

Quantitative Analyses of Flavonoids in Yellow Dye Plant Species Weld (*Reseda luteola* L.) and Sawwort (*Serratula tinctoria* L.)

Renate Kaiser

(Received March 25, 1993)

Summary

Quantitative analyses of flavonoids in the yellow dye delivering plant species weld (*Reseda luteola*) and sawwort (*Serratula tinctoria*) were carried out. The flavonoid content of single weld plants (n=418) showed normal distribution and ranged from 1% up to 2,9%; the average was 1,9%. In separate weld organs, an increasing flavonoid content from main- and side-stems (0,4% resp.), to floriferous-stems (1,1%), leaves (2%) and capsules (2,3%) was found.

Single sawwort plants (n=27) could be divided in two groups due to their size. The plants of normal size contained 2,8%, whereas those of large size 4% flavonoids. The flavonoid content of plant organs in sawwort was increasing in both plant groups from stems (1,3% or 2,2% resp.), to flowers (2,5% or 3,4% resp.) and leaves (5,5% or 6,8% resp.).

Introduction

In ancient times natural dyes have been used for many purposes, e.g. colouring natural fibres such as wool, cotton, linen and silk, as well as fur and leather goods. They served also to colour cosmetic products and to produce inks, water-colours and artistic paints.

Dye delivering plants were first collected by dyers in nature as wild types and later the most important ones as weld (*Reseda luteola*), woad (*Isatis tinctoria*), madder (*Rubia tinctorum*) and others were cultivated on farmers fields.

At the end of the 19th century natural dyes have been replaced more and more by synthetic ones. Nowadays, however, allergic reactions to synthetic dyes and changing consumer claims lead to the demand for natural dye stuff.

Weld (*Reseda luteola*) and sawwort (*Serratula tinctoria*) are sources of natural yellow dye. Under European climatical conditions, weld is of greater importance than any other yellow dye delivering plant, because it produces a brilliant colour of extreme fastness to light and of great stability.

Within the scope of renewing resources for industrial use, dye delivering plants can be characterized as specialist alternative crops. At present dye delivering plant species are still typical wild types or useful plant species respectively. For extended industrial use and agricultural cultivation, however, certain characters e.g. yield of dye have to be improved.

Whereas numerous informations on qualitative composition of these dyes are available, the content of dye in the whole plant or in the different parts of plants has never been analyzed (WICHTL, 1971). In this paper first results of quantitative analyses of flavonoids in weld and sawwort are presented.

Materials and Methods

Distribution and biology

Weld (*Reseda luteola*) is spread throughout the Mediterranean region and the middle of Europe. It belongs to the resedaceae and is a winter annual species. Before winter it grows into flat rosettes with lanceolate, undivided leaves developing flower stalks in the following season, being 100-150 cm high with long, slender, erect spikes, with numerous tiny, inconspicuous, yellowish flowers.

Sawwort (*Serratula tinctoria*) grows throughout Europe and South-Russia. At present this species is almost lost in the wild flora. It is a perennial one belonging to the asteraceae. The erect stalks are of 50-100 cm high, with more or less serrated leaves and cylindrical, purple coloured flowers, arranged in umbellate inflorescences.

Cultivation of plant material

With regard to record the variation of dye content, seeds of weld were collected from botanical gardens, genebanks and private companies. At the end of January 1992, the material was sown in the greenhouse. In early February seeds germinated and two weeks later, seedlings were transplanted in Jiffy pots. Plants of rosette stage were transferred at the beginning of April into sandy soil of a greenhouse. Inflorescences were developed at the end of May. Adapted to the development of single plants, weld was harvested just at the beginning of seed maturity (almost end of July).

Seeds of sawwort were germinated in the greenhouse and transplanted into the field in spring 1991, forming only one stalk per plant in the first season. In 1992, however, robust plants were developed with numerous stalks. A broad phenotypical variation was registered and typical plants with different growth habit were harvested by cut-off above ground.

Sample preparation

Before sample preparation, the dry matter weight and plant length was determined for each single plant in weld as well as sawwort. Later the plants were coarse cut in pieces of 2-3 cm length with a chaffcutter and then grinded for two minutes into powder using a Retsch laboratory mill. From the powder a sample of about 2 g was dried at 60°C for 48 hours.

Analysis of flavonoid content

The quantitative determination of flavonoid content was carried out using a spectrometric assay (HAIRI et al., 1991). Three replications of 100 mg each were extracted with 80% methanol at 60°C, shaken 20 minutes and then filtered. The filtrate was diluted 1:3 and 100 µl of the reactive solution (1% 2-amino-ethyl-diphenylborate) added. Spectrometric measurements were done at the maximum wavelength of 404 nm. Extract absorption was compared with that of a standard solution (2mg luteolin/100ml 80% methanol) resulting in the calculation of the total amount of flavonoids (HAIRI et al., 1991).

