BIOCHEMICAL MUTANT IN BARLEY RENDERS CHEMICAL STABILIZATION OF BEER SUPERFLUOUS

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The recessive mutant *ant*-13 isolated from Foma barley after a mutagen treatment with ethyl methanesulfonate is shown to be blocked in the formation of anthocyanins, catechins and proanthocyanidins (= anthocyanogens). The mutant has been propagated, malted on a pilot scale and the malt used for pilot brews of beer. Foma barley has been malted and brewed for comparison. Malt, wort and beer made from the mutant was free of the polyphenols that are found, when Foma is used as raw material. The beer brewed from the proanthocyanidin- and catechin-free mutant had satisfactory haze stability. In colour and flavour *ant*-13 beer was indistinguishable from Foma beer, but in haze stability the former was drastically superior to the latter.

1. INTRODUCTION

Permanent haze and chill haze in beer are caused by precipitation of proteins with polyphenols which derive from barley and hops (17). The polyphenols most prominent in this

respect are the proanthocyanidins – frequently called anthocyanogens – and the catechins which tend to form polymers. The simple phenols present in the barley grain such as the phenolic and hydroxycinnamic acids as well as

their esters and glycosides, and the coumarines contribute apparently little to haze formation (9). The proanthocyanidins, the anthocyanins and the catechins from the barley grain precipitate some proteins during the boiling of the wort and thereby participate in the formation of the break. The polyphenols remaining after removal of the break are primary sources for chill haze and permanent haze formation in beer.

In order to retard haze formation polyphenols can be removed in part with e.g. polyvinylpyrrolidone (PVP), its insoluble polymer Polyclar AT or nylon 66. The use of barley grains lacking proanthocyanidins and catechins is expected to yield beer with improved haze stability. TROLLE (26) has suggested to look for barley varieties with a low content of proanthocyanidins, but surveys for the content of proanthocyanidins and catechins in different barley cultivars including anthocyanin-free varieties have revealed little quantitative variations of these compounds (20, 22). We have therefore studied the flavonoid contents of a series of induced mutants affecting anthocyanin pigmentation in various plant parts (12). One mutant, named *ant*-13, in the variety Foma turned out to be practically free of proanthocyanidins and catechins in the grains. The mutant has been propagated, malted and used for brewing beer. The beer made from this anthocyanin-, proanthocyanidin- and catechin-free mutant revealed good haze stability.

2. MATERIALS

The flavonoid content was analysed in 53 recessive gene mutants affecting anthocyanin pigmentation contained in the collection of artificially induced mutants kept at the Swedish Seed Association, Svalöv. The mutants in this collection have been induced and isolated over the past 45 years by Å. GUSTAFSSON, N. NYBOM, L. EHRENBORG, U. LUNDQVIST and D. VON WETTSTEIN (cf. 12). For genes controlling anthocyanin pigmentation the designation *rubrum* and the symbol *rub* were proposed by

GUSTAFSSON, HAGBERG, LUNDQVIST and PERSSON in 1969 (12). Subsequently the International Committee for assigning barley gene symbols has agreed that gene symbols starting with the letter *r* should be used only for genes controlling resistance towards diseases. It is therefore proposed that the gene symbol *ant* be reserved for genes affecting the anthocyanin, anthocyanidin, and proanthocyanidin content in barley.

For the original screening, grains of the 53 mutants and their mother varieties were kindly supplied by Professor ÅKE GUSTAFSSON and Mrs. UDDA LUNDQVIST and grown in observation plots at the experimental farm at Allindemagle. Of the mutants, 42 have originated in the variety Foma, 10 in the variety Bonus and 1 in the variety Kristina after mutagen treatments with γ -rays, neutrons, different sulfonates, ethyleneimine or N-methyl-N-nitrosourea. As to anthocyanin pigmentation the mutants comprise 42 classified visually as anthocyanin-free or anthocyanin-poor, 4 as anthocyanin-rich, 6 as red awns, and 1 as red stem. A detailed genetic and chemical analysis of the 53 mutants will be presented by B. JENDE-STRID in a subsequent paper. Mutant *ant*-13 is not allelic to any of the other mutants and has been isolated in 1960 after a mutagen treatment of resting grains of Forma with a solution of 0.25% v/v ethyl methanesulfonate.

