

Chemical composition of barley varieties with different nutrient supplies

III. Concentration of tannins and β -glucans in two-year experiments

Bygsorters kemiske sammensætning ved varierende næringsstofforsørg

III. Koncentration af tanniner og β -glucaner i toårige forsøg

Ebbe Truelsen

Summary

Twenty barley varieties were grown in 1982 and 1983, and 21 varieties, including a breeding line Ca 700202, were grown in 1984 and 1985 in pots with increasing nitrogen supplies. The concentrations of tannins and soluble β -glucans were determined in the mature grains.

The contents of tannins and β -glucans were closely connected to the varieties, therefore highly significant correlations could be calculated between the two years of experiments.

Only a low positive reaction for tannin was found in the proanthocyanidin-free variety Galant, but Cerise, Carina, Ca 700202 and Claret also had fairly low tannin contents. Significant differences between the two years were found in tannin contents.

The lowest content of soluble β -glucans was found in the breeding line Ca 700202 and the varieties Triumph, Mandolin and Yriba. No significant differences could be found in the content of β -glucans between the two years.

Increasing supplies of nitrogen caused increases in the content of soluble β -glucans, whereas only small changes in the content of tannins were found.

Key words: Barley varieties, tannins, β -glucans, nitrogen nutrition, year variation.

Resumé

20 bygsorter blev dyrket i 1982 og 1983 og 21 sorter inklusive en høj-lysin mutant Ca 700202 i 1984 og 1985 i kar ved stigende kvælstofforsørg. Indholdet af tanniner og opløselige β -glucaner blev bestemt i de modne kerner. Indholdet af både tanniner og β -glucaner var tæt knyttet til de enkelte sorter, således at stærkt signifikante korrelationskoefficienter kunne beregnes mellem de to forsøgsår med hensyn til indholdene af tanniner og β -glucaner.

Der blev fundet et meget lavt tanninindhold i den proanthocyanidinfrige sort Galant, et indhold der formentlig skyldes positiv reaktion med andre stoffer i bygkernen ved den anvendte metode. Også Cerise, Carina, Ca 700202 og Claret havde et relativt lavt indhold, mens de højeste tanninkoncentrationer blev fundet i sorterne Ida, Mirjam, Anna, Taarn, Uffe og Gunnar. Der blev i forsøget fundet små, men

signifikante forskelle mellem de to forsøgsår. Med den benyttede analysemetode kunne det ikke klargøres, om forskellene skyldtes niveauændringer i tanninindholdet eller forskydninger mellem indholdet af de enkelte proanthocyanidiner.

De højeste indhold af opløseligt β -glucan, bestemt ved viskositetsmålinger, blev fundet i sorterne Magnum, Mirjam, Uffe og Zita, mens de sorter, der havde de laveste indhold var Ca 700202, Yriba, Triumph og Mandolin. Der blev ikke fundet signifikante årsvariationer i indholdet af opløseligt β -glucan.

Stigende kvælstofgødskning medførte stærk stigning i indholdet af opløseligt β -glucan, mens tanninindholdet ikke ændredes væsentligt.

Nøgleord: Bygsorter, tanniner, β -glucan, kvælstofnæring, årsvariation.

Introduction

Tannins and β -glucans are groups of compounds in the barley grain which are mentioned as reducing factors for the value of barley for fodder and brewing (see (13) for references).

The tannins belong in monocotyledons to the condensed tannins or proanthocyanidins (14) and are located, in barley, in the seed coat (testa) just outside the aleurone layer (3). Although the overall concentrations of tannins in the barley grain are low, the local concentration in the narrow band in the seed coat may be fairly high thereby creating protection of the wintering seed against soil microorganisms and other predators (14).

The tannins exert their effect by binding to proteins (6). Recently, it has been found that these interactions are highly specific for tannins as well as for proteins (4). Which proteins, the tannins in the barley grain prefer are uncertain, and the degree of harm they exert in monogastric animals during digestion has still to be found. A great variability in tannin contents has been found in different barley varieties (13). Smaller differences have been found between harvest years (8).

