GERMINATION INHIBITION IN WHEAT AND BARLEY DURING STEEPING, AND ALPHA-AMYLASE DEVELOPMENT IN THE PRESENCE OF GIBBERELLIC ACID[1]

FELIX BLOCH AND ARTHUR I. MORGAN, JR.

ABSTRACT

Germination of wheat and barley was inhibited by turbulent agitation during steeping. The formation of alpha-amylase was induced by addition of gibberellic acid. The agitative treatment combined with elevated temperatures shortened steeping time to about one-eighth that of conventional steeping. Yields were about 6% higher. Alpha-amylase developed at an increased rate to two to three times higher levels than in green malt steeped conventionally. The capacity of the equipment for germination was increased, not only because of the possible shorter "germination" time but also because, in the absence of growth of rootlets, no increase in bulk volume in the green malt stage took place. The process was demonstrated in a pilot plant designed for ⅓-bu. capacity.

The biochemical changes of grain to malt have been effected traditionally by germination. This plant-physiological process is inherently connected with losses of substance due to steeping, respiration, and growth of rootlets, which are an accessory by-product of germination and removed from the malt after drying (see table below). The substance lost varies with temperature, moisture

	%
Steeping loss	1.0–1.5
Respiration loss	4.5–8.0
Root loss	2.5–4.5
Total	8–14

content, oxygen supply, and duration of malting; the losses are larger when a higher degree of enzyme formation is to be attained.

Efforts to reduce malting losses have been made over the years, and numerous chemical and physical treatments for partial suppression of rootlet development have been suggested (1). Recent patents involve: physical impact during more than one-fifth of the total germination time (2), application of ammonia during the germination (3), and acidulation with sulfuric acid (4). Growth of the embryo was completely prevented by freezing of steeped barley, and enzyme development was induced by gibberellic acid (5).

The multiple-steeping method is being practiced in some malting plants. It employs alternating steeping, air-rest, and resteeping stages, some of which may use warm water (6). It is designed to allow the embryo to produce and secrete gibberellins into the endosperm after the initial steeping; subsequent re-

[1]Manuscript received May 26, 1966. Presented at the 51st annual meeting, New York City, April 1966. Contribution from the Western Regional Research Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif. 94710.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

steeping treatments suppress further germination during the final air-rest period.

Fungal gibberellic acid (GA3) is applied sometimes for increasing the rate of enzyme formation during the malting process. The growth of rootlets is not enhanced by exogenous gibberellic acid, and, therefore, for a comparable modification, germination times and malting losses are decreased.

The endogenous formation of gibberellins and their stimulating effect on alpha-amylase formation in germinating barley and wheat was first suggested by Yomo in 1958 (7). Since then a chain of investigations has demonstrated that the aleurone layers were the main sites of enzyme formation (8) and that amylolytic and proteolytic enzymes were formed in barley kernels detached from their embryo protions (9). Finally, the alpha-amylase formation was shown to represent a *de novo* synthesis taking place in the aleurone layer (10). The large field of plant-physiological effects of gibberellins was reviewed recently by Paleg (11).

Thus, the possibility was envisaged of malting without germination, provided that a technically feasible process for inhibiting germination were found (12,13). We are presenting in this paper such a process of inhibiting germination of wheat and barley by the effect of steeping under turbulent agitation, followed by treatment with gibberellic acid to induce enzyme fomation.

Equipment

The pilot plant was designed for malting of ⅓ to ½ bu. A steeping tank for conventional steeping and a malting drum are placed in a temperature-controlled room. Auxiliary equipment outside this room provides the moisture-saturated, chilled air for the malting drum. The tank for the agitative steeping treatment (Fig. 1) can be heated to controlled temperatures. The processing equipment is of stainless steel; it is described below in more detail.

Steeping Tank for Conventional Steeping. Diameter 16 in., height 18 in., to overflow 16 in.; equipped with air sparger (circle, 16 holes of 0.04-in. diameter). A 15¾-in.-diam. × 20-in. screen basket contains the grain during steeping and weighing.

Tank for Agitative Steeping (Fig. 1). Diameter 18 in., height 18 in., to overflow 15 in. Air sparger consists of three circles with a total of 84 holes of 0.04-in. diameter. The agitation is provided by a Lightnin' Mixer (Model D3, ⅓ h.p.), turning with 350 r.p.m. The 7-in. propeller, with ring guard, is screened with a 14-mesh, 0.02-in. wire screen. A steam coil provides controlled-temperature heating. The same screen basket as in the tank for conventional steeping holds the grain.

