Use of Ultrasonics

Evaluation of methods for extraction and analysis of wood resin in birch kraft pulp

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SUMMARY: Pre-treatment procedures and extraction techniques for analysis of lipophilic extractives in chemical pulps were compared on unbleached and bleached birch kraft pulps. Long air-drying of pulp caused oxidation of polyunsaturated compounds, such as linoleic acid and betulaprenols. Five different extraction techniques were compared: Soxhlet, Soxtec, reflux, ultrasonic extraction and Accelerated Solvent Extraction. Only small differences in the extract yield and composition were found between the different extraction methods for unbleached kraft pulp. However, in the case of bleached pulp containing a high proportion of saturated fatty acids the reflux extraction technique with addition of KH_2PO_4 gave a 30% higher yield of saturated fatty acids than the other extraction techniques.

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Lipophilic wood extractives, also called wood resin or pitch, normally constitute 0.5-3 % of the wood. The amount and composition of wood resin varies considerably depending on the wood species and the wood tissue. Birch wood contains 2-2.5% resin, which is mainly composed of triglycerides (fat), fatty acid esters of sterols, triterpenyl alcohols, and so-called betulaprenols (Paasonen 1967; Ekman, Holmbom 2000). Bark residues will also bring in resin to pulp mills. Birch bark resin contains a large amount of the triterpenyl alcohols betulinol and lupeol. Resin acids, present in most softwoods, do not occur in birch wood. However, it is common practice in birch pulp mills to add tall oil, containing resin acids, to the cook in order to enhance pulp deresination.

Removal of wood resin, is an important part of pulping and bleaching operations since residual resin negatively affects the pulp quality (Back, Allen 2000). Deresination is considerably more difficult in kraft pulping of birch wood than of softwoods. This is due to the high proportion in birch resin of neutral, unsaponifiable components. Moreover, process disturbances caused by wood resin are common in birch kraft pulp mills, foaming and deposition of wood resin on the surfaces of process equipment to be mentioned.

Standard methods for extraction of pulps and gravimetric determination of resin content have been developed (SCAN-CM 49:93, ISO 14453). The standard methods include extraction with Soxhlet or Soxtec apparatus with acetone as the solvent. This standard came to replace the toxic dichloromethane in extraction. The need for a critical evaluation of the extraction method became obvious when discrepancies were found in results, especially after detailed gas chromatographic analysis. Varying extraction parameters and procedures are still used (Gutiérrez et al. 1999; Wallis, Wearne 1999; Silvestre 2000). Some critical evaluations have been done, e.g. comparing Soxhlet and Soxtec extraction (Sitholé et al. 1991). Accelerated Solvent Extraction (ASE) has also been compared with Soxhlet for wood and pulps (Thurbide, Hughes 2000) and for extraction of natural products (Kaufmann, Christen 2002). Recently a round robin has been done to compare the use of different solvents in extraction of pulps (Gruber et al. 2002).

This work was done to compare different extraction techniques for determination of lipophilic extractives with special reference to chemical pulps. The influence of pre-acidification of the pulp was investigated, two different drying techniques (air-drying and freeze-drying) were studied, and five different extraction techniques were compared (Soxhlet, Soxtec, reflux, ultrasonic extraction and ASE). Three extraction solvents of different polarity were also compared.

Materials and methods

An overview of the experimental programme is presented in *Fig. 1*.

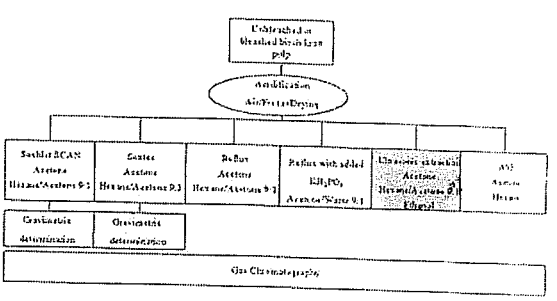

Fig. 1. Scheme of the evaluation programme.

Pulp samples

Unbleached and bleached birch kraft pulps from a Finnish pulp mill using ECF bleaching were used in the evaluation of the different extraction methods. The unbleached birch pulp was sampled before the oxygen delignification step and the bleached pulp was sampled after the last chlorine dioxide stage. The pulp consistencies were

about 20 % for both pulp samples. The pulps were stored in a freezer.