3. METHODS

3.1. Determination of anthocyanidins and proanthocyanidins

The analysis has been modified from that described by HARRIS and RICKETTS (14). After determination of the grains dry weight, 5.0 g freshly prepared barley flour (Prometer mill) was extracted while rotating with 50 ml of 60% v/v ethanol containing 1% w/v ascorbic acid for one hour. After centrifugation for 10 min at 4100 x g in a Sorvall refrigerated centrifuge, the sediment was re-extracted in the same way. A total of four successive extractions yielded 97-99% of the proanthocyanidins and anthocyani-

dins present in the grain. The volume of the combined extracts was adjusted to 250 ml with the extraction medium.

An aliquot of 10 ml of extract, wort or beer was pipetted into a 25 ml graduated centrifuge tube, 0.5 g of polyamide powder (Divergan) and the sample shaken for 40 min. After centrifugation for 5 min at 3000 x g the pellet consisting of polyamide powder (Divergan) with the absorbed proanthocyanidins and anthocyanidins was washed with distilled water and recentrifuged. For cleavage of proanthocyanidins into their anthocyanidin and catechin moieties, 20 ml of butanol and conc. HCl (5:1, v/v) were added and the sample was placed in a boiling water bath for 30 min. After cooling to room temperature, the volume was adjusted to 25 ml and the anthocyanidin concentration determined by measuring the extinction of the solution at 550 nm, which is close to the absorption maximum for cyanidin in butanol-HCl. The extinction was measured in a Zeiss PMQ III spectrophotometer against a blank of distilled water treated in the same way as the barley flour extract. Proanthocyanidin content of the solution was calculated using a calibration curve obtained with known amounts of leucocyanidin hydrate (Fluka) dissolved in butanol-HCl. Usually two samples were analysed in parallel. If the difference in extinction was greater than 0.010 a third analysis was made. The mean of the two closest values of the extinction was used for calculation of the proanthocyanidin content.

3.2. Simultaneous determination of proanthocyanidins and catechins in barley grains, malt, wort and beer

The analysis has been worked out by DADIC (7) and DADIC and MORRISON (8). 5.0 g freshly ground barley or malt (Prometer mill) was extracted by shaking with 100 ml of 50% v/v ethanol and 50 µl saturated vitamin E (D-tocopherolsuccinate, Valby Pharmacy) solution for one hour. After centrifugation for 10 min at 4100 x g in a Sorvall refrigerated centrifuge, the supernatant liquor was filtered on a Büchner funnel. The filtrate was adjusted to 100 ml with

50% ethanol and used immediately. 6.0 g of Nylon 66 powder was added to 100 ml of prepared extract. If wort or beer was analysed, 6.0 g of Nylon 66 was added to 60 ml of degassed beer or wort and 60 ml of distilled water. The suspension was stirred magnetically for 30 min and then filtered through a membrane filter (Millipore 1.2 microns). The filtrate was set aside for preparation of the blank. Transfer of Nylon 66 with the adsorbed proanthocyanidins and catechins from the adsorption vessel to the filter and subsequent washing was accomplished with 200 ml of distilled water. The washed Nylon 66 powder was air-dried for 15 min and then 90 ml of propanol and conc. HCl (5:1 v/v) or 90 ml of butanol and conc. HCl (5:1 v/v) was added. The mixture was refluxed for 30 min in a boiling water bath with magnetic stirring. After cooling to room temperature, the extinction of the solution was measured at 455 and 545 nm which is close to the absorption maxima for catechin and cyanidin on a Zeiss PMQ III spectrophotometer using a blank prepared of the filtrate from the membrane filtration. The blank was treated in the same way as the barley flour extract. Estimation of the proanthocyanidin and catechin content was made using calibration curves obtained with known amounts of cyanidin chloride and D-(+)-catechin dissolved in propanol-HCl or butanol-HCl. Binary analysis was applied to calculate the concentrations of proanthocyanidin and catechin.

