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Breeding of Lipoxygenase-1-less Malting Barley Variety CDC PolarStar and Effect of Lipoxygenase-1-less Trait on Beer Quality at Pilot and Commercial Scale Brewing

CDC PolarStar was successfully developed as the first lipoxygenase-1-less (LOX-less) malting barley variety in North America by successive backcross and molecular marker assisted selection (MMAS). The yield potential, the agronomic performance and the general malting quality of CDC PolarStar were equivalent to those of the recurrent parent CDC Kendall which was one of well evaluated and accepted two-rowed malting barley varieties in North America. These are very important findings to demonstrate that the LOX-less trait could be used for barley breeding to improve beer quality without affecting such characteristics. To evaluate effects of LOX-less trait on beer quality, several brewing trials were conducted at the pilot and the commercial scale facilities. The wort and beer analyses indicated that the LOX-less trait did not have much effect on the characteristics which are expected not to be affected by absence of LOX-1 activity. In contrast, the beers made of CDC PolarStar malt showed reduced levels of beer deteriorating substances, such as *trans*-2-nonenal (T2N) and trihydroxyoctadecenoic acid (THOD) compared to the beers made of the control malt. The sensory evaluations by well-trained panelists showed the significant superiority of CDC PolarStar beers in terms of staleness in most trials and the positive effect of LOX-less trait was more apparent in low malt beer conditions. These results indicate that LOX-less barley variety CDC PolarStar can improve flavor stability without affecting any other beer characteristics.

Descriptors: barley, lipoxygenase-1, LOX-less, flavor stability, *trans*-2-nonenal, trihydroxy-octadecenoic acid

1 Introduction

Lipoxygenase (EC.1.13.11.12) (LOX) catalyzes the hydroperoxidation of polyunsaturated fatty acid with 1, 4-*cis*-*cis*-pentadien structure. In germinating seeds of barley (*Hordeum vulgare* L.), two isozymes of LOX (LOX-1 and LOX-2) have been identified and characterized, and LOX-1 and LOX-2 mainly catalyze the formation of 9-hydroxyperoxide (9-HPOD) and 13-hydroxyperoxide (13-HPOD), respectively [1, 2]. From brewers' viewpoint, LOX-1 is of greater interest and importance since 9-HPOD is a precursor of beer deteriorating substances, such as *trans*-2-nonenal (T2N) and trihydroxyoctadecenoic acid (THOD) [3, 4, 5]. T2N is known as a component of cardboard flavor in stale beer [6] and THOD is considered to have negative effect on beer quality in terms of foam stability and flavor [7, 8].

Six LOX-less barley landraces were discovered by surveying the barley germplasm collected by the Institute of Plant Sciences and Resources, Okayama University, Japan. These landrace lines did not show any significant LOX-1 activity and they lacked the authentic LOX-1 protein [9]. Furthermore, the genetic analysis revealed that the LOX-less trait was governed by a single recessive gene. The polymorphism at an *Afa*I site of LOX-1 structural gene could be used for the cleaved amplified polymorphic sequence (CAPS) analysis and it could be applied for the MMAS of LOX-less gene. The pilot brewing trials using the malt made of the experimental LOX-less/normal (no LOX-less) barley populations or the barley itself as adjunct revealed that flavor and foam stability were improved by the reduction of T2N and THOD levels [10, 11].

North America is one of the major malt supplying regions to Asian brewing companies and stable supply of high quality malt from

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Table 1 Conditions of pilot and commercial scale brewing trials

Trial	Scale		Control	Beer type	Crop	Malt %	LOX-less Malt %	Adjuncts	LOX-less Barley %
Trial 1	Pilot	400 L	CDC Kendall	Beer	2006	100	0 or 100	0	0
Trial 2				Beer		71	0 or 71	29 % (Starch/ Corn/Rice)	0
Trial 3				Low malt beer		24	0	76 % (Barley)	0 or 76
Trial 4				Low malt beer		24	0 or 24	76 % (Sugar)	0
Trial 5	Pilot	5000 L	AC Metcalfe	Beer	2009	70	0 or 70	30% (Starch)	0
Trial 6	Commercial	285 hL	Commercial mixed malt	Beer	2007	74	0 or 74	26% (Cornsyrup)	0
Trial 7	Commercial	1000 hL	CDC Kendall	Beer	2009	100	0 or 100	0	0

North America is of great importance. Therefore, the joint breeding program among Sapporo Breweries, Ltd., the Crop Development Centre (CDC), the University of Saskatchewan, Canada and Prairie Malt Limited, Canada was established in 1994 for developing high quality malting barley varieties. In the joint breeding program, successive backcross and MMAS were adopted to introduce LOX-less trait and breed a first LOX-less barley variety quickly and efficiently.

