New Enzymes for Brewing: Promozyme, a Debranching Enzyme and SP-249, a Glucanase[1]

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ABSTRACT

Two newly developed polysaccharide-degrading enzymes were evaluated. One, a pullulanase, Promozyme™, can be used alone during mashing, wort treatment, or fermentation to increase the fermentability of wort. When used during fermentation in combination with a fungal α-amylase, Fungamyl®, apparent attenuations above 100% can be reached. The other enzyme, a multicomponent carbohydrase, SP-249, degrades the nonstarch polysaccharides of barley and malt, resulting in reduced wort viscosity and improved runoff. SP-249 is more efficient than commercial β-glucanases.

Key words: Low-carbohydrate beer, Multicarbohydrase, Runoff, Saccharifying enzymes, Wort viscosity

In the production of low-carbohydrate beer, the application of external saccharifying enzymes during mashing or fermentation has gained preference over traditional methods such as extension of the saccharification rest, use of high diastatic malt during fermentation, and addition of sugar or glucose syrup to the wort.

Glucoamylases, in particular, have been used extensively. Low dosages of glucoamylase added during fermentation hydrolyze all dextrins of the wort to glucose, which is fermented. However, the enzyme is relatively heat stable and will not be inactivated under the conditions normally used in the pasteurization of beer.

This paper describes laboratory studies with a recently developed pullulanase, Promozyme™. This pullulanase, in combination with a fungal α-amylase, Fungamyl®, has been added to wort during fermentation. The proportion of the two enzymes has been optimized on wort produced from a 100% malt grist. An apparent attenuation above 100% can be reached with this enzyme system. These enzymes are less heat stable than glucoamylase and are inactivated under reasonable pasteurization conditions. To be rendered inactive, the glucoamylase AMG requires approximately 1,200 pasteurization units (PU) whereas Promozyme and Fungamyl need only about 80 and 10 PU, respectively (4).

The effect on wort when AMG, Fungamyl, and Promozyme are applied separately is also discussed. Promozyme, in particular, has a significant effect because the content of nonfermentable sugars is reduced about 25% by a 1-hr treatment of the wort at 60°C.

The mode of action of the saccharifying enzymes (3,6) can be summarized as follows: Starches of the common brewing raw materials consist of amylopectin (75–85%) and amylose (15–25%) (Fig. 1). The 1,6-α-glucosidic linkages of amylopectin act as a barrier to the action of exoacting saccharifying amylases such as malt β-amylase. Limit dextrinases of malt are very thermolabile. Malt α-amylase, an endoacting enzyme, can bypass the branching points but cannot hydrolyze the 1,6-α-glucosidic linkage. Thus, several branched dextrins are present in wort unless external enzymes that can degrade the 1,6-α-linkage are used. AMG and Promozyme are two such enzymes.

AMG, an exoacting glucoamylase, hydrolyzes the 1,4-α-linkage, but it can also slowly hydrolyze the 1,6-α-linkage, the reaction product being glucose. Promozyme has a specific action on the 1, 6-α-linkages of amylopectin and branched dextrins, with a side chain having more than a single glucose unit. In combination with malt β-amylase or fungal α-amylase (eg, Fungamyl), Promozyme hydrolyzes dextrins to fermentable sugars, mainly maltose, but glucose and maltotriose are also formed.

Investigations of the use of AMG, Fungamyl, and a debranching enzyme (DBE) identical to Promozyme for production of low-carbohydrate beer have been published (5). The enzymes were added during mashing or during fermentation of a wort resulting from 50% malt and 50% barley. When AMG or Promozyme was used during mashing, a significant amount of the dextrins was hydrolyzed into fermentable sugars. The carbohydrate spectra contained 89% fermentable sugar, mainly glucose, when AMG was used, and 92% fermentable sugar, mainly maltose, when Promozyme was used.

The application of Promozyme, Fungamyl, and AMG during fermentation increased the apparent attenuation from 82% to 86, 90, and 104%, respectively. With a combination of Promozyme and Fungamyl, it was increased to approximately 103%. The dosages used to obtain a specific fermentability during fermentation generally are only 3–6% of those needed during mashing.

