 68 ppm and the C2 band is at 78 ppm, which could be covered by the carbohydrates signals.

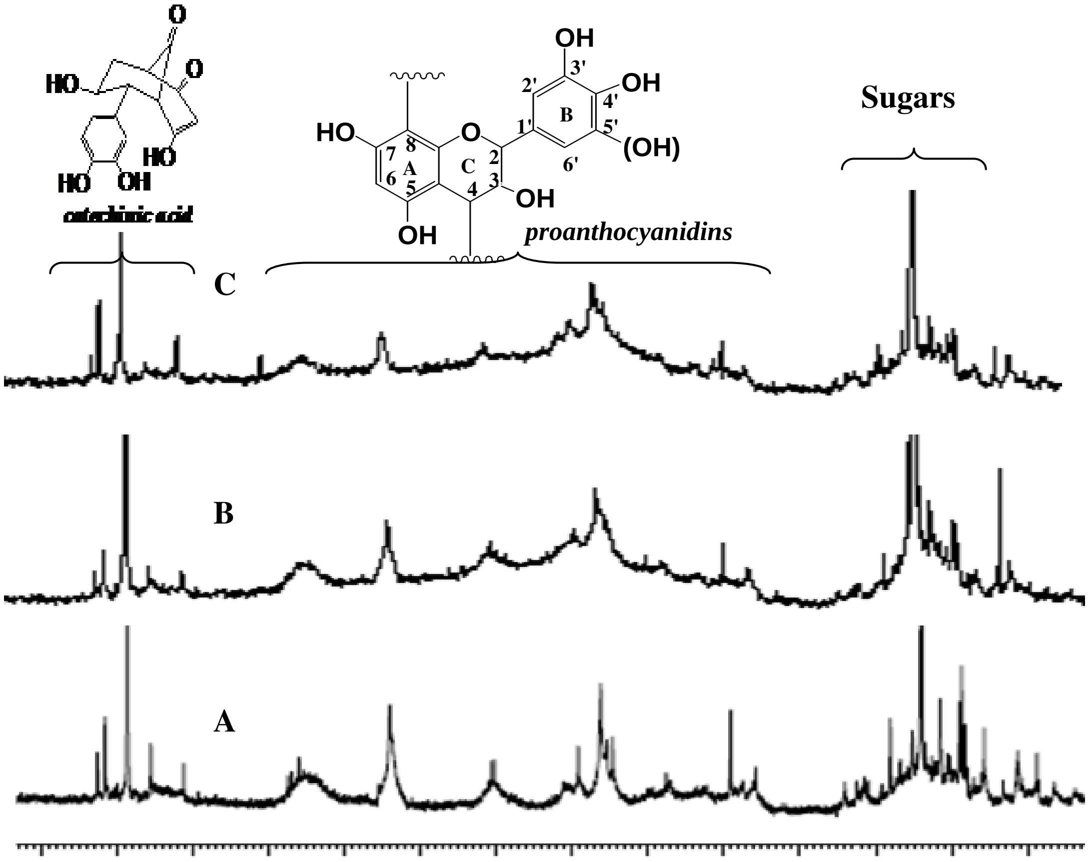


Fig.4. 6 ^{13}C-NMR spectra of grape pomace condensed tannins extracted by different extraction conditions

A comparison of ^{13}C-NMR spectra (Fig.4.7) of lyophilized tannins (A) (NaOH 2.5% (*w/w*)/ Na_2SO_3 2.5% (*w/w*)) and acidified tannins (D) (NaOH 5 %(*w/w*)) shows some different patterns in some bands.

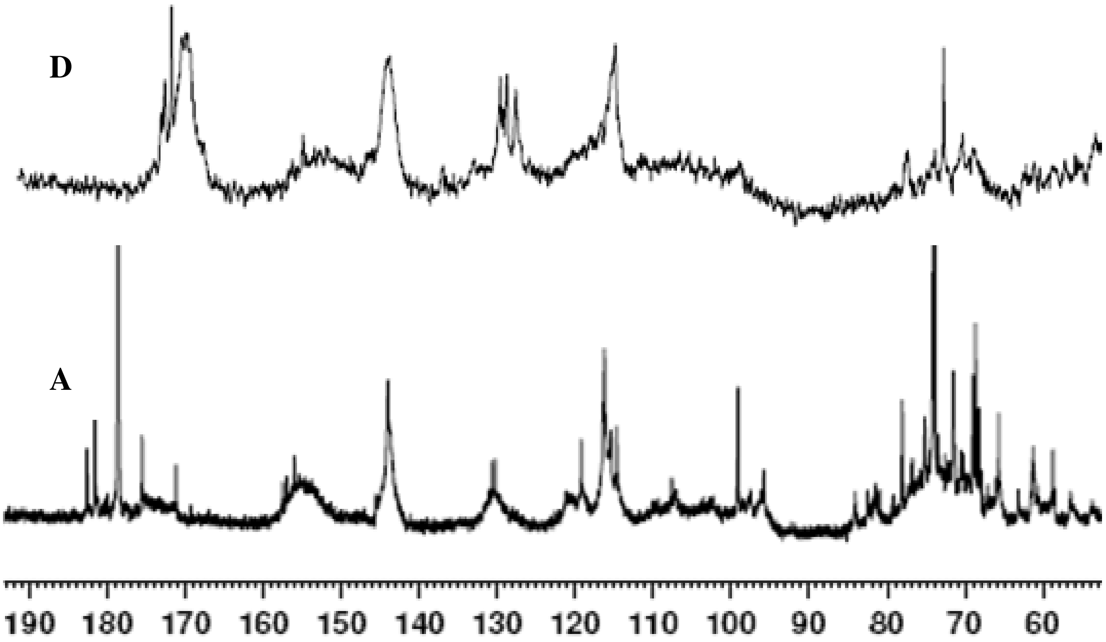


Fig.4. 7 ^{13}C-NMR spectra of grape pomace condensed tannins extracted by different extraction processes

-The carbonyl groups band at about 170-182 ppm in acidified tannins is stronger than lyophilized tannins. It could be rationalized by the catechinic acid rearrangement (Sears et al., 1974; Kiatgrajai et al., 1982) which would occur during the acidification step. The formation of catechinic acid could also explain the low water solubility of these tannins fractions.

-The unreacted C6 and C8 sites on A-rings at 96-98 ppm are a set of very sensitive bands indicating the polyflavonoid reactivity directly and the degree of polymerization of the tannins indirectly. Compared to acidified tannins, the lyophilized tannins present more intense signals in this region suggesting a higher reactivity and lower degree of polymerization than the former.

-Relatively high carbohydrate content could also be deduced by the high intensity of the peaks at 60 to 90 ppm in lyophilized tannins. This observation is in agreement with the important amount of sugars or sugar derivatives detected in the lyophilized tannins samples and low stiasny number results which were used to measure the polyphenol content of tannin extracts in previously study. The acidified tannin is 97% and the lyophilized tannin is 52%.

4.2.5 MALDI-TOF study of tannin extracts

Grape pomace is a natural polymer that consists of various types of polyphenolic oligomers. Fig.4.8 and Fig.4.9 show a typical MALDI-TOF analysis of acidified tannins (NaOH=5 %(*w/w*)) and lyophilized tannins (NaOH 2.5% (*w/w*)/ Na_2SO_3 2.5% (*w/w*)) extracted from grape pomace with the molecular weight ranging from 500 to 3000 Da. It could be concluded that acidified tannins contain oligomers up to 10 repeating units, and lyophilized tannins have oligomers up to 6 repeating units, based on the largest tannin oligomer formed in the samples which showed a peak at 2926.7 Da and 1924.2 Da respectively.

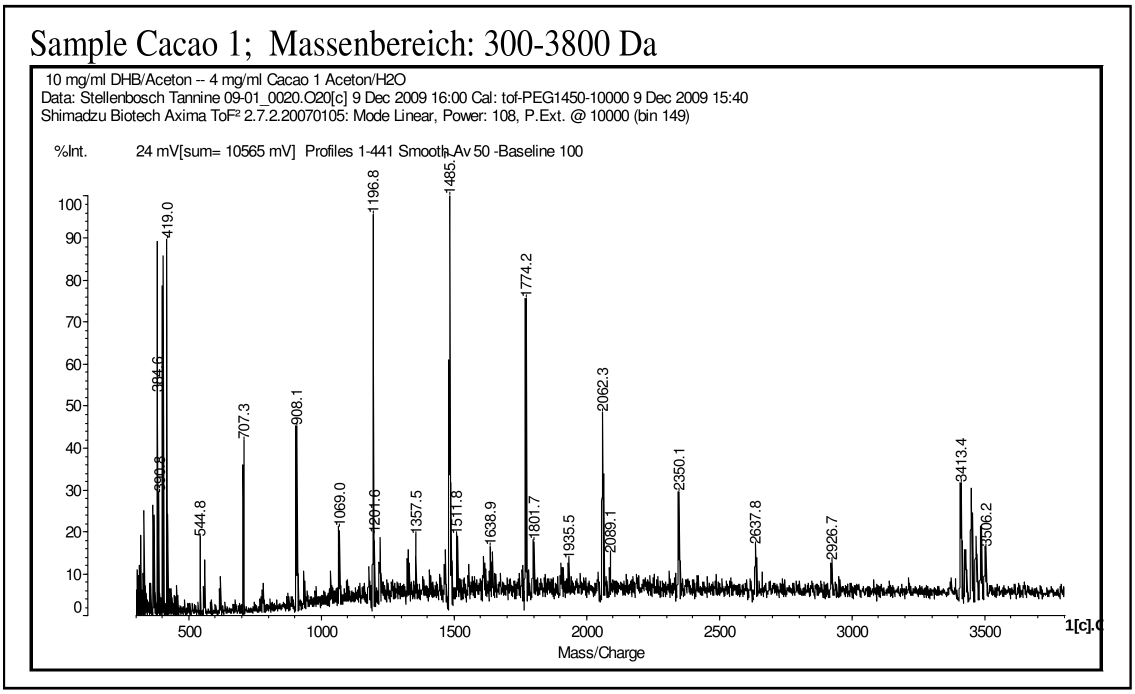


Fig.4. 8 MALDI-TOF mass spectrum of acidified tannins obtained from grape pomace

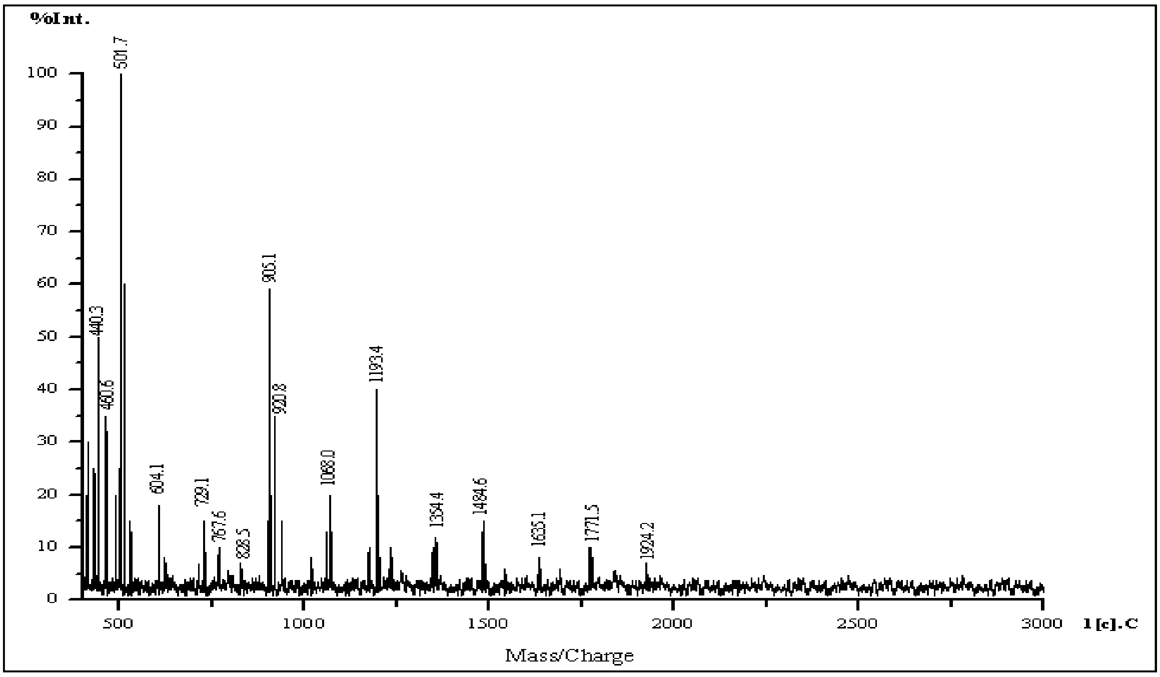
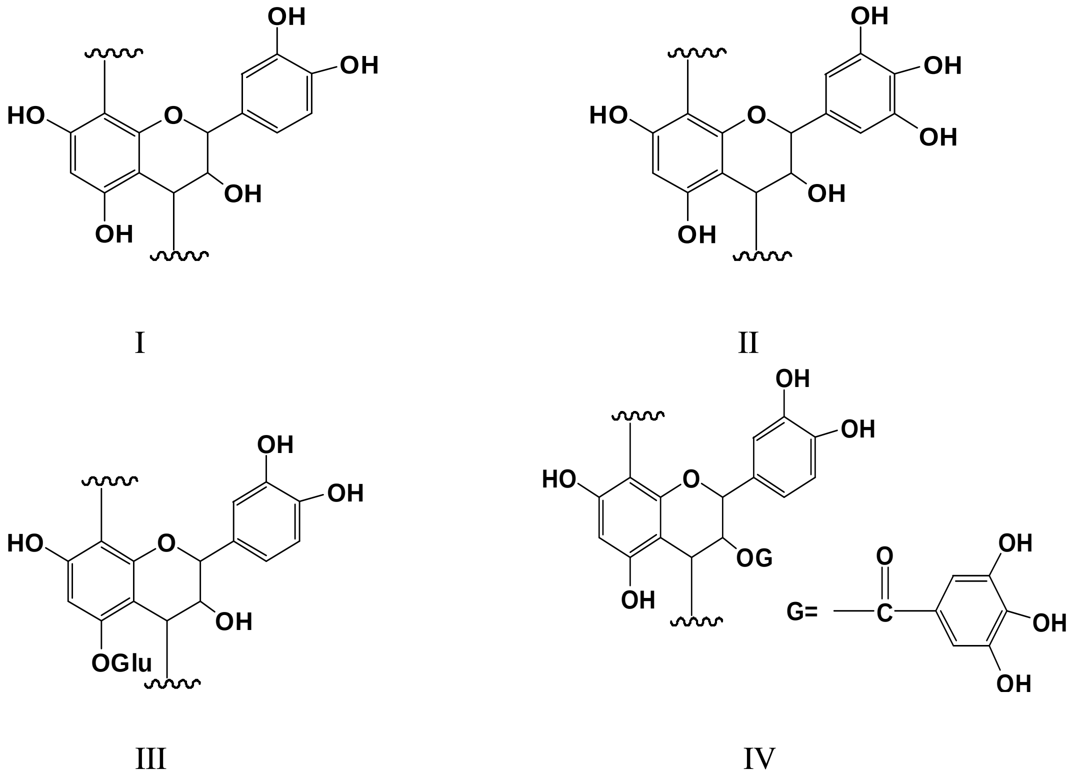


Fig.4. 9 MALDI-TOF mass spectrum of lyophilized tannins obtained from grape pomace



I

II

III

IV

Fig.4. 10 Polymer structure of grape pomace tannin repeating unit: (I) procyanidin, (II) prodelphinidin, (III) glucosylated procyanidin, (IV) gallated procyanidin.

The MALDI-TOF mass spectra showed clear repetitive patterns of peaks that allow for the identification of specific oligomer series present in the tannin samples. It showed that grape pomace oligomers prevalently contain repeating units of 288 Da. This proved that procyanidins composed of catechol B-ring were the main constituents of this polymer. It also indicated that the most common repeating units corresponded to (I) procyanidin, (II) prodelphinidin, (III) glucosylated procyanidin, (IV) gallated procyanidin repeating units, respectively (Fig.4.10) of 288.3 Da, 304.3 Da, 450.3 Da and 440.4 Da.

Based on the DHB/NaCl was used as the matrix in the MALDI-TOF study, the number of repeating units in each oligomer was calculated using the expression $M+Na^+=23.0(Na) + 2.0(endgroups, 2\times H) + 288.3A+304.3B+450.3C+440.4D$ with I, II, III and IV representing the four relevant monomers in the tannin oligomer. Table 4.4 and 4.5 showed the possible combinations of different repeating units and the degree of polymerization corresponding to acidified tannins and lyophilized tannins, respectively. These values were obtained based on the *m/z* value of types I, II, III and IV of tannin structures.

The dominant series of peaks at 908.1, 1196.8, 1485.7, 1774.2, 2062.3, 2350.1, 2637.8, 2926.7 Da for acidified tannins obtained from grape pomace were trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers, decamers (Fig.4.8) respectively. Besides that, the spectrum showed a second peaks series with a low intensity at 1069.0, 1357.5, 1638.9, 1935.5 Da corresponding to trimers, tetramers, pentamers, hexamers. The mass increment between the two peak series of trimers, tetramers and hexamers are 161 Da in the average indicated a glucose substitution, represents glucosylated flavonoid in the acidified tannins (Fig.4.10 III). This structure has already been observed in proanthocyanidins from sorghum (Gujer et al., 1986; Reed et al., 2005). The mass increment between the two series peak of pentamers is 152 Da. Based on the galloylated structures described by Rocardo-Da- Silva et al, galloylation always appears to occur on an epicatechin unit; to date no compound with catechin-O-gallate has been described. This peaks could be explained by the presence of epicatechin gallate units in which the gallic acid residue has been bonded on C3 (Fig.4.10 IV).

Concerning the lyophilized tannins, the spectrum displayed ultimately same tendency with two peak series (Fig.4.9). The dominant peaks at 604.1, 905.1, 1193.4, 1771.5 Da and the second peak series at 767.6, 1068, 1354.4, 1635.1, 1924.2 Da for dimers, trimers, tetramers, pentamers, hexamers. The possible combinations of repeating units can be seem in Table 4.5. the lyophilized tannins has oligomers up to 6 repeating units, and display a degree of polymerization lower than acidified tannins. This is in accordance with the ^{13}C-NMR results.