Pre-treatment procedures

Drying

The pulps were either air-dried or freeze-dried. About 500 g of acidified pulp and untreated pulp were air dried in a laboratory fume-hood for a week, until the dry matter content was more than 90%. Such a long time was required with this large amount of pulp. In a similar manner, a 500 g pulp sample was dried in a Heto CT 60e freeze dryer for 2 days, again until the dryness of the pulp exceeded 90%.

Acidification with acetic acid and phosphoric acid

The pulps were acidified according to the SCAN-CM standard 49:93. 1.4 l of acetic acid having a pH of 2.5 was added to 800 g dry-weight of the unbleached and bleached pulp. The samples were stirred for 10 min and then vacuum-filtered. The pulp was rinsed with the filtrate four times. The pH of the filtrate after rinsing was between 2.8 and 3.0. After acidification the pulp was either freeze-dried or air-dried. In a similar way acidification was performed with a phosphoric acid solution having a pH of 2. After acidification with the phosphoric acid the pH of the filtrate was between 2.1 and 2.5.

Extraction methods

Soxhlet

The pulp samples were extracted with Soxhlet equipment according to the SCAN-CM standard 49:93. Four parallel 3 g samples were weighed with an accuracy of 0.01 g in a pre-extracted cellulose thimble. A quantity of 150 ml acetone, or a 9:1 hexane/acetone mixture, was used as solvent. The samples were extracted for four hours. After cooling the extracts were diluted to 200 ml. A quantity of 2 ml of the unbleached pulp extract solution, and 10 ml of the bleached pulp extract solution was then used for GC analysis.

Soxtec

In a similar manner as described above, four parallel samples of 1 g pulp weighed with 0.01 g accuracy were extracted with Soxtec equipment. A quantity of 50 ml acetone, or a 9:1 hexane/acetone mixture, was used as solvent. The extraction time was 1 h followed by 30 min rinsing. After extraction the extract samples were diluted to 100 ml with acetone. A quantity of 2 ml of the unbleached pulp extract solution, and 10 ml of the bleached pulp extract solution was taken for GC analysis.

Reflux extraction

In a 250 ml flask 1 g pulp were weighed with 0.01 g accuracy. The extraction was performed with 150 ml acetone or hexane/acetone 9:1. A cooler was attached to the flask and the flask was heated. The pulp suspension was refluxed for four hours. After the extraction the flask was cooled to room temperature and the extract solution was filtered through a glass fiber filter (Schleicher & Schuell GF 50). The extract was diluted to 200 ml with acetone and a quantity of 2 ml was taken for GC analysis.

Reflux extraction with excess KH_2PO_4

The Reflux extraction with added KH_2PO_4 was done in a similar manner as described above for reflux extraction. Non-acidified pulp was used in this extraction. The difference in method were the addition of 150 mg KH_2PO_4 to the flask to obtain a saturated solution and the use of 150 ml acetone/water 9:1 as the extraction solvent. This method has been described previously by Sundberg et al (1997). The aliquots taken for further analysis were also extracted with methyl-*tert*-butyl-ether at pH 8 twice in order to remove all water from the extract.

Ultra-sonic extraction

Into a 25 ml E-flask 0.1 g pulp was weighed. 25 ml of solvent was added, either acetone, hexane/acetone 9:1 or ethanol. The samples were then extracted with a Sonics & Materials Inc. 600 W Vibra Cell™. The extraction time was 2 min with a sequence of 5 s extraction and 2 s pause. The amplitude setting was 25%. The extract solution was filtered through a glass fiber filter (Schleicher & Schuell GF 50). The extract was then diluted to 50 ml with acetone and a quantity of 8 ml was taken for GC analysis.

Accelerated Solvent Extraction

The extraction was performed in an ASE 200 Equipment (Dionex Corporation, California, USA). 1-2 g pulp was weighed with 0.01 g accuracy into a 33 ml extraction vessel. The extraction vessels were closed and put into the ASE apparatus. Acetone or hexane were used as solvents. The extraction was performed under 2000 psi pressure and at 100°C. After the extraction the extract volume was adjusted to 50 ml with acetone. A quantity of 2 ml extract was taken for analysis.

Analysis of extracts

Gravimetric determination

Gravimetric determination was made on all extracts, except for the ASE extracts. The extract solution was evaporated to a volume of 20-30 ml with a rotavapor, followed by evaporation using N_2-gas in pre-weighed test-tube. The extracts in the tubes were kept in a vacuum oven at 40°C for 2 h and stored in an exsiccator before weighing. For the Soxtec extraction, the extraction vessels were pre-weighed. After extraction and solvent evaporation the vessels were heated in the vacuum oven for 2 h in 40°C and stored in an exsiccator until weighing.