3.3 Determination of polyphenols in wort and beer

The method of determination of total polyphenols in wort and beer has been developed by JERUMANIS (18). The beer was degassed by shaking. Hazy wort and beer was clarified by centrifugation.

Reagents: CM/EDTA reagent: 1% w/v solution of low viscosity carboxymethylcellulose (British Drug House) containing 0.2% w/v disodiumethylene-diamine tetraacetate. Ferric reagent: 3.5% w/v green ammonium ferric citrate. Ammonia reagent: Conc. ammonia diluted with 2 volumes of distilled water.

Ten ml of sample and 8 ml of CM/EDTA reagent

gent were mixed and 0.5 ml of ferric reagent added and mixed. Then 0.5 ml of ammonia reagent was added and the solution thoroughly mixed. The volume was adjusted to 25 ml with distilled water and the solution well mixed again and left to stand for 10 min. The polyphenols of the sample reacted with the ferric iron in the alkaline solution and a red or red-brown colour was produced. The extinction was measured on a Zeiss PMQ II spectrophotometer at 525 nm against a blank prepared from the same solution as the test sample. The polyphenols had been removed from the blank by treatment with washed Polyclar AT (General Aniline and Film Co.). Otherwise the blank was treated in the same way as the test sample.

Concentration of polyphenols in ppm is given by: $E_{525} \times 630$.

3.4. Pilot malting

Portions of 14 kg of barley were steeped and germinated in a rotating drum made of stainless steel net and surrounded by a plastic housing. Steeping was carried out over 2 days with alternating periods of several hours in water and in air at 14–15°C until a moisture content of 41–43% was reached. During germination for 5–6 days at 12°C, the moisture content was maintained by adding water dropwise. Air with the desired temperature was supplied through the upper part of the housing. The kilning was carried out by placing the green malt in a heating cabinet. Over the first few hours the temperature of the malt increased to 50°C and was kept at this temperature up to 10 hours, whereafter the temperature was raised over 3 hours to reach 78°C. Curing was done at this temperature for additional 3 hours. The cured malt was re-placed in the drum and the rootlets were removed by fast rotation.

3.5. Pilot brewing

10 kg of ground malt was mashed in the pilot brewery (1) with 42 l of deionized water by infusion mashing without adjuncts. The fol-

lowing standard mashing procedure was employed:

30 min at 52°C, heating from 52°C to 63°C in 20 min, 60 min at 63°C, heating from 63°C to 78°C in 40 min, 10 min at 78°C. A lauter tub was used for the wort filtration at 73°C, and four spargings with 6+6+10+15 l of deionized water were performed. Total filtration time for 61–62 l of wort amounted to about 160 min. After addition of 8.7 g tannin-free hop extract from Horst Company, the wort was boiled for 90 min and cooled to 5°C by standing overnight in a wort holding vessel cooled with ice water. On the following day the cold wort was separated from the hot and cold break, transferred to two 30 l fermentation vessels and pitched with yeast, *Saccharomyces carlsbergensis* strain U, from which possibly adhering polyphenols had been removed by washing the cells six times with water. The amount of pitching yeast was 90 g per 28 kg of wort. The fermentation vessels were placed in a water thermostat at 10°C. After fermentation for one week, the beer was racked into two 25 l storage tanks with CO₂-pressure and placed in a refrigerated cabinet. During 6 weeks of storage, the beer temperature was decreased gradually from 5°C to –1°C. The beer was filtered through 20 x 20 cm Seitz K-7 sheets and the CO₂ content adjusted to 0.5%. Bottling and crowning were carried out manually, the bottles being knocked to reduce the air content in the headspace. The bottled beer was pasteurized at 62°C for 20 min.

3.6. Analyses of barley, malt, wort and beer

Determination of moisture content and sieving tests of the barley used for pilot malting was carried out according to the procedures recommended by the Analysis Committee of the European Brewery Convention (2). Germination of the barley was tested by placing 100 grains on moistened sand in a covered petri dish at 16–18°C and counting the number of germinated grains after three days.

