70

Contribution of malt kilning to the cardboard flavour of aged beers

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Descriptors:
Antioxidant, caramel malt, kilning, lipoxygenase, 2-nonenal, stale flavour

SUMMARY
This study aimed to examine whether malt kilning could be another significant source of wort nonenal potential, the indicator of how a beer will release (*E*)-2-nonenal during storage. We found that pale malts contain very few (*E*)-2-nonenal/protein adducts, which will be further degraded during mashing. Therefore, selecting pale malt with less nonenal potential does not significantly improve beer stability in terms of cardboard flavour. On the other hand, the increase of (*E*)-2-nonenal/protein adducts brought by 10 % of caramel malt remains significant after boiling, despite the lower lipoxygenase activity during mashing and the lower autoxidation during boiling.

Beitrag des Darrens zum Cardboard-Aroma von gealterten Bieren

Deskriptoren:
Alterungsgeschmack, Antioxidantium, Darren, Karamelmalz, Lipoxygenase, Nonenal-2

ZUSAMMENFASSUNG
Diese Studie hat sich die Untersuchung zum Ziel gesetzt, ob Darren eine andere signifikante Quelle für das Würze-Nonenal-Potenzial sein könnte, dem Indikator für die Ausschüttung von (*E*)-2-Nonenal während der Lagerung. Wir haben herausgefunden, dass helle Malze sehr wenige (*E*)-2-Nonenal-Proteinaddukte enthalten, welche während des Maischens weiter abgebaut werden. Deshalb wird durch die Auswahl heller Malze mit niedrigerem Nonenal-potenzial die Bierstabilität bezüglich des Cardboard-Aromas nicht signifikant verbessert. Auf der anderen Seite bleibt eine Erhöhung der (*E*)-2-Nonenal-Proteinaddukte, durch 10 % Karamelmalz eingebracht, auf signifikantem Niveau, trotz der niedrigeren Lipoxygenase-aktivität während des Maischens und trotz der niedrigeren Autoxidation während der Kochung.

Contribution du touraillage du malt à l'arôme de carton des bières vieillies

Descripteurs:
Antioxydant, flaveur d'éventé, lipoxygénase, malt caramel, 2-nonénal, touraillage

RESUME
L'objectif de cette étude était de déterminer si le touraillage du malt contribue au nonenal potential du moût, l'indicateur de la propension d'une bière à libérer du (*E*)-2-nonénal durant le stockage. Nous avons montré que les malts pâles contiennent très peu d'adduits (*E*)-2-nonénal/protéines, et que ceux-ci seront dégradés durant le brassage. En conséquence, la sélection d'un malt pâle contenant peu de nonenal potential ne permet pas d'améliorer significativement la stabilité de la bière en terme d'arôme de carton. Par contre, une partie des adduits (*E*)-2-nonénal/protéines apportés par 10 % de malt caramel résiste à l'ébullition. L'arôme de carton se voit dès lors intensifié, malgré une activité lipoxygénasique plus faible durant le brassage et une auto-oxydation plus faible durant l'ébullition.

La contribución del secado de la malta al flavor de cartón de las cervezas envejecidas

Palabras claves:
Antioxidante, flavor de cartón, lipoxigenasa, malta caramel, 2-nonenal, tostar

RESUMEN
Este estudio quería examinar si el secado de la malta podía ser otra fuente significativa del potencial de nonenal del mosto, indicador de cómo una cerveza liberará el (*E*)-2-nonenal durante su almacenamiento. Descubrimos que las maltas pálidas contienen muy pocos aductos proteína - (*E*)-2-nonenal, y que este contenido será degradado durante la maceración. Por este motivo, la selección de malta pálida con menos potencial de nonenal no mejora de forma significativa la estabilidad de la cerveza en términos del flavor a cartón. Por otra parte, el incremento de los aductos proteína - (*E*)-2-nonenal provocado por un 10 % de malta caramel continuaba siendo significativo después de la ebullición, a pesar de la menor actividad lipoxigenásica durante la maceración y la menor autoxidación durante la ebullición.