The polymeric β -glucans are, in the barley grain, constituents of the endosperm cell walls. They make viscous solutions whereby the absorption of nutrients can be decreased in monogastric animals (7). The content of soluble β -glucans in barley grain is known to be dependent on variety as well as growing conditions. Warm climatic conditions and early harvesting (10) and increasing

N-supplies (13) give rise to increasing viscosities, while rain induces a decrease in viscosity (2).

In a previous experiment it was found that the varietal differences concerning the content of tannins and β -glucans tended to be maintained when the barley varieties were grown at different localities (13). The present investigation was performed to find out if varietal differences were maintained in two-year experiments. Furthermore, to investigate whether the levels of the concentrations of tannins and β -glucans changed in the two years of experiments and finally to test the contents of these compounds in 40 barley varieties, including a breeding line with increasing N-supplies.

Materials and methods

Twenty barley varieties were grown in 1982 and 1983, and another 21 varieties, including a highlysine mutant, Ca 700202, were grown in 1984 and 1985. The experiments were carried out in cylindrical PVC-pots with 20 plants per pot and 2 replications. The pots were placed randomly in blocks with equal N-supplies. The variety Zita was used as a standard variety for all 4 years. The growing conditions and supplies of potassium, phosphorus and micro-nutrients were identical to a previous experiment (13). However, the nitrogen, in the present experiments, was supplied in the following amounts: 0.0 (0 N), 1.5 (1.5 N), 4.5 (4.5 N), and 9.0 (9 N) g of nitrogen per pot.

The content of tannins and β -glucans was determined as previously described (13, 15). However, the variety Galant, which is proanthocyanidinfree (12), behaved in a different manner at the tannin determinations. After mixing of the barley extract with DAC (11, 15), the colour intensity for this variety continued to rise, even after the time at which the other varieties had reached their highest absorbances. For comparison of the varieties, the absorbance of Galant was read at the same time as the other varieties after mixing of the reagents.

Results

The results from the tannin determinations are presented in Tables 1 and 2. For all years the contents generally decrease from 0 N to 4.5 N and then an increase from 4.5 N to 9 N can be seen. Moreover, from both Tables, great varietal differences can be seen.

The varieties Ida, Mirjam and Anna had the highest tannin content in 1982 and 1983 (Table 1). The lowest content in 1982 was found in Cerise and Carina and in 1983 in Carina and Mandolin. Generally, in the two-year experiments, the differences in tannin content from one year to the other were similar for the different varieties. The following coefficients of correlation in the tannin content between 1982 and 1983 was calculated for the different N-supplies: 0 N, $r = 0.84^{***}$, 1.5 N, $r = 0.86^{***}$, 4.5 N, $r = 0.79^{***}$, and 9 N, $r = 0.43$.

From Table 2 it can be seen that the highest tannin content in 1984 and 1985 was found in the varieties Taarn, Uffe and Gunnar. In the proanthocyanidin-free variety Galant a very low content was found, and also the mutant Ca 700202 and the variety Claret had a fairly low tannin content both years. Comparing 1984 and 1985 the tannin content was correlated even higher: 0 N, $r = 0.93^{***}$, 1.5 N, $r = 0.93^{***}$, 4.5 N, $r = 0.96^{***}$,

Table 1. Content of tannins in dry matter of barley seeds with different nitrogen supplies. 1982 and 1983.
Tanninindhold i bygkerner i tørstof ved varierende kvælstoftilførsel. 1982 og 1983.