Results

Variation of flavonoid content in whole weld plants

First of all the variation between three replications per plant were tested, because one sample or one replication consisted only 100 mg powder. The results of each replication have been very similar, indicating that there were no problems by analyzing such small amounts of plant flour. The flavonoid content was measured in 418 single weld plants of 39 accessions with varying number of plants. Among the single plants the flavonoid content varied between 1% as minimum up to 2,9% as maximum resulting in an average of 1,9% (Tab. 1). Considering the flavonoid content per accession 9 among 39 analyzed ones had a higher percentage compared with the average

value. Among these nine accessions plants with a flavonoid content below-average could be observed. There were two accessions, however, where all plants showed flavonoid content above-average and one accession (No.15) had the top value of even 2,5% flavonoids (Tab. 1). The latter plants had a decisive morphology because of their compact growth habit.

Tab. 1: Flavonoid content of weld accessions

Acc.-No.	Number of Plants	Minimum (in %)	Maximum (in %)	Average (in %)
1	18	1,3	2,7	2,0
2	12	1,6	2,9	1,9
3	14	1,3	2,2	1,8
4	3	1,6	1,9	1,8
5	2	1,9	1,9	1,9
6	22	1,3	2,5	1,9
7	19	1,0	2,6	1,9
8	3	1,3	1,6	1,4
9	11	1,5	1,9	1,7
10	8	1,3	2,1	1,8
11	5	1,1	1,9	1,5
12	17	1,5	2,4	1,9
13	17	1,5	2,6	1,9
14	2	1,7	1,7	1,7
15	18	2,0	2,8	2,5
16	16	1,2	2,5	1,8
17	17	1,4	2,6	2,0
18	14	1,4	2,8	2,2
19	19	1,7	2,9	2,2
20	4	1,6	2,1	1,9
21	18	1,2	2,8	1,8
22	10	1,4	2,2	1,8
23	17	1,2	2,3	1,7
24	18	1,2	1,9	1,6
25	8	1,5	2,2	1,8
26	6	1,5	2,4	2,0
27	1	1,9	1,9	1,9
28	9	1,6	2,1	1,8
29	3	1,8	2,0	1,9
30	16	1,4	2,3	1,9
31	5	2,0	2,5	2,2
32	14	1,4	2,0	1,7
33	15	1,4	2,0	1,7
34	10	1,3	1,9	1,6
35	4	1,6	1,9	1,7
36	4	1,7	2,2	1,9
37	3	1,8	2,0	1,9
38	10	1,6	2,2	2,0
39	6	1,8	2,3	2,0
Total	418	1,0	2,9	1,9

Except two accessions plants with above-average flavonoid content occurred randomly. Variation of the flavonoid content of weld from all accessions showed normal distribution (Fig. 1). Among 418 plants 64% showed average flavonoid content of 1,9% $\pm$ 0,3%, 19% below-average and 17% above-average.

Relation between flavonoid content and plant yield

The percentage of flavonoid content per plant may be one interesting parameter for further screening experiments, but from a practical point of view the yield of dye per plant or per area unit will be regarded as the main factor for agricultural production as well as industrial utilization. In literature (NENCKI, 1984) it was stated that the smallest plants have the highest dyeing capacity. To check this statement, the flavonoid content was related to plant length and weight of dry matter per single plant. There was no negative correlation as one should expect, neither between flavonoid content and plant length nor dry

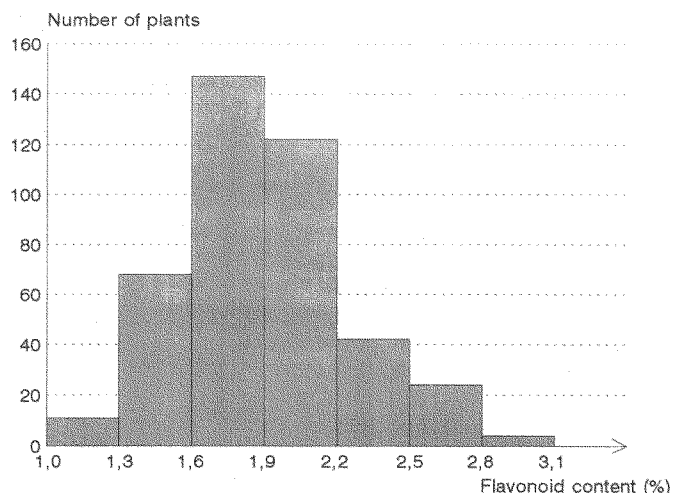


Fig. 1: Variation of flavonoid content in weld

matter weight. Selected plants with the highest weight of dry matter ($n=46$; > 100 g) and those with the lowest one ($n=186$; < 50 g) had the same average of flavonoid content (1,9%).