INTRODUCTION

As previously described by Noël et al. (1999b), the nonenal potential found in the wort before fermentation is a good indicator of how a beer will release (*E*)-2-nonenal during storage (Drost et al., 1990; Collin et al., 1997; Collin et al., 1999; Lermusieau et al., 1999; Noël et al., 1999a). According to Lermusieau et al. (1999), it derives from the retention of (*E*)-2-nonenal by wort amino acids and proteins (Noël and Collin, 1995). The so-obtained adduct protects (*E*)-2-nonenal from yeast reduction activity but can release it by acidic hydrolysis. Due to their Schiff base structure, we understand now how a low beer pH or a high storage temperature can intensify the cardboard flavour. On the other hand, sulphites can be used as a masking agent for aldehydes.
Although lipid autoxidation in the boiling kettle is a key determinant of the cardboard flavour of aged beers (Collin et al., 1999; Noël et al., 1999b; Lermusieau et al., 2000), recent studies showed that mashing is another significant source of wort nonenal potential (Liégeois et al., 2002). The presence of oxygen during mashing and, to a

lesser extent, high lipoxygenase activity, can enhance the stale cardboard flavour (Liégeois et al., 2002).
The aim of this study was to examine whether malt kilning could be another significant source of nonenal potential. First, pale and coloured malts were compared in terms of (*E*)-2-nonenal content, nonenal potential, antioxidant and LOX activities. The effect of a natural/artificial increase of nonenal potential in malt was then investigated in laboratory scale brewing trials.

ANALITYCAL METHODS

Antioxidant assay

The antioxidant activity was determined by the inhibition time (Tinh) of the AAPH-induced linoleic acid oxidation assay as described by Liégeois et al. (2000). Linoleic acid (0.16 mM) was incubated with 2 mM AAPH in 50 mM potassium phosphate buffer, pH 7.4, at 37 °C under air, in presence of wort samples (12 °P wort diluted 400 times in the assay) or methanolic malt extracts (final concentration in the assay = 133.3 mg/l). UV spectroscopy was used to monitor the appearance of oxidation products at 234 nm. The inhibition time, defined as the point of intersection between the tangents to the inhibition- and propagation-phase curves under precise oxidation conditions, varied according to the antioxidant and its concentration. All analyses were done in duplicate. All variation coefficients were under 3 %.

Lipoxygenase assay

Lipoxygenase (LOX) activity was determined polarographically on the crude malt extract obtained after extraction of malt flour with 10 volumes of cold 0.1 M potassium phosphate buffer, pH 7 and centrifugation at 14,000 g as described by Liégeois et al. (2002).

(*E*)-2-Nonenal analysis

(*E*)-2-Nonenal was extracted by vacuum distillation, transferred to dichloromethane, and concentrated before analysis by gas chromatography – mass spectrometry, as previously described by Lermusieau et al. (1999). All analyses were performed in duplicate, and their global variation coefficient was under 10 %.

Nonenal potential experiment (based on the method of Drost et al., 1990)

The pH of 1.5 l of wort or beer was adjusted to 4 with 85 % phosphoric acid. After being purged for 15 min with argon to reduce the oxygen level, the sample was heated at 100 °C for 2 hours in a 2 l closed vessel and then cooled with cold water prior to (*E*)-2-nonenal analysis. All analyses were performed in duplicate and their global variation coefficient was under 15 %. The malt nonenal potential was obtained by applying the same treatment directly on the deoxygenated flour/water mixture (0.05/1.5 : p/v).

Wort and beer production

Mashing was performed with either air or nitrogen or no bubbling. In some experiments, deoxygenated malt flour was used (deoxygenated in a vacuum freeze-drying apparatus after milling). Malt flour and mashing water (Millipore water containing 35 mg/l $CaSO_4.2H_2O$, 10 mg/l $MgCl_2.6\ H_2O$, and 30 mg/l NaCl) were mixed at the ratio of 1 kg for 2.5 l. The temperature program applied was 36 °C for 15 min., to 50 °C at 2.8 °C/min., 50 °C for 30 min., to 63 °C at 1.3 °C/min., 63 °C for 30