Year of experiment <i>Forsøgsår</i>	1982				1983			
Treatment <i>Forsøgsled</i>	0N	1.5N	4.5N	9N	0N	1.5N	4.5N	9N
Variety <i>Sort</i>	g of tannins per kg <i>g tannin pr. kg</i>				g of tannins per kg <i>g tannin pr. kg</i>			
Zita	1.05	1.07	1.03	1.09	0.96	0.90	0.88	0.93
Anna	1.17	1.19	1.15	1.20	1.10	1.10	1.03	1.05
Caja	1.12	1.13	1.02	1.09	1.04	0.92	0.87	0.94
Carina	0.98	0.92	0.89	1.01	0.84	0.81	0.74	0.79
Cerise	0.84	0.83	0.78	0.90	0.91	0.83	0.83	0.89
Emir	1.05	1.05	0.95	1.23	0.95	0.91	0.89	0.90
Europa	0.99	0.96	0.88	1.09	0.92	0.83	0.79	0.89
Gula	1.08	1.02	0.98	1.34	0.95	0.88	0.83	0.94
Havila	1.00	0.99	0.93	0.98	0.93	0.84	0.82	0.86
Ida	1.30	1.29	1.17	1.25	1.17	1.07	1.11	1.23
Jarl	1.00	1.00	1.03	1.21	0.89	0.88	0.89	0.92
Jonna	1.12	1.06	0.93	1.02	0.94	0.92	0.83	0.87
Koru	0.97	0.95	0.90	1.16	1.00	0.91	0.88	0.98
Magnum	1.04	1.03	0.94	1.00	0.98	0.96	0.92	0.92
Mandolin	0.95	0.94	0.90	1.12	0.90	0.85	0.77	0.82
Mirjam	1.21	1.21	1.08	1.25	1.10	0.98	0.89	0.89
Nery	1.16	1.17	0.99	1.31	1.06	0.97	0.86	0.92
Torkel	1.06	1.04	0.97	1.10	0.94	0.87	0.83	0.82
Triumph	1.01	0.99	0.94	1.16	0.91	0.89	0.83	0.89
Welam	1.08	1.07	0.97	1.12	0.99	0.95	0.82	0.87

Table 2. Content of tannins in dry matter of barley seeds with different nitrogen supplies. 1984 and 1985.
Tanninindhold i bygkerner i tørstof ved varierende kvælstoftilførsel. 1984 og 1985.

Year of experiment <i>Forsøgsår</i>	1984				1985			
Treatment <i>Forsøgsled</i>	0N	1.5N	4.5N	9N	0N	1.5N	4.5N	9N
Variety <i>Sort</i>	g of tannins per kg <i>g tannin pr. kg</i>				g of tannins per kg <i>g tannin pr. kg</i>			
Zita	1.04	1.01	1.00	1.00	1.11	1.12	1.02	1.15
Albert	1.03	0.96	0.93	0.97	1.28	1.12	1.05	1.03
Ca 700202	0.94	0.86	0.86	0.77	0.99	0.97	0.96	0.76
Claret	0.90	0.86	0.84	0.86	1.03	1.05	0.96	0.92
Galant	0.19	0.17	0.17	0.17	0.18	0.15	0.15	0.19
Golf	1.07	0.99	0.88	0.98	1.16	1.07	0.96	0.97
Gorm	1.14	1.02	1.02	1.06	1.27	1.17	1.21	1.26
Gunhild	1.09	1.06	1.03	1.09	1.13	1.16	1.13	1.26
Gunnar	1.26	1.19	1.13	1.36	1.41	1.35	1.31	1.71
Inga	1.13	1.10	1.08	1.33	1.29	1.24	1.20	1.25
Jenny	1.24	1.18	1.17	1.19	1.31	1.38	1.35	1.30
Odin	1.02	1.07	0.94	1.19	1.11	1.09	1.09	1.17
Pamina	1.03	0.93	0.90	0.95	1.24	1.08	1.03	0.97
Roland	1.03	0.95	0.92	0.86	1.15	1.06	0.98	0.98
Romi	1.25	1.18	1.17	1.13	1.33	1.27	1.33	1.33
Sune	1.09	1.06	0.95	0.97	1.22	1.16	1.13	1.37
Susan	1.23	1.14	1.08	1.05	1.33	1.24	1.25	1.17
Tyra	1.10	1.12	1.06	1.12	1.20	1.18	1.18	1.22
Taarn	1.42	1.30	1.31	1.47	1.50	1.47	1.45	1.53
Uffe	1.41	1.21	1.16	1.31	1.53	1.42	1.34	1.34
Yriba	1.05	0.97	0.91	0.92	1.13	1.11	1.04	1.13

and 9 N, $r = 0.84^{***}$. For these calculations the variety Galant was excluded because of the very low results found for this variety.

