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EFFECT OF STEEPING DEGREE, MALTING TEMPERATURE AND ADDITION OF GIBBERELLIC ACID ON THE ACCUMULATION OF α-AMYLASE IN MALT

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Key words: malting barley, α-amylase, giberellic acid.

Malting on a microtechnological scale was performed with the use of brewer's barley of Trumpf and Polon varieties and with the feed variety Diva, all from the 1982 crop. The highest α-amylase content was found in the Polon variety; this content in the Trumpf variety was lower by about 7%, while in the feed variety Diva it was lower by about 20%. The following conditions were found to favour the accumulation of α-amylase in malt containing no addition of gibberellic acid (g.a.): malted grain humidity — 46%, malting temperature — 18°C, time of malting — 7 days. When gibberellic acid was added in doses of 0.2-0.3 mg/kg barley and when pH of the water with g.a. ranged from 6.5 to 7.5, the mean increase of α-amylase content in the studied malt varieties was 54% as compared to control samples without g.a. In the case of the variety Diva this increase was 57%. The conditions of malting with an addition of g.a. that were worked out allow the production of brewer's malt from Diva barley.

The replacement of malt with larger additions of unmalted starch raw materials that is practiced in the brewing industry necessitated additions of enzymatic preparations of bacterial origin. Brewing industry seeking ways of eliminating the use of expensive enzymatic preparations. One such way is the production and application of malts with an increased α-amylase content. Among the principal technological conditions affecting the synthesis of α-amylase during the steeping and malting of barley are: the temperature of steeping water, intensity of aeration, degree of grain steeping, temperature of grain germination, time of malting, temperature of wilting and drying of malt, addition of germination stimulator (gibberellic acid).

The studies of Narziss et al. [13] demonstrated that the variety of barley also has a clear effect on the level of α-amylase in malt. Among the ten varieties studied by these authors there was also the variety Trumpf used in our experiments, and its α-amylase content was found to be medium as compared to the contents in the remaining varieties. The temperature of steeping water has a bearing on the accumulation of enzymes, but this dependence is not quite clear. Kretschmer [12], Pizło [14] and Bielawska [3] indicate 14°C as the optimum temperature. On the other hand, Fridrich and Narziss [9] claim that 21°C is the most favourable temperature for enzyme accumulation during air-water steeping.

According to Sommer [15] exact temperature control during grain steeping at 10-20°C is not necessary. Banasik [1] found a strict dependence between the intensity of grain aeration during steeping and enzyme activity in the obtained malt. The studies of Narziss and Fridrich [9] showed that the activity of α-amylase in grain steeped to 46% humidity is twice higher than in grain of 40% humidity. Godlewska et al. [10] found that 46% humidity is optimum for the accumulation of α-amylase in malt.

The studies of Dan [6] indicate that germination temperature 16-17°C is best for the accumulation of enzymes in grain.

The supply of oxygen is particularly important in the initial phase of barley germination. Malting in an atmosphere with large amounts of carbon dioxide leads to a drop of α-amylase content in malt [7].

The metabolic processes in plant cells during germination are stimulated by natural hormones. An artificial addition of plant hormone speeds up the growth and development of grains [11]. The plant hormone used in Polish brewing industry, gibberellin acid (g.a.), is produced by the "Polfa" Enterprise in Kutno under the brand name "Gibreskol".

The search for optimum doses of gibberellin acid and of germination inhibitors was the subject study of Dylkowski [8]. In report of Bielawska 3 stated that optimum α-amylase accumulation at 10°C of the last steeping water with g.a. and inhibitors, and pH of 6.1 and 7.1.

MATERIAL AND METHODS

Three varieties of barley from the 1982 crop were used to produce malts on microtechnological scale: Polon, Trumpf (brewer's barleys), and the spring barley Diva (Table 1).

The malting of 1-kg samples of barley was done in the micro-malt house of the Institute of Fermentation Technology and Microbiology o the Łódź Technical University (Fig.).