min., to 72 °C at 0.6 °C/min., and 72 °C for 30 minutes. After mashing, the temperature was raised to 80 °C at 0.6 °C/min for filtration on a 2001 mash filter (Meura, Belgium). The wort was diluted to 12 °P with mashing water, and boiled with liquid CO_2 hop extract (3.14 g α-acids/hl) for 90 minutes. After clarification for 20 minutes, the hot break was removed and the wort quickly cooled to 20 °C. After another adjustment to 12 °P with mashing water, 0.3 mg/l $ZnCl_2$ was added to the clarified wort. Fermentation was carried out in 3-l EBC tubes with an ale yeast (*Saccharomyces cerevisiae*, pitching rate: 7.5 x 10^6 cells/ml) at 20 °C for seven days and at 7 °C for seven days. Yeast cells were removed from the beer by continuous centrifugation (15000 rpm; Contrifuge 17RS, Heraus Sepatech). Accelerated ageing was carried out at 40 °C for 5 days.

RESULTS AND DISCUSSION

Screening of pale and coloured malts

Pale malts (table 1) contain very high levels of free (*E*)-2-nonenal (table 2), derived from an intense lipoxygenase activity during germination.

Table 1: Final temperature, moisture, antioxidant activity (Tinh) and LOX activity of pale malts.

Pale malt	Final kilning temperature above/below (°C)	Moisture (%)	Tinh (min)	LOX (nkat/g malt dry weight)
Pilsner	-	5.0	14.4	129.9
Astoria 75	75/82	4.4	14.8	184.4
Aspen 73	73/82	4.7	14.8	227.2
Scarlett 66	66/76	5.3	13.8	190.8
Scarlett 74	74/80	4.8	14.9	157.7

Table 2: (*E*)-2-nonenal (*E*2N) and nonenal potential (NP) contents of pale malts. Nonenal concentrations are expressed for an equivalent 12 °P wort.

Pale malt	(*E*)-2-Nonenal (ppb)	Nonenal potential (ppb)	NP/*E*2N
Pilsner	292.2	226.7	0.78
Astoria 75	495.0	281.5	0.57
Aspen 73	391.4	178.4	0.46
Scarlett 66	188.4	202.1	1.07
Scarlett 74	211.1	206.6	0.98

As previously shown by Noël et al. (1999b), the Drost's experiment removes only around 50 % of the initial (*E*)-2-nonenal. As a consequence, when the free (*E*)-2-nonenal level is very high (as it is the case here for pale malts), the nonenal potential value also includes a fraction of free (*E*)-2-nonenal. Our data indicate thus that very few (or no) (*E*)-2-nonenal/protein adducts are present in pale malts as depicted by the ratio NP/*E*2N below one.

Very different conclusions must be drawn for coloured malts (table 3). In that case, the roasting process rapidly reaches temperatures at which LOX are inactivated. As a result, the free (*E*)-2-nonenal level can be from 5 to 50 times lower than what is found in pale malts (table 4).

Table 3: Final temperature, moisture, antioxidant activity (Tinh) and LOX activity of coloured malts.

Coloured malt	Final kilning temperature (°C)	Moisture (%)	Tinh (min)	LOX (nkat/g malt dry weight)
Amber 50 °EBC	100-105	6.7	9.6	9.1
Cara 20 °EBC	110	8.8	13.9	6.1
Cara 50 °EBC	120	6.1	14.0	11.6
Cara 120 °EBC	130-135	4.3	15.2	5.4

Table 4: (*E*)-2-nonenal (*E*2N) and nonenal potential (NP) contents of coloured malts. Nonenal concentrations are expressed for an equivalent 12 °P wort.

Coloured malt	(*E*)-2-Nonenal (ppb)	Nonenal potential (ppb)	NP/*E*2N
Amber 50 °EBC	42.9	87.5	2.04
Cara 20 °EBC	10.1	28.0	2.77
Cara 50 °EBC	12.3	28.2	2.29
Cara 120 °EBC	17.4	47.5	2.73

For a same roasting process, the level increases with the final kilning temperature (as it is the case for Cara malts). This observation indicates that free (*E*)-2-nonenal in coloured malts derived mostly from the non-enzymatic pathway.
On the other hand, the higher roasting temperatures are favourable for creating (*E*)-2-nonenal/protein adducts. Consequently, the nonenal potential of coloured malts are much higher than the level of free (*E*)-2-nonenal. The ratio of NP/*E*2N is greater than 2 for all tested coloured malts (table 4).