Gas chromatography

Fatty acid 21:0 (heneicosanic acid), cholesterol and cholesteryl heptadecanoate were used as internal standards for the gas chromatographic analysis. After evaporation the extract aliquotes were silylated with 80 µl N,O-Bis-trimethylsilyl-trifluoracetamide (BSTFA) and 40 µl trimethylchlorosilane (TMCS), held in a 70°C oven for 1 h and analysed by GC and GC-MS. The extracts were analysed with a HP-1 column with the length of 5 m, inner diameter of 0.53 mm and film-thickness of 0.15

mm. According to the method developed in our laboratory (Örså, Holmbom 1994) the initial temperature was 100°C and the temperature gradient was 12°C/min. The injector temperature was 80°C and detector temperature 340°C. The same silylated extracts were also analysed by GC using a HP-1 column (25 m, 0.20 mm i.d.) and FID detection to analyse individual fatty acids, resin acids and unsaponifiable neutral components, mainly sterols and triterpenyl alcohols. The initial temperature was 150°C and the temperature gradient was 7°C/min. The injection temperature was 250°C and detector temperature 290°C. Fatty and resin acids were quantified against the 21:0 standard and the unsaponifiable components against cholesterol. Steryl esters were quantified against the cholesteryl heptadecanoate standard. No response factors were used.

Results

Pre-treatment

In the SCAN-CM 49:93 standard method it is recommended to use air-drying or oven-drying in a 40°C oven over night if the dry matter is less than 90% of the pulp. However, since pulp samples can contain much more water, such as after the acidification according to the standard method the drying time at room temperature can become extensively long. During one-week drying time, sensitive components in the pulp were oxidized (*Fig. 2.*). Linoleic acid (18:2) as well as squalene and the prenols were oxidized to some extent during air-drying. Oxidation was also noted for pulp that was not acidified but only air-dried. Oven drying at 40°C was not evaluated, but it is probably more oxidative than ambient air drying.

Effect of acidification

For acidified unbleached birch kraft pulp a significant difference was noted for the extraction of free fatty acids: 0.6 mg/g more was extracted compared to the non-acidified pulp. This is due to the fact that metal soaps of fatty and resin acids are transferred to their acidic form and become extractable. In *Fig. 3* the yields are given for the major components and groups for pulp acidified with acetic acid according to the SCAN standard method. In this case acidification is improving the extraction of fatty acids from the pulp. For unbleached pulp there was no difference between the two different acidification procedures used (acetic acid according to the standard or phosphoric acid.) Somewhat less resin acids was extracted from the acidified pulp. For betulinol, sitosterol and steryl esters no effect of acid pre-treatment was observed.

For bleached pulp, acidification with acetic acid or phosphoric acid did not give higher extraction yields of fatty acids (or other components). This is probably due to the low amounts of fatty acids in the bleached pulp, varying between 0.11 - 0.13 mg/g pulp for the saturated fatty acids and only 0.01 mg/g for the unsaturated fatty acids. In *Fig. 4* the results for the saturated and unsaturated fatty acids from Soxhlet and Soxtec extraction are shown also in comparison with both ASE and

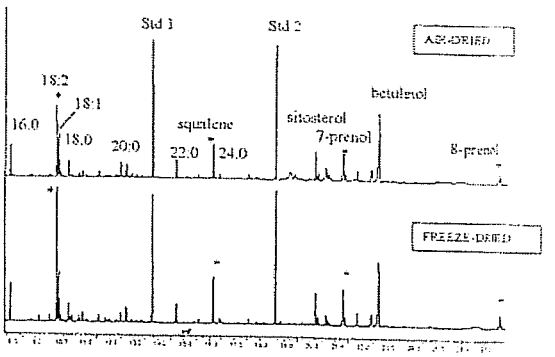

Fig. 2. Chromatograms of acetone extracts of acidified unbleached pulp. Standards used are Std1 as 21:0 and Std2 is cholesterol. The components noted to be diminished are marked with a *.

