Volume II

1st World Conference on Biomass for Energy and Industry

Proceedings of the Conference held in Sevilla, Spain, 5–9 June 2000

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Proceedings published by:
James & James (Science Publishers) Ltd
35-37 William Road
London NW1 3ER
UK

A catalogue record for this book is available from the British Library.

ISBN 1 902916 15 8

Printed in the UK by MPG Books Ltd.

Cover photos courtesy of Greenpeace/Morgan and Bioscan/Jeppe Michael Jensen, Peak Photo

1st World Conference on Biomass for Energy and Industry, Sevilla, Spain, 5–9 June 2000
Pages 1148–1151.

EXTRACTION AND ANALYSIS OF PLANT DYES FOR INDUSTRIAL USE

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ABSTRACT : In this paper we present some results on the extraction and analysis of plant dyes from Woad (*Isatis tinctoria* L.) Weld (*Reseda luteola*) and Madder (*Rubia tinctorum*). Optimal extraction conditions of indigo from woad as pH of extraction solvent, temperature and soaking time are discussed. On the other hand, the results of of extraction methods in hydroalcoholic medium of flavonoids pigments from weld and anthraquinone dyes from madder are shown. Analytic techniques by each group of compounds are mentioned . For quantifying pigments of woad we have used the Tin Layer Chromatography (TLC)/ Densitometry and for quantifying the derives flavonoids of weld and anthraquinones of madder we have used the High Performance Liquid Chromatography (HPLC).

1. INTRODUCTION

Since the beginnings, man used biomass to produce natural dyes. The first dyes had been extracted of plants and animals. Until the beginning of the 20th century, the totality of pigments had a natural origin. An important number of colors were obtained from molecules which have very different structures.
The mauvein synthesis in 1886 marked one of the greatest progress of the industrial chemistry, but also displaced the utilization of natural compounds.
The synthetic dyes, being less expensive that the natural ones, had known a great progress in a short period of time. Nevertheless, their utilization presents some risks for the consumers' health. Our days we are witness of a remarkable interest for the natural dyes. To optimize their production and to be able to offer them to more competitive prices, several laboratories in Europe have devoted their works. The laboratory of Agro-Industrial Chemistry has progressively opened up a specific area of research of natural dyes. Part of the human and equipment means set in place, directly contributes to the scientific and technology advancement of the natural dye production project.

2. BLUE INDIGO DYE.

Indigo is a blue colored dyestuff which have been derived from seceral plants. In temperate climates, the most commonly used species was *Isatis tinctoria* L. or Woad. Other species, e.g., *Polygonum tinctorum*, *Baphicacanthus cusia*, *Calanthe veratrifolia*, and *Indigofera* species, have been used for the indigo production.
In medieval Europe, a large industry grew around the production of indigo from woad. However, from the early 17th century, this industry declined in the face of competition from imported indigo derived from tropical *Indigofera* species. Since the 19th century woad and natural indigo have been virtually replaced by chemical derived synthetic indigo (1).
The medieval method of indigo extraction from woad was making *woadballs* or *cocagnes*. This was done by crushing the freshly harvested leaves, forming them into balls and allowing them to dry over a period of 4 – 6 weeks. The balls were then crushed, heaped into piles, water added and allowed to ferment over a period of 3 – 15 days. A mixture of vegetal matter and indigo was obtained. This mixture was used in the dyeing process (2). Today this technology is no longer used because it is not transferable to large-scale production.