Various process parameters can be altered during kilning to achieve the desired malt. The temperature and moisture profiles during kilning appeared to have the most profound effect upon the antioxidant activity (Chandra, 2001; Liégeois et al., 2001). For coloured malts, the production of antioxidant activity is independent of temperature until the grain moisture drops below a level of approximately 5 %. Once the grain has dried to beyond this limit, the temperature becomes the dominant factor and the antioxidant levels rise exponentially to a concentration dependent upon the temperatures applied (Chandra, 2001; Liégeois et al., 2001). The same behaviour was reported by Woffenden et al. (2002) for pale malts.
For our pale malts, the higher the moisture level, the lower the antioxidant activity. Probably also due to their high water content (close or above 5 %), our four coloured malts did not exhibit higher antioxidant activity than the pale malts.

Contribution of malt nonenal potential to the cardboard flavour of aged beer
Collin et al. (1997) previously reported that the higher the kilning temperature, the higher the nonenal potential of pale malt. The (*E*)-2-nonenal level measured in the

wort after 15 minutes of mashing at 36 °C under N_2 was proposed as an indicator of the malt nonenal potential.
In the present work, we artificially created nonenal potential by steeping green malt in a 10 ppm (*E*)-2-nonenal solution before kilning. As expected, nonenal potential measured at the 36 °C mashing stage revealed to be higher (table 5). However, whatever the steeping applied before kilning (in presence or absence of 10 ppm (*E*)-2-nonenal), similar concentrations were measured in the wort after filtration. As previously reported by Liégeois et al. (2002), the presence of oxygen during mashing (allowing high lipoxygenase activity) has the most profound effect upon the wort nonenal potential.

Table 5: Nonenal potential in wort samples produced from 100 % Cork malt, either steeping in a 10 ppm (*E*)-2-nonenal solution or in an aqueous solution before kilning. Values are expressed for an equivalent 12 °P wort. Mashing was performed under air bubbling from deoxygenated malt flour. Tinh in the filtered wort were 43.1 min and 41.5 min, respectively. Tinh in the clarified wort were 49.5 min and 46.4 min, respectively.

Steeping before malt kilning	in a 10 ppm *E*2N solution	in an aqueous solution
NP/ *E*2N		
After mashing at 36 °C for 15 min	0.57	0.43
Nonenal potential (ppb)		
After mashing at 36 °C for 15 min	14.6	9.7
Filtered wort	13.3	14.8
Boiled wort	6.4	6.7

In a second experiment, we attempted to assess the impact of a natural source of nonenal potential either by using the same variety of malt kilned at two different temperatures (66 and 74 °C) or by adding 8.8 % of Amber malt rich in (*E*)-2-nonenal protein adducts (see table 4). To avoid excessive oxidation linked to laboratory-scale experiments, malt flour was deoxygenated and mashing was carried out under nitrogen bubbling.
Slight increase in the ratio NP/*E*2N was observed at the 36 °C mashing stage for higher kilning temperatures (0.26 for 100 % Scarlett 66, 0.28 for 100 % Scarlett 74 and 0.36 for 8.8 % Amber). During mashing and filtration, the free (*E*)-2-nonenal content dramatically decreased for the three productions, as expected. On the other hand, a higher value of nonenal potential was still measured in the filtered wort containing 8.8 % Amber malt (3.8 ppb versus 2.0 ppb for 100 % pale malt).
Probably linked to its higher antioxidant activity, the coloured wort exhibited a very good resistance to lipid autoxidation, leading to only 3.3 ppb nonenal potential in the clarified wort.
To avoid excessive excretion of sulphites, a top fermentation yeast was used. During fermentation, yeast strongly reduced free (*E*)-2-nonenal (nonenal potential in less extend). Nonenal potential remained higher in the coloured wort (1.3 ppb versus 0.9 ppb for 100 % pale malt). This first proof that the use of coloured malt can increase the nonenal potential of fresh beer was further corroborated by the level of free (*E*)-2-nonenal measured in the aged beer (0.70 ppb versus 0.26 ppb for 100 % pale malt). In conclusion, (*E*)-2-nonenal/protein adducts created at high kilning temperature are able to contribute to the free (*E*)-2-nonenal level released during ageing.