Barley samples were steeped in 4 dm^3 cylindrical-conical vats equipped with a spiral coil pipe feeding air from a compressor. The samples were

Table 1. Results of analysis of Diva, Polon and Trumpf barleys

Characteristic	Diva	Polon	Trumpf
Humidity (%)	12.4	12.2	13.0
Extract (% dry mass)	75.5	78.9	78.4
Protein (% dry mass)	11.68	10.52	11.33
Starch (% dry mass)	56.0	59.6	58.7
Weight of 1000 grains (g dry mass)	52.0	46.6	50.8
Homogenens diameter of grain (%)	87.5	96.0	87.0
Germination energy after 3 days (%)	90	93	92
Germination energy after 5 days (%)	96	98	98

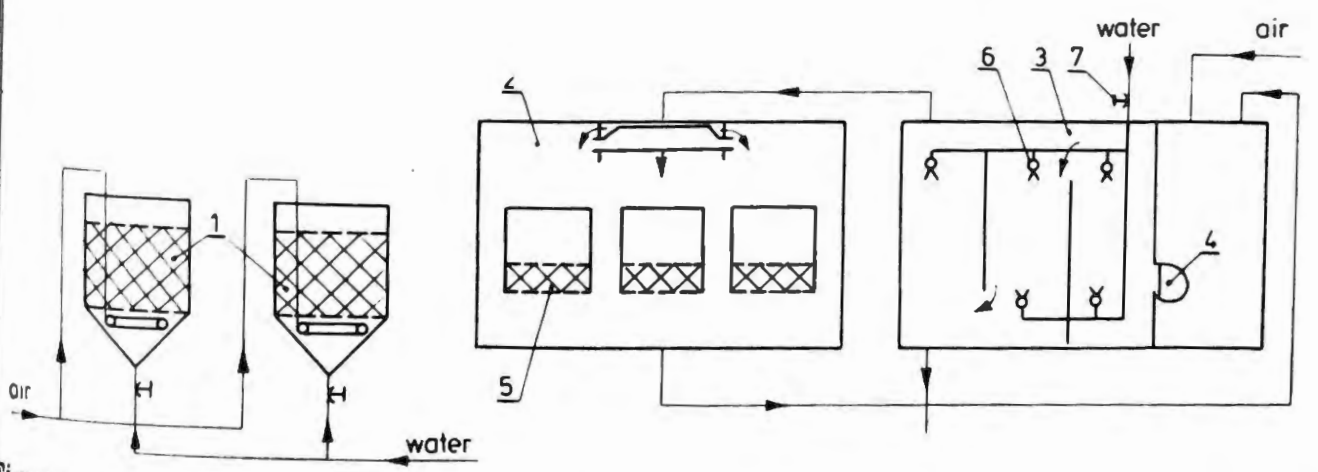

Figure. Scheme of micro-malt-house. 1 — infusion vats, 2 — germination chamber, 3 — air humidification chamber, 4 — ventilator, 5 — grain baskets, 6 — spray nozzles, 7 — valve

steeped by the air-water method in tap water at 12-14°C. Grain was aerated in the water phase every hour for 15 min, and continuously in the air phase. The grain was steeped to about 43% humidity, and in order to obtain 46% humidity, the soaking in water was prolonged by 2-6 h.

The steeped grain was transfered to baskets with perforated bottoms which were then placed in a germination chamber for 5, 7 or 9 days. A ventilator pumped air into the germination chamber through a compartment with water nozzles providing humidity. The amount of pumped air was adjusted by a valve.

The temperature in the malt-house was maintained with an XKO9L refrigeration unit coupled with a contact thermostat suspended inside the malt-house. Germinated samples were mixed by hand twice daily, at 9 a.m. and 6 p.m. Malts maintained at 14°C featured small amounts of cotyledons, while those maintained at 18°C had about 20% of cotyledons after 9 days of malting.

The malts were dried in a semi-automatic laboratory drier with thermoregulator and a temperature recorder. Drying commenced in the evening and the temperature of the drying air was kept at 35°C through the night; this caused grain humidity to drop to 15%. The process was

continued from 8 a.m. to 9 p.m. with the temperature raised gradually to 75°C. The total time of drying was 24 h.