The present study describes the characteristics and the performance of CDC PolarStar, which is the first LOX-less malting barley variety in North America, with the data of the breeding process and the positive effects of the LOX-less trait on beer quality which were verified in several brewing trials conducted at the pilot and the commercial scale facilities.

2 Materials and methods

2.1 Breeding and field trials

CDC Kendall is a high quality two-rowed malting barley variety developed by the CDC and it was widely distributed in Western Canada since the agronomic performance was accepted by malting barley growers and the malt quality was well evaluated by maltsters and brewers. Therefore, CDC Kendall was used as the recurrent parent for the successive backcross. OUI003, one of the six LOX-less landrace lines [9] was used as the nonrecurrent parent for the introduction of the LOX-less trait. In 2001, the first cross between OUI003 and CDC Kendall was made at the Bioresources Research and Development Department (BRDD), Sapporo Breweries, Ltd., Gunma, Japan. Five successive backcrosses and the MMAS using the CAPS marker for the LOX-less gene for the selection of the heterozygous individual plants at each generation (BC_XF_1) were made from 2002 to 2004 at the BRDD and the CDC. The BC_5F_1 plants were self-pollinated to BC5F2 and the single plants homozygous for the LOX-less gene were selected by MMAS. In 2005, the BC_5F_4 lines were tested in a multi-locational, randomized complete block yield trial with three replicates at six locations: Saskatoon, Saskatchewan (SK), Goodale, SK, Harris, SK, Wakaw, SK, Lacombe, Alberta (AB) and Taber, AB. These lines were also grown in disease nursery and assessed for resistance or susceptibility to typical barley diseases in North America. The one line which was the most similar to CDC Kendall was selected by the visual assessment and the malt quality evaluation. It was tested in the Western Canadian Cooperative Two-Row Barley Registration Tests as TR06918 during 2006 and 2007. The agronomic performance and the disease resistance were evaluated according to the protocols provided by the Prairie Recommending Committee for Oat and Barley in Canada. (http://www.pgdc.ca/committees_ob.html)

2.2 Micromalting and malt analysis

Micromalting and malt analysis in the Western Canadian Cooperative Two-Row Barley Registration Tests was conducted according to the protocols provided by each collaborator of the Western Canadian Cooperative Two-Row Barley Registration Tests. The barley samples of CDC PolarStar and other check cultivars from each trial site were malted on the same condition to compare malt quality. The one example of protocols which was applied for the micromalting is as follows:

Steeping: Steeping process comprised of 10 hours immersion, 18 hours air-rest, 8 hours immersion and 12 hours air-rest. Total steeping time was 48 hours and water temperature was 13 °C.

Germination: Total germination time was 96 hours and temperature was 15 °C.

Kilning: Kilning process was comprised of 6 hours increase from 30 to 48 °C , 16 hours at 48 °C , 8 hours increase from 48 to 66 °C , 10 hours at 66 °C , 2 hours increase from 66 to 85 °C and 6 hours at 85 °C. Total kilning time was 48 hours.

The protocol for malt analysis was based on the American Society of Brewing Chemists (ASBC) recommended methods [12].

2.3 Pilot scale malting for brewing trial and malt analysis

Pilot scale malting for brewing trial was conducted according to the standard methods of the Product & Technology Development

Center, Sapporo Breweries, Ltd., Japan as previously described [13]. The analysis of the malt produced at pilot scale malting and the commercial malt used for the trials was conducted at the BRDD and the following malt parameters were analyzed using the European Brewery Convention (EBC) recommended methods [14]: malt moisture, wort clarity, color, boiled wort color, extract (fine grind), total and soluble nitrogen, apparent attenuation limit, diastatic power, Hartong index (VZ 45°C), viscosity, friability and wort beta-glucan. The LOX assay was conducted as previously described [9, 10]. One unit of LOX activity was defined as the amount of enzyme that produced 1 nmol of hydroperoxide (with cumene hydroperoxide as the standard)/min/1 g of malt.

2.4 Pilot and commercial scale brewing

Pilot scale brewing at a 400 L or 5000 L apparatus was conducted according to the standard methods of the Product & Technology Development Center, Sapporo Breweries, Ltd., Japan. CDC Kendall or AC Metcalfe malt was used as control. To evaluate the effect of the LOX-less trait, CDC Kendall was used as control in most trials since it was used as the recurrent parent of the successive backcross and therefore the LOX-less variety should have highly similar genetic background to CDC Kendall except for the LOX-less gene. AC Metcalfe was used as the control since it is the most dominant malting barley variety in Canada. The commercial scale brewing trials were also conducted at our several breweries. The conditions of the pilot and the commercial brewing trials were summarized in Table 1. The T2N and the THOD concentrations were determined as previously described [10, 15].