2.1. The chemistry of indigo.

Blue indigo is not visible in natural state in the leaves of woad. Indeed its formation comprises an evolution from precursors colorless becoming colored molecules at the end of the extraction. Epstein *et al.*,(3) has identified the major precursor in woad and he has determined that it is a heteroside named **Indoxyl-5-ketogluconate** or Isatan B **[1]** (fig. 1), being different than **Indoxyl-β-D-glucoside** or Indican **[2]** which is the precursor in *Indigofera* species and *Polygonum tinctorium*. Strobel and Gröger (4) shown that woad contain also Indican but in less important quantity. These precursors are glucosides that are different between them by the nature of the sugar and by the type of bond between the carbohydrate moiety and the aglycone part. The bond ester of the Isatan B is very sensitive to the hydrolysis in alkaline medium, while the bond of Indican is more resistant. This necessitates therefore the action of enzymes or a fort alkaline (5).
Precursors are cleaved and liberating **Indoxyl [3]** that isomerise and two molecules combine to form **Indigo [5]**, or oxidize to form **Isatin [4]**. This last one can condense with a free indoxyl molecule and produce **Indirubin [6]**. The formation of indigo and indirubin from indoxyl takes place spontaneously in presence of oxygen. These molecules are insoluble in water and they precipitate after a while.

2.2. Extraction.

Woad leaves were harvested from the field in Castelnaudary-France, and brought to laboratory where they were cut into pieces and put into a reactor with double envelope. 100 g of leaves were put into 1000 ml of water. It was necessary to leave to soaking the leaves in water in order that the totality of precursors could exit. The soaking accomplished, the leaves were removed from extract that contains the precursors. It was added a sufficient quantity of NaOH 2N to obtain a pH value between 9.0 –12.0. The alkali medium was necessary for the hydrolysis of Isatan B. The extract changes color, it passes clear green to deep green. Indigo particles are visible once free indoxyl is in contact with oxygen of air.

In order to increase the formation of indigo it was needed to oxygenate. It was left to precipitate during 12 to 16 hours. The blue dough was recuperated and placed into a steamer at 50°C during 14 hours. This is the raw extract that will be analyzed.

2.3. Analysis.

To dose the content of pigments we have proceeded to dilute 10 to 20 mg of the sample in 25 ml of Dimethylformamid for the indigo and 50 to 100 mg in 25 ml of Chloroform for the indirubin. Samples were deposited on TLC silica plates 60 PF_{254} (MERCK).These deposits were undertaken with the help of an applicator DESAGA AS30 which allowed to deposit samples as well as standards. The standards that we used were the indigo SIGMA and the indirubin synthesized in the laboratory. The solvent system was Toluen-EtOAc (3:2).
After TLC separation, plates were processed with the help a densitometer DESAGA/HEIDELBER CD 60. Each sample was processed three times.

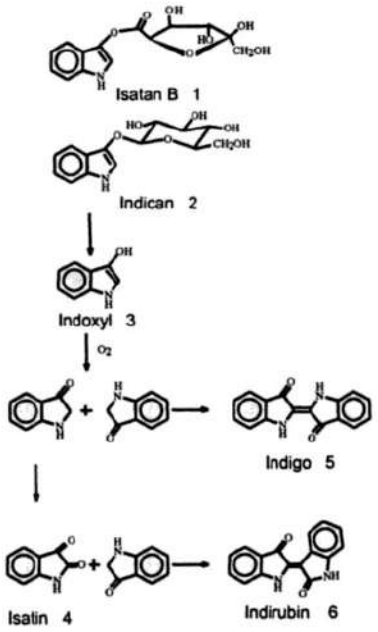

Figure 1 : Formation of the pigments from precursors present in woad leaves.

2.4. Results.

We have tried to determine the three factors that influence the woad pigments extraction. Namely, the solvent pH , the soaking time and the temperature. We have seen from preliminary works that indigo and indirubin yields depend on the properties of plant material and on extraction conditions.

2.4.1. Extraction solvent pH .

We have tried to extract the precursors with organic solvents and despite the good results, other compounds as chlorophyll were also extracted from leaves; those can be difficulty separated once the pigments are formed. Elsewhere, utilization of large quantities of organic solvents to industrial-scale extraction is not practical. Given the glucosides nature and their great solubility in water, we have found that a water extraction can be used. On the other hand, its availability and its physical and chemical properties makes water accessible for the woad extraction.
Using buffer solutions at several pH values, we have tested the effects on the indigo and indirubin yields. The best indigo yield occurred under acid conditions. This can be linked to the stability of isatan B that decompose rapidly without a pH control in the aqueous phase. When we have used a solution with a pH greater than 9.0 , indigo and indirubin were formed once the temperature exceeds 60 °C but most pigments remain fixed on the vegetal matter and their separation is very difficult. In this case, the crude extract become minimum.
Concerning the indirubin , we have found that its yield increases with the pH value .This can be explained by a possible increase in the isatin formation. Isatin can react with a free indoxyl molecule and produce indirubin.
We can conclude that optimal pH conditions for the indigo extraction are between 2.0 and 4.0.