Glucan-Hydrolyzing Enzymes

Polysaccharides, such as β-glucans and pentosans, known to be present in viscous worts, cause slow rates of runoff from the mash tun and create problems in the filtration of beer. The important β-glucan-degrading enzymes of the malt are very heat labile, so the degradation of β-glucans during malting is of vital importance unless an external β-glucanase is used. Barley pentosans contain a backbone of xylan to which side chains of single arabinose units are attached. They are sparingly degraded by pentosanases during malting (6).

Use of barley or malt in which the β-glucans are not fully degraded leads to a loss of extract. This is caused not only by the presence of the high-molecular-weight glucans and pentosans in the wort but can also be attributed to the remaining cell wall material, preventing a complete liberation and degradation of the starch. Therefore, if barley or undermodified malt is used in the mash, sufficient enzymes must be supplied in the mash tun. The application of bacterial and fungal β-glucanases has been practiced for the last 10 to 15 years.

The β-glucanases Cereflo® and Finizym™ and the cellulase Celluclast™ have been described previously (7). SP-249, a newly developed enzyme that degrades plant cell walls was only recently tested for brewing applications.

Whereas starch has α-links, the nonstarchy polysaccharides of

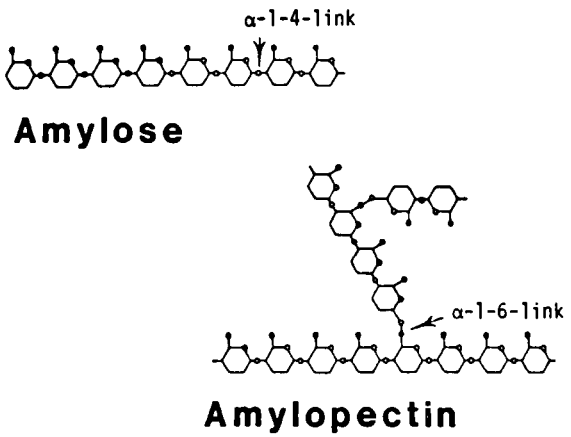

Fig. 1. Structure of amylose and amylopectin.

[1]Presented at the 49th Annual Meeting, Nashville, TN, April 1983.

malt and barley are β-linked polymers (2), (Fig. 2). Beta-glucan is essentially a linear polymer with 1,4-β- and 1,3-β-glucosidic linkages. About 70% of these links have the 1,4- link, whereas the remaining 30% have the 1,3 configuration. Barley pentosans contain a backbone of 1,4-β-linked xylose units, to which side chains of single 1,3-β-linked arabinose units are attached. Although the barley husk contains some β-glucans and pentosans, those that are important in liberating the extract are located in the cell walls of the endosperm. The main β-glucan-degrading enzymes of malt are endo-1,4-1,3-β-glucanases. Disaccharides, trisaccharides, and oligosaccharides are produced from the β-glucan. The action pattern of Finizym and SP-249 is similar, but SP-249 contain exo-β-glucanase activity as well, resulting in the formation of minor amounts of glucose. Cereflo and Celluclast contain only 1,4-β-glucanase activities. Celluclast contains endo- as well as exoacting β-glucanases, whereas Cereflo has no exoglucanase.

The external enzymes generally are more heat stable than the malt glucanases, which are heat labile (2). Besides the β-glucanase activities, Finizym and Celluclast contain minor xylanase activity. SP-249 contains significant xylanase activity as well as arabinase, galactomannanase, and pectolytic activities (1).

SP-249 has proved to be a superior glucanase in reducing the viscosity of the wort and in increasing the filtration rate.

