2(3)-BENZOXAZOLONE IN MALTING OF BARLEY[1]

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ABSTRACT

Experimental malting of barley showed that 2(3)-benzoxazolone (BOA), alone and together with a small quantity of gibberellin, increased malt yield by decreasing rootlet growth and respiration. Extract content and modification were improved. The activities of alpha- and beta-amylase remained unaffected or were somewhat increased. Best results were obtained using BOA together with gibberellin; with a short germination time it was possible to obtain malt of a high extract content and alpha-amylase activity. Furthermore, malt yield was increased by about 3%.

Several natural and synthetic compounds have been found to inhibit root growth during germination, e.g., indole-3-acetic acid (9), alpha-naphthylacetic acid (3), coumarin (6), and 2,4-dichlorophenoxyacetic acid (7). Kirsop and Pollock (5) have recently shown that the embryo can be removed after the first 3 days of germination without affecting properties of the resulting malt other than beta-amylase activity, which is decreased. These findings have given theoretical background for the use of root growth-restricting substances in malting. Plant-growth regulators of another type are gibberellins, which increase the speed of modification. Thus the time of germination can be reduced, and yet malt of better quality is obtained (11,12). Gibberellins can advantageously be used together with substances restricting root growth (7).

In a series of preliminary germination tests, 2(3)-benzoxazolone (BOA) gave some promising results in the restriction of rootlet growth (8). A further advantage of this compound is that mold growth is prevented or delayed. By proper treatment alpha-amylase and protease activities were not significantly lowered. However, in these preliminary studies malting conditions differed considerably from those of the technical process, and the samples were too small for a complete analysis of the final malt.

In the present study some further malting experiments were performed with BOA and gibberellin using a pneumatic-type experimental malting apparatus.

Materials and Methods

2(3)-Benzoxazolone was synthesized according to Bywater *et al.*

[1] Manuscript received April 28, 1960.

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(2). The barley varieties used were Pirkka, a Finnish 6-row variety, and Balder, a Swedish 2-row variety. The experimental malting apparatus had a capacity of 8×1 kg. of barley. The apparatus consisted of a steeping tank, a pneumatic-type germination chamber, and a kiln. The steeping tank was equipped with a pump for water circulation and with a small compressor for aeration. The temperature of steeping water could be controlled. The germination chamber was equipped for circulation of moist, temperature-controlled air through the grains. The quantities of fresh and recirculating air could be controlled with perforated plates. In the kiln, heated air was forced through the grains, and the temperature was automatically controlled and registered. A detailed description of the apparatus will be published elsewhere[3]. The following malting process was employed:

Steeping. Four different methods of steeping were used:

a. Normal steeping involving spraying with a solution of BOA after 31 hours. The grains were first steeped at 11°–12°C. for 23 hours in water, which was changed at intervals of 1, 6, and 16 hours. This was followed by dry-steeping for 8 hours. Then the grains were sprayed with 10 ml. of a solution of BOA (448 to 672 mg. BOA per 10 ml., corresponding to 500 to 750 mg. BOA per kg. barley, d.m.) in 80% ethanol (the control samples were sprayed with 80% ethanol). The process was then continued by dry-steeping only. The grains were sprayed with water at about 6-hour intervals to increase the moisture content. After a total steeping time of 55 hours the moisture content of the barley was 43%. Aeration was continuous during the whole process.

b. Normal steeping involving standing in a water solution of BOA at the end of the process. The grains were first steeped at 11°–12°C. for 20 hours in water, which was changed at intervals of 2, 4, 6, and 8 hours. After this followed dry-steeping for 9 hours, again steeping in water for 0.5 hour, dry-steeping for 6.5 hours, steeping in water for 0.5 hour, and dry-steeping for 8.5 hours. Finally the grains were allowed to stand for 2 hours in a 0.05% water solution of BOA (or water in the control sample), followed by dry-steeping for 8 hours. Thus the total steeping time was 55 hours. The final moisture content of barley was 45%. The aeration was continuous, except for the standing in BOA-solution.

c. Steeping without aeration. In this process there was no aeration during the steeping in water. The grains were first allowed to stand

[3] Linko, M., Experimental maltings in the Laboratory of Brewing, Helsinki. *Mallasjuomat* (to be published).

for 20 hours in a 0.05% water solution of BOA (or water in the control sample). After this followed dry-steeping with aeration for 9 hours, standing in BOA-solution for 7 hours, and finally dry-steeping for 9 hours. The total steeping time was 45 hours, temperature about 14°C., and the final moisture content of barley 44%.

d. Steeping by spraying. In this process the grains were first steeped in water for 2 hours, but after this the moisture content was increased only by spraying with 0.05% water solution of BOA at 6-hour intervals (the control sample was sprayed with water). The total steeping time was 60 hours, temperature 11°–12°C., aeration continuous, and the final moisture content of barley 43%.