2.5 Sensory evaluation

The sensory quality was evaluated for three aspects: off-flavor, stale taste and total staleness. The off-flavor which derived from staleness is sensed and recognized as papery (cardboard), whisky-like or caramel-like flavors [16]. The total staleness is defined as the overall impression of staleness. The sensory evaluation was conducted for fresh beers and aged beers by a group of several well-trained panelists. The off-flavor, the stale taste and the total staleness were rated from 0 (fresh) to 4 (strongly stale) at 0.5 intervals. The means of sensory ratings were statistically analyzed by paired t-tests to determine significant differences in terms of the staleness between the CDC PolarStar beers and the control beers.

3 Results and Discussion

3.1 Agronomic performance

In the Western Canadian Cooperative Two-Row Barley Registration Tests, the grain yield and the other agronomic parameters of

Table 2 Grain yield and agronomic trait data for CDC PolarStar and check cultivars from 2006 and 2007 Western Canadian Cooperative Two-Row Barley Registration Tests

Year		2006						2007				
Variety		Harrington	Xena	AC Metcalfe	CDC Kendall	CDC PolarStar	No. station	CDC Copeland	Xena	AC Metcalfe	CDC PolarStar	No. station
Grain yield	(kg/ha)	5065	6266	5334	5290	5424	16	5084	5629	5071	4933	15
as AC Metcalfe	(%)	95	117	100	99	102		100	111	100	97	
Days to head	(days)	58,0	57,8	58,4	59,2	58,7	13	59,0	56,6	56,6	57,9	11
Days to maturity	(days)	86,1	87,5	86,9	86,0	85,6	13	90,0	90,3	89,2	88,1	13
Height	(cm)	81,0	83,6	83,1	80,8	84,1	14	87,0	85,6	85,9	85,1	14
Lodging score[1]	1-9	6,0	4,8	5,7	6,8	7,3	2	4,5	3,8	4,2	4,7	2
Test weight	(kg/hl)	64,5	67,0	66,2	65,4	64,9	13	63,3	66,2	65,2	64,9	13
Kernel weight	(g $1000k^{-1}$)	42,6	49,8	44,7	43,4	44,1	13	43,8	47,2	42,7	42,9	12
Plump	(%) >2.44mm	88,0	93,4	89,9	92,7	91,4	10	88,0	89,0	87,8	92,0	11

1 1=no lodging; 9=completely lodging

2006 test grown at
Black soil zone: Brandon and Grenlea, Manitoba (MB), and Indian Head, Melfort, and Regina, Saskatchewan (SK).
Black and gray soil zone: Beaverlodge, Calmer, Fort Vermillion, and Lacombe, Alberta (AB).
Brown soil zone: Harris, Bieseker, Lethbridge, Saskatoon, Swift Current, Trochu, and Watrous (SK).
2007 test grown at
Black soil zone: Brandon (MB), and Indian Head, Melfort, and Regina (SK).
Black and gray soil zone: Dowson Creek, British Columbia (BC), and Beaverlodge, Calmer, Fort Vermillion, and Lacombe (AB).
Brown soil zone: Harris, Bieseker, Lethbridge, Saskatoon, Swift Current, Scott, and Watrous (SK).

CDC PolarStar were evaluated together with the recurrent parent CDC Kendall and other Canadian two-rowed malting and feed barley varieties including Harrington, Xena, AC Metcalfe and CDC Copeland during 2006 and 2007 (Table 2). The grain yield of CDC PolarStar was less than that of Xena which is a high yielding feed variety in North America. But it was equivalent to that of CDC Kendall in 2006 and CDC PolarStar showed comparable grain yields to AC Metcalfe during 2006 and 2007 in province-wide average. The plant height of CDC PolarStar was slightly longer than that of CDC Kendall in 2006 and equivalent to that of AC Metcalfe during 2006 and 2007. The other agronomic characteristics were similar between CDC PolarStar and CDC Kendall in 2006 and between CDC PolarStar and AC Metcalfe during 2006 and 2007. Disease resistance is summarized in Table 3. The total disease resistance of CDC PolarStar was similar to that of CDC Kendall and thus there is no major concern for growing this variety.