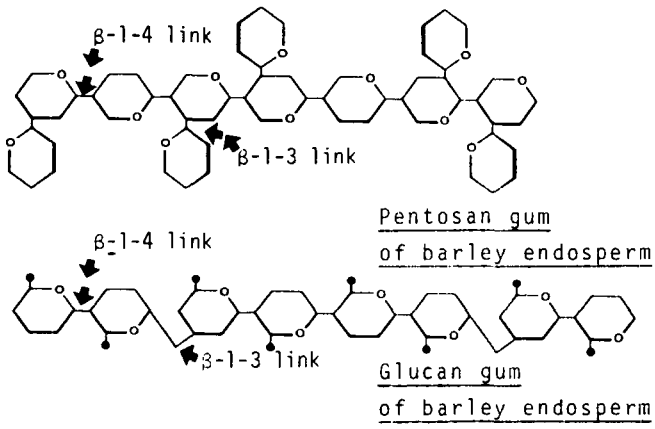

Fig. 2. Structure of barley endosperm gums.

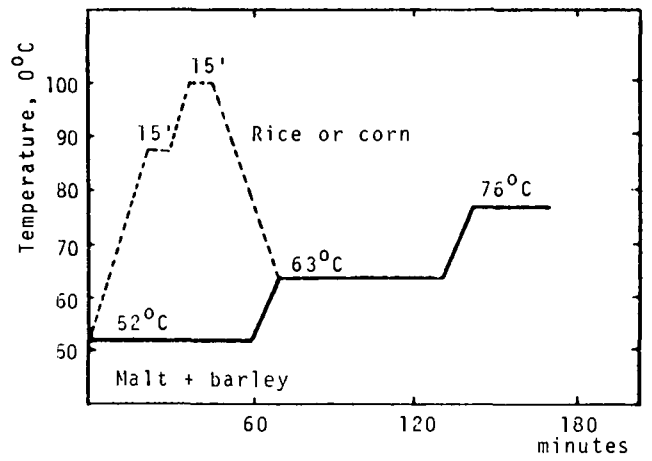

Fig. 3. Standard mashing procedure used for laboratory mashing trials.

EXPERIMENTAL

Enzymes

The saccharifying enzymes we used were Promozyme 200L, *Bacillus* sp. pullulanase (200 PUN/g);[2] Fungamyl 800 L, *Aspergillus oryzae* α-amylase (800 FAU/g);[2] AMG 150 L, *Aspergillus niger* glucoamylase (150 AGU/g).[2] The glucanases used were SP-249 *Aspergillus* sp. multicomponent carbohydrase Celluclast 1.5 L, *Trichoderma reesei* cellulase (1,500 NCU/g);[2] Cereflo 200 L, *Bacillus subtilis* β-glucanase (200 BGU/g);[2] Finizym 200 L, *Aspergillus nuger* β-glucanase (200 FBG/g).[2]

The above enzymes were produced by Novo Industri A/S. All are commercially available, except SP-249, which at present is an experimental product.

All our standard procedures were published previously (4,5,7). When rice or corn grits were part of the grist, they were liquefied separately with the thermostable bacterial α-amylase Termamyl before the malt mash was added (Fig. 3).

Two series of trials have been made with the saccharifying enzymes. In one trial, Fungamyl and Promozyme were added in different dosages at the beginning of the fermentation to a wort prepared from 100% malt (two-rowed barley malt). In another trial, AMG, Fungamyl, and Promozyme were added to the wort prepared from 100% malt or from 70% malt and 30% rice just after filtration. The reaction time was 1 hr at 65, 55, and 60°C, respectively. The enzymes were then inactivated by boiling for 10 min and the carbohydrate spectra determined by high-performance liquid chromatography.

Three series of trials with SP-249 have been made. In one series, SP-249 was added to a grist–water mash (1:5.5) consisting of malt or 50:50 malt and barley. In another series, the ratio of malt to water was 1:4.2. In the first and the second series of trials the enzyme was added at mashing-in, and our standard mashing procedure was used. In the third series of trials, the effect of SP-249 was compared to that of other glucanases. The enzymes were added either at mashing-in of our standard mashing, or they were added at mashing-off (ie, at 76°C). In this case the mashing-off was prolonged to 90 min. The barley used was Danish two-rowed malting barley. The malt was produced from the same type of barley. Our main consideration in these trials was viscosity, extract, and filtration rate of the wort produced.