Germination. The steeped barley was germinated for 5 to 7 days. The initial temperature was 12°C. In the beginning the temperature was raised by 1°C. per day, until 16°C. was reached. This temperature was then maintained through the rest of the germination period. Moisture content was adjusted to 43 to 44%, and the grains were turned by hand once a day.

Kilning. The temperature was first raised to 52°C. within 1 hour and maintained there for 3 hours. This was followed by a raise to 60°C. during 5 hours, which temperature was again maintained for 4 hours. Finally temperature was increased to 86°C. during 5 hours, and maintained there for 3 hours; total kilning time, 21 hours.

The rootlets were removed after kilning by brushing against a steel wire net, and resulting malts and rootlets were weighed.

Analysis of malt. The resulting malts were analyzed according to the standard methods of the European Brewery Convention (4), which do not essentially differ from those of the American Society of Brewing Chemists. Alpha-amylase activity was determined according to the ASBC method (1), and beta-amylase activity was calculated from the values of total diastatic power and the diastatic effect of alpha-amylase. This calculation was based upon the additivity of the diastatic effects of alpha- and beta-amylase (10).

The mashings of finely and coarsely ground malts and the respective determinations of extract content, as well as the determinations of moisture content, total nitrogen, Kolbach index, and acrospire length were performed in duplicate. According to earlier experience based on replicate maltings, differences exceeding 0.3% in the values of extract content, 0.5% in malt yield, and 0.3% in the loss by rootlets or by respiration can be considered as significant.

Results and Discussion

The results from the first series of maltings made with Pirkka bar-

TABLE I

THE EFFECT OF 2(3)-BENZOXAZOLONE (BOA) AND GIBBERELLIN ON MALT QUALITY AND MALT YIELD OF BARLEY VARIETY PIRKKA

(Steeping Procedure (*a*); 500 to 750 p.p.m. BOA added as an alcohol spray after 31 hours of steeping; 0.5 p.p.m. gibberellin added as a water spray after 33 hours of steeping; germination time 7 days)

BOA ADDED (p.p.m.):	0	750	0	500
GIBBERELLIN ADDED (p.p.m.):	0	0	0.5	0.5
Moisture, %	3.7	3.6	3.5	3.6
Extract, fine grind (d.m.), %	80.6	80.7	81.9	82.1
Extract, coarse grind (d.m.), %	76.4	76.4	78.9	79.5
Difference fine-coarse (d.m.), %	4.2	4.3	3.0	2.6
pH of the laboratory wort	5.75	5.70	5.65	5.70
Saccharification time, minutes	<10	<10	<10	<10
Clarity of the laboratory wort	clear	clear	clear	clear
Speed of filtration	normal	normal	normal	normal
Total nitrogen (d.m.), %	2.08	2.09	2.06	2.08
Kolbach index, %	59	61	67	69
Acrospire length: 0–¼	5	6	3	3
¼–½	4	4	7	6
½–¾	58	66	17	23
¾–1	33	24	64	56
overgrown	0	0	9	12
Average acrospire length	0.68	0.66	0.82	0.81
Malt yield (d.m.), %	89.1	90.1	89.4	89.6
Loss by rootlets (d.m.), %	4.1	3.6	3.8	3.5
Loss by respiration and steeping (d.m.), %	6.8	6.3	6.8	6.9
Malt yield × extract yield (d.m.), %	71.8	72.7	73.2	73.6

ley according to the steeping procedure *a* are presented in Table I. The results show that the quality of malt was not affected by BOA. Malt yield was increased by 1% owing to a 0.5% decrease in the growth of rootlets and to a 0.5% retardation in respiration. The acrospire growth was not affected, or was slightly reduced. The addition of gibberellin[4] caused a marked increase in the extract content, in the acrospire growth, and in the speed of modification, as could be expected, but the growth of rootlets was not increased. In these samples malt yield was almost unaffected by BOA, perhaps owing to the fact that the samples were overgrown. However, the modification was somewhat improved.

The next series of maltings was made with Balder barley according to steeping procedures *b, c,* and *d*. In two cases a small amount of gibberellin (1 p.p.m.) was added as water spray during the first day of germination. In these two samples germination time was shortened by 2 days. The amount of BOA in the grains was not determined, but according to earlier studies concerning BOA uptake it can be assumed to be of the order of 200 to 300 p.p.m. when steeping

[4] Purum grade, Fluka AG, Chemische Fabrik, Buchs SG, Switzerland.