Table 4. 4 Distribution of polyflavonoid oligomer by MALDI-TOF for acidified tannins from grape pomace

	M+Na$^+$ experiment	**M+Na$^+$ calculated**	**Unit type**			
			I	**II**	**III**	**IV**
Trimer(3)	908.1	905.9	2	1	0	0
	1069.0	1067.9	1	1	1	0
Tetramer (4)	1196.8	1194.2	3	1	0	0
	1357.5	1356.2	2	1	1	0
Pentamer (5)	1485.7[a]	1482.5	4	1	0	0
	1638.9	1634.5	3	1	0	1
Hexamer(6)	1774.2	1770.8	5	1	0	0
	1801.7	1802.8	3	3	0	0
	1935.5	1932.8	4	1	1	0
Heptamer(7)	2062.3	2059.1	6	1	0	0
Octamer(8)	2350.1	2347.4	7	1	0	0
Nonamer(9)	2637.8	2635.7	8	1	0	0
Decamer(10)	2926.7	2924.0	9	1	0	0

I =procyanidin,
II = prodelphinidin,
III = glucosylated procyanidin,
IV = gallated procyanidin.
[a] Dominant peak

Table 4. 5 Distribution of polyflavonoid oligomer by MALDI-TOF for lyophilized tannins from grape pomace

	M+Na$^+$ experiment	M+Na$^+$ calculated	Unit type			
			I	II	III	IV
Dimer(2)	604.1	601.6	2	0	0	0
	767.6	763.6	1	0	1	0
Trimer(3)	905.1[a]	905.9	2	1	0	0
	920.8	921.9	1	2	0	0
	1068.0	1067.9	1	1	1	0
Tetramer (4)	1193.4	1194.2	3	1	0	0
	1354.4	1356.2	2	1	1	0
Pentamer (5)	1484.6	1482.5	4	1	0	0
	1635.1	1634.5	3	1	0	1
Hexamer(6)	1771.5	1770.8	5	1	0	0
	1924.2	1922.8	4	1	0	1

I =procyanidin,
II = prodelphinidin,
III = glucosylated procyanidin,
IV = gallated procyanidin.
[a] Dominant peak

The dominant peak present in acidified tannins in 1485.7 Da assigned to the pentamers, on the other hand, the dominant peak in 905.1 Da assigned to the trimers in lyophilized tannins.

Concernint the peak at 729.1 Da, the lyophilized tannins extracted in this study introduced sodium sulfite group on the C2 position of flavonoid structure results in heterocyclic ring opening (Pizzi, 1982; Hoong et al., 2009). It could be predicted that one of the flavonoid units of a 905 Da trimer loses its catechol B-ring (-110) from a type I repeat unit as well as the $-SO_2^-$ group (-64) (Fig. 4.11). This phenomenon also has been observed by Pash et al (2001) in industrial quebracho tannin.

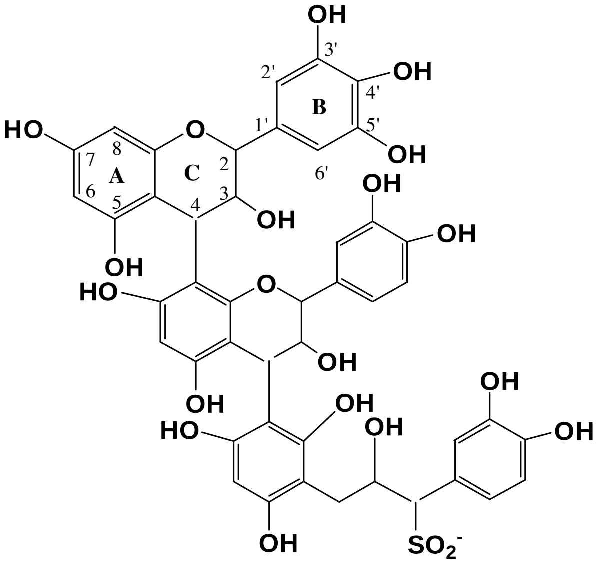


Fig.4. 11 The possible structure of peak at 729.1 Da of lyophilized tannins

It was shown by MADI-TOF experiments that grape pomace extracts are mainly composed of flavoinoid oligomers with dominant procyanidin units. This result is in accordance with ^{13}C-NMR and gel times analyses described in a previously study. In fact, we described that the grape pomace extracts exhibited gel times below 100 sec at pH 7 and then can be classified under the same group as the faster reacting procyanidin-prodelphinidin tannins such as pine bark and pecan nut pith extracts (Pizzi, 1994). Thus, a ring of grape pomace tannins possess only the phloroglucinol moity, much more reactive toward formaldehyde than a resorcinol-type structure 10 to 50 times higher than phenol (Kim et al., 2003), with important consequences for the use of these tannins in adhesive applications.

4.3 Conclusions

In this Chapter, polymer composition, chain length distribution and the oligomer repeating units as well as degree of polymerization of grape pomace condensed tannin extracts were examined by different techniques. The study indicated that:

1. The structure of grape pomace sulfited tannin extracts were established by elemental analysis and FT-IR. It did not present noticeable discrepancy exception of the ratio of the opening pyran ring which was different as a function of the catalyst used. The opening of pyran ring during the tannins extraction seem to be more important by using Na_2CO_3 and $NaHCO_3$ than when using NaOH. The bands assigned to aromatic ring vibration and carbonyl groups were stronger and no bands attributed to sulfited groups were tested in acidified tannins compared to lyophilized tannins.

2. We never observed noticeable difference among sulfited tannins extracts from TGA study. The weight lost of these tannin extracts were mainly composed of two steps. The first step starts from room temperature to 200 °C. It is attributed to the mass lost of water and some easy-degraded small low molecules. The secondly steps which is the mainly degradation step of tannin extract samples from 200 to 400 °C. Concerning acidified tannins, it never appeared apparent mass lost rate point in first step. The mass lost mainly occurred by the evaporated of water content.

3. The grape pomace tannins used in this study are crude extracts.The results from ^{13}C-NMR showed that condensed tannin extracts from grape pomace are consistent with dominant procyanidin units with a minor amount of prodelphinidin units that are linked together by a C4-C8 bond; relatively low carbonhydrate and high catechinc acid content were detected in acidified tannins compared to lyophilized tannins.

4. It was shown by MADI-TOF experiments that grape pomace extracts are mainly composed of flavoinoid oligomers up to 6 repeating units in lyophilized tannins and 10 repeating units in acidified tannins respectively, with dominant procyanidin units, a small proportion of substitution with glucose and gallic aicd in procyanidin units of polyflavonoid tannins. The degree of polymerization of acidified tannins is higher than lyophilized tannins. The difference between these two non-purified tannins has apparent effects on reactivity towards linking agents when it used as wood adhesive.

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Chapter 5. Utilization of condensed tannins from grape pomace in wood particleboard production

5.1 Introduction

Phenolic-type compounds obtained from natural resources have been used for the production of wood adhesives since 1970s for interior and exterior wood bonding of products such as particleboard, plywood, glulam and finger jointing (Tondi and Pizzi., 2009; Pizzi., 1994). Among the possible alternatives of the petroleum-derived phenolic compounds, condensed tannin is an excellent renewable resource (Kim et al., 2003, Sowunmi et al., 1996). It was demonstrated that condensed tannins are both chemically and economically interesting for the preparation of adhesives and that they could be successfully used as substitutes for phenol in the production of resorcinol-formaldehyde (RF), phenol-resorcinol-formaldehyde (PRF) and phenol-formaldehyde (PF).

Many researches have done on this adhesive field since the late of 20 th century.Vázquez et al (1996) studied the influence of solid/liquid ratio, extraction time and NaOH concentration on the yield and Stiasny number of tannin extracts from *Pinus pinaster* bark, this extracts was copolymerized with resol to afford a resin used to bond plywood, which was superior to commercial PF resin as regarded its tolerance to moisture in plies and adhesive. Lu and Shi (1995) evaluated the feasibility of 60 % Larch tannin was used to substitute phenol in the resin to produce exterior grade particleboard. It would be a desirable substituent as a low cost material and suitable for industrial application. Stefani et al (2008) used mimosa tannin extract substituted part of PF resol to produce Eucalyptus plywood panels which displayed good bonding properties. Moubarika et al (2009) studied the performances of cornstarch-quebracho tannin-PF resins as adhesive in the plywood production. The results showed that plywood panels bonded with cornstarch-quebracho tannin-PF resins (15:5:80, *w/w/w*) exhibited better mechanical properties than commercial PF resins. Bisanda et al (2003) used tannins and cashew nut shell liquid to modify UF resin, it has been found to reduce particleboard’s formaldehyde emission, improvement dimensional stability and water resistance. DSC results showed that tannin blends exhibit better thermal stability and have a higher glass transition temperature than UF resin. In addition, tannins could also replace of resorcinol in resorcinol-formaldehyde resin

(RF) and phenol-resorcinol-formaldehyde resin (PRF) based on its high reactive activity. Lee and Lan (2006) prepared Resorcinol–tannin–formaldehyde copolymer resins(RTF) by using the bark extracts of Taiwan acacia (*Acacia confusa*) and China fir (*Cunninghamia lanceolata*) to substitute part of the resorcinol. The RTF copolymer resins had cold-setting capability, higher viscosity and shorter gel time when compared to the RF resin. Li et al (2004) revealed that a mixture of procyanidins-type condensed tannin and polyethylenimine (PEI) was an excellent surrogate for the marine adhesive protein. A tannin-PEI mixture performed successfully as a formaldehyde-free wood adhesive. Two-plywood composites bonded with tannin–PEI adhesives had very high shear strengths and very well water resistant. This study will provide a new research thought different from the traditional tannins adhesive reaction principle.

In 1992, Nanjing Forestry University and Inner Mongolia YaKeShi tannin plant cooperated to develop the larch tannin instead of 60% PF resin, which displayed the equal performance compared to traditional PF resin (Sun et al., 2006). At present, larch tannin-PF adhesives has been extensively applied in poplar box board, pine board and bamboo plywood container floor and bamboo wood composite container floor, because it lower cost with the equal bonding properties compared to excellent PF resins (Huo., 2002; Zhang et al., 1991; Sun., 2000). Some progresses also made in wattle tannins adhesive used in particleboard and plywood production. Huang (1995) used wattle condensed tannin to prepare thermosetting resin, which had neutral layer, shorter press time and low cost. The quality of particleboard and plywood using this adhesive can reach the China standard level. Li (1994) prepared the resin with 80 % of wattle tannin extracts and 20 % of phenol, which was applied in plywood production. The quality can reach the grade standard. Zhao et al (1994) prepared tannin-based adhesive with PF and PUF as cross-linking agent instead of paraformaldehyde, and systematically studied the parameters such as coating load, dosage of cross-linking agent, pH value, hot press temperature and assembly time on effect of plywood. Huang (2003) modified the wattle tannin adhesive with sodium sulfite and the MUF so as to prepare out a tannin adhesive with good water-proof and better glue properties. The test result has showed that the modified adhesive is feasible to be developed for producing exterior medium density fiberboard.

Among all the researches, condensed tannins used in the previously described studies are generally extracted from the bark of various trees like mimosa, quebracho or pine (Fradinho et al., 2002, Panamgama, 2007, Vázquez et al., 2000); none has been studies so far on the analysis of the condensed tannins-based adhesive from grape pomace.

In this Chapter, condensed tannin extracts from grape pomace were applied to prepare condensed tannin-formaldehyde nonfortified formulation and fortified formulation with diisocynanates, which were applied to press wood particle board productions. The internal bond strength and formaldehyde emission of the productions were tested. The purpose is to investigate the possibility of using this grape pomace tannin as wood adhesive and the influence of different tannin extracts and formulations on the properties of particle board productions.

5.2 Results and discussions

Two kind of condensed tannin lyophilized extracts from grape pomace were studied in this section considering previously described in Chapter 3. The first tannin fraction is extracted by sodium hydroxide (NaOH), with the condition of 120 °C, 120 min. the second tannin fraction is extraxted by sulfited reagents using 2.5 % (*w/w*) or 5 % (*w/w*) of sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) in the presence of sodium sulfite (Na_2SO_3) at 100 °C during 120 min in an aqueous medium.

Two different adhesive formulations were investigated in this paper, the first formulation is nonfortified tannin-formaldehyde adhesive, the second formulation is fortified with a synthetic cross linker: polymeric 4, 4'-diphenyl methane diisocyanate (*p*-MDI).

5.2.1 TMA study of tannin adhesives

The hardening reaction of tannin adhesive system can be evaluated by TMA by the study of the rigidity of the wood-adhesvie joint as a function of temperature. For the first tannin fractions extracted by sodium hydroxide (NaOH), the influence of concentration of sodium hydroxide on the modulus of elasticity (MOE) of adhesive formulations was studied.

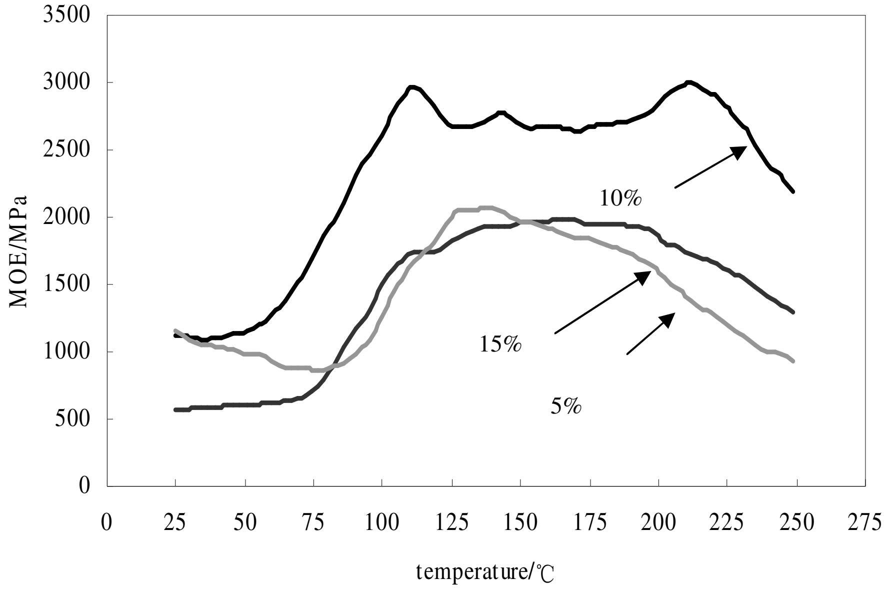


Fig.5. 1 TMA measuring MOE as function of temperature to describe the curing of resins of lyophilized grape pomace tannins (extracted by sodium hydroxide) /paraformaldehyde

The three lyophilized tannins solutions (30%) –5% (*w/w*) of paraformaldehyde (based on oven dry tannin weight) were scanned by thermomechanical analysis in bending according to a technique already reported (Laigle et al., 1998). The curves of the modulus of elasticity (MOE) as a function of increasing temperature for the three resins are given on Fig.5.1. The maximum MOE values are 2073 MPa when NaOH=5 % (*w/w*), 2996 MPa when NaOH=10 % (*w/w*), 1960 MPa when NaOH=15 % (*w/w*), respectively. The higher Modulus of Elasticity (MOE) was reached for NaOH =10 %, *w/w*. The value indicates that the tannins fraction isolated in these conditions gave relatively high wood-joint strength. This is in accordance with gel time results, demonstrating a good correlation between Stiasny number of the tannins extracts and the gelation properties of the resulting resins.

Moreover, concerning NaOH =10 %, *w/w* condition , a signal is observed at about 100 °C (Fig.5.1) which corresponds to a first cross-linking reaction through formation of a non stable cross linker. This behavior is a characteristic of several phenolic resins (Garcia and Pizzi, 1998). In this precise case, the resin quickly reticulated involving the more reactive tannins molecules with formation of unstable methylene-ether cross linkers ($-CH_2OCH_2-$). Because of their instability and the increase of the temperature, they break to form a more stable methylene cross linker ($-CH_2-$) with emission of formaldehyde able to react with phenolic material.

Concerning the second tannin fraction extracted by sulfited reagents, the influence of 3 parameters (i.e., nature of the base for the extraction of tannins, concentration of the chemical reagents and effect of the addition of a cross linking agent (polymeric 4, 4'-diphenyl methane diisocyanate, *p*-MDI)) on the properties of the adhesive formulations was examined.

The formulations of tannins solution- 5% (*w/w*) of paraformaldehyde (based on oven dry tannin weight) were scanned by thermo mechanical analysis in three points in bending according to a technique already reported (Laigle et al., 1998). The addition of 20 % ((*w/w*) of a cross linking agent (polymeric 4,4'-diphenylmethane diisocyanate, *p*-MDI) was also experimented in the formulations. The curves of the modulus of elasticity (MOE) as a function of increasing temperature are given in Fig. 5.2; the maximum MOE values at 195 °C are also given in Table 5.1.

F1, F7 and F11 correspond to the same formulations but using three different bases for the extraction of tannins (respectively NaOH, Na_2CO_3 and $NaHCO_3$, 2.5% *w/w*). It clearly appears that grape pomace tannins extracted using NaOH (F1) and $NaHCO_3$ (F11) gave lower performance (MOE < 2000 MPa) than those extracted with Na_2CO_3 (F7) with a MOE nearly 3500 MPa. The TMA results of the curing of F1 and F2, F11 and F12, F7 and F8 indicated that, in most of the essays, an increase in tannin concentrations used for the preparation of the formulation yielded noticeable improvements in the value of MOE. A comparison of the TMA profiles (given in the Fig.5.1) of F2 and F3, F5 and F6, F7 and F8, F9 and F10 shows that the addition of *p*-MDI did not yield an important increase in the maximum MOE value. On the other hand, concerning F12, it appears that addition of *p*-MDI has a positive effect: MOE values of F13 are still higher in the range of temperatures corresponding to industrial wood panel press conditions (190°C < T < 250°C).