RESULTS AND DISCUSSION

Trials with Saccharifying Enzymes

Table I shows the pH and temperature optima of the saccharifying enzymes under analytical conditions. The activities of the enzymes at 25 and 10°C are 10–20% and 5–10% of the maximum activities, respectively. Table II shows the enzyme combinations and dosages of Promozyme and Fungamyl used in our trials. Table III shows the apparent attenuation achieved with the different enzyme dosages. Neither Promozyme nor Fungamyl when used alone can increase the apparent attenuation above

[2] Enzymes were measured according to Novo's own methods, copies of which may be obtained on request.

TABLE I
pH and Temperature Optima of Saccharifying Enzymes Under Analytical Conditions

Enzyme	pH Range[a]	Temperature Range[a]
AMG	3.5–5.0 (55°C, 30 min)	65–75°C (pH 4.5, 30 min)
Fungamyl	4.0–6.0 (37°C, <20 min)	45–60°C (pH 4.7, <20 min)
Promozyme	4.3–6.0 (60°C, 30 min)	45–65°C (pH 5.0, 30 min)

[a] More than 80% of the potential enyzme activity was observed to be effective within the indicated range.

approximately 90%, because both enzymes are limited in their action on dextrins to the 1,6-α- and 1,4-α-amylase activities, respectively. However, when added together they increase the fermentability of the beer significantly, and apparent attenuations above 100% can be reached. The apparent attenuation of wort not enzyme treated is 83.5.

Figure 4 illustrates our results graphically. It shows that the highest degree of apparent attenuation was achieved with 24.0 g of Promozyme 200 L and 2.5 g of Fungamyl 800 L. Based on weight, the optimum ratio of Promozyme 200 L to Fungamyl 800 L is 9–10:1. If an apparent attenuation of 100% is desired, it can be obtained with ratios of Promozyme 200 L to Fungamyl 800 L between 3:1 and 12:1. The optimum ratio still is 9–10:1, as it gives 100% apparent attenuation by addition of 15.6 g of Promozyme 200 L + 1.6 g of Fungamyl 800 L grams per hectoliter.

The effect of the saccharifying enzymes during treatment of wort is illustrated in Figs. 5 and 6. The enzymes were allowed to react for 1 hr at their optimum temperatures on a wort produced from 100% malt or from 70% malt and 30% rice. On wort produced from all malt, Fungamyl at low dosage rates converted more dextrins to fermentable sugars than did AMG and Promozyme, whereas AMG and Promozyme at all dosage rates were superior to Fungamyl on wort from malt and rice. Promozyme generally was superior to AMG and Fungamyl. However, compared to the conversion when adding the enzymes during fermentation, the effect during wort treatment under the applied conditions was rather limited. About 25% of the dextrins were saccharified with Promozyme at a dosage five times higher than that used when the enzymes were added during fermentation. However, a prolonged reaction time would undoubtedly increase the formation of fermentable sugars.

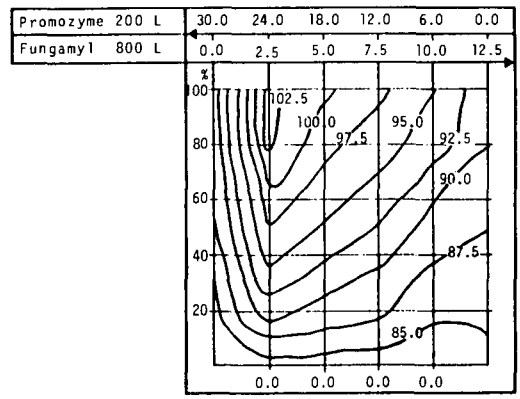

Fig. 4. Apparent attenuation as a function of enzyme combination and dosage. The apparent attenuation of wort not enzyme treated is 83.5.