LOX-null genes have been discovered in several other crops, for example soybean [17, 18] and rice [19]. The LOX-null soybean lines showed normal growth and agronomic performance compared with check cultivars [20]. In barley, it was concluded that the LOX-less trait did not cause adverse effects on the general quality of malt, wort and beer, however the effect on the agronomic performance was not reported [10,11]. The results in terms of the agronomic performance of CDC PolarStar and CDC Kendall in the present study indicates that the LOX-less barley showed normal growth and the LOX-less trait did not have much impact on the agronomic performance. This is very important finding

Table 3 Disease reaction summaries for CDC PolarStar and check cultivars from 2006 and 2007 Western Canadian Cooperative Two-Row Barley Registration Tests

Year		2006					2007			
Variety		Harrington	Xena	AC Metcalfe	CDC Kendall	CDC PolarStar	CDC Copeland	Xena	AC Metcalfe	CDC PolarStar
WSNB	102[1]	10[7]	10	9	5	5	6	9	9	8
	858[1]	10	10	10	9	8	9	10	9	9
	857[2]	9	3	5	3	1	5	5	5	3
MNB		7,5	2,0	4,5	1,5	1,0	2,0	1,0	3,5	1,0
BSB		7,5	7,0	5,5	6,5	4,5	4,5	5,0	5,0	4,5
MSB		4,5	5,0	3,5	4,0	4,5	5,0	4,5	3,5	4,3
SSB		6,5	5,0	4,5	4,8	6,0	5,5	4,5	3,5	4,5
WSB	1903[3]	7[8]	6	6	6	5	6	7	6	4
FHB[4]		2,3	1,3	2,3	2,5	2,3	1,8	2,2	2,2	2,8
DON[4]		5,9	3,7	5,6	5,4	5,9	1,9	1,7	5,9	3,1
WSS	1998[5]	S[9]	S	S	S	S	n.a.	n.a.	n.a.	n.a.
	1493[6]	S[9]	S	S	S	S	S	S	S	S
ES	Aug. 9	1,5	2,5	0,5	2,0	4,0	3,0	2,0	2,0	1,5
LS	31. Jul	6,5	7,0	6,0	4,0	5,5	4,0	3,5	3,5	3,5
SCS		S[10]	S	R	MR	MR	MR	S	R	S
WS	*U. nuda*	29%	81%	0%	81%	81%	94%	89%	0%	n.a.
	U. hordei	10	3	3	3,5	0	0,5	0	0	0
	U. nigra	22,5	40	7,5	26,5	tr	0	0	0	0
LCRR		89%	89%	89%	87%	89%	96%	57%	80%	80%
		n.a.	n.a.	n.a.	n.a.	n.a.	S[11]	MRMS	MS	MS

WSNB, Winnipeg seedling net blotch; MNB, Melfort net blotch; BSB, Brandon spot plotch; MSB, Melfort spot blotch; SSB Saskatoon spot blotch; WSB Winnipeg spot blotch; WSS1998, Winnipeg seedling Septoria 1998; WSS1493, Winnipeg seedling scald 1493; ES, Edmonton scald; LS, Lacombe scald; SCS, Saskatoon covered smut; WS, Winnipeg smut, LCRR, Lacombe common root rot

1 *Pyrenophora teres* net-form isolates
2 *Pyrenophora teres* spot-form isolate
3 *Cochliobolus sativus* isolate
4 FHB, Fusarium head blight; DON, deoxynivalenol; FHB rating scale=0-5 (0=best)
5 *Septoria passerinni* isolate
6 *Rhyncosporium secalis* isolate
7 10=very susceptable, 9=susceptable, 7=moderately susceptable, 5=moderately resistant-moderately susceptable, 3=moderately resistant, 1=resistant.
8 0-9 scale: 0=no visible lesions, 4=small, 9=very large, coalescing.
9 R, resistant; MR, moderately resistant; MS, moderately susceptable; MSS, moderately susceptable-susceptable; S, susceptable.
10 I, immune; R, resistant; MR, moderately resistant; S, susceptable; HS, highly susceptable.
11 MR, moderately resistant; MRMS, moderately resistant-moderately susceptable; MS, moderately susceptable.
n.a.: data not available

to demonstrate that the LOX-less trait could be used for barley breeding to improve beer quality without affecting agronomic characteristics.

The successive backcross was applied to various crops for introducing only a certain gene or trait into existent cultivars [21]. The successive backcross breeding was adopted to develop CDC PolarStar as the first LOX-less line since the original LOX-less landrace OUI003 is quite different from two-rowed malting barley in morphological, agronomic and quality aspects. OUI003 is six-rowed barley (http://www.shigen.nig.ac.jp/barley/) and has week straw strength based on our observations (unpublished data) and malt quality relatively poor according to the previous study [11]. Therefore it was supposed that the successive backcross was the most efficient method to develop a first LOX-less line with acceptable agronomic properties for malting barley producers and quality for malting and brewing purposes. The agronomic performance of CDC PolarStar is similar to that of the recurrent parent CDC Kendall and AC Metcalfe which is the most dominant and well-accepted variety for malting barley producers in Canada. Therefore these results suggest that CDC PolarStar shows a good and acceptable agronomic performance for malting barley growers and the backcross breeding strategy was effective and practical at the initial stage of LOX-less barley breeding.