Germination Drum. Twelve-inch-diameter, 24 in. long, equipped with ⅓ h.p. variable-speed drive, set for 1 r.p.h. The aeration system is stationary in the bed, while the drum is turning. The germination drum, including the drive, is placed on a platform scale so that the operator can observe any weight changes during the germination period.

Equipment for Supply, Cooling, and Moisture-Saturation of Air. A spray column, 3 in. in diameter, 6 ft. high, and a column, 6 in. in diameter, 6 ft. high, packed with ceramic Raschig rings, were used to humidify the air stream passed into the drum. A refrigerated water bath, 50-gal. capacity, ¼ h.p. refrigeration, supplied chilled water to the column.

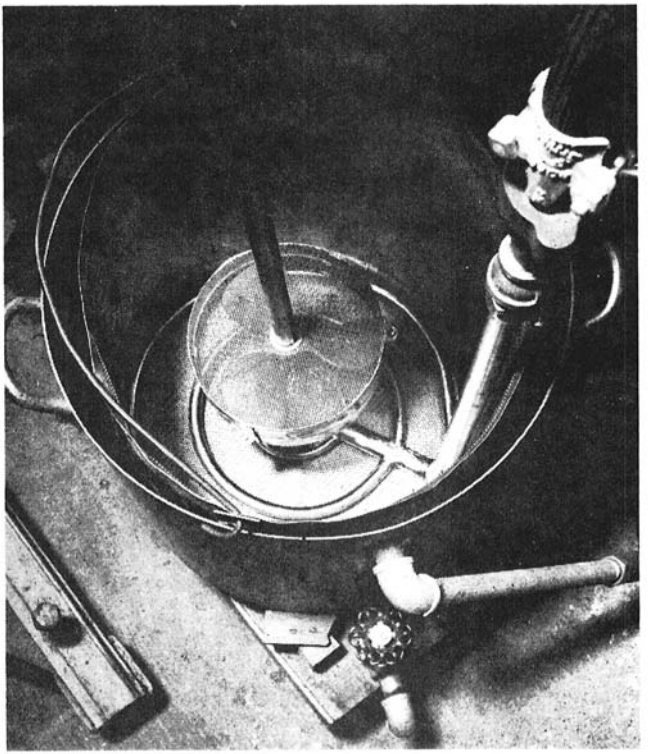

Fig. 1. Tank for steeping under turbulent agitation.

The oil-free air first flows through the spray column against a spray of tap water and then through the packed column against refrigerated water. The moisture-saturated air leaving the packed column is controlled to ± 1°F.

Materials

The wheat and barley used for malting and some analytical data are listed in Table I.

The gibberellic acid was applied as potassium salt of GA3, a commercial product of fungal origin ("Gibrel" 80%, Merck & Co.). An aliquot part of an aqueous solution of 60 mg. in 1,000 ml. was applied where indicated.

The water for the processing came from the Municipal District supply ranging in pH from 9.5 to 8.6; the total dissolved solids varied from 24 to 120 mg./liter. The water temperature varied over the year from 54° to 70°F.

Sodium hypochlorite N.F. (4–6% NaOCl) was applied for chlorination during steeping as indicated.

TABLE I
WHEAT AND BARLEY. ANALYTICAL DATA

		Moisture	Test Weight	1,000-Kernel Weight	Nitrogen (as-is basis)
		%	lb./bu.	g.	%
Hard red winter wheat	Kansas	11.0	62.7	32	2.11
Hard red spring wheat	North Dakota	10.2	61.6	...	2.65
White club wheat	Washington	11.2	63.2	31	1.48
Hannchen barley	Klamath, Calif.	11.1	54.2	39	1.97
Winter Tennessee barley	California	9.3	52.3	...	1.35

Analytical Methods

AACC methods were applied unless indicated otherwise (14). The assays of alpha-amylase in green malt were modified by performing the grinding and extraction for 2 min. in the Waring Blendor. The yields of malt were estimated from the weight and solids content of the dried malt, obtained by air-drying the green malt in about 7 hr., using the following drying schedule: 110°F. for 2 hr.; 135°F. until about 10% moisture, that is, for 2–2.5 hr.; and 165°F. until about 6% moisture, that is, for 2.5–3 hr.