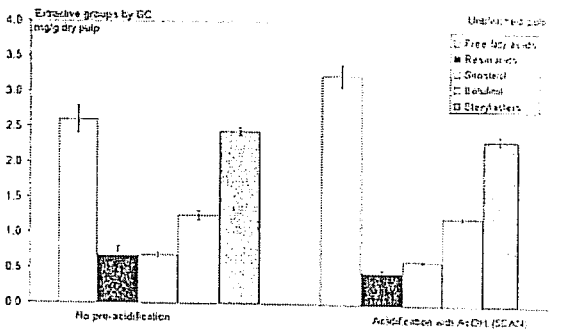

Fig. 3. Effect of acidification on extraction yield of the unbleached birch kraft pulp. Samples Soxhlet extracted.

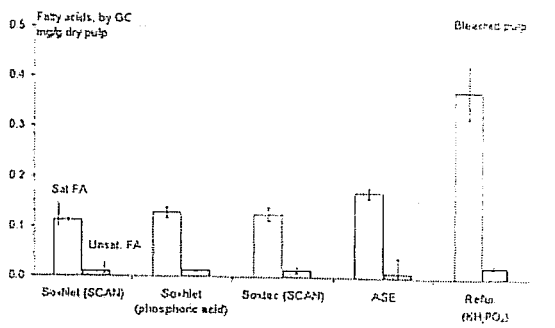

Fig. 4. Extraction yield of saturated and unsaturated fatty acids in bleached birch kraft pulp.

reflux extraction. The reflux method with excess KH_2PO_4 gave significantly higher yields for fatty acids and also somewhat higher yields for resin acids (not shown in fig).

Extraction methods

In the extraction of unbleached birch pulp only small differences were found between the extraction methods, only ASE gave a slightly higher yield. The repeatability of the extractions was good, (± 3 %) especially with hexane/acetone (*Fig. 5*). The differences come almost exclusively from more extracted fatty acids. The ultrasonic extraction gave similar yields as the Soxhlet and Soxtec extractions.

In *Fig. 6* the different extraction methods are compared

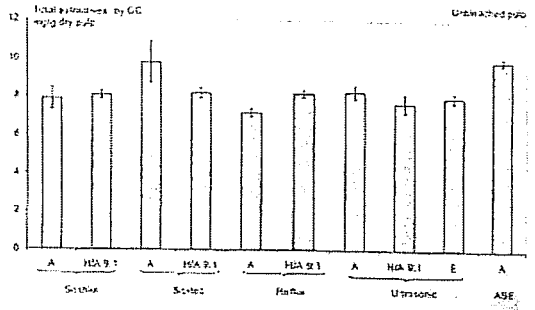

Fig. 5. Total extracts of acidified and freeze-dried unbleached birch pulp with different extraction methods and solvents. The standard deviation for four parallel analyses marked in the figure. A: Acetone, H/A: Hexane Acetone (9:1) and E: Ethanol.

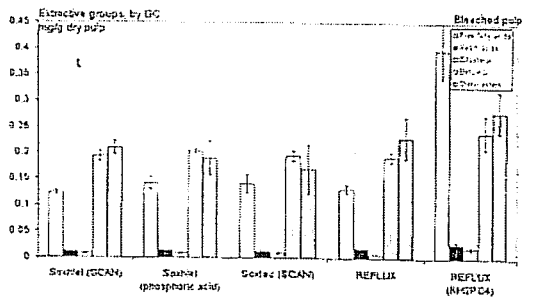

Fig. 6. Comparison of different acidification and extraction methods with bleached birch kraft pulp.

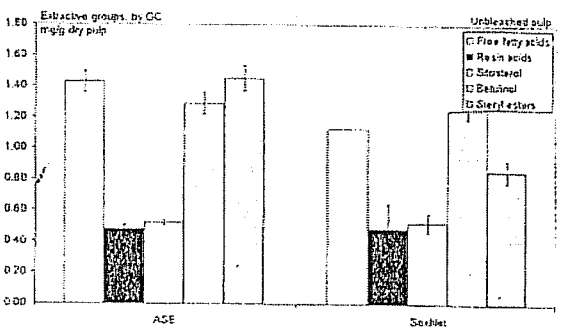

Fig. 7. Extraction yields with acetone of acidified freeze-dried unbleached birch pulp with ASE and Soxhlet.

for bleached pulp regarding different compound groups analysed by GC. Reflux with excess KH_2PO_4 gave much higher yield of saturated free fatty acids than the other acidification methods. The yield was slightly higher also for the other extractives. For unsaponifiable neutral compounds the reflux and ASE (not shown in fig.) gave better yields. For the sterols the difference for reflux with excess KH_2PO_4 comes with more extracted sitosterol, slightly higher yield was seen for sitostanol with the same extraction method. Since the resin acids might be isomerised by the acidification, the reflux technique extracts somewhat more of isopimaric acid compared to the other methods. Also betulinol is extracted somewhat more than by the other methods.