3.2 Malting Quality

The malting quality of CDC PolarStar was tested in the Western Canadian Cooperative Two-Row Barley Registration Tests during 2006 and 2007 together with the recurrent parent CDC Kendall, and two-rowed malting varieties Harrington, AC Metcalfe and CDC Copeland. CDC PolarStar showed similar malting quality to CDC Kendall in 2006 and comparable quality to AC Metcalfe in these three trials (Table 4). The analysis of malt used for the pilot and the commercial brewing trials also showed no major difference between CDC PolarStar malt and other control malts except for soluble nitrogen and Kolbach index of Trial 5 (Table 5). These results suggested that LOX-less trait did not much affect the general malting quality and the backcrossing was successful. In the previous report [10], it was clarified that the general malting quality was similar between LOX-less and normal F_4 bulk materials which were grouped by genotyping with the CAPS markers for the LOX-1 gene and it was consistent with the results in this study.

In contrast to the general malting quality, the LOX activity of CDC PolarStar malt and CDC Kendall malt used for Trial 1 to 4 showed a clear difference (Table 5). This result was consistent with the result about the comparison between LOX-less and normal F_4 bulk populations in the previous report [10] and the slight LOX activity detected in CDC PolarStar malt is regarded as the LOX-2 activity.

3.3 Pilot and commercial scale brewing

No particular problem was found in each pilot and commercial brewing trial using CDC PolarStar. The general beer quality was analyzed and there was no major difference between the CDC PolarStar beers and the control beers (Table 6). The general quality of the CDC PolarStar beers was totally acceptable compared to control beers considering the beer analyses. The results suggest that CDC PolarStar is suitable for brewing.

3.4 T2N concentration and flavor stability

The T2N concentrations were determined in the fresh beers and in the beers which were stored under the conditions shown in Table

Table 4 Malting quality for CDC PolarStar and check cultivars from 2006 and 2007 Western Canadian Cooperative Two-Row Barley Registration Tests

Year			2006					2007			
Variety			Harrington	AC Metcalfe	CDC Kendall	CDC PolarStar	No. station	CDC Copeland	AC Metcalfe	CDC PolarStar	No. station
Plump		(%)	93,0	97,3	97,0	95,8	2	94,1	92,8	96,3	3
Kernel Weight		(g 1000k^{-1})	45,7	47,8	45,2	45,0	2	43,1	42,3	42,5	3
Protein		(%)	11,8	11,9	12,2	12,2	2	10,2	10,7	11,1	3
Germination Energy	4ml	(%)	100	99	100	98	2	97	98	99	3
	8ml	(%)	99	93	95	93	2	96	96	94	3
Fine Extract		(%)	80,5	80,9	80,4	80,7	2	81,1	81,7	80,9	3
Soluble Protein		(%)	5,0	4,8	4,9	5,0	2	4,6	4,8	4,8	3
Diastatic Power		(°L.)	108	130	150	149	2	105	123	137	3
Alpha- Amylase		(D.U.)	58,4	62,3	59,6	60,4	2	52,0	68,0	69,1	3
Beta-Glucan		(ppm)	150	81	61	80	2	80	87	97	3
Viscosity		(cps)	1,45	1,43	1,42	1,44	2	1,43	1,42	1,42	3
Friability		(%)	89,7	90,0	91,6	92,4	1	98,4	99,4	97,1	2
Peeled		(%)	3,4	2,1	2,6	2,2	1	5,7	4,8	3,0	2

7. The T2N concentrations of the fresh beers were nearly equal between the test and the control beers, and the T2N concentrations increased during the storage in both the test and the control beers. However, the T2N increase in the test beers was smaller than that in the control beers in all of the trials. These results were consistent with the previous studies [10, 11].