The yield on malt sprouts (if any) was estimated by drying the green malt, and separating the sprouts on a vibrating shaker.

Steeping losses were estimated by evaporating to dryness 250-ml. aliquots of the steep water in a thin film evaporator (water bath temperature: 52°C., vacuum: 29 in.), and weighing the residue.

Processing Methods

Conventional Steeping. Wheat (10–20 lb.) was steeped under aeration for 20–24 hr., to 42–43% moisture. During the first hour, the wheat was washed in running water; then, sodium hypochlorite solution was added (70–150 p.p.m. chlorine in the steeping water). Water of ambient temperatures was used for steep-in and washing; steeping temperatures of 59°F. were reached as the room temperature was controlled to 57°–59°F.

Barley (10–20 lb.) was steeped under aeration for 43–46 hr., to 45% moisture. Water changing and chlorination at the start were the same as with wheat, and were repeated after 24 hr.

Steeping under Turbulent Agitation. We observed that when turbulent agitation was applied during the steeping procedure, the germination of the grain was delayed or inhibited; at elevated temperatures the inhibition was achieved in shorter time.

The following experiment illustrates the germination-inhibiting effect of the agitative treatment.

Hard red winter wheat was steeped for 17 hr. at 54°–58°F., to 38.9% moisture, and then subjected to turbulent agitation at 54°–61°F. Samples

were removed after 0.5, 1, 1.5, and 2 hr. of agitation, placed between blotters in Petri dishes, and kept moist for germination at 72°F. The turbulent agitation inhibited the development of rootlets in proportion to the duration of the agitation (Fig. 2); acrospire growth was impaired less.

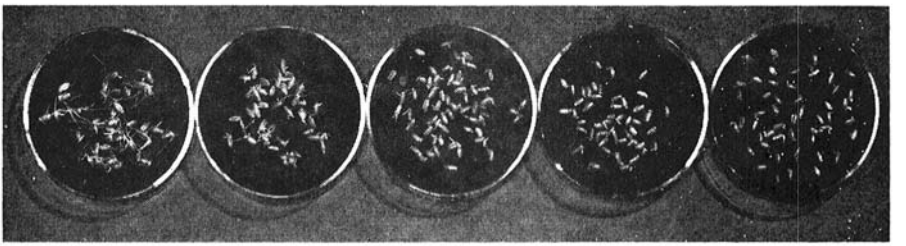

Fig. 2. Effect of turbulent agitation on sprouting, after 3 days of germination. Wheat was steeped to 39% moisture, subsequently agitated for (L to R) 0, 0.5, 1, 1.5, and 2 hours, and placed for germination between moist filter papers.

The agitative treatment as part of the steeping process can be shortened by raising the temperature. There is an upper temperature limit, as shown by the fact that a winter Tennessee barley agitated at 135°F. for 3 hr. to 44.3% moisture and treated with gibberellic acid (2 p.p.m.) did not show any cytolytic modification, and did not produce any alpha-amylase during 4 days in the germinating drum at 57°F.

When the turbulent agitation was started immediately with the steep-in, some abrasion was observed; barley is less subject to abrasion than wheat. The abrasion can be practically eliminated and the agitative treatment shortened by steeping first to about 40% moisture before agitation begins.

A longer agitative treatment and higher temperatures were necessary to suppress the germination of barley, as compared to wheat.

After the agitative steeping, gibberellic acid (2 p.p.m. on barley or wheat basis) was added, generally as a spray after the steeped wheat (or barley) was transferred to the germination drum. The green malt was aerated (5 c.f.m.) continuously while in the drum for 4 to 9 days.

Results

After the agitative treatment, the total solids content in the steep water from both wheat and barley was higher by about 25%. The increase in steeping loss from 1 to 1.25% is insignificant and, because of the extracting effect on components of the husk, may even be beneficial.

The alpha-amylase formation was chosen as index for the progress of malting; the endosperm became friable, giving evidence for the progress of modification. The sprouting was inhibited.

Figure 3 shows the development of alpha-amylase in green malt of hard red spring wheat after conventional steeping, and after agitative steeping with and without addition of gibberellic acid. The onset of germination was observed only after 4 days in the green malt steeped with agitation at 110°F. for 2 hr.