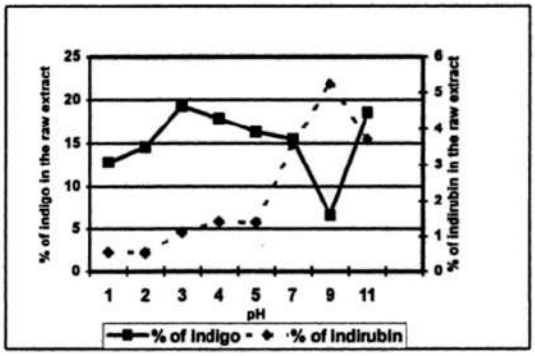

Figure 2. Effect of pH on indigo and indirubin yields.

2.4.2. The soaking time.

The soaking is the critical stage of extraction because it is the moment where precursors exit leaves and react with compounds that exit with them. The soaking time is important as show our results.
At 60°C we have found that a soaking time of 20 minutes is sufficient to reach the best indigo yield. If it exceeds this time, the yield falls. We can explain this behavior by telling that prolongation of soaking entails an increase quantity of compounds in the extraction juice that react with free indoxyl and decrease probabilities that two molecules of indoxyl can meet and form indigo.

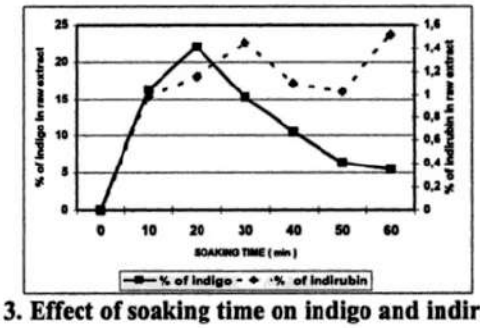

Figure 3. Effect of soaking time on indigo and indirubin yields.

2.4.3. Temperature.

It is well known that temperature plays an important role in the pigments extraction from woad. Temperature can allocate the barrier that represents the leaves epidermis by facilitating the exit of cell compounds.
Our results show that a temperature of 60 to 70 °C gives the best indigo yield. Indirubin increases if the temperature exceeds 70°C.

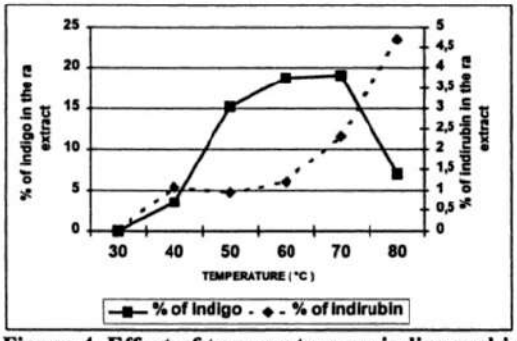

Figure 4. Effect of temperature on indigo and indirubin yields.

3. EXTRACTION, IDENTIFICATION AND QUANTITATIVE DETERMINATION OF WELD'S NATURAL DYES.

Weld (*Reseda luteola*) was an important European yellow dye delivering plant and was considered as the most light fastness yellow colouring matter for textile use.
Weld dyes are flavonoids compounds, and notably luteolin's glucoside derived (6-10).

3.1. Extraction.

Flavonoids were extracted with methanol : water (8 : 2) solution from dried aerial parts of weld, at room and at boiling temperature, for different extraction times ranging from 5 mn to 240 mn, in order to determine the adequate method for weld's flavonoids extraction.

3.2. Analysis.

The three main weld flavonoids were identified by Reverse Phase HPLC / diode array detector at 350 nm (Fig; 6).