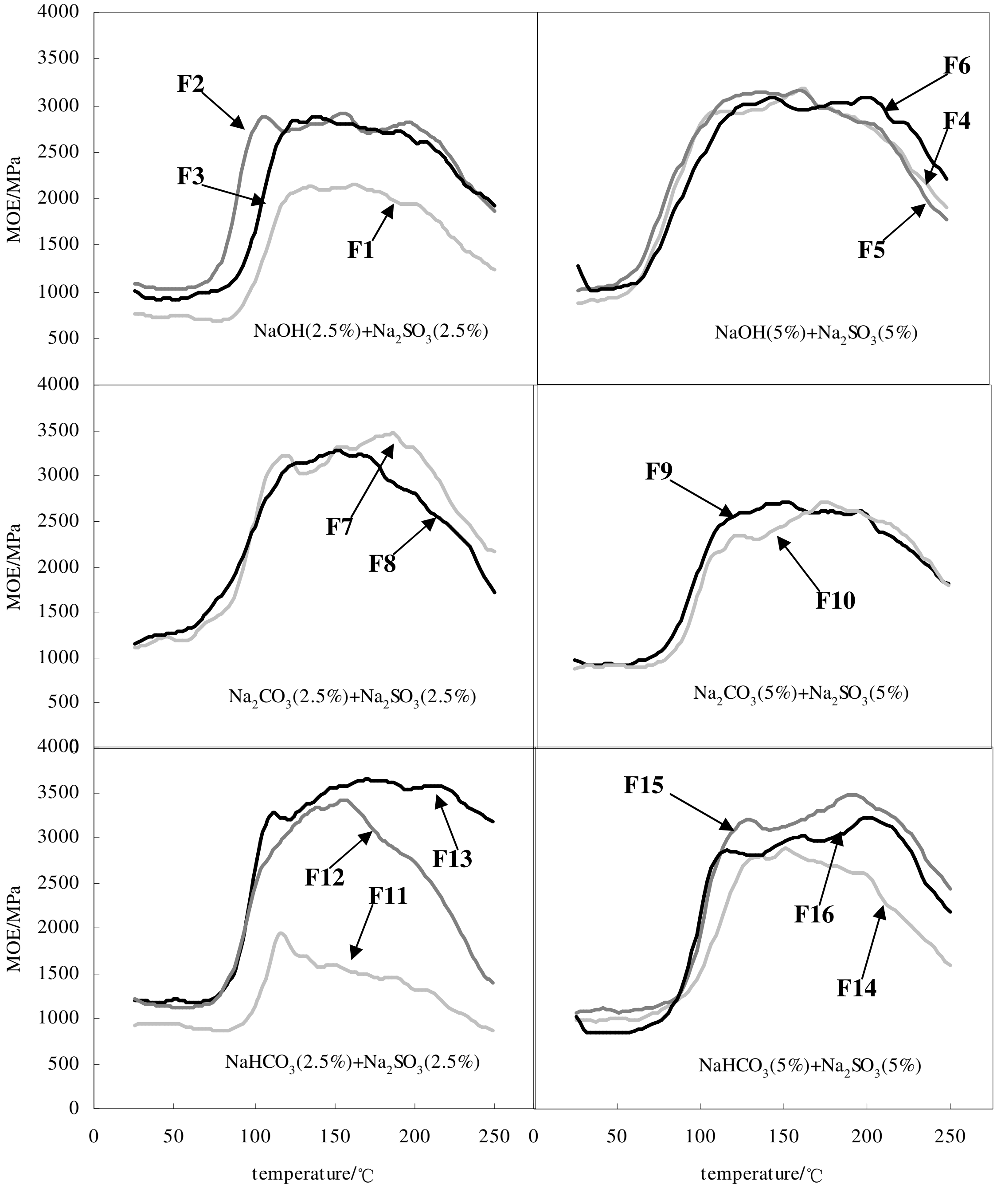


Fig.5. 2 TMA measuring MOE as a function of temperature to describe the curing of resins of lyophilized grape pomace tannins extracted by sulfited reagents

5.2.2 Mechanical testing for particle boards

Grape pomace tannins based adhesive were used to press one layer wood particleboards. In this study, two different formulations (the nonfortified tannin adhesive and fortified with diisocyanate adhesive) were analysed. The internal bond (IB) strength of a panel is a direct measure of the performance of the adhesive. The results are shown in Table 5.1.

The internal bond strength of a particleboard usually influences both by the adhesive bonding quality and the choice of hot press properties such as pressure, pressing time and pressing temperature. Before the determination of the IB value, the distribution vertical density profile of particleboard was used to evaluates the hot press parameters. Normally, the distribution of vertical density of a particleboard presents a saddle shape. The densities in the two surface sides are higher than average value and the density in the middle of the panel lower than average value. The middle density will be lower and the gap between the surface and middle will become huge when the hot press parameters are not suitable. The distribution of vertical density profile of all particleboards presents a good result. The values of the minimum density value in the middle of panel compared to the average density of panel are up to 75%. This shows that the hot press parameters we choosed are suitable for the elaboration of tannin-based panels. Fig. 5.3 gives an example of the distribution of vertical density profile of particleboards we pressed.

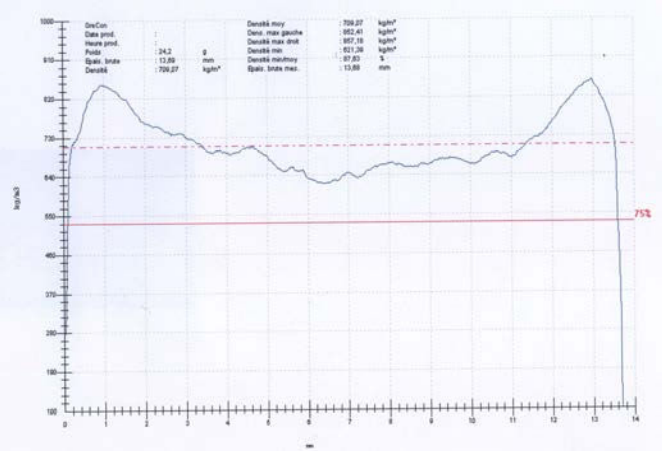


Fig.5. 3 The distribution of vertical density profile of a particleboard pressed by tannin-based adhesive

Table 5. 1 Adhesive formulation compositions, thermomechanical analysis and particle board testing

Extraction reagents [a]	FN[b]	Composition Tannin / H_2CO/pMDI	TC %[c]	TMA	Particle board	
				MOE[d] at 195°C(MPa)	Density (kg/m^3)	IB[e](MPa)
NaOH(2.5%) Na_2SO_3(2.5%)	F1	20/1/0	30	1937.1		
	F2	20/1/0	40	2812.5	696.3	0.23 ± 0.01
	F3	20/1/5	40	2662.5	693.7	0.28 ± 0.01
NaOH(5%) Na_2SO_3(5%)	F4	20/1/0	30	2846.9		
	F5	20/1/0	40	2455.1	702.0	0.28 ± 0.02
	F6	20/1/5	40	3062.8	698.4	0.32 ± 0.03
Na_2CO_3(2.5%) Na_2SO_3(2.5%)	F7	20/1/0	30	3322.9	706.1	0.45± 0.06
	F8	20/1/5	30	2833.5	710.4	0.68± 0.08
Na_2CO_3(5%) Na_2SO_3(5%)	F9	20/1/0	30	2179.4	711.5	0.36 ± 0.03
	F10	20/1/5	30	2605.9	706.9	0.60 ± 0.04
$NaHCO_3$(2.5%) Na_2SO_3(2.5%)	F11	20/1/0	30	1369.7		
	F12	20/1/0	45	2776.8	692.6	0.26 ± 0.02
	F13	20/1/5	45	3520.7	708.5	0.36 ± 0.02
$NaHCO_3$(5%) Na_2SO_3(5%)	F14	20/1/0	30	2932.9		
	F15	20/1/0	45	3878.8	705	0.38 ± 0.03
	F16	20/1/5	45	3589.0	703.4	0.34 ± 0.02

[a] % based on weight of dry pomace

[b] formulation number

[c] tannin concentration in water, % based on weight of dry pomace

[d] Modulus of elasticity

[e] Internal bond strength

The IB strengths demonstrate that the nature of the extraction reagent (NaOH, Na_2CO_3 or $NaHCO_3$) greatly impacts the value of panel IB strength. It can be seen that formulations F1 to F6 using NaOH for the tannins extraction yielded low panel performances and did not satisfy the IB strength results required (‡0.35 MPa) even with addition of *p*-MDI in the formulation (F3 and F6). In the same way, the utilization of $NaHCO_3$ for the tannins extraction (F12) led to an adhesive bearing poor properties; however, with addition of 20% of pMDI in the formulation, the adhesive F13 yielded a good internal bond strength (IB = 0.36). These results are in accordance with the TMA experiments previously exposed.

The best results were obtained from Na_2CO_3 extracts and it is noteworthy that F7 and F8 yielded very good internal bond strength results (IB = 0.45 and 0.68 respectively). Concerning F7, it is important to note that for the first time a resin formulation composed of 95% of pomace extracts solution, 5% formaldehyde and without *p*-MDI, yielded good internal bond strength which passed relevant international standard specifications for interior-grade panels (IB > 0.35 MPa).

5.2.3 Formaldehyde emission of particle boards

Formaldehyde emission has become one of the major concerns in wood-based panels industry, especially the interior wood bonding products, such as particle boards used as a major component in the production of decoration and furniture materials.

In this study, the best IB value performance particleboards (F7 to F10) were chosen to determine the emission levels of formaldehyde. The formaldehyde emissions from the particleboards were determined used UV spectrophotometer at 412 nm. The specification curve was drafted by the standard formaldehyde solution in different concentrations. The result shows in section 2.5.4. The results in Table 5.2 show that the formaldehyde emission of the particleboards was very low, largely below the European Norm requirements (≤ 6.5 mg/ 100g panel).

Table 5. 2 Formaldehyde emission of particleboards

Extraction reagents [a]	Formulation number	Composition Tannin / H_2CO/pMDI	Tannin concentration% [b]	Formaldehyde emission (mg/100g panel)
Na_2CO_3(2.5%)	F7	20/1/0	30	2.01± 0.19
Na_2SO_3(2.5%)	F8	20/1/5	30	1.24± 0.14
Na_2CO_3(5%)	F9	20/1/0	30	1.62± 0.15
Na_2SO_3(5%)	F10	20/1/5	30	1.05± 0.20

[a] % based on weight of dry pomace

[b] tannin concentration in water, % based on weight of dry pomace

5.2.4 Discussions

Tannin-formaldehyde adhesives that are not fortified by the use of a synthetic resin are the most commonly used adhesives in particleboard application, some application for plywood are also on record. Non-fortified tannin adhesives formulations used today can be divided into two main classes: formulations based on chemical modification of the tannin extract preceding its use, and formulations based on the non-modified, the latter were introduced with the advent of the fast-reacting procyandin-prodelphinin tannins.

Fortification of tannin adhesives with diisocyanates first appeared in 1981. It was introduced as a consequence of difficulties in utilizing pine tannin extract for particleboard adhesives. In the wood adhesive field, there appears to be a preconception that a nonemulsified MDI can not be used in water systems, due to it reacts with water to form polyureas and is mostly lost to urethane formation with polyols that are present in water solution. Pizzi (1994) studied to determine what happens when tannin adhesive fortification with diisocyanates use in practice using model compounds react with MDI. The rate of reaction of MDI with water is several orders of magnitude greater than the reaction with the phenolic hydroxyl groups of catechin and with the protonated formaldehyde. However the reaction of formaldehyde onto catechin to form methylol groups is the faster one which dominates the entire system initially. In such a system the reaction of MDI with water is relegated and the deactivation of MDI by water is small.

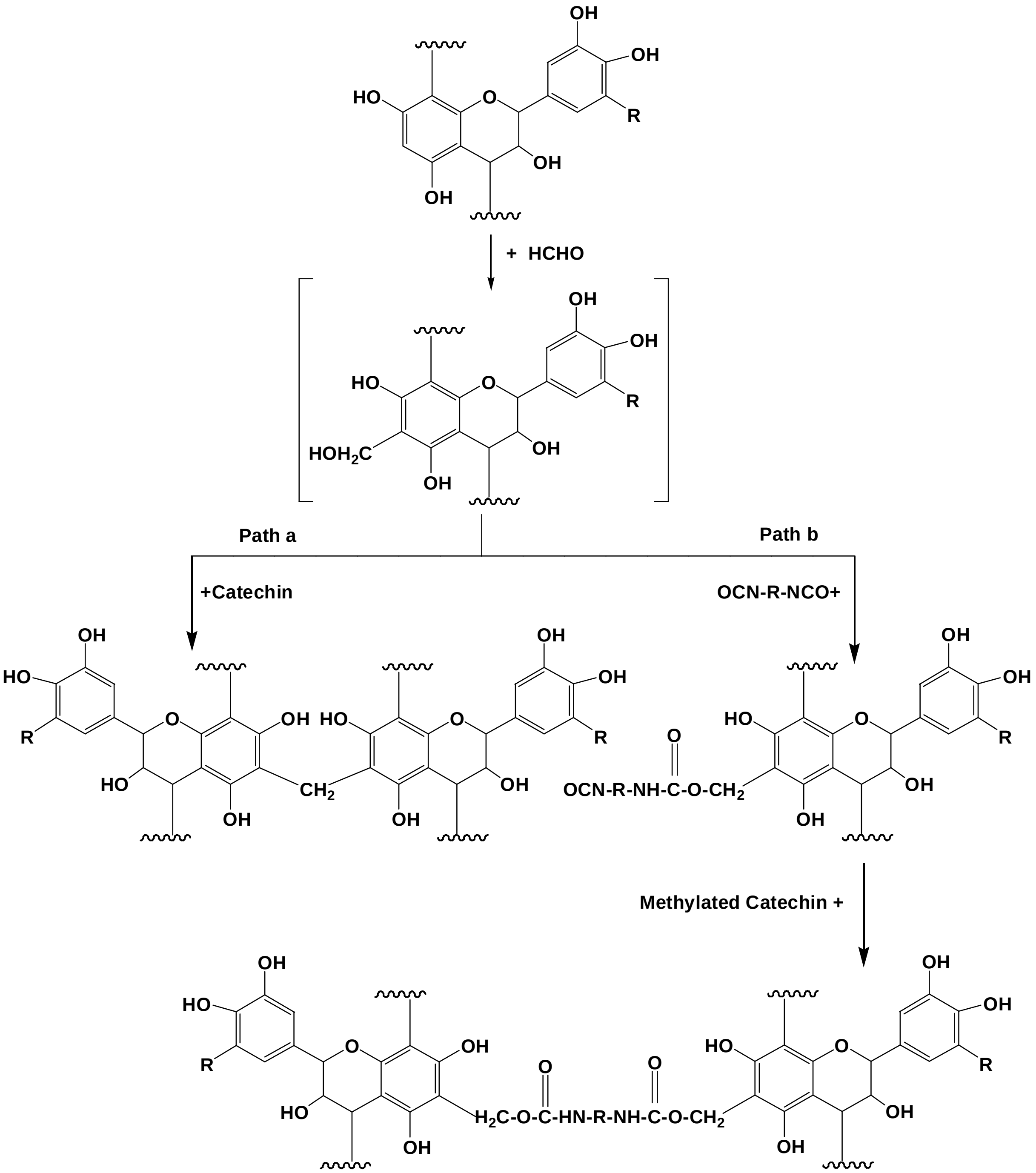


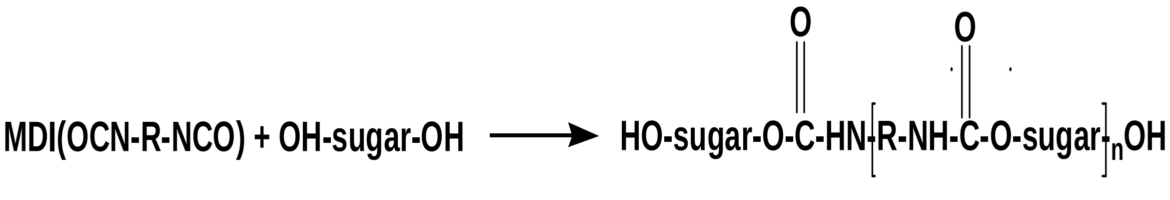
Fig.5. 4 Underlying mechanisms proposed for the grape pomace tannins adhesive system

Based on the results of the ^{13}C-NMR and MADI-TOF, grape pomace condensed tannin extracts is a kind of faster reacting procyandin-prodelphinidin tannins much more reactive towards formaldehyde. The high nucleophilicity and reactivity of the grape pomace condensed tannins can explain the good to very good properties of the extracts isolated in the present study for adhesive applications. The underlying mechanism proposed for the grape pomace tannin nonfortified adhesive and fortified with diisocyanates adhesive are described in Fig.5.4. The catechin-formaldehyde condensates are formed through methylene bridges ($-CH_2-$) (path a). Concerning adhesives fortified with diisocyanate, the methylolated catechins undergo two reactions of almost equal rate. The first is the standard condensation reaction according the path a (Fig.5.4); the second is the attack of the *p*-MDI on the hydroxyl of the methylol group ($-CH_2OH$) formed onto the catechin moities (see Fig.5.4, path b).

The grape pomace tannins used in this study are crude extracts, with a considerable proportion of nonphenolic materials, mainly simple sugars and polymeric carbohydrates. Relatively high carbohydrate contents were previously deduced by from ^{13}C-NMR spectrum and low Stiasny numbers were obtained from the extracts (~ 0.5-0.6). The presence of non phenolic compounds in the formulations can explained:

(1) The poor results obtained in terms of mechanical properties of the panels for some of the extracts;

(2) The improved performance for some of the adhesive formulations prepared with *p*-MDI. In fact, the reactive isocyanate group of *p*-MDI can rapidly react with the hydroxyl groups of the monomeric and polymeric carbohydrates (see Fig.5.5). The formation of sugar-*p*-MDI polyurethane could enhance the joint performance of grape tannin fortified adhesives.



$$\text{MDI(OCN-R-NCO)} + \text{OH-sugar-OH} \longrightarrow \text{HO-sugar-O-}\overset{\overset{\displaystyle O}{\|}}{C}\text{-HN}\left[\text{R-NH-}\overset{\overset{\displaystyle O}{\|}}{C}\text{-O-sugar}\right]_n\text{OH}$$

Fig.5. 5 The mechanism proposed for *p*-MDI reacted with hydroxyl groups(-OH) of the carbohydrates

A low formaldehyde emission of particleboard productions were observed in this study is can be rationalized:

(1) The reaction rate of condensed tannin towards formaldehyde which have a 10-50 times higher than phenol does;

(2) Condensed tannins is kind of high molecular polymer, Tannin-formaldehyde adhesive don't need to reaction like PF resin reacts from phenol and formaldehyde monomer. Less formaldehyde was used.