TABLE II
Enzyme Combinations and Dosages[a] of Promozyme 200 L and Fungamyl 800 L Added at Start of Fermentation

Percentage[b]		Enzyme Combinations					
100%	Promozyme	30.0	24.0	18.0	12.0	6.0	0.0
	Fungamyl	0.0	2.5	5.0	7.5	10.0	12.5
80%	Promozyme	24.0	19.2	14.4	9.6	4.8	0.0
	Fungamyl	0.0	2.0	4.0	6.0	8.0	10.0
60%	Promozyme	18.0	14.4	10.8	7.2	3.6	0.0
	Fungamyl	0.0	1.5	3.0	4.5	6.0	7.5
40%	Promozyme	12.0	9.6	7.2	4.8	2.4	0.0
	Fungamyl	0.0	1.0	2.0	3.0	4.0	5.0
20%	Promozyme	6.0	4.8	3.6	2.4	1.2	0.0
	Fungamyl	0.0	0.5	1.0	1.5	2.0	2.5
0%	Promozyme	0.0	0.0	0.0	0.0	0.0	0.0
	Fungamyl	0.0	0.0	0.0	0.0	0.0	0.0

[a] Dosages are in grams per hectoliter of wort.
[b] Percentage of dosages mentioned as 100%.

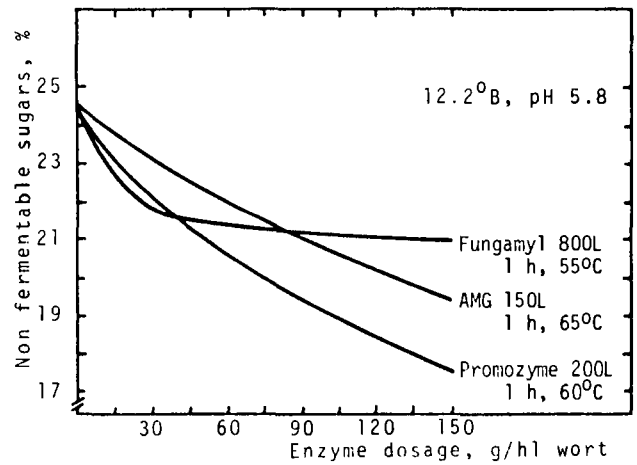

Fig. 5. Effect of saccharifying enzymes on wort produced from 100% malt.

TABLE III
Apparent Attenuation[a] of Beer Treated with Promozyme and Fungamyl During Fermentation

Percentage[b]	Wort[c] (11.93°B, from 100% malt)					
100	88.2	103.0	100.5	98.0	94.9	90.0
80	87.2	103.0	98.2	96.1	93.6	90.1
60	87.1	98.8	96.0	93.7	90.0	88.4
40	85.2	95.7	92.7	90.6	87.6	86.7
20	83.7	91.0	88.7	88.0	85.3	86.1
0	83.5	83.5	83.5	83.5	83.5	83.5
Promozyme[d]	30.0	24.0	18.0	12.0	6.0	0.0
Fungamyl[d]	0.0	2.5	5.0	7.5	10.0	12.5

[a] Apparent attenuation: (SG Wort − SG Beer)/(SG Wort − 1,000) × 100.
[b] Percentage of dosages mentioned as 100%.
[c] Fermented at 25° C for five days with 3 g of yeast per 250 ml of wort.
[d] Grams per hectoliter.

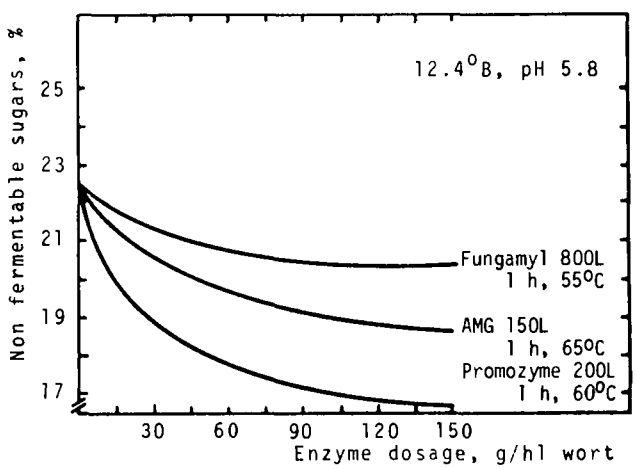

Fig. 6. Effect of saccharifying enzymes on wort produced from 70% malt and 30% rice.

