

JÓZEF SURMIŃSKI  
STANISŁAW MASIOR  
TADEUSZ KUCHCIAK

## EFFECT OF TIME OF GIBBERELIC ACID ADDITION ON ACCUMULATION OF $\alpha$ -AMYLASE IN MALT. Part II

Institute of Fermentation Technology and Microbiology,  
Technical University, Łódź

Key words: gibberellic acid, barley malt,  $\alpha$ -amylase activity, endo- $\beta$ -glucanase, endopeptidase.

The effect of the time of gibberellic acid (g.a.) addition (8 and 20 h) to the final water in barley steeping on the  $\alpha$ -amylase content in malt was studied. The brewer's barley varieties Grit and Polon and feed variety Diva from the 1983 crop were malted. The prolongation of g.a. action from 8 to 20 h increased the activity of  $\alpha$ -amylase, endo- $\beta$ -glucanases and endopeptidases in malts from all three barley varieties. It was found that specific properties of barley from the given crop have a strong effect on the increase of  $\alpha$ -amylase content in malt when g.a. is added.

In our previous publication [5] we have described the effect of steeping degree, malting temperature and gibberellic acid (g.a.) addition on the  $\alpha$ -amylase accumulation in malt. In this research the effect of the time of g.a. addition to the final water in barley steeping on the  $\alpha$ -amylase content in malt is studied. The basic literature of the subject is given in [5]. The study of prolonged addition of g.a. to barley was encouraged by the preliminary results reported in Bielawska's Doctor's thesis [2]. Bielawska extended the time of g.a. addition from 4 to 8 h and the activity of  $\alpha$ -amylase clearly increased, by 18 units, in the Wisa barley variety; in the Damazy variety this increase was slight (2 units).

### MATERIALS AND METHODS

The time of g.a. additions, in doses of 0.2 mg/kg, with the final steeping water was set at 8 and 20 h, and the temperature of second drying of malt at about 79°C. The remaining malting parameters were based on the previous experiments [5]. Air-water steeping was done in water of 14—

16°C, with aeration in both phases; the pH of the water with which g.a. was added was 6.5. The grain was steeped to 46% ( $\pm 0.2\%$ ) of humidity. The steeping schedules for the 8 and 20-h g.a. additions are given in Tables 1 and 2.

The germination of barley took place at 18°C for 5 days in the case of samples with g.a., and for 6 days for control samples without g.a.; the course of steeping is given in Table 1. The malted barley was of the brewer's varieties Grit and Polon and of the feed variety Diva of the

Table 1. The schedule of steeping barley with 8-h gibberellic acid addition

Time	Activity
08.50-09.00 a.m.	Filling of vats with water and introduction of raw material
09.00-11.00 a.m.	Preliminary washing in 1st water with aeration
11.00 a.m.-01.00 p.m.	Washing with 2nd water with NaOH addition
01.00-03.00 p.m.	Steeping with aeration
03.00-05.00 p.m.	Steeping in 3rd water
05.00-08.00 p.m.	Steeping with aeration
08.00 p.m.-08.00 a.m.	Steeping in 4th water
08.00 a.m.-08.00 p.m.	Steeping with aeration
08.00 p.m.-08.00 a.m.	Steeping in 5th water
08.00 a.m.-10.00 a.m.	Steeping with aeration
10.00 a.m.-06.00 p.m.*)	Steeping in 6th water with g.a.
06.00-08.00 p.m.	Steeping with aeration
08.00-08.30 p.m.	Weighing of samples, transference into sprouting vessels

\* The grain was steeped to about 46% humidity, so the time of final steeping could be subjected to variation

Table 2. The schedule of steeping barley with 20-h gibberellic acid addition

Time	Activity
08.50-09.00 a.m.	Filling of vats with water and introduction of raw material
09.00-11.00 a.m.	Preliminary washing in 1st water with aeration
11.00 a.m.-01.00 p.m.	Washing with 2nd water with NaOH addition
01.00-03.00 p.m.	Steeping with aeration
03.00-05.00 p.m.	Steeping in 3rd water
05.00-08.00 p.m.	Steeping with aeration
08.00 p.m.-08.00 a.m.	Steeping in 4th water
08.00 a.m.-08.00 p.m.	Steeping with aeration
08.00 p.m.-04.00 p.m.*)	Steeping in 5th water with g.a.
04.00 p.m.-08.00 p.m.	Steeping with aeration
08.00-08.30 p.m.	Weighing of samples, transference of samples into sprouting vessels

\* The grain was steeped to about 46% humidity, so the time of final steeping could be subjected to variation

Table 3. Results of analyses of barleys of Diva, Grit and Polon varieties

Determination	Diva	Grit	Polon
humidity (%)	10.6	11.0	10.7
extract (% dry substance)	75.5	77.4	76.7
protein (% dry substance)	11.7	11.1	10.7
starch (% dry substance)	58.6	63.5	59.5
weight of 1000 grains (g dry substance)	37.7	37.9	34.2
germination energy after 3 days (%)	96	96	94
germination energy after 5 days (%)	97	97	95
size and uniformity of grain (%)	73.4	74.8	72.1

1983 crop. The chemical composition of the investigated barley varieties is given in Table 3.