It is noteworthy to mention that the formaldehyde emission of particleboards is slightly lower using fortified formulations than non-fortified formulations. This phenomenon can explain that more unstable methylene ether bridges ($-CH_2-O-CH_2-$) are generated used non-fortified formulation, which could break to form a more stable methylene cross-linker ($-CH_2-$) with emission of formaldehyde.

5.3 Conclusions

In this Chapter, Three different sulfited grape pomace condensed tannin extracts and two different tannin-based adhesive formulations (nonfortified tannin adhesive and fortified with addition of 20% of polymeric 4,4'-diphenyl methane diisocyanate (*p*-MDI)) are compared. The observation showed that:

1. It has been proved that the nature of the extraction (NaOH, Na_2CO_3 or $NaHCO_3$) greatly impacts the MOE value of the adhesive and IB strengths of the particle board productions. The grape pomace condensed tannin fractions extracted by Na_2CO_3 base obtained the best performance compared the other bases. The addition of ***p***-MDI did not yield an important

increase in the maximum MOE value. On the other hand, the addition of *p*-MDI improved the IB strength.

2. It was noteworthy that the best results were obtained from Na_2CO_3 extracts yield a very good internal bond strength results which passed relevant international standard specifications for interior grade panels with low formaldehyde emission below the European Norm requirements (≤ 6.5 mg/ 100g panel).

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Chapter 6. General conclusions and perspectives

General conclusions

Grape pomace is by-product of winemaking industry, which retains high level of condensed tannins mainly proanthocyanidins composed by procyanidin and prodelphinidin. Until now, most of the grape pomace which is not exploited but only used for soil fertilizer or animal feed. Therefore, the purpose of this study is to extract condensed tannins from grape pomace and use as tannin-based adhesive for particleboard productions in wood industry. A system research was done on this study on extraction process, structure characterization and application, which mainly composed by three parts.

The first part of the thesis was dedicated to extraction of condensed tannins from grape pomace. Two different extraction processes and the influence of 4 parameters (i.e., temperature, reaction time, chemical reagents and concentration of the chemical reagents) on the tannin extracts yield, properties and the composition of the solid residue and liquid phase were studied.

It was demonstrated that it is possible to extract reactive tannins from grape pomace in basic medium using harsher extraction conditions with higher alkali concentration and higher reaction temperature. The tannin extracts obtained by precipitation in acidic conditions display a high phenolic content (Stiasny number higher 95%) but low water solubility and low reactivity toward formaldehyde because of the formation of catechinic and phlobatannins acid during the acidification step. The tannins extracts obtained by lyophilization of the liquid, despite of their lower phenolic contents, displayed promising properties for adhesive applications.

The optimum temperature was 100 °C; the optimum reaction time was 120 min; the best concentration of reagent was 10% when reactive polyphenol content of tannins reaches the highest value.

Sulfitation of tannins is the most useful method in flavonoid chemistry and can be useful in the preparation of tannin-based adhesive. The introduction of a sulfonic group through sulfitation increased both tannin solubility and reactivity as a result from the opening of the heterocyclic ring during extraction.

The second part was dedicated to the characterization of condensed tannins from grape pomace. The grape pomace condensed tannin extracts were characterized using nuclear magnetic resonance spectroscopy (^{13}C-NMR), Fourier transform-infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA) and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS).

The structure of grape pomace sulfited tannin extracts did not present noticeable discrepancy exception of the ratio of the opening pyran ring which was different as a function of the catalyst used. The opening of pyran ring during the tannins extraction seem to be more important by using Na_2CO_3 and $NaHCO_3$ than when using NaOH. The FT-IR bands assigned to aromatic ring vibration and carbonyl groups were stronger and no bands attributed to sulfited groups were tested in acidified tannins compared to lyophilized tannins.

It never showed noticeable difference among sulfited tannins extracts from TGA study. rephraseThe weight lost of these tannin extracts were mainly composed of two steps. The first step starts from room temperature to 200 °C. It is attributed to the mass lost of water and some easy-degraded small low molecules. The secondly steps which is the mainly degradation step of tannin extract samples from 200 to 400 °C. Concerning acidified tannins, it never appeared apparent mass lost rate point in first step. The mass lost mainly occurred by the evaporated of water content.

The grape pomace tannins used in this study are crude extracts, this kind of condensed tannin extracts from grape pomace consistent with dominant procyanidin units with a minor amount of prodelphinidin units that are linked together by a C4-C8 bond. We observed a relatively low carbonhydrate and high catechinc acid content in acidified tannins compared to lyophilized tannins. Grape pomace extracts in this study are mainly composed of flavoinoid oligomers up to 6 repeating units in lyophilized tannins and 10 repeating units in acidified tannins respectively, with dominant procyanidin units, displaying a small proportion of substitution with glucose and gallic aicd in procyanidin units of polyflavonoid tannins. The degree of polymerization of acidified tannins is higher than lyophilized tannins. The difference between these two non-purified tannins has apparent effects on reactivity towards linking agents when it used as wood adhesive.

The third part concerns the utilization of condensed tannins from grape pomace in wood particleboard production. Two different formulations (nonfortified tannin adhesive and fortified with addition of 20% of polymeric 4,4'-diphenyl methane diisocyanate (*p*-MDI)) were used to press one layer particle board. It was shown that the nature of the extraction reagent (NaOH, Na_2CO_3 or $NaHCO_3$) greatly impacts the properties of the resins and the mechanical properties of the panel internal bonding strengths. The particleboards bonded by the tannins extracted using Na_2CO_3 as catalyst give the best performance and were good enough to pass relevant international standard specifications for interior grade panels. Formaldehyde emission of these panels was below the European Norm requirements (≤ 6.5 mg/ 100g panel).

Perspectives

This thesis proposed an economically valuable utilization of grape pomace, a by-product currently largely underexploited, for the production of an environment friendly adhesive for particleboards productions. Although we obtained some important and interesting results but there are still some limitations. It is still need to do more work to improve it in the future work.

1, sulfitation is the most useful reaction in flavonoid chemistry to improve tannin extraction yield and properties. Sodium sulfite (Na_2SO_3) is the common use sulfited reagent. The usage ranges from 2% to 10 % have been described in the literature to extract tannins from barks. In this study, we only tried one usage about 5%. Maybe we can try different usage in the future work to compare its influence on the properties of crude tannin extracts.

2, characterization is the fundamental to tannin extract application and formulation as adhesive. The characterization done in this study is crude tannin extract. The purity affected the result in a certain degree. It is better to find new methods to puritify the crude tannin extract before characterization.

3. The adhesive formulations used in this study are prepared used formaldehyde and MDI as cross linker agent. The use of formaldehyde exist formaldehyde emission problem, and the price of MDI is higher than other resin such as PF and UF. We can do more work on use of no volatile hardener such as Hexamethylenetetramine and glyoxal, on use grape pomace tannin extracts replace part of phenol in preparation of PF resin and as filler or additives on PF and UF resin modification.

4. This study was performed starting from always the same batch. It is important now to check the effect of variability bof the raw material on the yield /properties of the extracts.

Annexes

Annex 1. The extraction of condensed tannins from grape pomace; composition and yields of the residue, purities and gel times of the extracts

Treatment condition				Solid residue[a]				Lyophilised tannins		
test	Reagents %	Tem °C	Time min	KL[b]	SL[c]	Glu[d]	OS[e]	Yield %[f]	SN[g]	T-Gel (pH)
1	NaOH 5 %	120	120	41.9	1.65	13.29	4.95	23.8	46	174(6.7)
2	NaOH 10 %	120	120	28.8	1.29	5.05	1.95	45.1	55	53(8.7)
3	NaOH 15 %	120	120	24.2	1.21	7.15	2.49	58.8	50	100(9.2)
4	NaOH 20 %	120	120	16.9	0.62	6.87	2.91	71.1	49	138 (9.6)
5	NaOH 30 %	120	120	7.6	0.36	8.99	2.49	88.4	39	1111 (10.7)
6	NaOH 40 %	120	120	7.7	0.65	7.32	1.75	101.5	32	∞ (12.9)
7	NaOH 5 %	100	120	41.6	1.43	15.57	5.60	26.3	48	55(7.8)
8	NaOH 10 %	100	120	27.9	0.99	13.15	5.14	46.0	59	34(9.3)
9	NaOH 15 %	100	120	19.5	0.86	11.69	4.09	56.1	55	62(10.0)
10	NaOH 5 %	70	120	44.0	1.67	16.19	6.00	22.6	44	No-gel
11	NaOH 10 %	70	120	32.7	1.30	12.85	5.78	37.2	55	No-gel
12	NaOH 15 %	70	120	23.3	1.11	12.58	5.24	47.0	54	No-gel
13	NaOH 5 %	100	60	41.4	1.61	14.37	5.41	25.3	52	37(8.0)
14	NaOH 5 %	100	180	40.3	1.48	13.67	4.47	25.9	42	57(7.8)
15	NaOH 2.5 % Na_2SO_3 2.5 %	100	120	37.1	1.98	15.52	5.22	27.2	52	111 (6.0)
16	NaOH 5 % Na_2SO_3 5 %	100	120	29.8	1.54	14.25	5.33	37.0	56	67 (7.4)

17	NaOH 7.5 % Na_2SO_3 7.5 %	100	120	24.5	1.48	12.36	5.09	47.0	45	35 (8.8)
18	NaOH 2.5 % Na_2SO_3 2.5%	100	60	40.4	1.94	13.46	4.28	23.2	46	95 (5.9)
19	NaOH 2.5 % Na_2SO_3 2.5 %	100	180	37.8	2.04	15.23	5.21	27.3	58	127 (6.1)
20	NaOH 2.5 % Na_2SO_3 2.5 %	100	300	37.9	2.03	15.00	5.04	28.0	50	151 (5.9)
21	Na_2CO_3 2.5 % Na_2SO_3 2.5 %	100	120	41.6	2.41	16.57	5.29	22.0	48	108 (6.3)
22	Na_2CO_3 5 % Na_2SO_3 5 %	100	120	30.4	1.47	13.79	5.44	39.8	62	72 (7.4)
23	Na_2CO_3 7.5 % Na_2SO_3 7.5 %	100	120	25.0	1.63	12.89	4.87	45.3	53	24 (8.7)
24	Na_2CO_3 2.5 % Na_2SO_3 2.5 %	100	60	41.7	2.18	15.88	4.91	20.3	45	93 (6.2)
25	Na_2CO_3 2.5 % Na_2SO_3 2.5 %	100	180	41.9	2.23	16.39	5.64	24.6	50	127 (6.1)
26	Na_2CO_3 2.5 % Na_2SO_3 2.5 %	100	300	41.2	1.87	15.63	5.12	24.5	49	173 (6.3)
27	$NaHCO_3$ 2.5 % Na_2SO_3 2.5 %	100	120	40.7	2.67	15.90	5.36	20.3	46	206 (5.9)
28	$NaHCO_3$ 5 % Na_2SO_3 5 %	100	120	36.6	2.01	15.71	5.29	25.9	52	114 (6.3)
29	$NaHCO_3$ 7.5 % Na_2SO_3 7.5 %	100	120	31.2	1.98	15.20	5.54	33.1	52	68 (7.3)
30	$NaHCO_3$ 2.5 % Na_2SO_3 2.5 %	100	60	45.2	2.73	16.27	5.57	16.7	39	158 (6.0)
31	$NaHCO_3$ 2.5 % Na_2SO_3 2.5 %	100	180	40.5	2.27	15.70	4.93	23.6	47	262 (5.7)
32	$NaHCO_3$ 2.5 % Na_2SO_3 2.5 %	100	300	40.9	2.05	15.40	4.90	23.3	51	324 (5.8)

[a] % based on weight of dry pomace ;

[b] Klason lignin ; [c] Soluble lignin ;

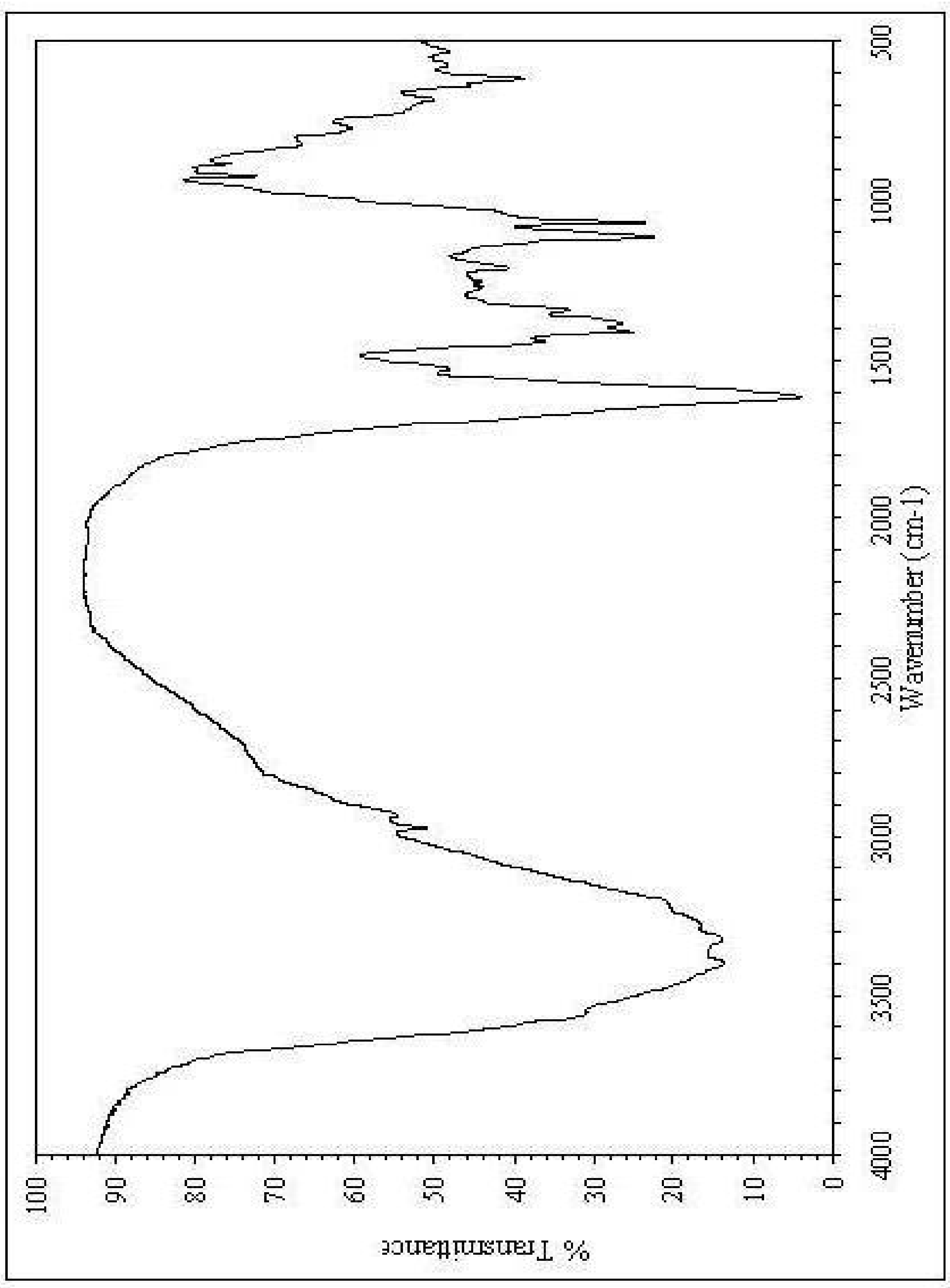
[d] glucanes;

[e] other sugars: arabinanes, xylanes, mannanes, galactanes ;

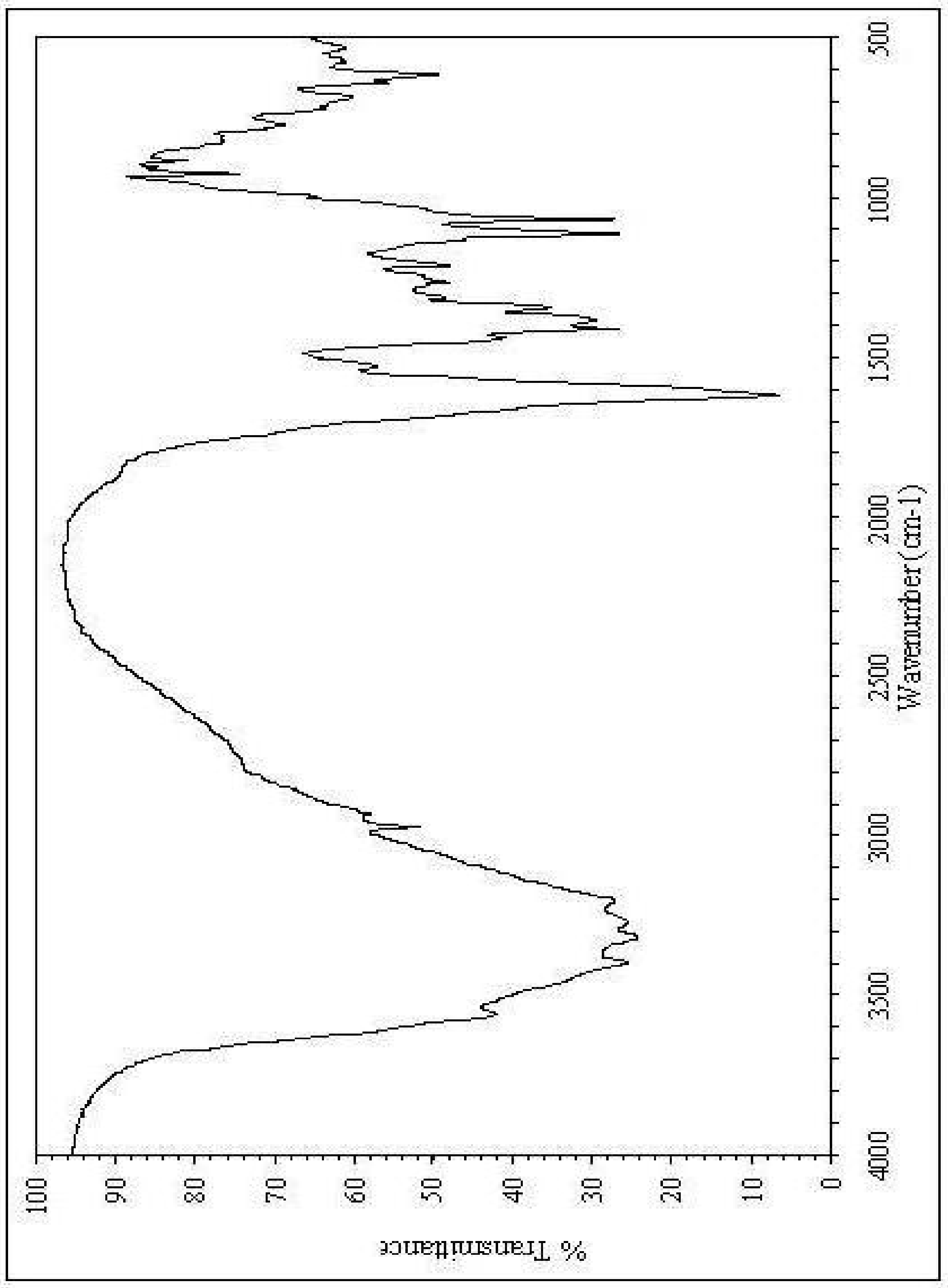
[f] yield calculated from the raw material (oven dry mass);