Trials with the β-Glucanases

Table IV shows the pH and temperature optima of the β-glucanases under analytical conditions. Figures 7 and 8 show the effect of SP-249 on the viscosity, extract, and filtration rate of worts produced from 100% malt and from malt–barley (50:50). The enzyme reduces the viscosity of the worts considerably: 100% malt wort was reduced from about 1.45 centipoise (cP) to 1.3 cP. Correspondingly, the viscosity of the 50:50 malt–barley wort is reduced from 1.55 cP to 1.25 cP. The extract yields are somewhat increased, and the filtration rates are increased significantly.

Table V shows the results of adding SP-249 to a malt and a 50:50 malt–barley mash with increased ratio of grist to water (1:4.2), as well as of adding both SP-249 and a bacterial glucanase, Cereflo. When used alone, SP-249 increased filtration rates and reduced viscosity of the worts. When used with the bacterial enzymes, extract increased considerably. The combinations of enzymes were superior to any of the enzymes used alone, because the enzymes had different specificities.

A similar but less pronounced effect was observed with Celluclast and Cereflo.

Figure 9 illustrates the results of our comparison of different glucanases for their temperature stability when added to a mash of 20% malt and 80% barley at different stages during mashing. The results show that SP-249 is superior to the other glucanases in reducing the viscosity of wort when added at mashing-in, whereas Celluclast and Cereflo appear to be more active and stable when

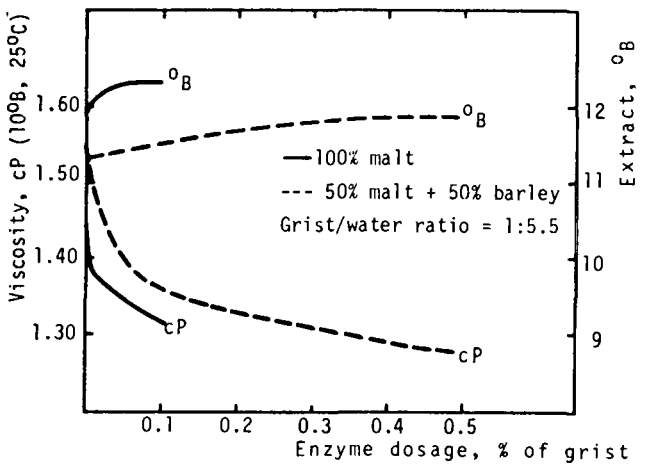

Fig. 7. The influence of SP-249 on the viscosity (cP) and extract (° B) of wort produced from 1:5.5 grist-to-water mash.

TABLE IV
pH and Temperature Optima of β-Glucanases Under Analytical Conditions

Enzyme	pH Range[a]	Temperature Range[a]
SP-249	3.8–5.8 (30°C, 30 min)	45–65°C (pH 5.0, 30 min)
Celluclast	4.2–6.8 (50°C, 20 min)	55–70°C (pH 4.8, 20 min)
Cereflo	5.8–8.5 (30°C, 30 min)	30–70°C (pH 7.5, 30 min)
Finizym	3.4–5.8 (30°C, 30 min)	45–65°C (pH 5.0, 30 min)

[a]More than 80% of the activities measured optimized within the ranges shown for pH and temperature.

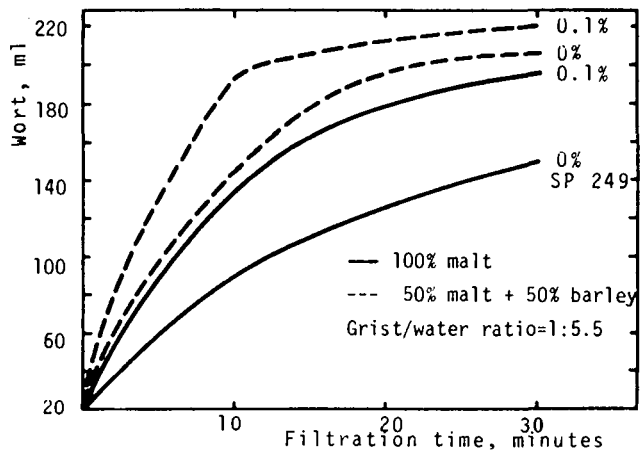

Fig. 8. The influence of SP-249 on the filtration rate of wort produced from a 1:5.5 grist-to-water mash.