It should be mentioned that the 1983 barleys were marked by particularly small grain, low extractivity and 1000-grain weight, and were difficult to steep.

The malting of 1-kg barley samples was carried out in a micro-malt-house [5] in which the temperature (18°C) was maintained with XKL9L refrigeration units coupled with contact thermometers. During the germination cycle, the samples were manually mixed twice daily, in the morning and in the evening.

The "wet" malts were characterized by well developed root germs and infrequent sprouts. The malt was dried in a semiautomatic laboratory drier equipped with a thermoregulator and temperature recorder. Drying was begun in the evening and for 12 h (nighttime) the temperature of the

Table 4. Chemical analysis of malts produced of Grit barley steeped with gibberellic acid for different periods (second-drying temperature — about 79°C)

Determination	Control sample	Experimental samples	
		8 h with g.a.	20 h with g.a.
extract in flour (% dry substance)	79.1	80.0	80.5
losse of firmness (% dry substance)	2.2	1.2	1.8
wort colour (EBC units)	3.0	4.0	4.5
total protein (% dry substance)	10.0	10.5	10.8
Kolbach number (%)	45.7	41.0	47.3
wort viscosity (mPa · s)	1.522	1.512	1.492
diastatic force (W-K units in dry substance)	480	500	510
$\alpha$ -amylase (F-S units) (g dry substance)	733.7	791.2	953.7
endo- $\beta$ -glucanase (units /100 g dry substance)	206	232	310
endopeptidases (units/100 g dry substance)	11.9	14.2	17.1

Table 5. Chemical analysis of malts produced from Polon barley steeped with gibberellic acid for different periods (second-drying temperature — about 79°C)

Determination	Control sample	Experimental samples	
		8 h with g.a.	20 h with g.a.
extract in flour (% dry substance)	78.9	78.1	80.3
losse of firmness (% dry substance)	2.8	2.2	1.9
wort colour (EBC units)	4.5	6.0	5.5
total protein (% dry substance)	10.1	10.2	10.4
Kolbach number (%)	34.3	40.2	45.6
wort viscosity (mPa · s)	1.564	1.532	1.488
diastatic force (W-K units in dry substance)	490	420	440
$\alpha$ -amylase (F-S units/g dry substance)	606.9	626.4	666.0
endo- $\beta$ -glucanases (units/100 g dry substance)	229	288	328
endopeptidases (units/100 g dry substance)	10.1	12.8	17.2

drying air was maintained at 35°C, bringing the grain humidity to about 15%. Drying was continued during the following day, with the temperature increased gradually to about 79°C, until grain humidity dropped to 5—6%.

The barleys and experimental barleys were analysed as in [5]. The  $\alpha$ -amylase content in malt was determined by Briggs' maltose method [4] with 3-5-dinitrosalicylic acid, the content of endo- $\beta$ -glucanases (cellulase) according to Bernat [1] with carboxymethylcellulose sodium salt, and endopeptidases by the method of Breit et al. [3].

The mean values of results of chemical analyses of malts produced from the three barley varieties, steeped with gibberellic acid in various times and second-dried at about 79°C are given in Tables 4—6.

Table 6. Chemical analysis of malts produced of Diva barley steeped with gibberellic acid for different periods (second-drying temperature — about 79°C)

Determination	Control sample	Experiment samples	
		8 h with g.a.	20 h with g.a.
extract in flour (% dry substance)	78.2	78.4	79.1
losse of firmness (% dry substance)	3.8	3.3	3.5
wort colour (EBC units)	3.5	4.5	5.0
total protein (% dry substance)	11.3	11.3	11.2
Kolbach number (%)	35.8	40.5	39.8
wort viscosity (mPa · s)	1.543	1.520	1.505
diastatic force (W-K units in dry substance)	280	270	320
$\alpha$ -amylase (F-S units/g dry substance)	512.8	524.6	587.1
endo- $\beta$ -glucanases (units/100 g dry substance)	189	205	239
endopeptidases (units/100 g dry substance)	8.5	11	16.6

## DISCUSSION OF RESULTS

The highest  $\alpha$ -amylase content after malting without g.a. was found in malts from Grit barley, with Polon malt coming in second place; the malt from the feed variety Diva had the lowest  $\alpha$ -amylase content. After 20-h g.a. introduction (Tables 2—4), the  $\alpha$ -amylase content in the experimental malts increased by 23.8% in Grit barley, by 9.8% in Polon barley and by 14.4% in the Diva variety (all figures in relation to malts without g.a. additions).