The following was determined in the barleys taken for experiments: humidity, extractivity by the method of Pawłowski, total protein, starch polarimetrically, the weight of 1000 grains, with homogenous % of grain diameter germinative energy after 3 and 5 days.

The analysis of the experimental malts concerned: humidity, extractivity in flour and grist, total protein, tannin protein according to Lundin, wort colour in Hellige's neocomparator, Kolbach number, total tannins in wort by the colorimetric method with Fe ions, wort viscosity in a Hoepler viscosimeter, α-aminoacid nitrogen by the ninhydrin method, α-amylase content by Briggs' maltose method [5] with 3,5 dinitrosalicyllic acid, diastatic activity according to Windisch-Kolbach, content of endo-β-glucanases (cellulase) according to Bernat [2] with carboxymethylcellulose sodium salt, endopeptidases content by the method of Breit et al. [4].

In the preliminary phase of experiments we checked the effect of grain steeping, and of malting time and temperature on the contents of α-amylase, endo-β-glucanases and endopeptidases in the malts. Barley samples were steeped until their humidity increased to 43 and 46%. Malting was performed at 14 and 18°C for 7 and 9 days. The results are given in Tables 2 and 3.

Subsequent experiments were intended to determine the best dose of the Polish gibberellic acid (g.a.) for the studied barley varieties. Doses of 0.2, 0.3 and 0.5 mg g.a./kg barley were investigated. The acid was introduced into the final steeping water in the form of an alcohol solution (0.2 mg g.a. per cm^3). The barley was steeped till it attained 46% humidity. The pH of the water was adjusted to 6.5 before adding the g.a. Previous studies have demonstrated that pH 6.5-7.5 is the most advantageous to g.a. activity. Table 4 shows exemplary results obtained for the Trumpf variety.

During steeping, g.a. remained in contact with barley for 14-16 h. The steeped samples were malted for 5 days at 18°C. Control samples without g.a. were malted for 7 days. The results for various doses of g.a. are given in Table 5.

RESULTS AND DISCUSSION

Looking at the results of experiments collected in the Tables we notice the following: The content of α-amylase in comparable malts clearly depends on the variety of barley, with the highest content being in the Polon variety; Trumpf contains less α-amylase, and the least amount is present in the feed variety Diva.

For example, Polon barley steeped to 46% humidity and malted for

Table 2. Chemical analysis of malts produced from grain steeped to 43 and 46% of humidity and malted for 7 days at 14°C

Characteristic	Barley variety					
	Diva — humidity		Polon — humidity		Trumpf — humidity	
	43%	46%	43%	46%	43%	46%
Humidity (%)	5.35	5.85	6.07	5.46	5.67	5.01
Total protein (% dry mass)	10.67	10.11	10.33	10.36	10.86	11.11
Kolbach number (%)	40.6	40.1	45.7	45.3	43.5	44.0
Tannin protein (% dry mass)	0.84	0.85	1.19	1.18	1.10	1.08
α-aminoacid nitrogen (mg/dm³)	151	168	170	195	175	191
Extract in grist (% dry mass)	74.9	74.5	79.4	79.2	77.9	78.1
Extract in flour (% dry mass)	80.8	81.2	81.9	82.1	81.3	81.4
Loosening (% dry mass)	5.9	6.7	2.5	2.9	3.4	3.3
Diastatic activity (W-K units in d.m.)	210	240	220	270	220	250
Colour (EBC units)	3.5	3.5	4.0	4.0	4.0	4.0
Viscosity (mPa · s)	1.82	1.79	1.70	1.66	1.69	1.64
Tannins (mg/dm³)	26.4	25.8	36.9	41.0	32.8	36.7
α-amylase (FS units/g dry mass)	400	460	470	570	460	530
Endo-β-glucanases (units) 100 g d.m.	93.9	115	100.0	162	95.0	120
Endopeptidases (units) 100 g d.m.	14	11.2	16	17.6	14.8	16.8