TABLE V
Effect of SP 249, Alone and in Combination with Other Glucanases

Mash[a]	Enzyme Added	Percent of Total Grist	Wort Filtration Rate[b] (ml wort/indicated time) 10 min	20 min	30 min	Final	Wort Extract °B	Wort Viscosity (cP)
100% Malt	None	...	80	115	128	182	15.7	1.38
	SP-249	0.01	100	128	153	188	15.7	1.37
		0.05	130	145	170	185	15.7	1.33
	Cereflo 200 L	0.1	80	119	140	185	15.8	1.36
	SP-249 + Cereflo 200 L	0.05 + 0.1	106	145	160	195	16.0	1.30
	Celluclast 1.5 L + Cereflo 200 L	0.02 + 0.1	95	145	160	185	15.8	1.33
Malt/barley (50:50)	None	...	79	112	126	175	15.2	1.59
	SP-249	0.05	135	150	165	185	15.1	1.37
		0.1	120	165	178	190	15.3	1.37
	Ceremix[b]	0.15	97	141	163	200	15.5	1.42
	SP-249 + Ceremix	0.01 + 0.15	109	154	171	210	15.8	1.39
		0.05 + 0.15	107	150	169	205	15.7	1.30
		0.1 + 0.15	128	168	181	200	15.8	1.31

[a]Grist to water ratio = 1:4.2.
[b]Ceremix is a mixture of Cereflo and Neutrase (neutral bacterial proteinase).

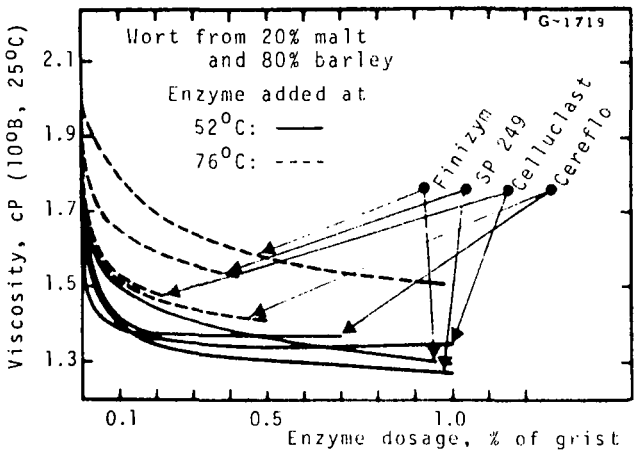

Fig. 9. Effect of various enzymes and dosages on viscosity.

added during mashing-off. Similar results were obtained with rates of filtration. In practical brewing, adding the enzymes at mashing-in appears to be the method of choice.

CONCLUSION

Apparent attenuations over 100% can be achieved when combinations of the two saccharifying enzymes Fungamyl (fungal α-amylase) and the recently developed Promozyme (pullulanase) are added during fermentation. Since the enzymes are inactivated under reasonable pasteurization conditions, they present an attractive alternative to the present use of AMG (glucoamylase) in production of low-carbohydrate beer.

Addition of the enzymes during fermentation is more economical than mash or wort treatment, because of the lower dosages required to achieve a specified attenuation.

Furthermore, the results of a comparison between a newly developed multicomponent carbohydrase SP-249 and other commercial glucanases indicate that SP-249 is a more potent enzyme for degradation of barley and malt glucans and pentosans.

ACKNOWLEDGMENT

Figures and tables are used with the kind permission of Novo Industri A/S, Copenhagen.

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