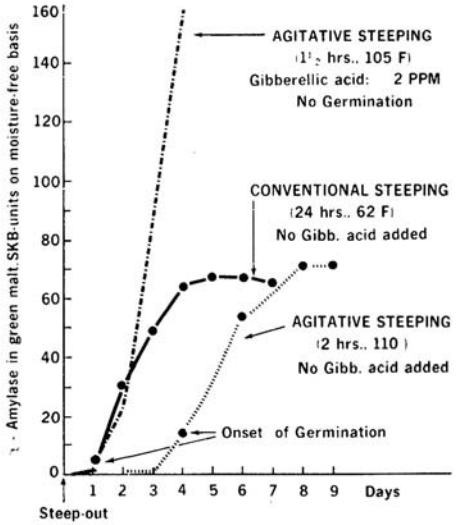

Fig. 3. Alpha-Amylase development in green malt (HRS wheat).

The effect of the 2-p.p.m. gibberellic acid spray is striking: in 4 days, while the germination was inhibited, 2.5 times more alpha-amylase formed than in conventional green malt after 7 days of germination, 8 days from steep-in. The higher rate of alpha-amylase formation induced by gibberellic acid after germination inhibition by the agitative steeping technique was found in every case.

Data on malting a barley without germination are presented in Table II.

TABLE II
MALTING OF BARLEY WITHOUT GERMINATION

	Moisture	Maltose Equivalents[a]	Alpha-Amylase[a]
	%		*SKB units*
Barley (Hannchen)	11.1	57	...
After 4-hr. steeping (110°-114°F.)	42.2	...	...
After 4-hr. agitation (additional) (110°-114°F.)	45.5	...	...
Green malt (days after steep-in)			
1	45.1	53	...
2	45.3	79	3
3	44.0	154	35
4	43.9	231	72
5	43.3	279	99

[a]Moisture-free basis.

Table III lists the results of malting experiments with several classes of wheat and with barley, using different temperatures and sequences in the agitative steeping process, in comparison to conventional malting. By combination of steeping and turbulent agitation at elevated temperatures, the steeping times were shortened to a fraction as little as one-fifteenth of conventional

TABLE III
INHIBITION OF GERMINATION BY STEEPING UNDER TURBULENT AGITATION: EXOGENOUS GIBBERELLIC-ACID-INDUCED ENZYME FORMATION

	Steeping Treatment			G.A.	Germination at 60°F.	Yield of Malt	Yield of Sprouts	Alpha-Amylase in Green Malt
		hr.	°F.	*p.p.m.*	*days*	*% d.b.*	*% d.b.*	*SKB units (d.b.)*
HWR wheat	1 Steeped	23	61	0	5	89	4	76
	2 Agitated	4	55–63					
	Steeped	3	62	2	4	96	0	101
	3 Steeped	1	56					
	Steeped	3	105					
	Agitated	1	105	2	4	96	0	131
HRS wheat	4 Steeped	24	58–62	0	5	90	4	50
	5 Agitated	1.5	105	2	4	96	0	158
White club wheat	6 Steeped	20	58	0	6	90	3.5	43
	7 Agitated	0.5	55					
	Agitated	1	108	2	4	95	0	131
Hannchen barley	8 Steeped	4	110–114					
	Agitated	4	110–114	2	4½	96.4	0	99

steeping times. Up to three times more alpha-amylase formed in 4 days than in 5 days on conventional germination. With no sprout formation, the yield of malt of much higher alpha-amylase level was increased by an average of 6%. If it were desired to produce malt of the alpha-amylase level of conventional malt, the yield increase could be still higher, or the "germination" time still shorter. The shorter steeping and "germination" times can be of special advantage in a continuous malting process.

While the bulk volume of conventional green malt increases by more than 50% during the germination because of the growth of rootlets, the volume did not increase when the sprouting was inhibited; therefore, the capacity of the germination equipment became accordingly higher.

Discussion

Though the germination-inhibiting effect of the turbulent agitation during steeping became evident, the mode of the inhibiting action is not clear.

The following factors could have taken part in the inhibition: 1) elevated temperature, 2) lack of oxygen, 3) mechanical damage to the embryo, 4) effect of chemical germination inhibitors, and 5) loss of essential materials.

1. Though elevated temperatures during the steeping agitation could be advantageously used, the agitative treatment at normal steeping temperature was also effective (see Table III, example 2).

2. The germination inhibition by the agitative steeping took place with or

without aeration during the turbulent agitation; the air sparger (Fig. 1) is capable of supplying an excess of air for saturation of the steep water even without agitation.