TABLE II

EFFECT OF 2(3)-BENZOXAZOLONE (BOA) AND GIBBERELLIN ON MALT QUALITY AND MALT YIELD OF BARLEY VARIETY BALDER

BOA Added	−	+	+	−	+	−	+	+
Gibberellin Added (p.p.m.)	0	0	1	0	0	0	0	1
Steeping Procedure (See Text)	*b*	*b*	*b*	*c*	*c*	*d*	*d*	*d*
Germination Time (days)	7	7	5	7	7	7	7	5
Moisture, %	3.7	3.5	3.6	3.6	3.6	3.6	3.6	3.6
Extract, fine grind (d.m.), %	78.6	79.1	80.7	79.0	79.7	78.6	78.5	80.0
Extract, coarse grind (d.m.), %	75.4	76.4	77.3	75.8	77.2	74.4	74.5	76.4
Difference fine-coarse (d.m.), %	3.2	2.7	3.4	3.2	2.5	4.2	4.0	3.6
pH of the laboratory wort	5.85	5.95	5.80	5.90	5.95	6.00	6.05	6.00
Saccharification time, minutes	>10	>10	>10	>10	>10	10–15	>10	>10
Color,°EBC	3.1	3.4	5.3	2.7	4.1	3.4	3.4	4.1
Clarity of the laboratory wort	clear	clear	clear	clear	clear	clear	clear	clear
Speed of filtration	normal	normal	normal	normal	normal	normal	normal	normal
Total nitrogen (d.m.), %	1.77	1.76	1.79	1.74	1.79	1.76	1.77	1.74
Kolbach index, %	45	47	57	44	49	43	42	53
Alpha-amylase, 20° D.U.	34	37	44	33	35	28	32	40
Beta-amylase, W.K.	240	250	240	210	210	210	220	200
Acrospire length, %: 0–¼	1	2	1	1	4	2	2	2
¼–½	2	1	3	2	4	8	2	9
½–¾	84	86	77	86	85	63	83	59
¾–1	13	11	16	11	7	27	11	29
overgrown	0	0	3	0	0	0	2	1
Average acrospire length	0.65	0.65	0.67	0.65	0.62	0.67	0.67	0.67
Malt yield, %	88.7	89.7	92.1	89.6	91.6	89.8	90.5	92.7
Loss by rootlets (d.m.) %	5.2	4.3	3.2	4.6	3.1	4.7	4.2	3.2
Loss by respiration and steeping (d.m.), %	6.1	6.0	4.7	5.8	5.3	5.5	5.3	4.1
Malt yield × extract yield (d.m.), %	69.7	71.0	74.3	70.8	73.0	70.6	71.0	74.2

procedures *c* and *d* were used, and about 20 p.p.m. when the steeping procedure *b* is used. The results are presented in Table II.

The most striking results were obtained with steeping procedure *c,* without aeration during the water steep. In these samples the growth of rootlets began very slowly as compared with other samples steeped with efficient aeration. No rootlets could be seen after the first day of germination in grains steeped in BOA-solution (Fig. 1).

Fig. 1. The effect of 2(3)-benzoxazolone (BOA) on the growth of rootlets during malting of barley, variety Balder. Steeping procedure *b* (see text for details). Top left: control sample after first day of germination; top right: control sample after second day of germination; bottom left: sample steeped in BOA after first day of germination; bottom right: sample steeped in BOA after second day of germination.

However, once started, growth continued almost normally. In these maltings BOA caused a 2% increase in the malt yield. The loss by rootlets was decreased by 1.5% and the respiration loss by 0.5%. Furthermore, the quality of malt was improved; the extract content was increased by 0.7%, and the modification was better as judged from the smaller fine/coarse grind extract difference and the increased Kolbach index (wort soluble N as percent of total N). The increase in the extract content results, no doubt, at least partly from the retardation of respiration, although manometric techniques with a fairly short measuring time failed to detect any decrease in carbon di-

oxide production (8). The lack of aeration did not impair the quality of malt, as can be seen by comparing the control sample with other control samples.

In the samples steeped only by spraying (procedure *d*), the effect of BOA was small as compared to the samples steeped according to the procedure *c,* although the BOA uptake must have been of the same order. This seems to be associated with the more aerobic conditions, which might cause metabolizing of BOA.

In the samples steeped according to the procedure *b* the effect of BOA was stronger than could be expected. These samples were standing in BOA-solution only for 2 hours, and the uptake of BOA was respectively less than one-tenth as compared with the other samples. Despite this, there was an increase of 1% in malt yield and 0.5% in the extract content. The fine/coarse grind extract difference was decreased by 0.5%, showing a better modification.

In all cases acrospire growth remained unaffected or was slightly reduced by BOA. Respiration was somewhat retarded, and the activities of alpha- and beta-amylase slightly increased or maintained at the same level as in the control samples. In these respects the effects of BOA are different from those of 2,4-D, which stimulates acrospire growth, does not affect respiration, and decreases alpha-amylase activity (7).

The best results were obtained by using BOA together with a small quantity of gibberellin (1 p.p.m.). Owing to the fact that germination time could be reduced by 2 days, malt yield was increased appreciably (about 3%). Furthermore, the extract content of the resulting malt was materially higher (1.5 to 2.1%). In spite of the shortened germination time, alpha-amylase activity increased about 30%. Proteolytic activity was also high as judged from the Kolbach index, but beta-amylase activity was not significantly changed.

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