For the results presented in Table 1 as well as in Table 2, some differences in tannin content between the two years were found. For both tables at each N-supply the differences were highly significant ($P < 0.001$). For the standard variety Zita the highest tannin content was found in 1985 and the lowest in 1983.

Tables 3 and 4 show the results from the viscosity determinations which are strongly related to the content of soluble β -glucans (1). From all experiments it can be seen that the viscosity increases from 0 N up to 4.5 N. From 4.5 N to 9 N there may be an increase or decrease depending on variety as well as year. Great varietal differences can also be seen for the content of soluble β -glucans.

From Table 3 it is seen that the varieties Magnum and Mirjam had the highest viscosities in 1982 and 1983 and Triumph and Mandolin the lowest. In 1984 and 1985 (Table 4) the highest viscosities were found in the varieties Uffe and Zita, and in 1984 the lowest were found in Ca 700202, Yriba and Claret and in 1985 in Ca 700202, Galant and Yriba.

The year variations, for the content of soluble β -glucans, were similar for the different varieties. Because of the logarithmic relation between the viscosity and content of soluble β -glucans (1), the logarithm of the viscosities was used for the calculations of the coefficients of correlation. Comparing 1982 and 1983 the correlations were as follows: 0 N, $r = 0.84^{***}$, 1.5 N, $r = 0.85^{***}$, 4.5 N, $r = 0.94^{***}$, and 9 N, $r = 0.79^{***}$. For 1984 and 1985 the calculated logarithms are presented in Fig. 1. The correlations were: 0 N, $r = 0.91^{***}$, 1.5

Table 3. Viscosities of extracts from barley varieties with different nitrogen supplies. 1982 and 1983.
Viskositet af ekstrakter fra bygsorter ved varierende kvælstoftilførsel. 1982 og 1983.

Year of experiment <i>Forsøgsår</i>		1982				1983			
Treatment <i>Forsøgsled</i>		0N	1.5N	4.5N	9N	0N	1.5N	4.5N	9N
Variety <i>Sort</i>		<i>centipoise</i>				<i>centipoise</i>			
Zita		3.01	4.00	6.18	5.70	3.12	4.11	5.59	5.44
Anna		2.70	4.06	5.51	5.88	2.33	3.33	5.00	5.45
Caja		2.06	3.12	4.69	4.71	2.17	2.79	4.81	5.21
Carina		1.98	2.64	3.41	2.83	1.92	2.53	3.53	4.14
Cerise		2.29	3.26	5.07	3.97	2.55	3.72	5.92	6.17
Emir		2.68	3.80	4.90	3.23	2.49	3.38	4.53	5.43
Europa		1.99	2.61	3.61	2.67	2.02	2.90	3.87	4.03
Gula		2.28	3.19	4.06	2.31	2.29	2.67	3.89	4.30
Havila		2.61	3.44	4.22	5.34	2.27	3.06	4.18	5.36
Ida		3.08	3.74	4.28	3.42	2.67	3.29	4.13	3.42
Jarl		2.96	3.76	5.15	4.73	2.58	3.90	5.66	5.33
Jonna		1.90	2.65	4.45	4.74	2.23	3.22	4.91	5.45
Koru		2.30	3.43	5.34	4.09	2.48	3.70	4.76	6.34
Magnum		2.84	4.19	6.38	7.90	3.59	5.05	6.07	7.77
Mandolin		1.77	2.44	3.25	2.32	1.77	2.45	3.09	3.68
Mirjam		2.78	4.66	6.60	4.88	2.89	4.29	6.00	6.34
Nery		3.08	4.31	6.45	4.35	2.75	4.10	6.02	6.47
Torkel		2.04	3.21	3.96	3.51	1.98	2.91	4.10	4.21
Triumph		1.83	2.13	2.82	2.28	1.61	2.12	3.00	3.23
Welam		2.02	2.98	4.23	3.67	1.87	2.83	3.88	3.98

N, $r = 0.94^{***}$, 4.5 N, $r = 0.96^{***}$, and 9 N, $r = 0.80^{***}$.