Table 3. Chemical analysis of malts produced from grain steeped to 46% himidity and malted for 7 and 9 days at 18°C

Characteristic	Barley Variety					
	Diva-days		Polon-days		Trumpf-days	
	7	9	7	9	7	9
Humidity (%)	5.53	5.94	4.64	4.92	4.93	5.16
Total protein (% dry mass)	10.45	10.29	9.96	10.46	11.14	11.03
Kolbach number (%)	40.0	39.6	46.9	46.5	44.8	44.8
Tannin protein (% dry mass)	0.89	0.84	1.15	1.04	1.02	0.90
α-aminoacid nitrogen (mg/dm^3)	134	140	168	177	166	173
Extract in grist (% dry mass)	74.8	74.6	79.3	79.1	77.7	78.1
Extract in flour (% dry mass)	80.6	80.4	82.0	82.3	80.9	81.0
Loosening (% dry mass)	5.8	5.8	2.7	3.2	3.2	2.9
Diastatic activity (W-K units in d.m.)	240	250	270	270	250	260
Colour (EBC units)	3.4	4	4	4	4	4.5
Viscosity (mPa · s)	1.75	1.74	1.64	1.60	1.60	1.59
Tannings (mg/dm^3)	28.2	32.8	45.1	45.5	40.1	41.0
α-amylase (FS units/g dry mass)	470	480	580	580	550	570
Endo-β-glucanases(units/100 g d.m.)	156	170	222	273	158	172
Endopeptidases (units/100 g d.m.)	10.0	12	12.8	14	13.6	14.8

Table 4. Chemical analysis of malts produced from Trumpf barley of 1981 crop with the use of final steeping water of variable pH. Additions of gibberellic acid (0.2 mg/kg) + $CaCl_3$ (3 g/kg) and $KBrO_3$ (0.03 g/kg). Humidity — 43%, malting duration — 5 days, temperature of malting — 14°C

Characteristic \ Sample		Control without g.a.	pH = 5.5	pH = 6.5	pH = 7.5	pH = 8.5
Humidity	(%)	6.08	5.24	4.97	5.30	5.18
Extract in flour	(% dry mass)	78.8	77.9	78.5	78.8	78.9
Extract in grist	(% dry mass)	74.6	72.3	74.0	74.0	73.8
Loosening	(% dry mass)	4.2	5.6	4.5	4.8	5.1
Wort colour	(EBC units)	4.5	3.5	4.0	4.5	4.5
Total protein	(% dry mass)	12.38	12.28	12.26	12.30	12.33
Tannin protein	(% dry mass)	1.08	1.02	1.06	1.09	1.09
Kolbach number	(%)	43.5	45.2	45.8	46.1	45.3
Tannins in wort	(mg/dm^3)	51.6	58.9	58.2	61.2	62.2
Viscosity of wort	(mPa · s)	1.50	1.50	1.51	1.61	1.59
Diastatic activity	(W-K units in dry mass)	450	450	450	460	450
α-amylase	(FS units/g dry mass)	710	640	930	900	750
Endo-β-glucanases	(units/100 g dry mass)	57.3	49.7	62.2	57.8	42.8
Endopeptidases	(units/100 g dry mass)	13.12	13.79	14.25	10.52	10.90

Table 5. Chemical analysis of malts produced from Polon, Trumpf and Diva barleys with various addition of gibberellic acid (0.2, 0.3 and 0.5 mg/kg). Humidity — 46%, malting duration — 5 days, temperature — 18°C. Control sample without g.a. was malted for 7 days