Total nitrogen and protein in barley, malt, wort and beer was determined by Kjeldahl analysis in a Kjeld-Foss Automatic 16.210 (A/S

Figure 1: Scheme for flavonoid biosynthesis according to HARBORNE (13), GRISEBACH (11) and WEINGES et al. (27).

N. Foss Electric, Hillerød). 1.000 g of barley or malt, or alternatively 5.0 ml of wort or beer were digested with 40 ml conc. H₂SO₄, 10 ml 35% H₂O₂, 0.75 g HgO and 15 g K₂SO₄ for 35 min. Distillation was carried out with a solution

containing 400 g NaOH and 80 g Na₂S₂O₃ per l. The distillate was collected in 2% boric acid and titrated with standard acid. Protein is given as total nitrogen x 6.25.

Analysis of malt for moisture content, extract

Table I

Content of proanthocyanidins and catechins in grains of Foma barley and its mutant *ant-13* in mg/100 g dry weight

Line	<i>ant-13</i>					Foma			
Year of harvest	1969	1973	1974	1975	1976	1968	1973	1974	1975
Proanthocyanidins (modif. Harris and Ricketts)	2	3	3	2	-	164	161	150	156
Proanthocyanidins (Dadic)				1	1				120
Catechins (Dadic)				2	4				117
Total polyphenols (Dadic)				3	5				237

content, extract difference, diastatic power, Kolbach index and colour was done with the procedures recommended by the Analysis Committee of the European Brewery Convention (2). Alpha amylase was determined according to BLOM and BAK (3).

Wort was analysed for extract content, attenuation limit, bitterness and colour according to Analytica-EBC (2). Free Alpha amino nitrogen was estimated spectrophotometrically with 2,4,6-trinitrobenzene-1-sulfonic acid according to SATAKE et al. (23). Viscosity was determined with a Haake Kugelfall Viskosimeter (Haake, K. G., Berlin-Steglitz). Beer analyses were done according to Analytica-EBC specifications (2) except for CO₂, which was measured after BLOM (4), head retention which was determined after BLOM (5), air in headspace, which was done according to SØRENSEN (25) and haze stability, which was also analysed according to CHAPON (6).

4. RESULTS

Proanthocyanidins are dimers of two catechin molecules, which upon hydrolysis give rise to one molecule of catechin and one molecule of anthocyanidin (27). The proanthocyanidin of barley has been assigned by SILBEREISEN and KRAFFCZYK (24), the structure depicted in Figure 1. The biosynthetic pathway of flavonoids is well established up to dihydroflavonol as outlined in Figure 1 (11, 13), whereas the subsequent steps to anthocyani-

Table II

Analyses of the barley used for pilot malting

	<i>ant-13</i>	Foma
Moisture content, %	12.0	12.1
Protein content, % of dry weight	17.2	16.2
Germination after 3 days, %	100	100
Sieving test:		
above 2.8 mm, %	19	46
" 2.5 mm, %	60	44
" 2.2 mm, %	19	9
below 2.2 mm, %	2	1

dins, catechins and proanthocyanidins are less well understood. A branch point is envisioned at the hypothetical flavenol intermediate and its existence is well supported by the analysis of the 42 anthocyanin-free or -poor mutants in barley. In 41 of them, the content of proanthocyanidins was the same as in their respective wild type in spite of the reduction or absence of the anthocyanidins. Consistently mutant *ant-13* was deficient in proanthocyanidins, catechins and anthocyanidins in the grain (Table I), as well as in leaves, roots and stems. This mutation thus appears to block the conversion of dihydroflavonol to a common intermediate for anthocyanidins and catechins.