No significant differences could be found in the logarithm of the viscosities from one year to another in the two-year experiments for 0 N, 1.5 N and 4.5 N. For 9 N, however, significant differences could be calculated. 1982–1983 ($P < 0.001$) and 1984–1985 ($P < 0.05$). In the standard variety Zita higher viscosities were generally found in 1982 and 1983 than in 1984 and 1985, but the differences were small and not significant.

Discussion

The tannin content (Tables 1 and 2) varies between 0.74 and 1.71 g per kg for the four-year experiments, apart from the variety Galant. This is similar to the results from a previous experiment (13) and to other tannin determinations in barley

grain with newer methods (see (15) for references). The variety Galant yielded a small positive response by the present method. Because it is proanthocyanidin-free (12) and behaved otherwise at the tannin determination, the positive response is probably not caused by tannins, but most likely by other compounds in the grain (11).

The decrease and then increase in tannin content with increasing N-supplies may mainly be caused by the differences in grain weight, which generally are lowest at 0 N and 9 N. In a previous experiment (13) it was found that the tannin content of a certain variety tended to be the same when grown at different localities. From the present experiment it can be established that the differences between the varieties were maintained in the two-year experiments, so the tannin content in the barley grain may be a varietal characteristic.

Table 4. Viscosities of extracts from barley varieties with different nitrogen supplies. 1984 and 1985.
Viskositet af ekstrakter fra bygsorter ved varierende kvælstoftilførsel. 1984 og 1985.

Year of experiment <i>Forsøgsår</i>	1984				1985			
Treatment <i>Forsøgsled</i>	0N	1.5N	4.5N	9N	0N	1.5N	4.5N	9N
Variety <i>Sort</i>	<i>centipoise</i>				<i>centipoise</i>			
Zita	2.42	3.48	5.22	5.53	2.21	3.39	4.87	6.62
Albert	1.85	2.49	3.27	3.79	1.72	2.44	3.14	3.40
Ca 700202	1.48	1.54	1.53	1.67	1.53	1.63	1.71	1.98
Claret	1.72	1.74	2.08	2.27	1.91	2.22	2.90	3.41
Galant	1.73	2.05	2.16	2.26	1.66	1.94	2.36	2.36
Golf	1.97	2.27	3.08	3.68	1.85	2.54	3.37	4.94
Gorm	2.18	3.27	3.86	3.52	2.25	3.35	4.32	4.21
Gunhild	1.89	2.33	3.28	3.55	1.82	2.31	3.48	4.10
Gunnar	1.96	3.06	4.27	3.23	1.99	3.00	4.21	2.51
Inga	1.73	2.97	4.11	2.64	1.74	2.46	3.71	3.61
Jenny	2.00	3.01	4.37	4.69	1.89	3.06	4.40	5.56
Odin	1.57	2.19	3.04	2.87	1.57	2.14	2.85	3.21
Pamina	1.84	2.44	3.48	4.04	1.76	2.49	3.34	4.05
Roland	1.93	2.70	3.15	4.00	1.80	2.56	2.99	3.60
Romi	1.91	2.81	3.80	3.82	1.85	2.72	4.07	4.84
Sune	2.44	3.39	4.52	5.31	2.21	3.19	4.82	3.76
Susan	2.10	2.71	4.13	4.98	1.87	2.86	3.86	4.47
Tyra	2.11	3.04	4.59	5.88	2.15	2.75	4.16	5.97
Taarn	1.85	2.56	4.13	2.78	1.98	2.76	4.32	4.15
Uffe	2.85	4.38	5.68	4.10	2.58	3.91	6.05	5.93
Yriba	1.68	1.68	2.06	2.26	1.88	1.99	2.31	2.55

Compared to the varietal differences the year variations were small, yet significant. By the present method of analysis (15) it cannot be concluded, whether the year variations are caused by an equal variation of all proanthocyanidins or whether a displacement has taken place between them. A displacement between the different proanthocyanidins has been found in a two-year experiment despite the sum of them being identical (5).

The viscosities (Tables 3 and 4) varied from 1.48 to 7.90 cP. Especially in the high-lysine mutant Ca 700202 very low viscosities were found for all N-supplies. The same has been found for another high-lysine mutant, Risø 1508 (1, 13). The low viscosity in Risø 1508 has been explained as a different structure of β -glucan (1) and this could also be the case for Ca 700202.