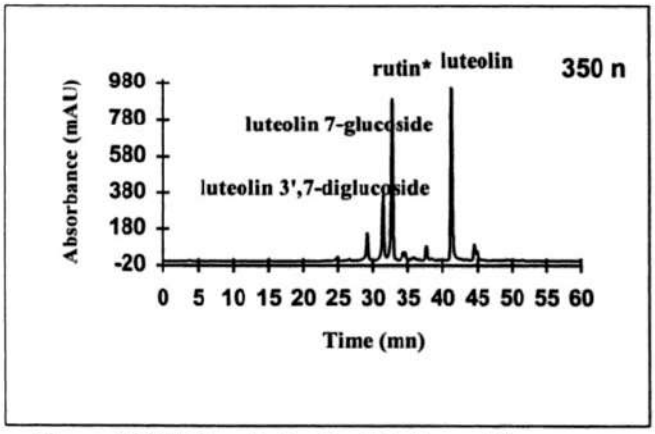

Figure 5. HPLC analysis of weld's flavonoids.

3.3. Results.

Luteolin, luteolin 3',7-diglucoside and luteolin 7-glucoside were identified by HPLC analysis in methanol extracts of weld. Rutin was used as internal standard.
Appropriate extraction time and temperature for weld dyes were determined by HPLC quantification of luteolin and luteolin 7-glucoside in the different extracts obtained. Each extraction was repeated four times (fig. 6).

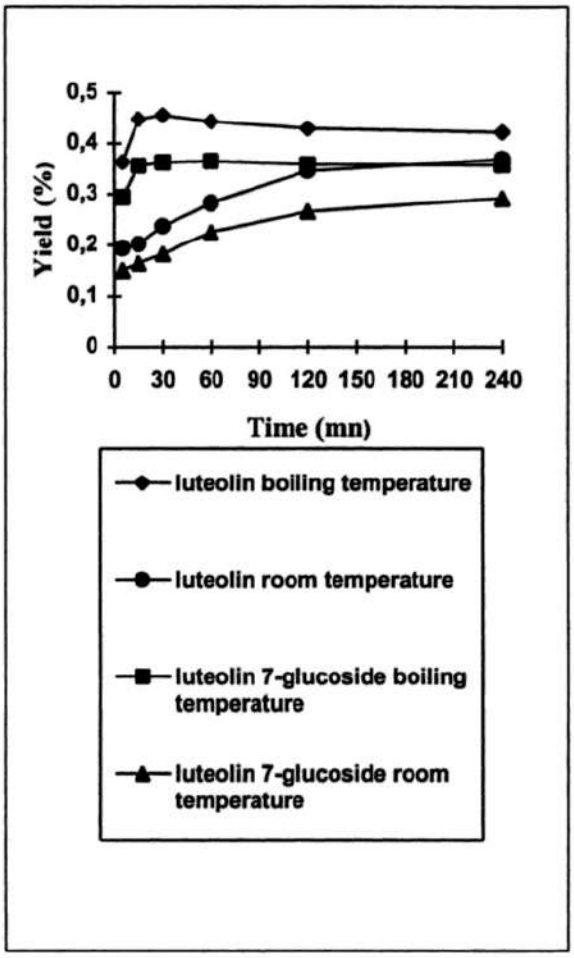

Figure 6. HPLC quantification of luteolin and luteolin 7-glucoside in different extracts.

4. EXTRACTION, IDENTIFICATION AND QUANTITATIVE DETERMINATION OF MADDER'S NATURAL DYES.

Madder (*Rubia tinctorum*) was extensively used until the end of the 19th century for the textile industry. Natural coloring matters of this plant are anthraquinoids compound, and mainly alizarin and lucidin and their glucoide derivatives (11-16).

4.1. Extraction.

Madder natural dyes were extracted from dried roots with methanol, at room and at boiling temperature, for extraction's times ranging from 15 mn to 240 mn, in order to determine the optimum time and temperature for dyes obtention.

4.2. Analysis.

Main madder anthraquinone were identified par Reverse Phase HPLC / diode array detector at 420 nm (fig. 7).