[g] Stiasny number

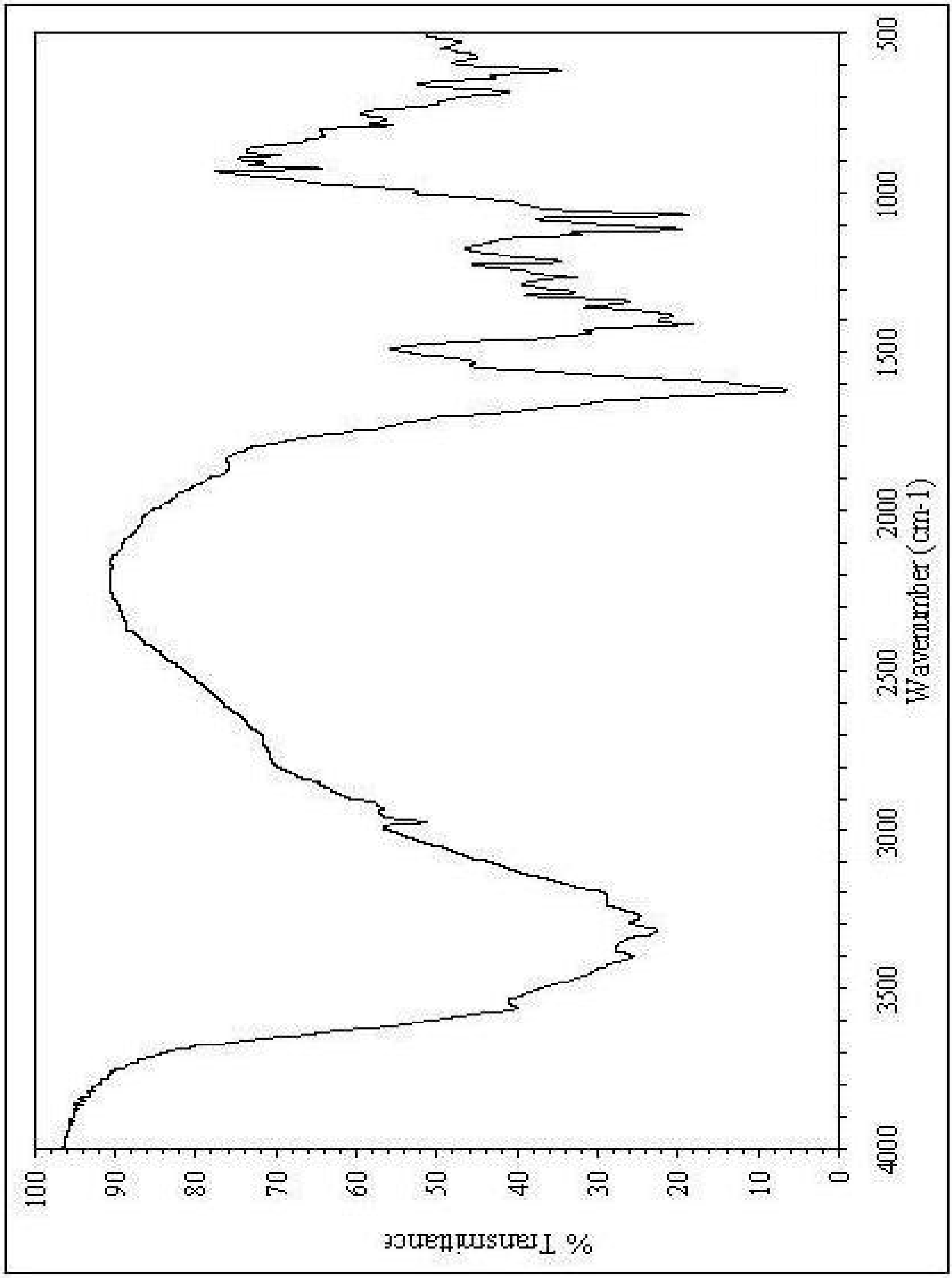
Annex 2. FT-IR spectrum of lyophilized condensed tannins from grape pomace extracted by NaOH (2.5%)/Na_2SO_3 (2.5%)



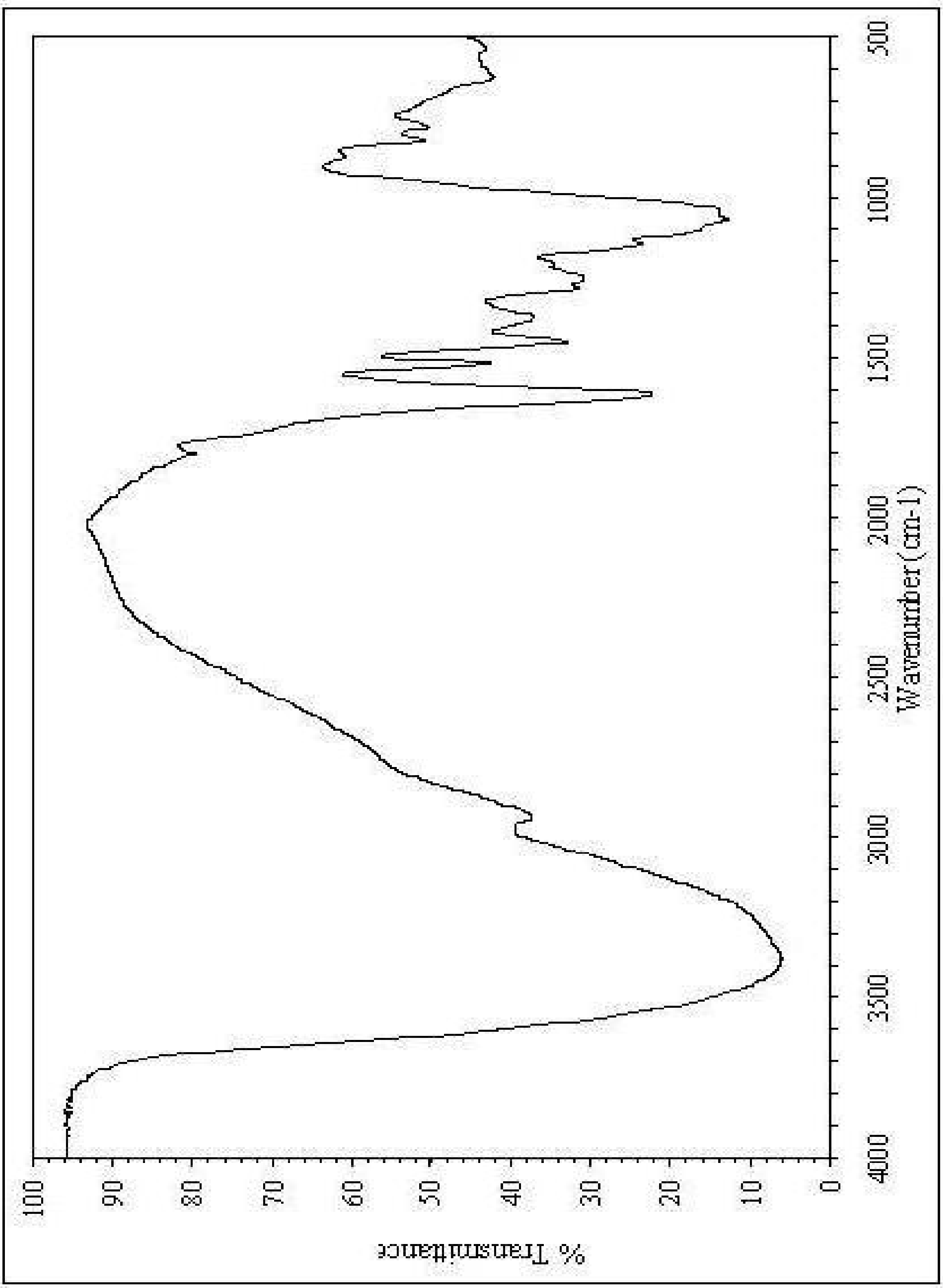
Annex 3. FT-IR spectrum of lyophilized condensed tannins from grape pomace extracted by Na_2CO_3 (2.5%)/Na_2SO_3 (2.5%)



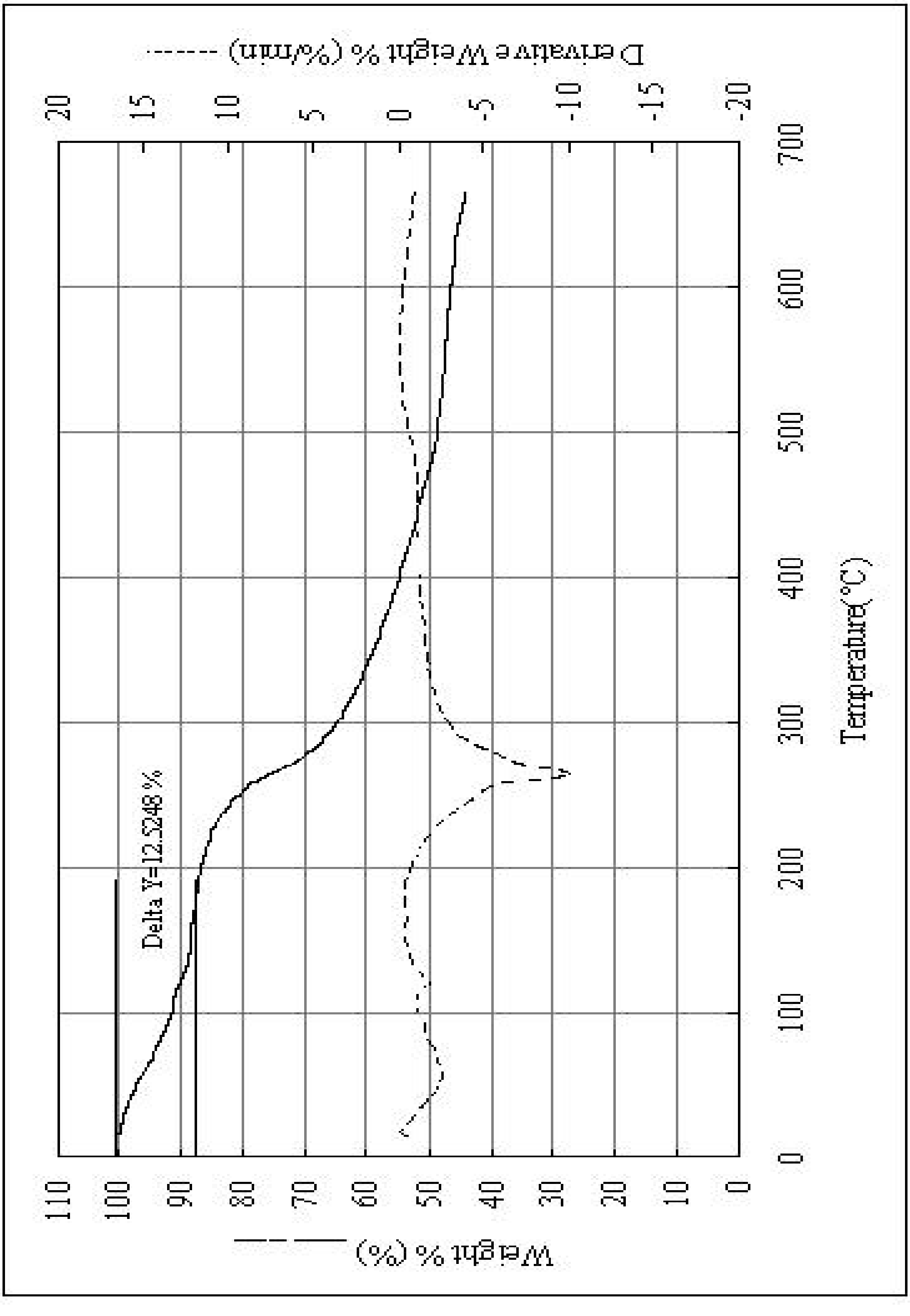
Annex 4. FT-IR spectrum of lyophilized condensed tannins from grape pomace extracted by $NaHCO_3$ (2.5%)/Na_2SO_3 (2.5%)



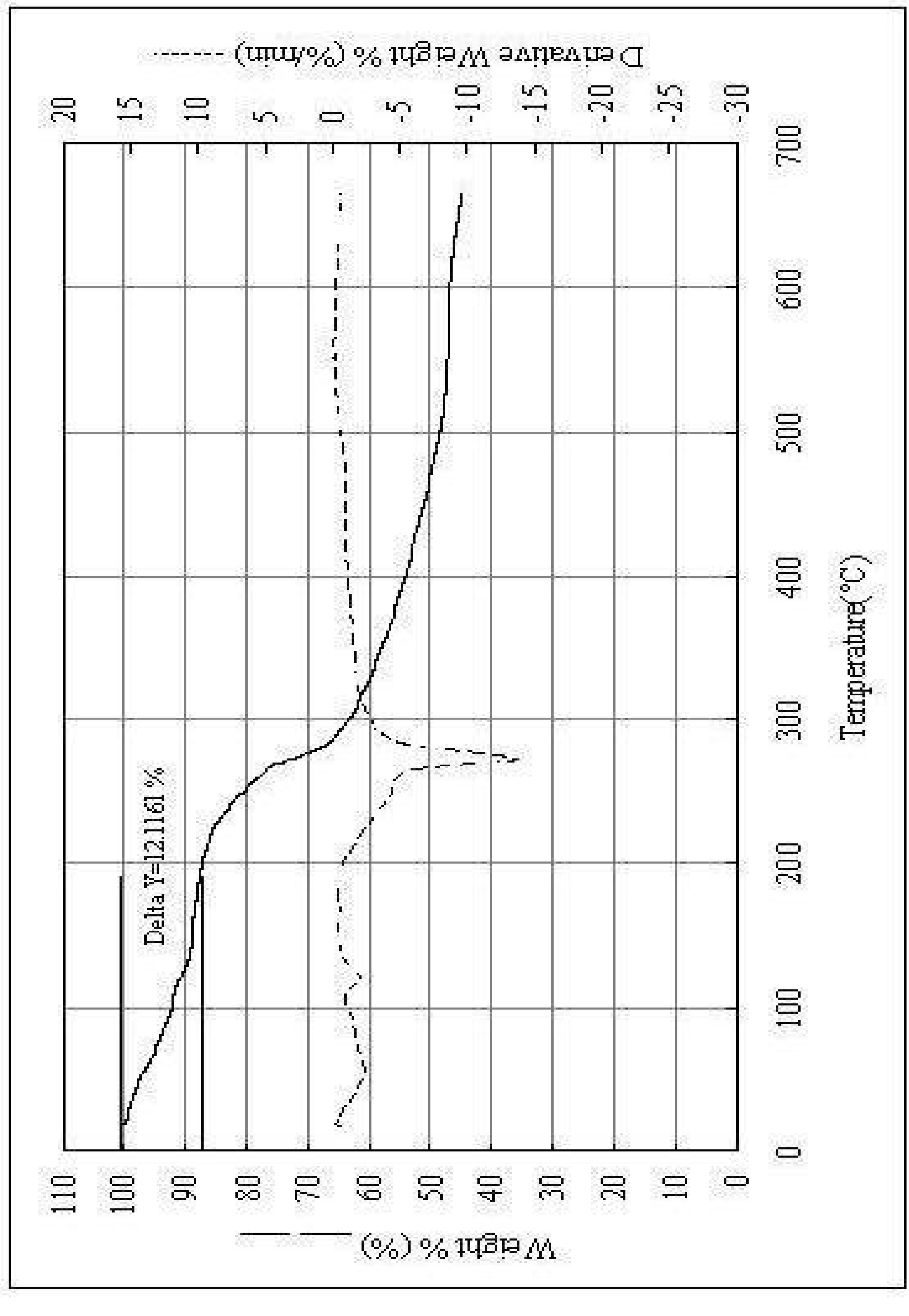
Annex 5. FT-IR spectrum of acidified condensed tannins from grape pomace extracted by NaOH (5%)