Generally, the varieties with low viscosities also have a low increase in viscosity with increasing N-supplies and varieties with high viscosities increase considerable. The reason why the viscosities increase up to 4.5 N for all varieties and why some varieties have a decrease in viscosity from 4.5 N to 9 N while other varieties have an increase is not known, but there is a tendency that varieties with low grain weights at 9 N also yield small viscosities. The two-year experiments show clearly that the content of soluble β -glucans is strongly correlated to the varieties, and supports a previous finding (13) that barley varieties grown at different localities maintained their varietal characteristics in viscosity.

It has been found that climatic conditions could influence the viscosities of the barley grain. Rainy weather, especially during maturity may lead to a

ln (extract viscosity) 1984
 ln (viskositet af ekstrakt) 1984

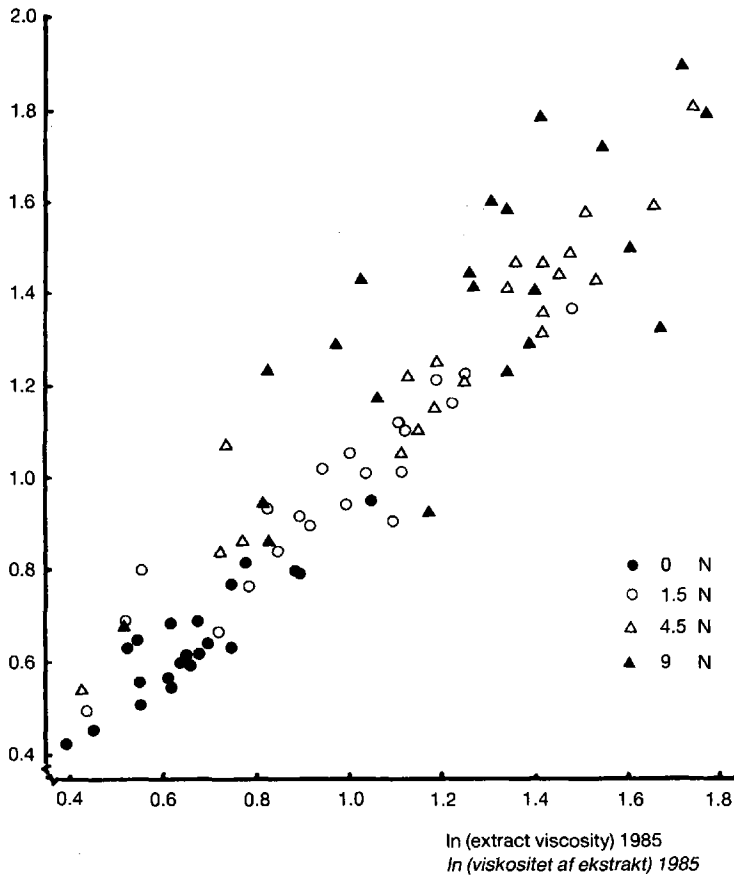


Fig. 1. The logarithmic relationship between the viscosities of barley extracts from 1984 and 1985.
 Den logaritmiske sammenhæng mellem viskositeter i bygekstrakter fra 1984 og 1985.

decrease in the viscosity (2, 10). However, from a three-year experiment, no significant differences in viscosity could be measured (10) and differences in the β -glucan content in a two-year experiment in Australian barleys were explained by different nitrogen contents (9).

In the present experiments no significant differences in the content of soluble β -glucans between the two years could be seen, apart from 9 N, where the differences are probably caused by differences in grain weight. One reason why no sig-

nificant differences were found, could be the use of an automatic watering system (13), which maintains a continued water supply during the whole growing season.

Conclusion

Considerable differences in the content of tannins and soluble β -glucans can be found in the grain of different barley varieties. In two-year experiments these varietal differences appear to be maintained. For each variety only small differ-

ences in the content of tannins and β -glucans occurred between the two years. Increasing nitrogen supplies caused increases in viscosity while the tannin content was fairly stable.

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