The  $\alpha$ -amylase increments in the case of 20-h additions of g.a. are much greater than those for 8-h additions. It must be stressed, however, that these increments for 1983 barley were in general much lower than for barleys from the 1982 crop which were of the order of 50% [5].

In comparison to the 8-h g.a. addition, the endo- $\beta$ -glucanases content at 20-h g.a. additions was higher by 23% in the Grit variety, by 17% for the Polon barley, and by 6% for the Diva variety.

The endopeptidases content in the 20-h g.a. addition cycle was higher than in the 8-h cycle by 27% in the Polon variety, by 8% in the Grit variety, and by 46% in the Diva variety.

## CONCLUSIONS

1. The prolongation of the time of gibberellic acid addition from 8 to 20 h led to an increase of the content of  $\alpha$ -amylase, endo- $\beta$ -glucanase and endopeptidases in malts from three barley varieties (Grit, Polon, Diva). The highest  $\alpha$ -amylase increment in the 20-h g.a. introduction was demonstrated by malt from the Grit barley.

2. It was found that the specific properties of barley from the given crop have a strong effect on the increase of  $\alpha$ -amylase content in the malt with g.a. addition.

3. The increase of enzymatic activity of malts had a positive effect on their basic qualitative properties such as extractivity, Kolbach number and losse of firmness.

## LITERATURA

1. Bernat J. A.: Vremiennyje jedynyje metody opredielenija celulloliticzeskoj aktywnosti. Centr. Isled. Inst. Piszczewoj Prom. WNR 1966.
2. Bielawska M.: Wpływ warunków wprowadzania kwasu giberelinowego i inhibitorów dla nagromadzenia enzymów w słodzie piwowarskim. PHD thesis. Technical University, Łódź 1979.
3. Breit J. and others: Malt Biotechnik 1974, 5 (4), 198.
4. Briggs D. E.: I. Ins. Brewing 1961, 5, 427.

5. Surmiński J., Masiór S., Kuchciak T.: *Acta Alimentaria Polonica* 1986, 12 (XXXVI), (2).

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*Authors address: 90-924 Łódź, Stefanowskiego 4/10*

*J. Surmiński, S. Masiór, T. Kuchciak*

## WPLYW CZASU WPROWADZANIA KWASU GIBERELINOWEGO NA NAGROMADZENIE $\alpha$ -AMYLAZY W SŁODZIE, cz. II

Instytut Technologii Fermentacji i Mikrobiologii, Politechnika, Łódź

### Streszczenie

Badano wpływ czasu wprowadzania kwasu giberelinowego (K. G.) do ostatniej wody w procesie moczenia jęczmienia na zawartość  $\alpha$ -amylazy, endo- $\beta$ -glukanaz i endopeptydaz w słodzie oraz na jakość słodu. Stosowano 8 i 20 h wprowadzanie K. G. (dawka 0,2 mg/kg). Do słodowania w skali mikrotechnicznej użyto browarowych odmian jęczmienia: Grit i Polon oraz odmianę paszową Diva ze zbiorów 1983 r. Przy wprowadzaniu 8 i 20 h K. G. stosowano zróżnicowany harmonogram moczenia jęczmienia (tab. 1 i 2). Kiełkowanie jęczmienia przebiegało w temp. 18°C w ciągu 5 dni dla prób z K. G. i 6 dni dla prób kontrolnych bez K. G. Słód dosuszano w ok. 79°C.

W doświadczalnych jęczmieniach oznaczano: wilgotność, ekstraktywność, białko ogółem, skrobię, wagę 1000 ziarn, celność ziarna, energię kiełkowania. Analiza słodów obejmowała: ekstraktywność w mące i śrucie, rozluźnienie, barwę brzezki, białko ogółem, liczbę Kolbacha, lepkość brzezki, siłę diastatyczną, zawartość  $\alpha$ -amylazy (metodą maltozową Briggsa), zawartość endo- $\beta$ -glukanaz i endopeptydaz. Przedłużenie czasu wprowadzania K. G. z 8 do 20 h wywołało wzrost zawartości enzymów:  $\alpha$ -amylazy, endo- $\beta$ -glukanaz i endopeptydaz w słodach z trzech odmian badanych jęczmieni (tab. 4-6). Najwyższy przyrost zawartości  $\alpha$ -amylazy wykazywał słód z jęczmienia Grit (tab. 4).

Stwierdzono, że cechy specyficzne jęczmienia z danego roku zbioru wywierają silny wpływ na zwiększenie się zawartości  $\alpha$ -amylazy w słodzie przy dodatku K. G. Zwiększenie zawartości enzymatycznej słodów wpłynęło dodatnio na ich podstawowe cechy jakościowe, jak ekstraktywność, rozluźnienie, liczba Kolbacha (tab. 4-6).