Flavonoid content in different organs of weld plants

Besides quantitative analysis of flavonoid content in whole weld plants, 16 plants have been dissected in organs. From main- and side-stems (0,4% resp.), to floriferous-stems (1,1%), leaves (2%) and capsules (2,3%) an increasing flavonoid content was observed (Tab. 2).

Tab. 2: Flavonoid content in organs of weld

Plant-No.	Flavonoid-Content (in %)				
	main stem	side stem	floriferous stem	leaf	capsule
1	0,2	0,2	1,3	1,8	2,6
2	0,4	0,4	1,4	1,9	1,9
3	0,4	-	0,9	1,4	1,6
4	0,4	-	1,1	1,9	0,8
5	0,3	-	0,7	1,9	2,5
6	0,5	-	1,1	1,5	1,8
7	0,5	1,5	1,7	2,3	2,1
8	0,5	0,3	1,5	1,8	2,8
9	0,4	0,2	0,8	1,6	2,6
10	0,6	0,3	1,4	3,2	2,7
11	0,5	-	1,0	1,5	2,1
12	0,2	-	1,0	1,8	3,0
13	0,2	0,2	1,2	3,1	3,9
14	0,3	0,6	0,8	2,5	2,3
15	0,5	-	0,8	1,9	1,7
16	0,2	0,3	1,1	2,2	2,2
Total	0,4	0,4	1,1	2,0	2,3

Flavonoid content in pure seeds of weld

There are a lot of references (e.g. NENCKI, 1984; WIESNER, 1927) indicating the importance of seeds as the main source for dyestuff. Therefore it was recommended to take care at harvest time and drying to avoid loss of seeds. In contrast to this, the most interesting result was the very low flavonoid content (0,6%) of a pure seed sample. This could be confirmed also by dyeing experiments of wool where seeds gave only quite a pale yellow colour compared with the dyestuff of the whole plant.

Variation of flavonoid content in whole sawwort plants

In sawwort a broad phenotypical variation was found and especially four plants were conspicuous due to their above-average size. In plants of normal size (90 cm in average) the flavonoid content ranged from 1,3% up to 4% and the average was 2,8%. The large sawwort plants (180 cm in average), however, contained 4% flavonoids in average, ranging from a minimum of 3,6% up to a maximum of 4,4% (Tab. 3).

Flavonoid content in different organs of sawwort plants

In both groups of sawwort plants, the flavonoid content (Tab. 3) was increasing from stems (1,3% or 2,2% resp.), to flowers (2,5% or 3,4% resp.) and leaves (5,5% or 6,8% resp.)

Tab. 3: Flavonoid content in whole plants and organs of sawwort

Plant-No.	Flavonoid-Content (in %)			
	whole plant	leaf	stem	flower
plants of normal size				
1	3,1	5,7	1,0	2,2
2	3,1	5,0	1,5	3,6
3	2,2	5,3	1,1	1,4
4	3,0	4,3	1,4	2,9
5	2,6	4,8	1,1	2,5
6	2,9	4,7	1,2	4,0
7	1,3	6,0	1,2	2,2
8	3,6	5,3	1,8	3,5
9	2,8	5,8	1,2	2,3
10	3,3	6,1	1,1	3,2
11	2,3	5,8	1,1	1,6
12	3,0	6,9	1,2	2,4
13	2,9	6,0	1,4	1,7
14	3,6	7,3	2,6	1,9
15	4,0	7,0	3,0	2,5
16	2,9	5,9	1,1	3,2
17	3,0	5,2	1,7	3,0
18	2,0	4,3	0,9	2,4
19	2,6	5,7	1,0	1,8
20	3,1	6,2	1,3	1,9
21	2,5	4,6	1,2	2,3
22	2,2	2,6	0,9	2,4
23	1,8	6,3	0,8	2,7
	2,8	5,5	1,3	2,5
plants of above-average size				
1	4,4	7,1	2,5	4,0
2	3,6	6,3	2,1	2,8
3	4,0	6,5	2,6	3,2
4	3,8	7,4	1,5	3,6
	4,0	6,8	2,2	3,4

Discussion

Most of the yellow dye plants contain dye stuff from the group of hydroxyflavones, which belong to the class of flavonoids. The combination of components, however, is specific for each plant species (ROTH et al., 1992). The yellow colour of weld consists of the flavonoids luteolin, isorhamnetin, kaempferol, apigenin and luteolinglucoside (ROTH et al., 1992; SCHWEPPE, 1993).

For sawwort it is somewhat difficult to describe the components of the yellow dye. Earlier references (WIESNER, 1927) stated the yellow dyestuff as "serratulins", which is the product of a process referring a colourless substance called "serratulan" present in the cells and changing during drying or under influence of alkali. The chemical constitution of such substances has never been analyzed. CARDON and

CHATENET (1990), however, point to the existence of apigenin, kaempferol and other flavonoids.