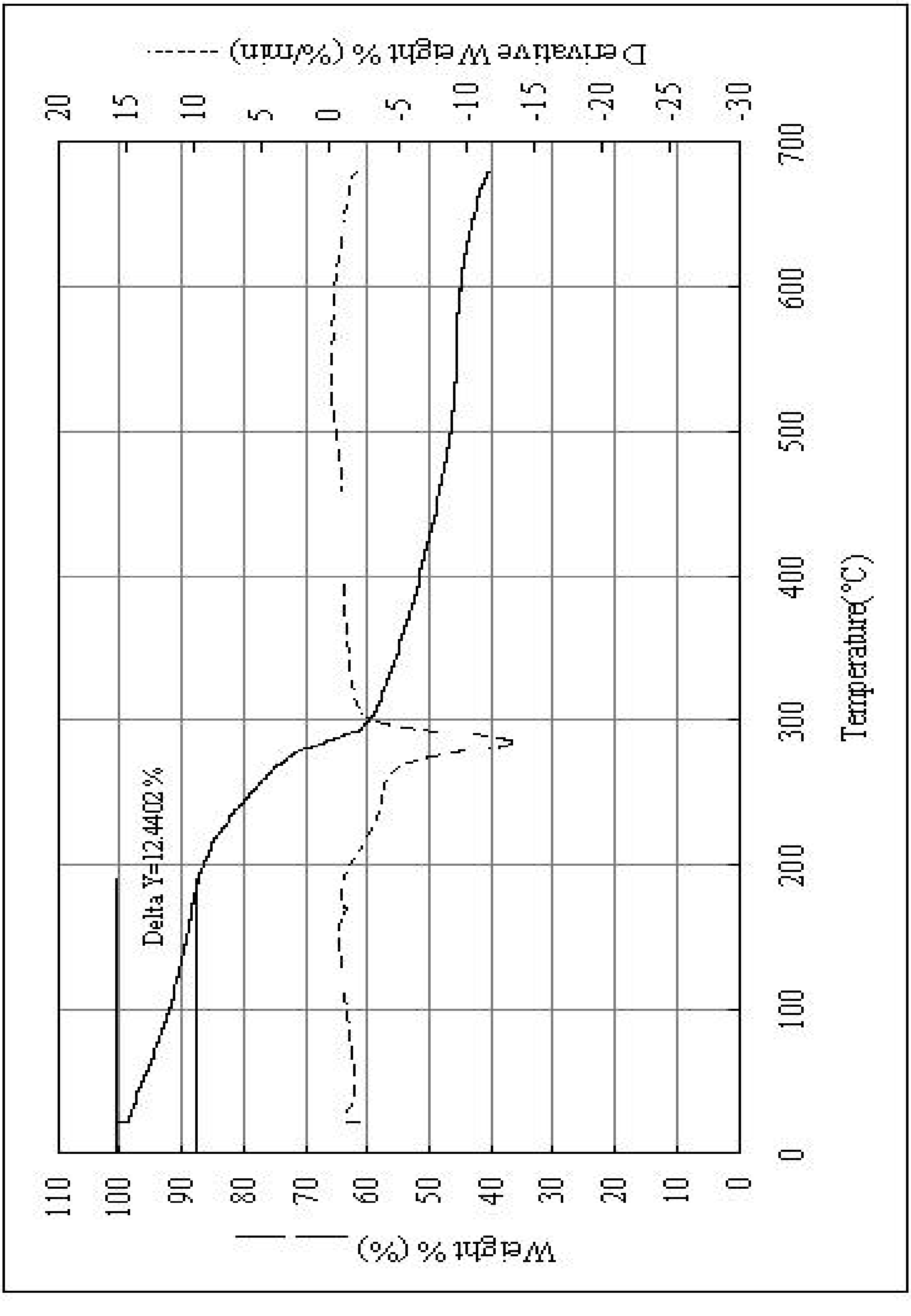
Annex 6. TGA and DTG curves of lyophilized condensed tannins from grape pomace extracted by NaOH (2.5%)/Na_2SO_3 (2.5%)



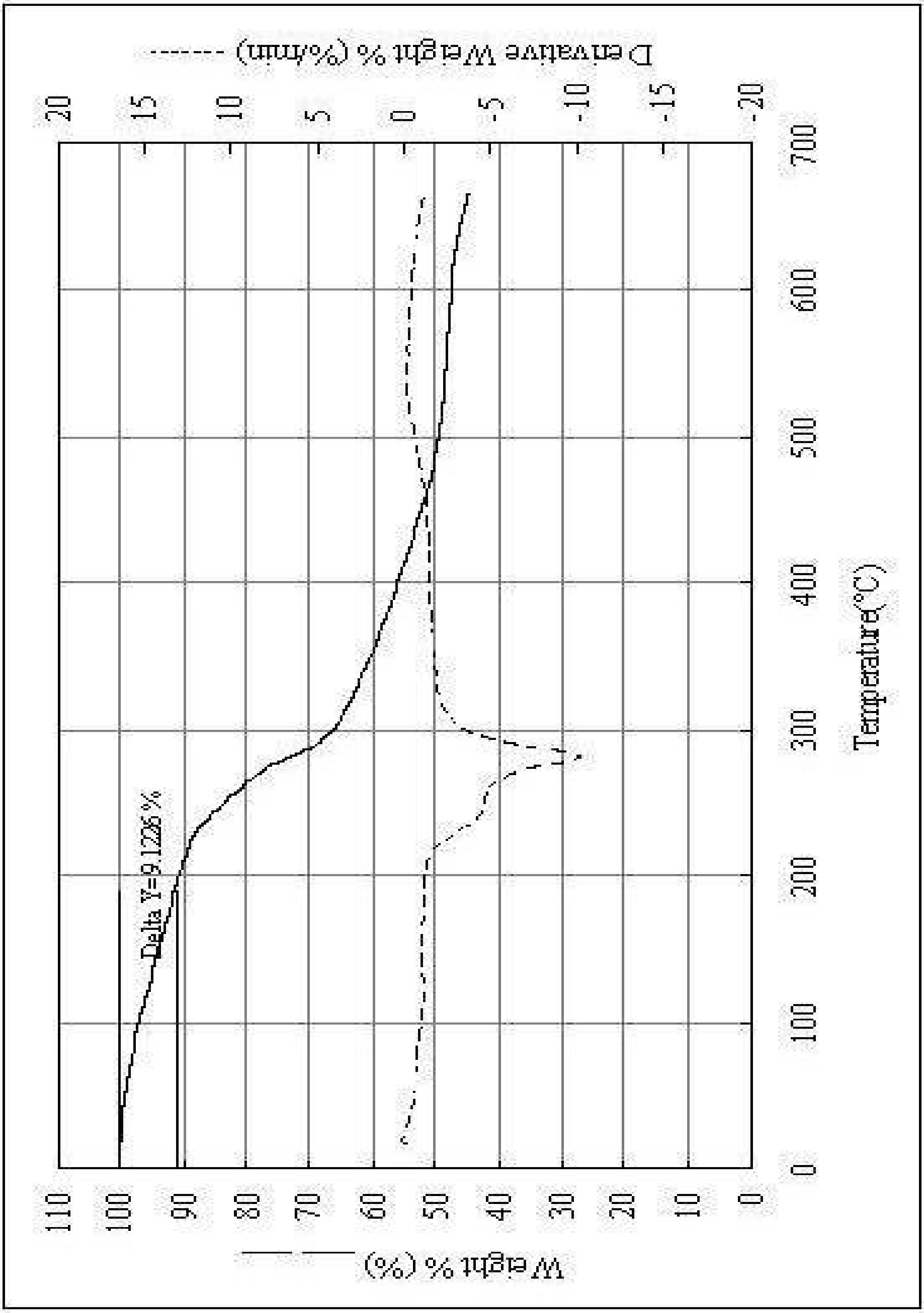
Annex 7. TGA and DTG curves of lyophilized condensed tannins from grape pomace extracted by Na_2CO_3 (2.5%)/Na_2SO_3 (2.5%)



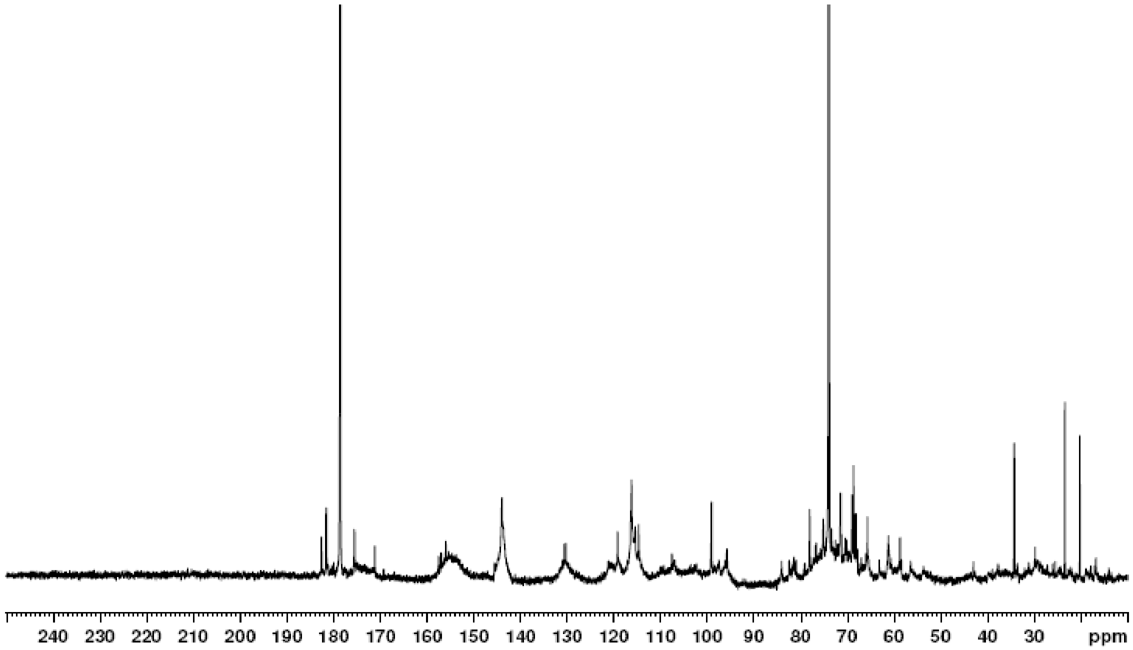
Annex 8. TGA and DTG curves of lyophilized condensed tannins from grape pomace extracted by $NaHCO_3$ (2.5%)/Na_2SO_3 (2.5%)



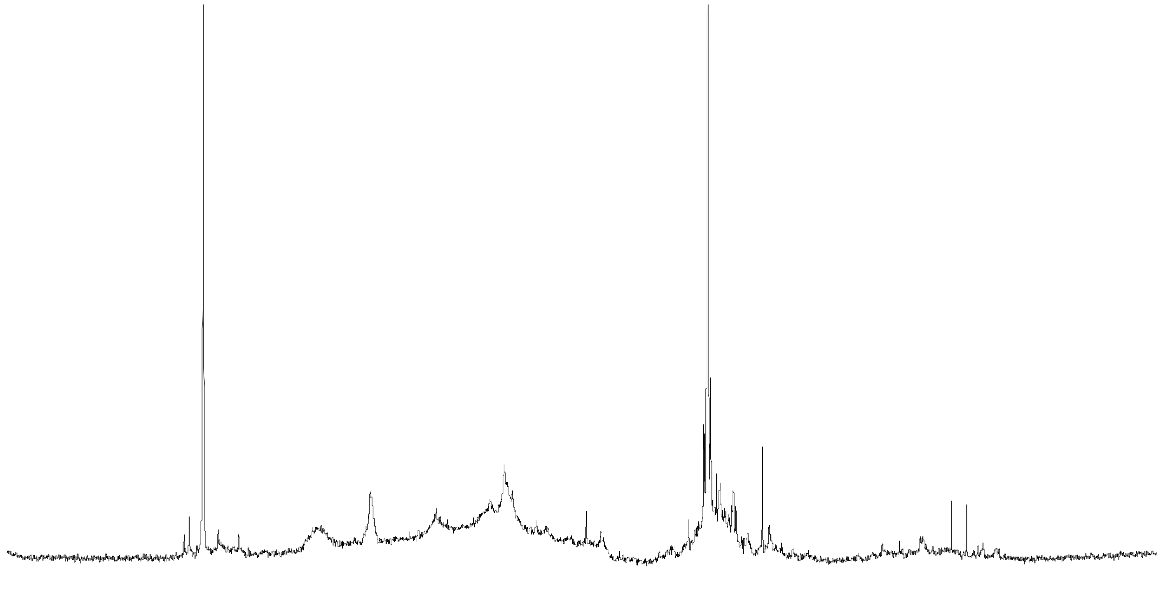
Annex 9. TGA and DTG curves of acidified condensed tannins from grape pomace extracted by NaOH (5%)



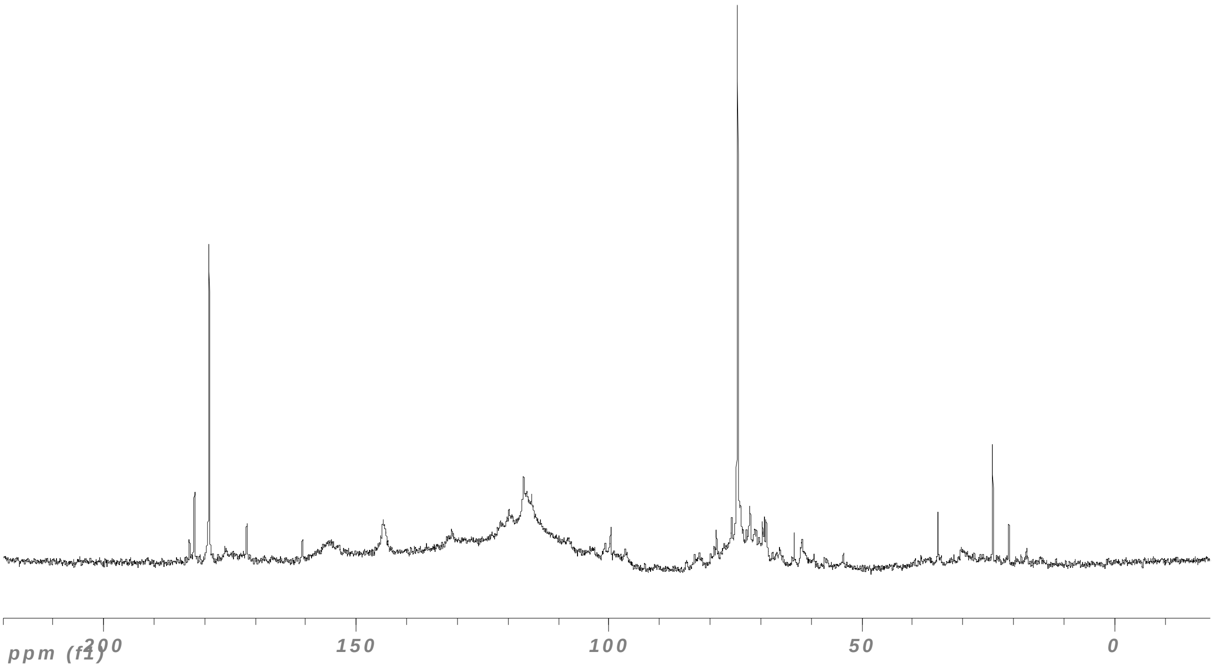
Annex 10. ^{13}C-NMR spectrum of lyophilized condensed tannins from grape pomace extracted by NaOH (2.5%)/Na_2SO_3 (2.5%)



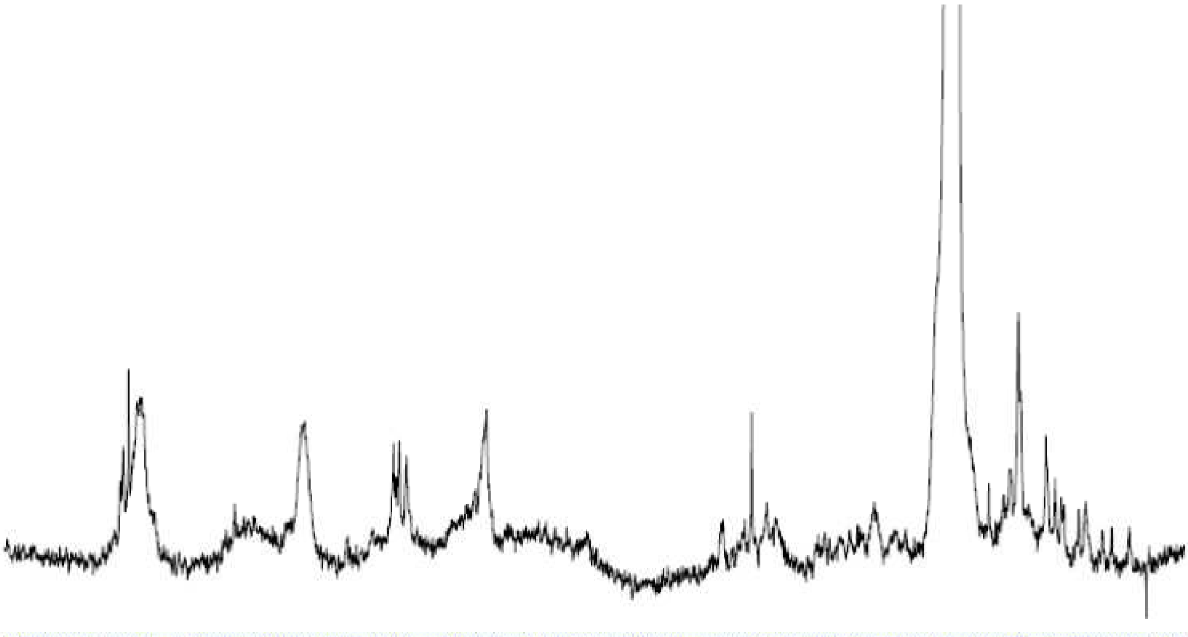
Annex 11. ^{13}C-NMR spectrum of lyophilized condensed tannins from grape pomace extracted by Na_2CO_3 (2.5%)/Na_2SO_3 (2.5%)



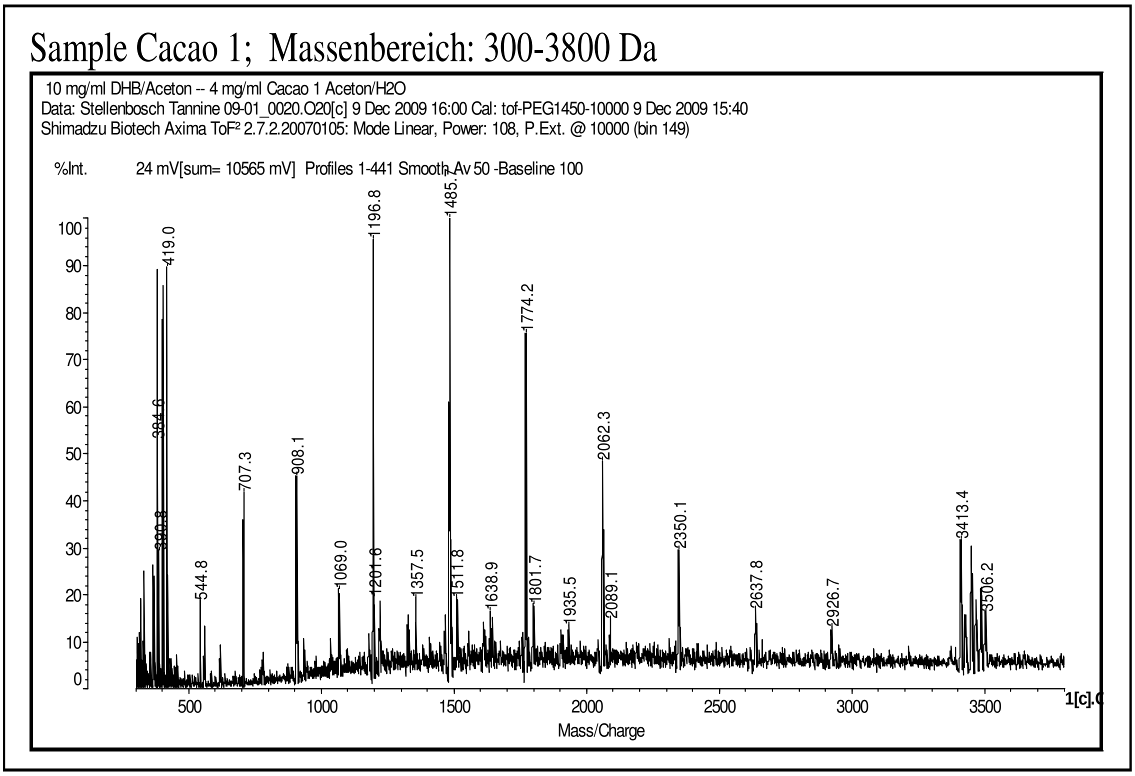
Annex 12. ^{13}C-NMR spectrum of lyophilized condensed tannins from grape pomace extracted by NaHCO$_3$ (2.5%)/Na$_2$SO$_3$ (2.5%)