3. Mechanical damage of the shoot-root axis of the embryo by the turbulent agitation must be suspected. However, agitation of shorter duration at elevated temperatures was more inhibitive than longer agitation at lower temperatures. Mechanical damage should be inflicted in direct relation to the duration of the treatment.

4. Germination inhibitors may be present in the outer layers of barley (15,16). In fact, in germination tests between layers of moist filter paper, after soaking the kernels for 30 min. in steep water concentrates (1:25), we observed a decline of germination energy. During the turbulent agitation, because of the increased rate of diffusion, these inhibitors may find their way to susceptible organelles essential to normal germination.

5. A loss of essential materials by increased elution, or by a higher metabolic rate due to the agitation and elevated temperatures, cannot be precluded.

Thus, the cause of the germination-inhibiting effect of the turbulent agitation is obscure. The increased rate of diffusion may produce conditions analogous to "water sensitivity" which may interfere with the uniform, rapid germination desired in conventional malting. Though the causes of water-sensitivity in malting have not been elucidated in spite of extended investigations, the difficulties it produces have been overcome in conventional malting technology by selection of malting barleys and a design of imbibition with alternating periods of steeping, aeration, and resting without water, so that the steeped kernel attains a water distribution optimal for fast germination.

Perhaps the turbulent agitation process may be considered a design for inhibiting germination by purposely generating conditions analogous to water-sensitivity.

Acknowledgments

The analytical work was performed by Nelson J. Tennis and members of an analytical group of this laboratory.

Literature Cited

1. POLLOCK, J. R. A. The nature of the malting process. *In* Barley and malt, ed. by A. H. Cook, chap. 8, pp. 303–398. Academic Press: New York and London (1962).
2. BECKORD, L. D., and FLECKENSTEIN, J. G. Malting process utilizing physical impact of kernels. U.S. Patent 3,174,909 (1965).
3. BECKORD, L. D., and FLECKENSTEIN, J. G. Malting process. U.S. Patent 3,134,724 (1964).
4. LUCHSINGER, W. W., and FLECKENSTEIN, J. G. Malting process. U.S. Patent 3,085,945 (1963); KNEEN, E., and FLECKENSTEIN, J. G. Malting process. U.S. Patent 3,149,053 (1963).
5. SMITH, L. F., LINKO, M., ENARI, T-M., and DICKSON, A. D. Modification of barley after embryonic destruction by freezing. Am. Soc. Brewing Chemists, Proc. 1964, pp. 86–89. (Abstr., J. Inst. Brewing **72**: 208. 1966.)
6. LUBERT, D. J., and POOL, A. A. Studies in barley and malt. **XXII.** Effect of

elevated temperatures during multiple steeping. J. Inst. Brewing **70:** 145–155 (1964).
7. YOMO, J. Barley malt. Sterilization of barley seeds and the formation of amylase by separated embryos and endosperms. Hakko Kyokaishi **16:** 444–448 (1958). (Chem. Abstr. **54:** 1669e.)
8. MACLEOD, ANNA M., and MILLAR, A. S. Effects of gibberellic acid on barley endosperm. J. Inst Brewing **68:** 322–332 (1962).
9. PALEG, L. G., and SPARROW, D. H. B. Gibberellic acid and the malting of embryoless barley. Nature **193:** 1102 (1962).
10. VARNER, J. E., and RAM CHANDRA, G. Hormonal control of enzyme systems in barley endosperm. Proc. Nat. Acad. Sci. U.S. **52**(1): 100–106 (1964).
11. PALEG, L. G. Physiological effects of gibberellins. Ann. Rev. Plant Physiol. **16:** 291–322 (1965).
12. FLEMING, J. R., and JOHNSON, J. A. Some recent advances in the chemistry of malting. Cereal Sci. Today **9:** 67, 68, 88 (1964).
13. LAUFER, S. New concept regarding mechanism of malt modification. Am. Brewer **97:** 75–77 (1964).
14. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minn. 1962).
15. VAN LAER, M., and FROSCHEL, P. Les substances freinantes et leur role dans la germination de l'orge. Rev. Ferm. Ind. Alim. **1:** 177–181 (1946).
16. FROESCHEL, P. Le procede de freinage biologique en malterie. Congr. Intern. Ind. Ferment., Ghent, Belg., pp. 195–201 (1947).