Extraction with ASE gave significantly higher yield than with Soxhlet (which had a similar yield as the other methods). Extraction with ASE gave higher yields than Soxhlet especially for free fatty acids, betulinol and steryl esters (*Fig. 7*) for unbleached birch kraft pulp. For bleached pulp ASE was not as effective as the reflux method with excess KH_2PO_4 (instead, the yields were similar to the other extraction methods evaluated).

Solvents and gravimetrical results

Unbleached birch kraft pulp was extracted with four different solvents: acetone, acetone/water 9:1, ethanol, and hexane/acetone 9:1. The differences both in the yield and the composition of the extracts were small. The extracts gave almost identical chromatograms both qualitatively and quantitatively. When acetone and hexane/acetone 9:1 were used as solvents the chromatograms obtained were identical, both total amounts and composition, although acetone has a significantly larger solvation capacity for polar compounds than hexane. Evidently, the kraft pulps studied did not contain polar extractable compounds. For all the extractions the gravimetric determination gave higher total yields than the yields obtained with gas chromatography. Total amount of extractives eluted from GC were between 50 - 80 % of the yields from the gravimetrical determination, for ultrasonic extraction only 50 % was eluted.

Discussion

Analysis of extractives is normally performed according to the standard method SCAN-CM 49:93 with acetone using Soxhlet or Soxtec equipment. According to this method the samples should be air-dried before extraction. However, air-drying involves risks for oxidation of sensitive extractive compounds. Instead, freeze-drying is recommended, especially if analysis of extractives is of interest.

Acidification is recommended for unbleached chemical pulp samples. The higher yield obtained after acidification is most probably due to improved extraction of fatty and resin acids, being converted to free form by the acid treatment. In the analysis of bleached chemical pulp the acidification according to the standard method was not effective enough to get all the fatty and resin acids to the acidic form, not even with a stronger acid such as phosphoric acid. A better exchange is obtained using the reflux method with acteone/water (9:1) with an addition of excess KH_2PO_4. During reflux, the phosphate ion binds the metal ions and the sample is in contact with the solvent during the whole extraction.

There were no essential differences in the amounts extracted from unbleached pulp between the different extraction methods. In the end, it is practical aspects that influence the choice of extraction method, e.g. time to perform the extraction, how much extract is needed, and it is also a question of cost of solvents and apparatus. Ultrasonic extraction has the advantage of being very fast, and is suitable for analysis of numerous samples. However, gravimetrical analysis is not feasible, due to the small sample amounts. Gravimetrical analysis is also to some extent inexact since the gravimetric value also

includes other compounds not eluted on GC which was seen comparing the total amounts eluted from GC analysis with results from gravimetrical analysis. For Soxhlet extraction the equipment is standard laboratory equipment and the method is much used and inexpensive. On the other hand, the Soxtec-method is faster and consumes less solvent, but the apparatus is more expensive. Reflux does not require any special equipment, but equally large amount of solvent is needed as in Soxhlet. ASE is a novel method and the most technically advanced. It is fast and easy to use and requires less solvent than Soxhlet and Reflux. The yield is also somewhat better than for the Soxhlet extraction method.

For bleached pulp, the reflux method with excess KH_2PO_4 gave somewhat better yields, especially for saturated fatty acids. The advantage of this method is that the sample does not need pre-treatment by acidification. The phosphate binds the calcium ions and releases the fatty and resin acids thus becoming extractable.

Conclusion

The evaluation done showed that extensive air-drying is not recommended since sensitive extractive compounds such as unsaturated fatty acids can be oxidised during prolonged air-drying. Acidification is necessary for unbleached chemical pulp samples. In the analysis of bleached chemical pulp the acidification according to the standard method was not sufficiently effective. Acidification increases the yield of fatty acids but not sufficiently for complete extraction of all saturated fatty acids. A better exchange is possible using the reflux method with acteone/water (9:1) with an addition of excess KH_2PO_4. The evaluation was done specifically on birch kraft pulps. Even though the wood extractives in birch contain more unsaponifiable compounds and to some extent more saturated fatty acids than do softwood extractives, and since resin acids are added to the process the conclusions can be considered general since softwood pulp do not include any other extractive compounds that would be difficult to extract.

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