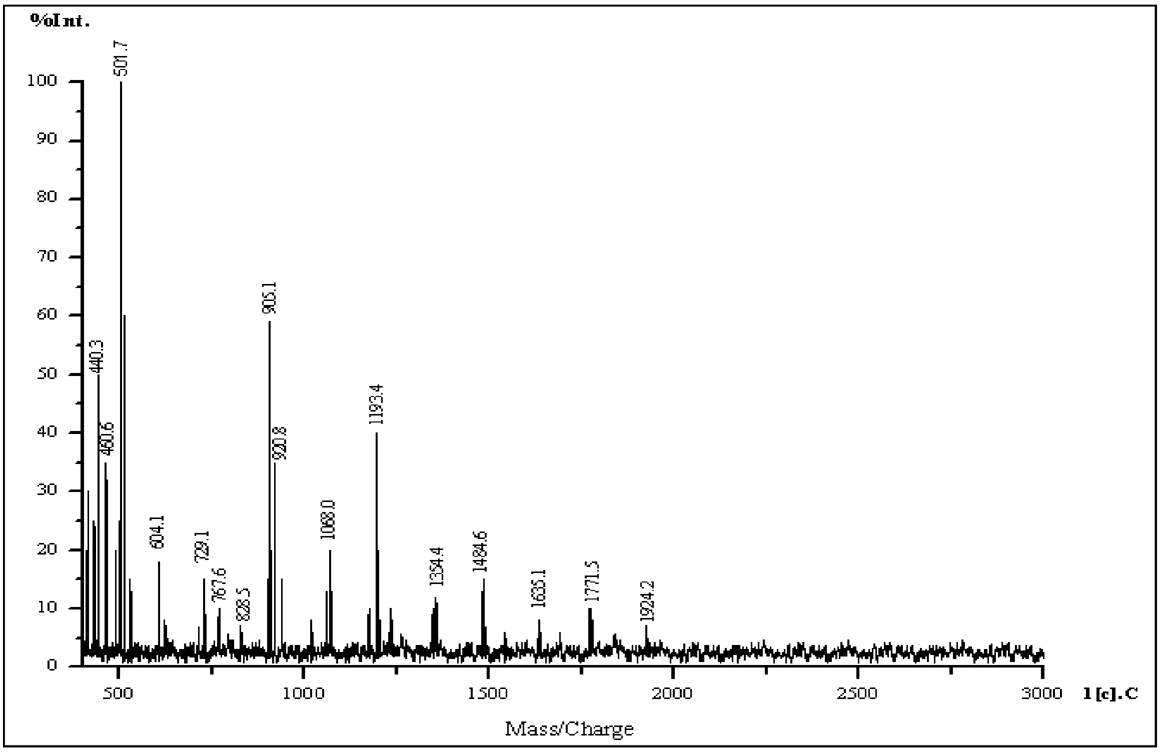
Annex 13. ^{13}C-NMR spectrum of acidified condensed tannins from grape pomace extracted by NaOH (5%)



Annex 14. MALDI-TOF mass spectrum of acidified tannins obtained from grape pomace



Annex 15. MALDI-TOF mass spectrum of lyophilized tannins obtained from grape pomace



List of Publications

Nicolas Brosse, Lan Ping, Poulomi Sannigrahi, Arthur Ragauskas. Evaluation of grape stalks as a bioresource. Industrial crops and products.2011, 33: 200-204.

Lan Ping, Nicolas Brosse, Laurent Chrusciel, Paola Navarrete, Antonio Pizzi. Extraction of condensed tannins from grape pomace for use as wood adhesives. Industrial crops and products. 2011, 33: 253-257.

Lan Ping, Nicolas Brosse, Antonio Pizzi, Zhou Ding Guo. Condensed tannins extraction from grape pomace: characterization and utilization as wood adhesives for wood particleboard. Industrial Crops and Products. 2011, 34: 907-914.

Lan Ping, Roland El Hage, Antonio Pizzi, Zhou Ding Guo, and Nicolas Brosse. Extraction of Polyphenolics from Lignocellulosic Materials and Agricultural Byproducts for the Formulation of Resin for Wood Adhesives. Journal of Biobased Materials and Bioenergy. 2011, 5: 1- 6.

Lan Ping, Antonio Pizzi, Zhou Ding Guo, Nicolas Brosse. Condensed tannins from grape pomace: Characterization by FTIR and MALDI TOF and production of environment friendly wood adhesive. Industrial Crops and Products. 2012, 40: 13-20.

Université de Lorraine
Faculté des Sciences et Technologies
Ecole Doctorale (Sciences et Ingénierie) Ressources, Procédés, Produits et Environnement (RP2E)

RESUME DE THESE (en français)

Discipline : Sciences du bois et des fibres

Soutenue à Nanjing le 19 octobre 2012

Présentée par
Lan Ping

Directeurs de thèse : Pr. Nicolas BROSSE[1] et Pr. Zhou Ding Guo[2]

EXTRACTION DE TANNINS CONDENSES A PARTIR DE MARC DE RAISIN : CARACTERISATION ET UTILISATION POUR LA PRODUCTION D'ADHESIFS POUR PANNEAUX DE PARTICULES

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SOMMAIRE

Résumé en français :

Les marcs de raisin issus de la viniculture constituent un gisement de ressource naturelle abondant, sous valorisé et riche en polyphénols. Une méthode d'extraction de ces tannins condensés a été développée et optimisée en vue d'une application en adhésif pour les produits dérivés du bois. Les tannins ont été caractérisés par Résonance Magnétique Nucléaire, spectroscopie Infra Rouge (FTIR), spectrométrie de masse (MALDI TOF) et Analyse Thermomécanique (TMA). Des panneaux de particules ont été fabriqués à l'aide des colles élaborées en laboratoire et testés suivant les normes françaises en vigueur, plusieurs dépassent largement les valeurs seuils.

Mots clés :

Marcs de raisin, tannins condensés, adhésifs à base de tannins, panneau de particules de bois, émission de formaldéhyde.

INTRODUCTION

Ce rapport en français est une synthèse du mémoire de thèse de Mme LAN Ping qui a été rédigé en anglais et qui traite de la valorisation chimique de marcs de raisin. Le mémoire de thèse en question s'articule autour de 6 parties. Dans un premier chapitre sont présentées les principales données relatives à l'industrie du vin et de la culture du raisin en France et à travers le monde. La gestion des déchets de l'industrie vitivinicole est ensuite abordée afin de mettre en relief l'intérêt de valoriser les marcs de raisin. En particulier, un état des lieux est fait sur la recherche scientifique traitant de la production d'adhésif à base de tannins.

Le deuxième chapitre est consacré aux dispositifs et aux protocoles expérimentaux utilisés dans le cadre de ces travaux de thèse. Les différentes méthodes permettant d'extraire les tannins des marcs de raisin ainsi que les dispositifs d'analyses chimiques sont ensuite décrites. Un mode de valorisation des produits extraits est proposé : la formulation de résine pour des panneaux de particules de bois.

Dans le troisième chapitre sont présentés les résultats expérimentaux relatifs à la caractérisation de la matière première étudiée puis à l'extraction des tannins condensés : teneurs en tannins des extraits, optimisation des méthodes d'extractions et cinétique d'extraction de ces tannins. Les résultats relatifs à la caractérisation des tannins condensés extraits des marcs de raisin sont ensuite exposés dans le quatrième chapitre.

Le cinquième chapitre est dédié à l'utilisation des tannins condensés pour la production de panneaux de particules : les principales caractéristiques des panneaux produits sont décrites. Le dernier chapitre est une conclusion générale. Quelques perspectives sont proposées quant à la suite des travaux de recherche présentés dans ce mémoire de thèse.

1. ETUDE BIBLIOGRAPHIQUE

1.1. CONTEXTE ET PROBLEMATIQUE

Le raisin est l'un des fruits les plus produit au monde, avec plus de 67 millions de tonnes en 2009 (données FAO). Une part importante de cette production est utilisée pour l'industrie vinicole. L'Union Européenne occupe une place prépondérante dans le marché mondial du vin : la France, l'Espagne et l'Italie produisent chaque année entre 40 et 50 millions d'hectolitres chacun. Les principaux producteurs de vins dans le monde et les surfaces dédiées à la viniculture à travers le monde sont présentés sur la Figure 1 et le tableau 1.

Ces surfaces dédiées à la production de raisins ont toutefois des rendements de production variables dépendant de la variété de raisins cultivés, des pertes liées aux maladies et attaques diverses.

Les raisins produits sont destinées à différents marchés et, parmi tous les débouchés, celui relatifs à la production de vin est, de loin, le plus importants. En effet, plus de 50 % de la production mondiale de raisins est absorbé par l'industrie du vin.

Pour information, la production mondiale de vin représentait en 2010 environ 300 millions d'hectolitres.

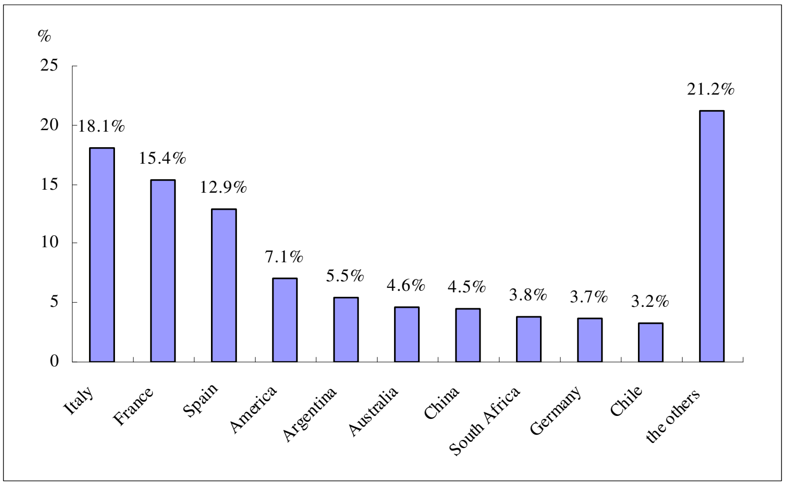

Figure 1. Les dix principaux pays producteurs de vins

Tableau 1 : Principaux pays vinicoles (surface des vignobles en milliers d’hectares)

Pays	2000	2001	2002	2003	2004	2005	2006	2007
Espagne	122.9	121.1	120.1	120.7	120.0	118.0	117.4	116.9
France	**90.7**	**90.0**	**89.8**	**88.8**	**88.9**	**89.4**	**88.7**	**86.7**
Italie	90.8	79.8	79.7	79.1	78.7	80.2	80.2	80.0
USA	41.2	42.6	41.5	41.5	39.8	39.9	40.6	40.9
Chine	**30.4**	**35.9**	**42.1**	**45.5**	**46.0**	**48.5**	**49.0**	**49.0**
Iran	29.2	30.1	30.2	30.0	32.9	33.8	33.8	-
Turquie	57.5	56.4	57.0	57.0	55.9	55.5	52.7	52.5
Portugal	24.6	24.8	24.9	24.9	24.7	24.8	24.9	24.8
Roumanie	24.8	24.7	24.3	24.0	22.2	21.7	21.3	20.5
Argentine	20.1	20.4	20.8	21.1	21.3	21.9	22.3	23.0
Chili	17.4	18.1	18.4	18.5	18.9	19.3	19.5	19.7
Afrique du Sud	12.4	12.6	12.9	13.2	13.3	13.4	13.4	13.5
Australie	14.0	14.8	15.9	15.7	16.4	16.7	16.9	17.4
Moldavie	14.7	15.5	15.3	14.9	14.6	14.7	14.7	14.7

1.2. DECHETS DE L’INDUSTRIE VINICOLE

La production vinicole génère de grandes quantités de coproduits puisque le marc de raisin représente environ 13% de la grappe après pressurage [1]. Le marc de raisin récupéré après la vinification est alors transféré vers les distilleries.

Les distilleries vinicoles sont des industries spécialisées dans la valorisation des marcs de raisin, qui se composent de rafles, de pulpes et de pépins (cf figure 2). Les principales matières récupérées dans ces industries sont l’éthanol obtenu après fermentation des pulpes, le tartrate contenu dans le raisin, les polyphénols et l’huile des pépins.

Les marcs épuisés, résidus de ces étapes, sont actuellement valorisés sans réel intérêt économique ou environnemental en tant que fertilisants pour les cultures ou composant d'alimentation animale. Ils sont cependant encore riches en tannins condensés, ce qui peut poser problème pour ces utilisations [2].

On trouvera sur la Figure 1 les principales molécules composant la baie de raisin.

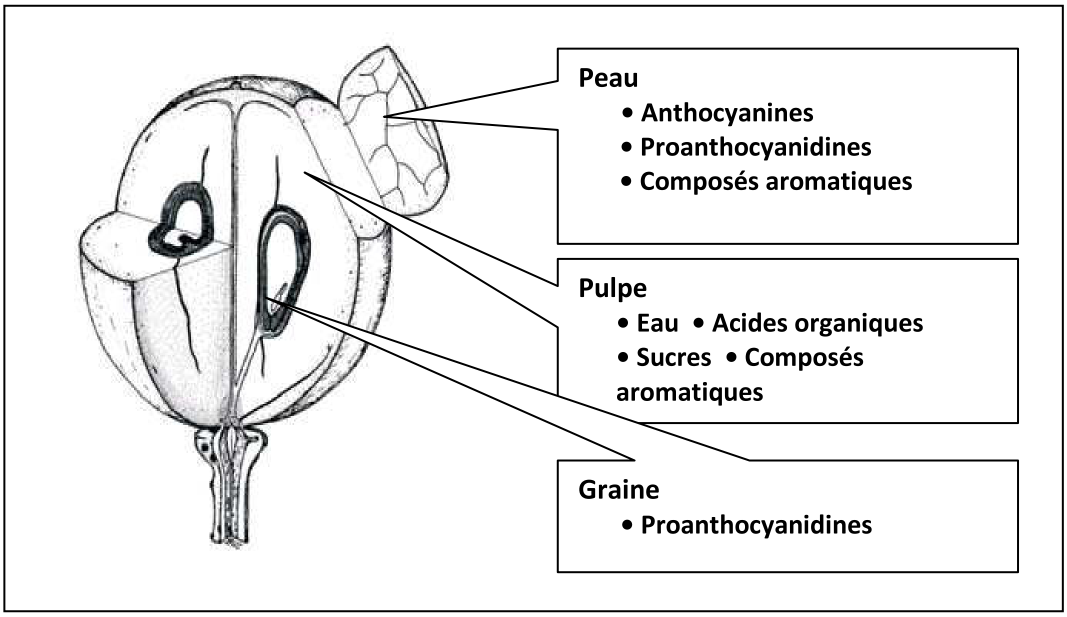

Figure 2. Principales molécules composant la baie de raisin

1.3. VALORISATION DES TANNINS

Les tannins sont des métabolites secondaires de plantes. Ils sont utilisés depuis longtemps dans l'industrie du tannage des cuirs. On distingue les tannins hydrolysables des tannins condensés dont la structure chimique est représentée sur la figure 3.

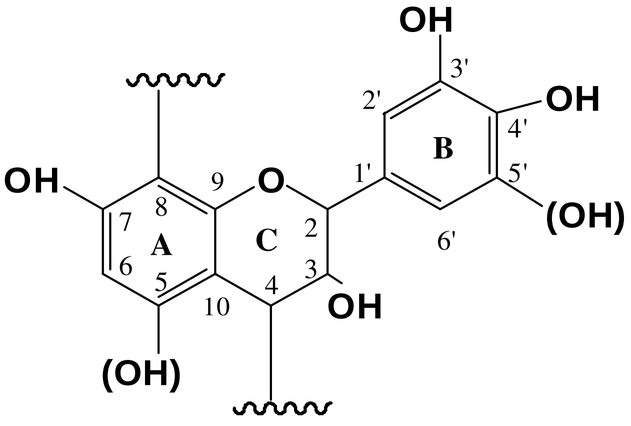

Figure 3. Structure chimique des tannins condensés

Les tannins condensés sont extraits depuis de nombreuses années d'écorces comme le mimosa ou le quebracho dans l'hémisphère sud depuis le début des années 70 pour des applications en tant qu'adhésif pour les matériaux dérivés du bois [3].

Une méthode inspirée de ces extractions a été développée au sein du LERMAB pour l'appliquer sur les marcs de raisin. Une première étude a permis de déterminer que la meilleure voie de récupération de ces tannins condensés après extraction était la lyophilisation plutôt que la précipitation en milieu acide pour l'application colle [4]. Une seconde a démontré l'importance d'ajout de Na_2SO_3 pour l'extraction de tannins condensés, ainsi que la formation d'acide catéchique à haute température [5].

1.4. OBJECTIF ET ASPECTS INNOVANT DE CETTE THESE

Le principal objectif de cette étude est d'extraire des tannins condensés de marc de raisin pour la production de panneaux de particules de bois. Il s'agit d'obtenir un adhésif naturel dont la production est écologie, du traitement de la matière première jusqu'aux techniques d'extraction puis la formulation de la résine en question.