Characteristic	Control			Barley variety								
				Polon			Trumpf			Diva		
	Polon	Trumpf	Diva	0.2 mg g.a.	0.3 mg g.a.	0.5 mg g.a.	0.2 mg g.a.	0.3 mg g.a.	0.5 mg g.a.	0.2 mg g.a.	0.3 mg g.a.	0.5 mg g.a.
Humidity (%)	4.64	4.93	5.53	5.22	5.22	5.32	5.44	5.33	5.72	5.69	5.83	5.69
Total protein (% dry mass)	9.96	11.14	10.45	10.08	10.19	10.21	11.06	10.92	10.89	10.42	10.38	10.35
Kolbach number (%)	46.9	44.79	40.0	47.5	47.8	48.2	46.50	48.40	49.10	42.20	43.90	44.10
Tannin protein (% dry mass)	1.15	1.02	0.89	1.10	1.08	1.07	1.01	0.98	0.92	0.92	0.85	0.80
α-aminoacid nitrogen (mg/dm^3)	1.58	166	134	209	212	222	211	226	227	165	174	193
Extract in grist (% dry mass)	79.3	77.7	74.8	80.4	80.7	81.1	77.7	78.7	78.9	76.5	77.9	78.2
Extract in flour (% dry mass)	82.0	80.9	80.6	82.9	83.0	83.2	80.5	80.8	80.9	81.0	81.6	81.8
Loosening (% dry mass)	2.7	3.2	5.8	2.5	2.3	2.1	2.8	2.1	2.0	4.5	3.7	3.6
Diastatic activity (W-K units in dry mass)	270	250	240	350	320	300	320	310	280	300	330	280
Colour (EBC units)	4	4	3.5	4.5	4.5	5	4	4	4	3.5	3.5	3.5
Viscosity (mPa. s)	1.64	1.60	1.75	1.54	1.51	1.49	1.59	1.51	1.49	1.68	1.64	1.64
Tannins (mg/dm^3)	45.1	40.1	28.2	47.5	48.3	48.9	39.2	39.8	41.0	28.9	30.3	31.8
α-amylase (Fs units/g dry mass)	580	550	470	880	780	690	840	770	640	710	740	700
Endo-β-glucanases (units/100 g dry mass)	222	158	156	233	248	250	160	169	150	161	168	172
Endopeptidases (units/ /100 g dry mass)	12.8	13.6	10.0	13.1	13.7	13.8	13.8	14.3	14.8	10.1	10.9	11.8

7 days at 14°C gives a malt containing 7% more α-amylase than the malt from the Trumpf variety and about 20% more than malt from the Diva variety. Steeping the barley to 46% humidity prior to malting increases the content of α-amylase by about 13% compared to when the humidity stands at 43%. An increase of malting temperature from 14 to 18°C also increases the α-amylase content but to a lesser extent. The prolongation of malting from 7 do 9 days results in a small increment of α-amylase content but the the obtained malt contains about 30% grains with sprouts exceeding the length of the grains.

The content of endo-β-glucanases, similarly as that of α-amylase, was highest in Polon malt and lowest (29% less) in the malt from Diva barley. Barley with 46% humidity gives about 30% more glucanases than that with 43% humidity. The malting temperature of 18°C also boosts the amount of enzymes.

The content of endopeptidases was similar in Polon and Trumpf malts, and was lowest in the Diva malt.

An addition of malting stimulant, gibberellic acid (g.a.) clearly increases α-amylase content in the malt as well as its diastatic activity. The strongest reaction to this stimulant was displayed by the Diva variety. The optimum g.a. dose of 0.3 mg/kg of Diva barley increases α-amylase content by about 57% and the diastatic activity by about 34%. The optimum addition of 0.2 mg g.a./kg Polon barley increased α-amylase content by about 53%, the figure for Trumpf barley being about 52%.

A small increase of endo-β-glucanases and endopeptidases was found in Polon and Trumpf malts with g.a. doses of 0.2 mg/kg and in Diva malt with 0.3 mg g.a./kg. Greater differences, of about 12%, occurred in malts with 0.5 mg g.a./kg.

The results in Table 5 show that and addition of 0.3 mg g.a. per kg of Diva barley markedly improves the technological properties of the resultant malt, making it suitable for beer production under certain conditions.