In 1974 the mutant was propagated and 26 kg of it used for the first pilot malting and brewing. The malt, wort and beer produced in this first experiment lacked measurable amounts of proanthocyanidins and the beer had drastically improved haze stability. A new series of 4 pilot maltings and brewings were therefore performed from the harvest of 1975 and

Table III

Analyses of the four malt portions from *ant-13* and Foma

	<i>ant-13</i>		Foma	
	a	b	c	d
Moisture content, %	4.5	4.5	4.6	4.8
Extract content, fine grind, % Plato	78.1	78.0	78.8	78.9
Extract difference, % Plato	5.1	5.0	4.3	4.6
Protein content, % of dry weight	15.3	14.9	14.1	13.6
Alpha amylase, arbitrary units	149	158	177	191
Diastatic power, Windisch-Kolbach units	400	415	469	440
Kolbach index, %	41	39	39	38
Wort colour, EBC units	5.0	5.0	4.5	4.0

Table IV

Analyses of the four portions of wort produced by pilot brewing from *ant-13* and Forma malt

	<i>ant-13</i>		Foma	
	a	b	c	d
Extract content, % Plato	9.72	10.87	10.22	10.00
Attenuation limit, real, %	59	59	62	61
Bitterness units	30	28	31	33
Total-N content, mg/l	–	1398	–	1258
Free alpha amino-N content, mg/l	333	380	333	349
pH	5.6	5.8	5.7	5.7
Colour, EBC units	11	10	10	10
Viscosity, mPa·s	1.86	1.87	1.92	2.02

Table V

Analyses of the beers produced by pilot brewing from *ant-13* and Foma malt

	<i>ant-13</i>		Foma	
	a	b	c	d
Original extract, % Plato	9.7	9.7	10.3	10.1
Attenuation, real, %	59	58	61	61
Bitterness units	21	20	19	20
Total-N content, mg/l	1172	1153	1070	1050
Free alpha amino-N content, mg/l	203	239	239	222
pH	4.7	4.6	4.7	4.7
CO ₂ content, %	0.46	0.51	0.49	0.51
Colour, EBC units	8.5	7.5	7.5	7.5
Head retention, seconds	109	114	111	108
Air in headspace, ml/bottle	1.7	1.6	1.3	1.3
Flavour profile: United Breweries' taste panel	Within acceptable range for pilot brews		Within acceptable range for pilot brews	

Table VI

Polyphenols in barley, wort and beer

	<i>ant</i> -13		Foma	
BARLEY:				
Proanthocyanidin content, mg/100 g dry weight (modif. Harris and Ricketts)	0		115	
Catechin content, mg/100 g dry weight (Dadic)	0		109	
WORT:	a	b	c	d
Proanthocyanidin content, mg/l (modif. Harris and Ricketts)	0	0	38	43
Catechin content, mg/l (Dadic)	38	31	97	69
Total polyphenol content, mg/l (Jerumanis)	18	16	105	102
BEER:				
Proanthocyanidin content, mg/l (modif. Harris and Ricketts)	0	0	31	29
Catechin content, mg/l (Dadic)	37	40	61	61
Total polyphenol content, mg/l (Jerumanis)	7	7	83	86

Table VII

Haze stability of the beers produced by the four pilot brewings

	<i>ant</i> -13		Foma	
	a	b	c	d
Haze after bottling, EBC units	0.9	0.8	0.9	0.9
Chill haze after 5 days at 60°C and 1 day at 0°C, EBC units	1.6	1.9	7.2	12.0
Haze after 40 min. at -8°C, EBC units (Chapon)	3.3	3.5	9.8	>12.5
Air in headspace, ml/bottle	1.7	1.6	1.3	1.3

analyses of these are presented in Tables II to VII.

With the exception of a decrease in α -amylase activity, the two malt portions of *ant*-13 are very similar to the two portions made from the feed and malting barley Foma (Table III). The malt analyses are within the normal specifications of the products from the pilot malting plant.

Likewise, the comparison of the wort analyses from the two portions of *ant*-13 with the two portions from Foma did not reveal significant differences (Table IV). Possibly the total N-content is slightly higher in the wort produced from *ant*-13 malt. This might be due

to the absence of proanthocyanidins, which are believed to precipitate major amounts of proteins in the form of the break. It is surprising that the absence of proanthocyanidins did not increase the nitrogen content more.