The sensory evaluations for the staleness of the test and the control beers were conducted by well-trained panelists (Table 7). In the trials except Trial 1, the test beers showed significant superiority in more than one of three criteria for sensory evaluation. Many substances and pathways involved in beer staling have been studied and suggested [22, 23]. The staling reaction during storage can be simplified and integrated as oxidative or non-oxidative conversions of precursors [24] and countermeasures against beer staling are suggested: reduction and/or removal of precursors, control of conversion rate from precursors to compounds related to stale flavor, and masking of staleness. In the modern breweries, several measures against beer oxidation were applied and they showed certain effects on improvement of flavor stability [25, 26]. T2N is considered to cause stale flavor in beer [6] and LOX-1 in malt catalyzes the formation of 9-HPOD which is a precursor of T2N. To reduce the precursors of T2N by decreasing the LOX activity, several approaches have been suggested [27]. However, the utilization of LOX-less barley and malt for reducing the T2N level was thought to be the most practical way since it can be applied for any type of beers and any brewing facilities. In the present study, we showed the effectiveness of LOX-less barley for reducing the T2N concentration in the stale beer and improving flavor stability. The T2N concentrations of the beers stored at 30 °C for 1 month were 0.05-0.37 ppb in the test beers and 0.10-0.44 ppb in the control beers (Table 7). The difference of T2N concentrations between the test and the control beers in Trial 1 to 4 was larger in low malt beer conditions (Trial 3 and 4). In the previous study [10], the brewing trial was conducted using lower percentage (24 %) of malt and corn syrup (76 %). The result also showed the relatively larger difference of the T2N concentration between the test and the control beer. Several reasons for this tendency could be speculated. Firstly, the concentration of antioxidant substances such as malt polyphenols in low malt beer is less than that in normal beer and therefore flavor stability of low malt beer is relatively lower than that of normal beer. Secondly, the difference of the LOX activity in wort should be greater in low malt beer conditions using LOX-less and normal barley as adjunct than in normal beer conditions using LOX-less malt and normal malt since malt is kilned with relatively higher temperature and therefore barley has higher LOX activity than malt. As a result, the effect of LOX-less trait on T2N concentration could be more apparent in low malt beer than in normal beer.

3.5 THOD concentration and foam stability

The THOD concentrations of the test and the control beers were analyzed in all of the trials. As expected, the THOD concentrations of the test beers (0.1-1.1 ppm) were definitely lower than those of the control beers (0.3-2.2 ppm) in all of the trials (Table 7).

THOD is known to have an adverse effect on foam stability. In the previous report, the involvement of LOX-1 in the formation of THOD was clarified [4] and di- and trihydroxyoctadecenoic acid (THOD) were decisive compounds to determine the total Foam Damaging Effect (FDE) [8]. In the previous study [10], the reduction of the THOD concentrations and the improvement of foam stability were verified at the same time and the THOD concentrations were 1.7 ppm and 3.6 ppm, and foam stability (NIBEM value) were 260 sec and 239 sec for the test and control beer, respectively. However,

Table 5 Malting quality of CDC PolarStar and control malts for pilot and commercial scale brewing trials

Trial		Trial 1-4		Trial 5		Trial 7	
Variety		CDC Kendall	CDC PolarStar	AC Metcalfe	CDC PolarStar	CDC Kendall	CDC PolarStar
Malt Moisture	(%)	8,0	4,3	4,7	4,2	3,9	4,2
Color	(°EBC)	3,9	3,8	3,8	4,5	3,8	3,5
Boiled Wort Color	(°EBC)	6,9	7,0	7,2	8,7	6,2	6,9
Extract	(% d.b.)	79,8	78,2	83,0	82,5	81,8	82,5
Soluble Nitrogen	(%)	0,762	0,756	0,785	0,861	0,734	0,736
Total Protein	(%)	11,8	a11,7	11,6	11,3	11,0	10,5
Kolbach Index	(%)	40,4	40,4	42,3	47,6	41,6	43,7
Apparent Attenuation Limit	(%)	82,9	82,3	81,0	83,2	82,8	83,4
DP	(°WK)	n.a.	n.a.	553	481	n.a.	n.a.
Hartong Index (VZ45°C)		43,1	42,8	n.a.	n.a.	n.a.	n.a.
Viscosity	(mPa•S)	1,40	1,49	1,50	1,46	1,46	1,46
Friability	(%)	n.a.	n.a.	86,0	86,0	n.a.	n.a.
Wort Beta-glucan	(mg/L)	50	59	59	39	28	37
LOX activity	(unit)	15,1	0,3	n.a.	n.a.	n.a.	n.a.

n.a.: data not available

the foam stability of the test beers in the series of the trials in the present study seemed not to be clearly improved (Table. 7). It is possible to speculate reasons for the less improvement for foam stability. Kobayashi et al. reported that there were positive correlations between the FDE values which were calculated from the concentrations of various free fatty acids including THOD and the NIBEM values [8]. The range of THOD concentrations in one of the correlation shown in the report was from 0.0082 mol/m^3 (2.7 ppm) to 0.02 mol/m^3 (6.6 ppm) and it was higher than the concentrations reported in this study. Because of this lower range