Les aspects innovant de cette thèse seront donc les suivants :

- La matière première (tannins condensés extraits de marc de raisin) est peu chère et abondante. Les travaux de recherche doivent conduire à l'amélioration de l'usage industriel de sous-produits actuellement sous exploités.

- Les procédés développés pour l'extraction des tannins utilisent de l'eau et des équipements simples ce qui rend une future application industrielle possible.

- L'application proposée consiste en la formulation de résine adhésive écologique à base de tannins pour la production de panneaux de particules de bois. Cette valorisation est économiquement viable et éco-responsable.

2. DISPOSITIFS ET PROTOCOLES EXPERIMENTAUX

Le procédé d'extraction se fait uniquement en milieu aqueux, les contraintes industrielles ne permettant pas d'utiliser un solvant organique. Il est choisi à l'échelle laboratoire de travailler avec un ratio d'une masse de matière sèche pour huit masses d'eau.

Le principal dispositif expérimental est un réacteur fermé en acier inoxydable pouvant opérer jusqu'à 250 °C et 70 bar. Ce réacteur (cf. figure 4) est équipé d'une agitation et d'un dispositif de chauffage dont la puissance est régulée en fonction de la température opératoire mesurée par un thermocouple placé dans un doigt de métal inséré dans le réacteur.
Le refroidissement peut être accéléré en fin de cycle grâce à un serpentin dans lequel circule de l'eau froide.

Figure 4. Mini réacteur fermé et son équipement

Deux modes d'extraction des tannins sont réalisées (cf. figure 5). Pour le premier mode, de l'acide chlorhydrique est ajouté afin d'ajuster le pH de la phase liquide à une valeur de 1,5. Cet ajustement du pH se traduit par une précipitation des tannins acidifiés qui sont ensuite récupérés par centrifugation et séchage à l'air.

Pour le second mode, le liquide est directement concentré dans un évaporateur rotatif à 60 °C. On procède par la suite à une lyophilisation pour obtenir ce qui, par la suite, est dénommés des tannins lyophilisés.

Les réactifs utilisés pour favoriser l'extraction des tannins sont l'hydrogénocarbonate de sodium et le carbonate de sodium à différentes concentrations, en présence de sulfite de sodium. Ces extractions sont réalisées à une température de 100°C pendant deux heures.

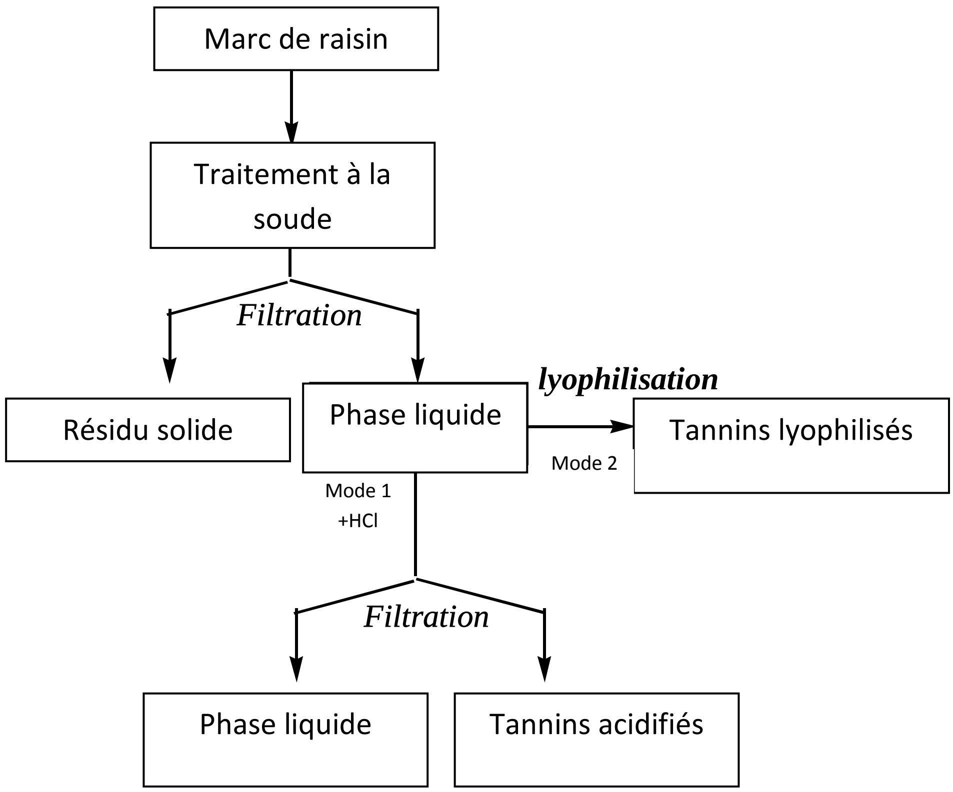

Figure 5. Représentation schématique des deux modes de traitement

3. CARACTERISATION DE LA MATIERE PREMIERE

Les extraits de tannins isolés dans la première partie de ce travail ont été analysés par différentes techniques analytiques :

- L'analyse élémentaire
- La spectroscopie infra rouge
- Des techniques thermogravimétriques (TG, DTG)
- La RMN du carbone
- La spectrométrie MALDI TOF

L’analyse par RMN du carbone 13 des extraits de tannin, présentée dans la figure 6, montre des pics caractéristiques des proanthocyanidines. Les carbones de l’anneau A apparaissent entre 160 et 150 ppm. Les carbones de l’anneau B sont visibles sur le spectre à 142-145 ppm ainsi qu’à 129-132 ppm [7], [8].

Les signaux à 170-182 ppm supposent la présence de groupes carbonyles qui peuvent être expliqués par un réarrangement catéchinique qui se produit pendant le processus d’extraction [9].

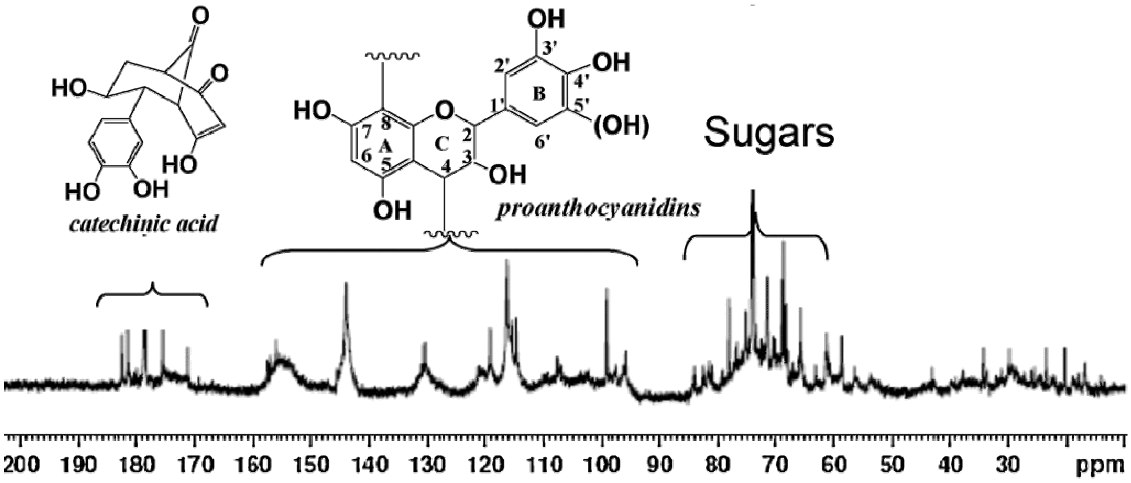

Figure 6. Spectre RMN ^{13}C d’un extrait de tannin dans D_2O.

Une analyse d’extraits par MALDI TOF a été réalisée et a permis de démontrer que les extraits de marcs étaient composés d’oligomères (procyanidine et prodelphinidine) contenant jusqu’à 6 unités (cf. figure 7).

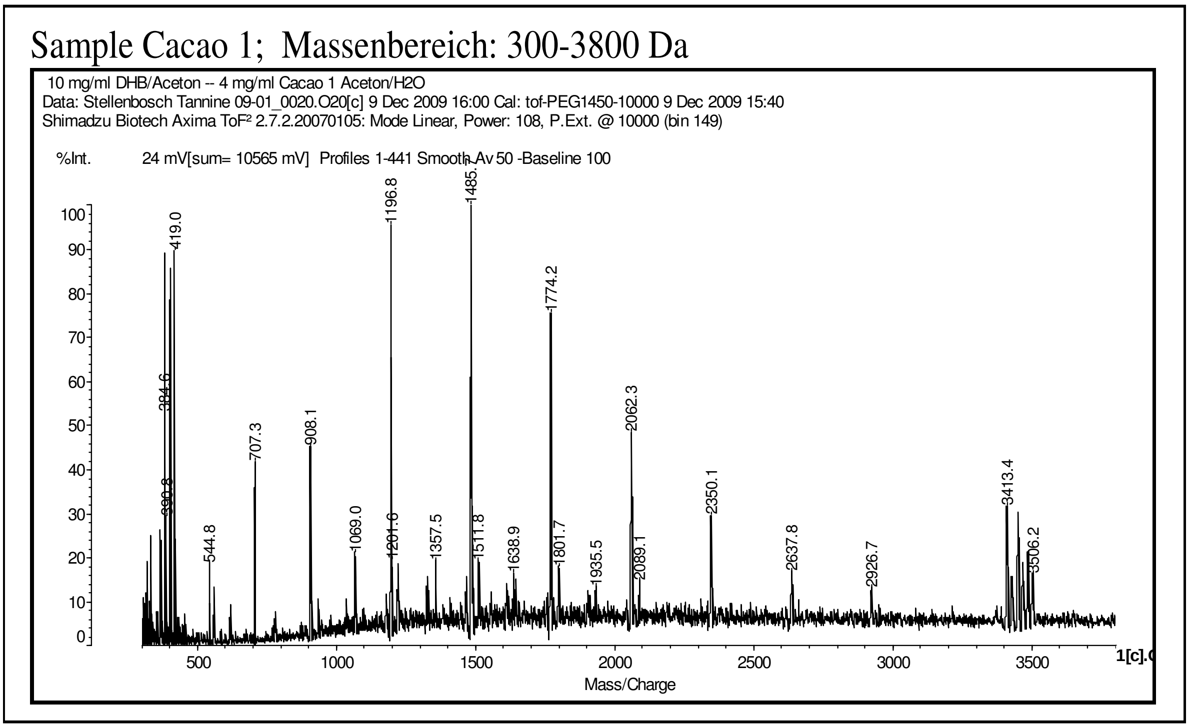

Figure 7. Spectre MALDI TOF d’un extrait de tannin

4. UTILISATION EN TANT QU’ADHESIF POUR PANNEAUX DE PARTICULES

Des formulations adhésives à base d’extraits de marcs de raisin et de formaldéhyde comme agent réticulant ont été préparées, analysées et comparées par analyse thermomécanique (TMA).

Au cours de ces analyses, le Module d’Elasticité est mesuré alors que la température de l’échantillon augmente progressivement. Ces mesures permettent donc de déterminer, dans un premier temps, la température de prise d’une résine et la valeur maximum du Module d’Elasticité puis de caractériser le domaine de température dans lequel on peut observer une dégradation thermique de la résine.

La figure 8 donnée ci-dessous est un exemple de mesures du Module d'Elasticité de la résine en fonction de la température de la température. Trois formulations ont été testées.

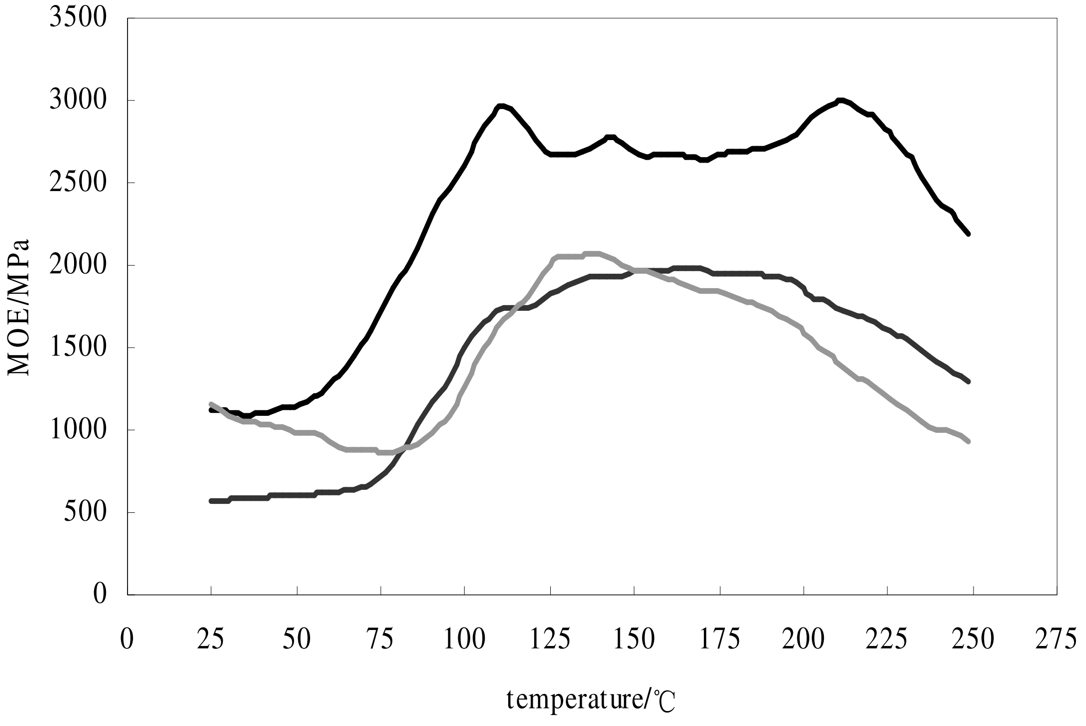

Figure 8. Analyse TMA de résines à base d'extraits de marcs de raisin

Les extraits réalisés à l'aide de $NaHCO_3$ nécessitent une concentration plus importante (45% contre 30% pour les extraits obtenus avec Na_2CO_3) pour des résultats de module d'élasticité équivalents à 195°C (température de la presse pour les panneaux de particule).

D'après les résultats présentés dans le tableau 2, l'ajout de pMDI dans les formulations ne permet une hausse de la résistance de la colle mesurée par la TMA que dans le cas de l'essai A2. Cette valeur n'est pas toujours représentative de la réponse donnée à chaque température.

Tableau 2. Analyse thermomécanique des différentes formulations

	Conditions d'extraction	**Formulation**		**TMA**
essai	Réactifs [a]	Composition Tannin/H_2CO/pMDI	Tannin %[b]	MOE[c] at 195°C (MPa)
A1	$NaHCO_3$ 2,5% Na_2SO_3 2,5%	20/1/0	45	2776,8
A2	$NaHCO_3$ 2,5% Na_2SO_3 2,5%	20/1/5	45	3520,7
B1	$NaHCO_3$ 5% Na_2SO_3 5%	20/1/0	45	3878,83
B2	$NaHCO_3$ 5% Na_2SO_3 5%	20/1/5	45	3588,96
C1	Na_2CO_3 2,5% Na_2SO_3 2,5%	20/1/0	30	3322,88
C2	Na_2CO_3 2,5% Na_2SO_3 2,5%	20/1/5	30	2833,47
D1	Na_2CO_3 5% Na_2SO_3 5%	20/1/0	30	2606,07
D2	Na_2CO_3 5% Na_2SO_3 5%	20/1/5	30	2605,91

[a] % base sur la masse sèche de marc de raisin ; [b] dans de l'eau ; [c] Module d'élasticité

Les panneaux de particules à base d'extraits de marcs de raison ont été fabriqués à partir des mêmes formulations que celles du tableau 3. La norme française impose un seuil de tolérance de 10 % pour la masse volumique des panneaux. La masse volumique des panneaux souhaitée est de 700 kg/m^3, ils répondent donc tous à la norme française.

La cohésion interne pour un usage sec en intérieur de 14 mm d'épaisseur doit, d'après la norme française relative, être d'au moins 0,35 MPa. Les cohésions internes des panneaux de particules A1, A2, B2 et D1 présentées dans le tableau 2 ne passent pas la norme.

Il est remarquable que l'ajout de pMDI n'abaisse la cohésion interne que pour la formulation B, de manière peu significative. Toutes les autres formulations voient leurs performances très fortement améliorées.

Tableau 3. Masse volumique et cohésion interne des panneaux de particules

essai	Composition Tannin/H_2CO/pMDI	Tannin (%) [a]	Masse volumique (kg/m^3)	CI [b] (MPa)
A1	20/1/0	45	692,60	0,26±0,02
A2	20/1/5	45	708,50	0,36±0,02
B1	20/1/0	45	704,97	0,38±0,03
B2	20/1/5	45	703,45	0,34±0,02
C1	20/1/0	30	706,06	0,45±0,06
C2	20/1/5	30	710,38	0,68±0,07
D1	20/1/0	30	711,54	0,36±0,03
D2	20/1/5	30	706,91	0,60±0,04

[a] % en masse dans de l'eau ; b Cohésion Interne

CONCLUSION – PERSPECTIVES

Après l'obtention d'extraits de tannins des marcs de raisins sous différentes conditions, ceux-ci ont été testés dans le but d'une application en tant que résine adhésive. Les principaux résultats de cette étude sont :

- des résultats comparables lors de l'analyse thermomécanique de toutes les formulations adhésives testées ;
- une mise en évidence de la présence de proanthocyanidines par spectrométrie de masse et de sucres et d'acides catéchiques par la RMN du carbone 13 ;
- une validation des masses volumiques des panneaux de particules fabriqués à partir des formulations adhésives par la norme relative ;
- plusieurs formulations passent la norme en termes de cohésion interne, même sans ajout d'agent réticulant (pMDI).

Ces résultats encourageants conduisent à la poursuite de cette étude en plusieurs points :

- une validation à l'échelle pilote des paramètres d'extraction, puis la vérification des propriétés des extraits obtenus pour l'application colle ;
- une adaptation de ce protocole en industrie, en procédé discontinu dans un premier temps, puis en continu compte tenu des quantités de matière à traiter annuellement.
- des essais de fabrication de panneau de particule à une échelle pilote puis industrielle avec les extraits de tannins obtenus en industrie.

REMERCIEMENTS

Nous remercions vivement la Région Lorraine pour son soutien financier ainsi que le CPER 2007-2013 « Structuration du pôle de Compétitivité Fibres Grand Est ».

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