Very close analytical results are also found for the two lots of beers made from *ant*-13 barley and Foma (Table V). The absence of proanthocyanidins in the beer (Table VI) did not affect the taste, as both beers were classified as within acceptable range for pilot brews by the United Breweries taste panel. With regard to Table VI it should be pointed out that the total polyphenol content is determined according to the JERUMANIS

procedure, which clearly is not commensurable with the DADIC procedure. The catechin values observable in the wort and beer of *ant-13* cannot be due to catechins from barley or malt as these lacked catechins according to the DADIC analysis. Experiments have shown that the tannin-free hop extract used in the brewings can contribute with no more than 3 mg catechin/l. It is possible that melanoidins formed during wort boiling interfere with catechin determinations and simulate the presence of catechins.

Comparison of haze stability between the beer produced from *ant-13* and Foma is presented in Table VII. No difference is observed for the amount of haze present immediately after bottling. The chill haze developed after 5 days at 60°C and 1 day at 0°C is very satisfactorily reduced in the beer of *ant-13* when compared to that of Foma. Likewise, the CHAPON-test gave a highly significant reduction of chill haze developed in the beer from *ant-13* barley as compared to beer from Foma barley. The use of *ant-13* barley resulted in a beer with a haze stability that can be obtained with Foma barley only after treatment with chemical stabilizers. The selective elimination of proanthocyanidins from malt, wort and beer to the extent possible by malting mutant *ant-13* and brewing beer from it cannot be achieved even by the use of very large quantities of Polyclar AT, as such treatments also remove most of the colour and flavour components from the beer. It can therefore be concluded that the selective removal of proanthocyanidins and catechins through biochemical mutations in barley can provide satisfactory haze stability and shelf life of beer without infringing on the possibilities to retain the desired colour and flavour components of the beer (28).

5. DISCUSSION

We have chosen in Figure 1 to reproduce the formula proposed by SILBEREISEN and KRAFFCZYK (24) for the proanthocyanidin of barley, as these authors have investigated this

structure of proanthocyanidins of barley grains in detail. With regard to the interflavan linkage this formula is at variance with the formulas now accepted for the A-2 type and B type proanthocyanidins. The latter formulas were established for proanthocyanidins of *Aesculus carnea*, *Vaccinium vitis idaea*, *Acacia*, *Humulus* as well as others and are considered typical for the majority of plant proanthocyanidins (15, 16). A re-investigation of the presence and amounts of the different proanthocyanidins in barley seems appropriate. Especially the presence of the B type proanthocyanidins together with the group containing an oxygen linkage according to SILBEREISEN and KRAFFCZYK and the absence of the A-2 type requires confirmation.

Measuring polyphenols in beer and brewing materials is difficult because of the large number of different compounds present including products of various degrees of polymerization, e.g. among proanthocyanidins (10). Several methods for quantitative estimation of proanthocyanidins, catechins and total polyphenols are in use. All quantitative estimations of proanthocyanidins are based on their conversion to red anthocyanidins upon heating in acidic solutions. This conversion is not quantitative as substantial amounts of brown phlobaphene-like polymers are formed along with the anthocyanidins. The yield of anthocyanidins has been reported to amount from c. 10% to as high as 27% depending on the solutions employed. In alcoholic solution the conversion yields about 18% (7). The majority of the methods for estimation of proanthocyanidins are based on the isolation of these compounds on a polyamide resin (Nylon-66, perlon or Polyclar AT), followed by heating of the adsorbate with alcoholic hydrochloric acid and spectrophotometric estimation of the resulting anthocyanidins (14, 19). The loss incurred by the formation of large amounts of polymers is generally compensated for by using an appropriate standard such as leucocyanidin. These methods consider the proanthocyanidins, but not the catechins present in the sample.

Catechins and phenolic acids are adsorbed together with proanthocyanidins on the poly-

midic resins but do not give anthocyanidins upon heating with dilute acids. Both catechins and proanthocyanidins react with vanillin in acidic media to form a red coloured complex and the determination of catechins is often based on this reaction. MCFARLANE et al. (21) used vanillin in conc. hydrochloric acid to estimate proanthocyanidins and catechins. The catechins were separated from the proanthocyanidins by chromatography on Polyclar AT. This method determines only free catechins and not those undergoing polymerization. DADIC (7) described a method for simultaneous determination of anthocyanidins and catechins based on their specific absorption coefficients in the green and the blue. After heating the alcoholic hydrochloric acid solution containing proanthocyanidins and catechins, the absorbancies at two suitable wavelengths (545 and 455 nm) are measured and from them the concentrations determined by application of binary analysis. The determinations made according to Dacic's method give, however, large standard deviations with replicate samples. This is in part due to the binary analysis used to calculate catechin and proanthocyanidin content as small variations in the absorbancies are reflected in large differences of both catechin and proanthocyanidin values.