Table 6 Analyses of CDC PolarStar and control worts and beers

Trial		Trial 1		Trial 2		Trial 3		Trial 4		Trial 5		Trial 6		Trial 7	
Variety		CDC Kendall	CDC PolarStar	CDC Kendall	CDC PolarStar	CDC Kendall	CDC PolarStar	CDC Kendall	CDC PolarStar	AC Metcalfe	CDC PolarStar	Commercial mixed malt	CDC PolarStar	CDC Kendall	CDC PolarStar
Wort															
Extract	(%)	11,36	11,36	11,17	11,17	10,06	10,14	12,24	12,15	12,27	12,23	n.a.	n.a.	12,65	12,69
Final Attenuation	(%)	69,3	70,3	72,1	73,3	74,7	73,2	70,1	70,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Apparent Attenuation Limit	(%)	85,5	86,8	88,9	90,2	92,1	90,3	86,9	87,2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
pH		5,63	5,65	5,57	5,54	5,43	5,46	5,9	5,94	5,25	5,35	n.a.	n.a.	5,24	5,22
Color	(°EBC)	9,5	9	6,8	6,6	4,8	4,7	3,6	3,7	9,7	10,2	n.a.	n.a.	10,5	10,8
BU		36,2	34,2	35,9	34,9	38,6	37,1	33,5	34,7	34,8	33,7	n.a.	n.a.	31,8	31,6
Polyphenol	(mg/L)	233	213	183	181	207	194	96	93	178	170	n.a.	n.a.	n.a.	n.a.
Free Amino Nitrogen	(mg/L)	204	197	148	143	93	97	59	59	163	160	n.a.	n.a.	n.a.	n.a.
Beer															
Original Gravity	(%)	11,11	11,11	10,67	10,75	8,33	8,57	12,1	12,01	11,08	11,08	10,71	10,46	11,87	11,87
Final Extract	(%)	3,54	3,49	3,05	3,03	2,15	2,32	3,75	3,68	3,57	3,43	3,29	3,02	3,57	3,75
Final Attenuation	(%)	68,1	68,6	71,4	71,8	74,2	72,9	69	69,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Apparent Extract	(%)	1,68	1,62	1,2	1,15	0,66	0,81	1,5	1,72	1,73	1,56	1,47	1,21	1,54	1,77
Apparent Attenuation Limit	(%)	84,9	85,4	88,8	89,3	92,1	90,5	85,9	86,3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Alcohol	(vol %)	4,98	5	4,98	5,05	3,97	4,02	5,52	5,5	n.a.	n.a.	4,86	4,86	5,45	5,34
Alcohol	(w/w %)	3,91	3,93	3,92	3,97	3,13	3,17	4,34	4,32	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
pH		4,61	4,62	4,55	4,5	4,63	4,59	3,47	3,47	4,26	4,24	4,3	4,26	4,56	4,56
BU		20,7	21,4	23,3	22,5	20,3	18,5	15,3	14,4	19,8	20	19,1	18,3	18,9	20
Polyphenol	(mg/L)	201	190	153	146	123	120	88	84	139	129	132	126	192	151
Free Amino Nitrogen	(mg/L)	132	118	80	74	25	28	12	11	73	64	n.a.	n.a.	80	91
Acetoaldehyde	(mg/L)	1,4	2,8	2,8	3,4	1,2	1,1	1,5	1,3	2,3	0,8	1,2	1,6	0,7	0,4
Acetone	(mg/L)	0,7	0,9	1	0,8	0,4	0,6	0,9	0,7	0,6	6	0,6	1,3	1,1	0,4
Ethyl Acetate	(mg/L)	22	24	23	22	23	24	26	23	17	17	13	13	20	28
Isoamyl Acetate	(mg/L)	1,4	1,6	1,7	1,8	1,8	1,6	1,5	1,5	1,1	1,3	0,7	0,7	1,2	1,6
n-Propanol	(mg/L)	10	12	11	11	11	12	11	10	12	12	17	12	14	16
Isobutanol	(mg/L)	7	9	9	10	9	10	7	7	8	10	14	12	10	10
Isoamyl Alcohol	(mg/L)	47	53	55	52	59	81	66	59	52	58	67	69	59	58
Ethyl Caproate	(mg/L)	0,14	0,17	0,19	0,23	0,13	0,13	0,17	0,17	0,2	0,21	n.a.	n.a.	0,17	0,18

n.a.: data not available

of the THOD concentrations in this study, the positive effect of the THOD difference between the test and the control beers on foam stability might not have been apparent. The reason for this lower THOD range is unknown, however the difference of the genetic background of the materials used for the brewing trials might have been associated with it.