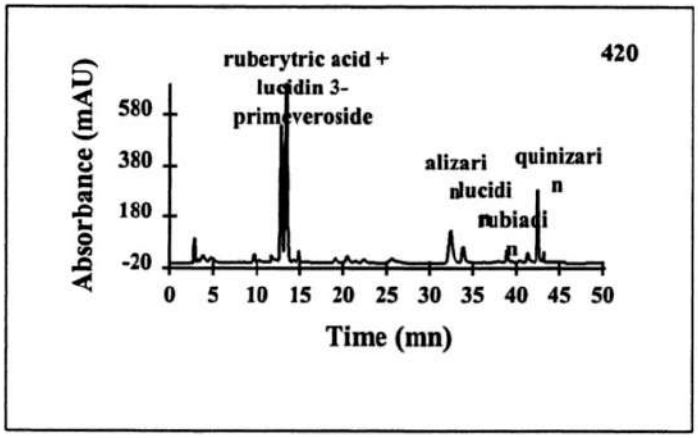

Figure 7. HPLC analysis of madder's antraquinones.

4.3. Results.

Five anthraquinones were identified by HPLC in the different extracts:

- ruberythric acid,
- lucidin 3-primeveroside,
- alizarin,
- lucidin and
- rubiadin.

Quinizarine used as internal standard.

Appropriate extraction time and temperature for madder dyes were determined by HPLC quantification of ruberythric acid+ lucidic 3-primeveroside and alizarin in the different extracts obtained. Each extraction was repeated four times (fig. 8).

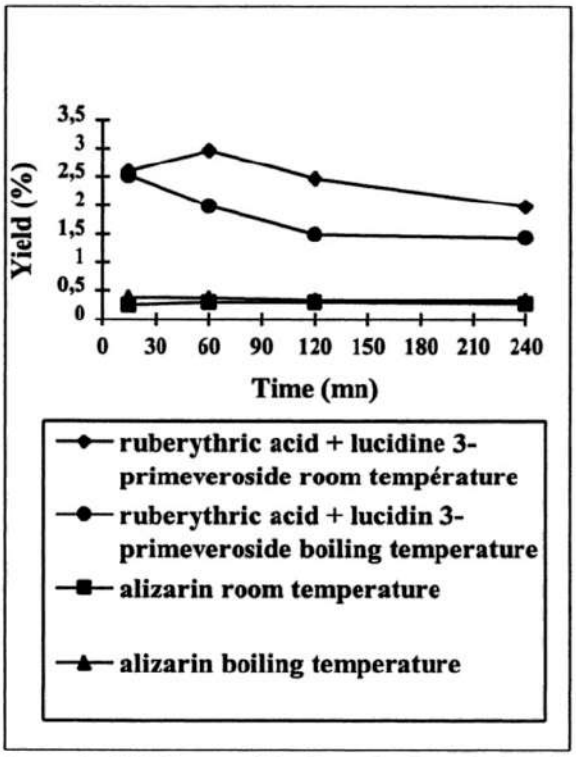

Figure 8. HPLC quantification of ruberythric acid + lucidin 3- primeveroside and alizarin .

5. CONCLUSIONS.

In case of woad, we can conclude that the pH of the extraction solvent, the soaking time and the temperature have effects on indigo and indirubin extraction yields. Among them, the pH is the more important, its influence being probably linked to isatan B stability, results in agreement with those of Epstein *et al.*

For weld , best yields of luteolin and luteolin 7-glucoside, determined by statistical analysis (Bonferroni test), were obtained for the extraction at boiling temperature for 15 mn with methanol : water (8 : 2) solution.

Finally, for madder , the best global yield of the three compounds (alizarin, ruberythric acid and lucidin 3-primeveroside), determined by statistical analysis (Bonferroni test), were obtained for extraction at room temperature for 60 mn, with methanol.

These results are a part of a bigger industrial project. Toward the 2000 and year 2001, the project will evoluate following different directions. First of all, by increasing the surfaces of the grown dye plants. During this period, the conditions of the industrial extraction must be improved, keeping in mind the possibility of recycling of the plant extract matter through new valorizations methods.

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