Jerumanis (18) used the colour reaction of ferric ammonium citrate with polyphenols to measure their amounts. The limitation of this method is that ferric salts also react with compounds such as phenolic acids in wort and beer. As the colour yield from different compounds depends on their chemical structure it is difficult to calibrate the method adequately.

If the same material is analysed with the different methods discussed, the results are not commensurable (Tables I, VI and also DADIC (7)). The individual method used on different materials provides adequate comparisons on the contents of proanthocyanidins and catechins. This is supported by the comparisons of grain extracts from *ant-13* and Foma (Table I). It is doubtful if the calculated amounts of proanthocyanidins and catechins in a sample will stand up to a precise chemical analysis.

Mutation of the gene *ant-13* has blocked the

formation of anthocyanidins, flavan-3-ols and condensation products of flavan-3-ols. The pilot brews with this barley have revealed that the elimination of catechins and proanthocyanidins improves haze stability without adverse effects on taste and colour. A more refined assessment of the role of different polyphenols in haze formation will be possible, when mutants become available that only block the dimerization of flavan-3-ols and others that block the conversion of the hypothetical intermediate flavenol into flavan-3-ols. Such mutants are expected to form anthocyanidins and may accumulate the hypothetical intermediate in the grains in which the pathway leading to anthocyanidins is largely suppressed.

Mutant *ant-13* has in field trials yielded 20-25% less than its mother variety Foma, possibly due to an extreme susceptibility towards powdery mildew. By crossing with high yielding varieties it is attempted to move the gene into more favorable genetic backgrounds and thereby to produce high yielding malting barley varieties containing this gene. Elimination of anthocyanidins, catechins and proanthocyanidins in all organs of the barley plant may limit seriously the disease resistance and yield which can be obtained. In such a case organ specific mutants which restrict the elimination of these flavonoids to the grain can be looked for. Finally, we screen for other mutant alleles of the *ant-13* gene with the aim of isolating an allele with less severe pleiotropic effects on yield.

REFERENCES

1. AHRENST-LARSEN, B.: Nyt forsøgsbryggeri på Tuborg (in Danish with English summary). Brygmesteren 24, 109-128 (1967)
2. Analytica-EBC, Analysis Committee of European Brewery Convention, T.-M. ENARI ed. Schweizer Brauerei-Rundschau, 3rd ed., Zürich, 87 pp. (1975)
3. BLOM, J. & A. BAK: Über die Bestimmung von Amylasen durch Verflüssigung von Stärkekleister. Z. Physiol. Chem. 256, 197-207 (1938).
4. BLOM, J.: Bestimmung von Kohlensäure im Bier. Brauwelt 95, 645-646 (1955)