Beer foam stability is dependent on interaction of number of components, including hop iso-alpha-acids, beer proteins and polypeptides. In beer proteins, lipid transfer protein I (LTP1) is known as one of the key malt-derived proteins affecting positively on foam retention which act as a binding protein for foam damaging free fatty acid. Several previous studies have indicated that only denatured form LTP1 could have foam stabilizing properties [28, 29, 30]. On the other hand, Van Nierop et al. concluded that LTP1 denaturation reduced its ability to bind free fatty acids [31]. It was also reported that considerable variation in the level of LTP1 in barley grain was observed and the variety selection might be opportunity for improving foam quality [32]. Whichever denatured form or non-denatured form can act as a binding protein for free fatty acid, variation in level of LTP1 in malt may cause variation in foam stability. Even if there is definite difference for THOD concentrations, the improvement of foam stability could not be apparent if concentration of LTP1 is much more than enough for binding free fatty acids both in CDC PolarStar (lower THOD concentration) beers and in CDC Kendall (higher THOD concentration) beers. In the brewing trials with the BC_5 line developed using CDC Copeland which is also one of dominant Canadian two-rowed malting barley as the recurrent parent, the beer with LOX-less malt showed clearly higher NIBEM values compared with the control beer in the 400 L pilot brewing trial (unpublished data). In contrast to CDC Kendall and CDC PolarStar, CDC Copeland may contain lower LTP 1 concentration resulting in lower binding capability for THOD and it may cause the clearer difference of the NIBEM values.

At any rate, the reduction of THOD level by the absence of LOX-1 was clearly verified in this study and it was consistent with the previous study [10, 11]. Now we are breeding other LOX-less lines by same strategy and by conventional breeding using CDC PolarStar as a parent in the breeding programs for North America and other countries. To clarify reasons for the different effect on improvement of foam stability, further investigations using such lines are necessary in the near future.

4 Conclusion

In the present study, CDC PolarStar was successfully developed as the first LOX-less malting barley variety in North America by successive backcross and MMAS. The analyses of the agronomic performance, the general malt quality and the general beer quality did not show any major difference between CDC PolarStar and the controls including CDC Kendall. The result indicates that the LOX-less trait does not have any adverse effect on these characteristics. The effect of LOX-less trait on the reduction of T2N and THOD concentrations was clearly shown in this study. In the sensory evaluations, the positive effects of LOX-less trait were confirmed and it was more apparent in low malt beer conditions.

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Table 7 THOD and T2N concentrations, NIBEM values and sensory evaluations of CDC PolarStar and control beers

Trial	Crop	THOD (ppm)		NIBEM (%)		T2N (ppb)				Sensory evaluation (0-4)									Number of panelists
						Fresh beer		Stale beer		Off-flavor			Stale taste			Total staleness			
		Control	Test	Control	Test	Control	Test	Control	Test	Control	Test		Control	Test		Control	Test		
Trial 1	2006	1,5	1,1	100	100	0,08	0,08	0,33	0,32	1,7	1,6	n.s.	1,6	1,6	n.s.	1,1	1,1	n.s.	>11
Trial 2	2006	1,4	1,0	100	96	0,07	0,07	0,23	0,22	2,0	1,4	*	2,1	1,6	*	2,2	1,6	*	>11
Trial 3	2006	1,5	0,6	100	99	0,1	0,08	0,19	0,08	2,3	1,7	n.s.	2,6	1,8	*	2,7	1,9	*	>11
Trial 4	2006	0,3	0,1	100	98	0,02	0,02	0,44	0,37	1,9	1,7	*	2,0	1,5	*	2,2	1,7	*	>11
Trial 5	2009	0,7	0,3	100	96	0.08[a]	0.05[a]	0.25[b]	0.1[b]	-	-	-	-	-	-	2,3	1,6	**	7
Trial 6	2007	0,9	0,3	100	101	0,17	0,14	0,27	0,18	2,2	1,6	*	2,4	2,0	**	2,4	1,9	**	11
Trial 7	2009	2,2	0,8	100	105	0,06	0,04	0,10	0,05	-	-	-	-	-	-	1,2	0,9	*	6

The T2N analyses and the sensory evaluations were conducted for the beers stored at 30 °C in 1 month except Trial 5

[a] 5 °C at 1month
[b] 37 °C at 14 days
* Significantly different at the 5 % probability level
** Significanly different at the 1 % probability level
n.s.: not significant

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