CONCLUSIONS

The results of the present research justify the following conclusions:

1. As reported in literature, the variety of barley has a clear bearing on the α-amylolytic value of malt. The highest enzyme content was in the Polon variety, Trumpf followed next, and the smallest content was in the feed barley Diva.

2. Steeping of barley to 46% humidity increases α-amylase content in malts. The following conditions were found to favour α-amylase accumulation in malt without gibberellic acid: humidity of malted grain — 46%, malting temperature — 18°C, malting duration — 7 days.

3. An addition of 0.2 mg g.a./kg grain is enough to ensure high accumulation of α-amylase in malt from Polon and Trumpf barleys. For the feed barley Diva the most effective dose was 0.3 mg g.a./kg grain. The mean increase of α-amylase content in the studied barley varieties was 54% for g.a. additions of 0.2-0.3 mg/kg, and for pH of the water with g.a. ranging from 6.5 to 7.5, as compared with control samples without g.a. It must be stressed that the results are for barley from the 1982 crop (differences between the various years have been noted). The dose of 0.5 mg g.a./kg did not cause a further increase of α-amylase content, but increased the amounts of endo-β-glucanases and endopeptidases.

4. In general, the changes in endo-β-glucanases and endopeptidases contents in the experimental malts were much smaller than changes of α-amylase, and were not corelated with the α-amylase level.

5. The feed barley Diva proved the most susceptible to the stimulating effect of the g.a. dose of 0.3 mg/kg (the α-amylase content exceeded that in the control sample by 57%).

6. In the established malting conditions with an addition of gibberellic acid, the feed barley Diva may be used in the production of brewer's malt in case of a shortage of suitable brewer's barley.

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WPŁYW STOPNIA NAMOCZENIA, TEMPERATURY SŁODOWANIA I DODATKU KWASU GIBERELINOWEGO NA NAGROMADZENIE α-AMYLAZY W SŁODZIE

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Streszczenie

Do słodowania w skali mikrotechnicznej użyto krajowe odmiany browarne jęczmienia: Trumpf i Polon oraz odmianę paszową Diva ze zbioru 1982 r. Określano wpływ zmienności warunków słodowania: namoczenie jęczmienia do 43 i 46%, czas słodowania 7 i 9 dni, pH wody z dodatkiem kwasu giberelinowego (K.G.) od 5,5 do 8,5, dawki K.G. w zakresie 0,2, 0,3 i 0,5 mg/kg. W doświadczalnych jęczmieniach oznaczano: wilgotność, ekstraktywność, białko ogółem, skrobię, wagę 1000 ziaren, celność ziarna i energię kiełkowania. Analiza słodów obejmowała: wilgotność, ekstraktywność w mące i śrucie, białko ogółem, białko taninowe, barwę brzeczki, liczbę Kolbacha, garbniki ogółem, lepkość brzeczki, azot α-aminokwasowy, zawartość α-amylazy (metodą maltozową Briggsa), siłę diastatyczną według Windisch-Kolbacha, zawartość endo-β-glukanazy i zawartość endopeptydaz. Najwyższe zawartości α-amylazy (tab. 2) dawała odmiana Polon (570 j. FS), niższe Trumpf (o ok. 7%), najniższe odmiana paszowa Diva (o ok. 20%). Za korzystne warunki nagromadzania α-amylazy w słodzie bez dodatku kwasu giberelinowego (K.G.) uznano: namoczenie ziarna do wilgotności ok. 46%, temperaturę słodowania ok. 18°C i czas słodowania 7 dni (tab. 2 i 3). Przy ilościach dodawanego kwasu giberelinowego od 0,2 do 0,3 mg/kg jęczmienia i pH wody z K.G. od 6,5 do 7,5 średni przyrost zawartości α-amylazy dla badanych odmian wynosił 54% w porównaniu z próbami kontrolnymi bez K.G. (tab. 5). Dla odmiany Diva przyrost ten wynosił 57%. Opracowane warunki słodowania z dodatkiem K.G. stwarzają możliwości produkcji z jęczmienia odmiany Diva słodu piwowarskiego.