5. BLOM, J.: Über die Bestimmung der Haltbarkeit des Schaumes. Eu. Brew. Con. Proc. Cong., Copenhagen 1957, 51-56 (1957)
6. CHAPON, L. & M. CHEMARDIN: Etude sur le trouble au froid. Eu. Brew. Con. Proc. Cong., Madrid 1967, 389-405 (1968)
7. DADIC, M.: Analytical methods for polyphenols in brewing. II: A new method for determination of anthocyanogens and catechins (tanninogens) in wort and beer. Am. Soc. Brew. Chem. Proc. 1971, 159-170 (1971)
8. DADIC, M. & N. M. MORRISON: Analytical methods for polyphenols in brewing III. Simultaneous determination of anthocyanogens and catechins (tanninogens) in brewing materials. Am. Soc. Brew. Chem. Proc. 1972, 50-56 (1972)
9. GRAMSHAW, J. W.: Beer polyphenols and the chemical basis of haze formation I. Phenolic constituents of beer and its raw materials. Tech. Q. Master Brew. Ass. Am. 6, 239-251 (1969)
10. GRAMSHAW, J. W.: Beer polyphenols and the chemical basis of haze formation III. The polymerization of polyphenols and their reaction in beer. Tech. Q. Master Brew. Ass. Am. 7, 167-181 (1970)
11. GRISEBACH, H.: Recent investigation on the biosynthesis of flavonoids. In Recent Advances in Phytochemistry. T. J. MABRY, R. E. ALSTON & V. C. RONECKLES eds. Vol. 1. Appleton-Century-Crofts, New York, pp. 379-406 (1968)
12. GUSTAFSSON, Å., A. HAGBERG, U. LUNDQVIST & G. PERSSON: A proposed system of symbols for the collection of barley mutants at Svalöv. Hereditas 62, 409-414 (1969)
13. HARBORNE, J. B.: Flavonoids. In Phytochemistry Vol. 2. L. P. MILLER ed. Van Nostrand-Reynold Co., pp. 364-371 (1973)
14. HARRIS, G. & R. W. RICKETTS: Beer haze – the problem and a solution. Eu. Brew. Con. Proc. Cong., Rome 1959, 290-302 (1959)
15. HASLAM, E.: Natural proanthocyanidins. In The Flavonoids. J. B. HARBORNE, T. J. MABRY & H. MABRY eds. Chapman and Hall, London, pp. 505-559 (1975)
16. HASLAM, E.: The chemistry and biochemistry of plant proanthocyanidins. In Topics in Flavonoid Chemistry and Biochemistry. L. FARKAS ed. Proc. 4th Hungarian Bioflavonoid Symp. Elsevier, Amsterdam, pp. 77-99 (1975)
17. HOUGH, J. S., D. E. BRIGGS & R. STEVENS: Malting and brewing science. Chapman and Hall, London, p. 362 (1971)
18. JERUMANIS, J.: Simplification et amélioration de la méthode de dosage des polyphénols totaux en brasserie. Bull. Ass. Anc. Etud. Ec. Super. Brass. Univ. Louvain 64, 223-240 (1968)
19. MCFARLANE, W. D.: Determination of anthocyanogens. J. Inst. Brew. 67, 502-506 (1961)
20. MCFARLANE, W. D. & P. T. SWORD: Determination of anthocyanogens. III. Analysis of barley and malt. J. Inst. Brew. 68, 344-350 (1962)
21. MCFARLANE, W. D., P. T. SWORD & G. BLINOFF: Catechins – tannins in brewing. Eu. Brew. Con. Proc. Cong., Brussels 1963, 174-181 (1964)
22. POLLOCK, J. R. A., A. A. POOL & T. REYNOLDS: Anthocyanogens in barley and other cereals and their fate during malting. J. Inst. Brew. 66, 389-394 (1960)
23. SATAKE, K., T. OKUYAMAT, M. OHASHI & T. SHINODA: The spectrophotometric determination of amines, amino acids and peptides with 2,4,6-trinitrobenzene-1-sulfonic acid. J. Biochem. (Tokyo) 47, 654-660 (1960)
24. SILBEREISEN, K. & F. KRAFFCZYK: Isolierung und Struktur biflavanoider Proanthocyanidine (Anthocyanogene) der Gerste. Monatschr. Brau. 20, 217-223 (1967)
25. SØRENSEN, J. A.: Penetration of gases into bottled beer sealed with crowns with discs of cork and plastic. Eu. Brew. Con. Proc. Cong., Rome 1959, 220-226 (1959)
26. TROLLE, B.: Bryggerimæssige betragtninger vedrørende maltbyg. (In Danish). Brygmesteren 17, 45-55 (1960)
27. WEINGES, K., W. BÄHR, W. EBERT, K. GÖRITZ & H.-D. MARX: Die dimeren Proanthocyanidine. In Fortschritte der Chemie organischer Naturstoffe, Vol. 27. Springer Verlag, Wien, pp. 234-245 (1969)
28. Danish Patent Application no. 4640/75.