The main aim of the above described experiments was to get informations on the variation of the flavonoid content in single plants from different sources. Among the single weld plants a broad variation was found. This evaluation was the first step as a basis for improvement by plant breeding if the high flavonoid content will be confirmed in 1993.

Some unexpected results were the low flavonoid content in weld seeds contrary to informations from literature (NENCKI, 1984) as well as the high dye content in leaves and capsules. Furthermore, there was a relative constant percentage of flavonoids in the dry matter of the weld plants independent from the weight of dry mass and plant size respectively being also in contrast to descriptions in literature (NENCKI, 1984).

Flavonoid content of whole plants in sawwort was higher than in weld. A direct comparison, however, is not possible, because sawwort plants have been grown up in the experimental field, whereas weld was cultivated under greenhouse conditions.

The most interesting result in sawwort is the different flavonoid content of plants with different size. Plants of above-average size with the highest flavonoid content could be interesting for further screening experiments.

Sawwort is recommended to be harvested just before flowering (CARDON and CHATENET, 1990; NENCKI, 1984). In these examinations, however, plants were harvested while flowering and it is interesting to note, that the flavonoid content of flowers is quite high in compare to stems.

With the above described analysis of flavonoid content various accessions have been evaluated. In this context it may be of certain interest to investigate also the influence of other parameters as environmental conditions (e.g. soil, temperature, fertilizer, sowing-time, number of plants per area unit) on flavonoid content.

At present weld and sawwort can be designated still as wild and/or useful plant species. Seed shattering in weld is a typical wild type character. In the case of increasing application of natural dyes in future both species have to be changed into cultivated plant species.

Zusammenfassung

Flavonoid-Gehalte bei Färberwau (*Reseda luteola*) und Färbescharte (*Serratula tinctoria*)

Der Färberwau (*Reseda luteola*) und die Färbescharte (*Serratula tinctoria*) enthalten gelbe Farbstoffe aus der Klasse der Flavonoide. Beim Wau ist der Flavonoid-Gehalt der 418 untersuchten Einzelpflanzen normal verteilt und bewegt sich zwischen einem Minimum von 1% bis zu einem Maximum von 2,9%, wobei der Durchschnitt 1,9% beträgt.

In verschiedenen Pflanzenorganen des Färberwau steigt der Flavonoid-Gehalt von den Haupt- und Seitenstengeln (0,4%), über die blütenträgenden Ästchen (1,1%) und Blätter (2%) bis hin zu den Kapseln (2,3%).

Bei der Färbescharte konnten die 27 untersuchten Einzelpflanzen entsprechend ihrer Länge in zwei Gruppen eingeteilt werden. Die kleineren Pflanzen enthalten durchschnittlich 2,8% und die größeren 4% Flavonoide.

In Pflanzenorganen beider Gruppen ist ein zunehmender Flavonoid-Gehalt vom Stengel (1,3% bzw. 2,2%) über Blüten (2,5% bzw. 3,4%) und Blättern (5,5% bzw. 6,8%) zu beobachten.

Acknowledgements

Thanks are due to the Federal Ministry of Food, Agriculture and Forestry for supporting the research project "Screening of dye delivering plants". Particular thanks are also due to Prof. Dr. Claude Andary (University of Montpellier) for introduction in the chemical method and Heike Ackermann for conscientious technical assistance.

References

- CARDON, D. and CHATENET, G., 1990: Guide des Teintures Naturelles - Plantes, Lichens, Champignons, Mollusques et Insectes. Delachaux et Niestlé, Paris.
- HAIRI, B., SALLÉ, G., and ANDARY, C., 1991: Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (*Viscum album* L.). *Protoplasma* 162, 20-26.
- NENCKI, L., 1984: Die Kunst des Färbens mit natürlichen Stoffen - Geschichte, Methoden, Rezepte. Verlag Paul Haupt, Bern, Stuttgart.
- ROTH, L., KORMANN, K., and SCHWEPPE, H., 1992: Färbepflanzen - Pflanzenfarben - Botanik, Färbemethoden, Analytik, Türkische Teppiche und ihre Motive. Ecomed Verlag, Landsberg.
- SCHWEPPE, H., 1993: Handbuch der Naturfarbstoffe - Vorkommen, Verwendung, Nachweis. Ecomed Verlag, Landsberg.
- WICHTL, M., 1971: Die pharmakognostisch-chemische Analyse - Untersuchung und Wertbestimmung von Drogen und Galenischen Präparaten. Akademische Verlagsgesellschaft, Frankfurt/Main.
- WIESNER, v. J., 1927: Die Rohstoffe des Pflanzenreiches. Verlag Wilhelm Engelmann, Leipzig.

Address of the author:

Dr. Renate Kaiser, Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, D-76833 Siebeldingen.