Table 6: Nonenal contents, nonenal potential and antioxidant activity (Tinh) in wort and beer samples produced from, either 100 % Scarlett 66, or 100 % Scarlett 74, or 91.2 % Scarlett 74 and 8.8 % Amber 50 °EBC. Nonenal concentrations are expressed for an equivalent 12 °P wort. Mashing was performed under nitrogen bubbling from deoxygenated malt flour. Fresh beers contain less than 1 ppm sulphite. * NP/*E*2N after mashing for 15 min at 36 °C.

	Scarlett 66 100 %		Scarlett 74 100 %		Scarlett 74 / Amber 50°EBC 91.2 % / 8.8 %	
(*E*)-2-Nonenal (ppb)		*		*		*
After 15 min at 36 °C	60.8	0.26	76.5	0.28	100.6	0.36
Filtered wort	0.31		0.36		0.61	
Clarified wort	0.25		0.21		0.49	
Fresh beer	< 0.03		< 0.03		< 0.03	
Aged beer – 5 days at 40 °C	0.19		0.26		0.70	
Nonenal potential (ppb)						
After 15 min at 36 °C	15.5		21.6		36.1	
Filtered wort	3.0		2.0		3.8	
Clarified wort	2.8		2.0		3.3	
Fresh beer	0.9		0.9		1.3	
Tinh (min)						
Filtered wort	36.0		35.4		40.4	
Clarified wort	41.4		43.8		49.8	

To confirm this contribution, we followed in a third experiment the nonenal potential of a 10 % Caramel malt – based wort, in comparison to the corresponding pale wort (table 7). Again, the increase of (*E*)-2-nonenal/protein adducts brought by 10 % Caramel malt is measurable until the final beer.
In that case, the antioxidant activity was measured all along the brewing process (figure 1). As expected, the use of 10 % Caramel malt lead to a 5 % increase of the wort antioxidant activity. Wort filtration revealed to be the most important step for antioxidant extraction. In our experiment, around 70 % of the malt antioxidants were extracted in the wort and survived until the final beer.

Table 7: Nonenal contents and nonenal potential in wort and beer samples produced from 100 % pale malt or 90 % pale malt and 10 % Caramel 152 °EBC. Nonenal concentrations are expressed for an equivalent 12 °P wort. Mashing was performed without specific bubbling. Fresh beers contain less than 1 ppm sulphite.

	Pale malt 100 %	Pale malt / Caramel 152 °EBC 90 % / 10 %
Nonenal potential (ppb)		
Filtered wort	5.5	6.6
Clarified wort	5.8	7.3
Fresh beer	0.9	1.1

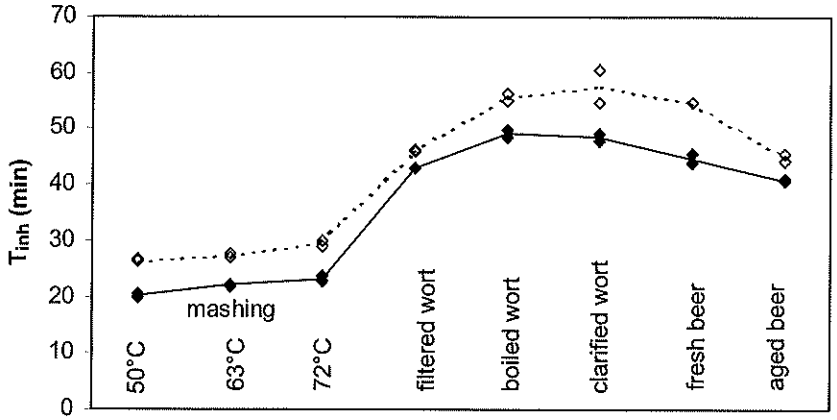

Figure 1: Antioxidant activity (Tinh) profile during the brewing process. (—) mashing of 100 % pale malt; (---) mashing of 90 % pale malt and 10 % Caramel 152 °EBC. Mashing was performed without specific bubbling. Antioxidant activity measured on a methanolic extract of malt (133.3 mg/l) reported following Tinh: 16.3 min for the pale malt and 24.8 min for the Caramel malt.

CONCLUSIONS

Pale malts contain very few (*E*)-2-nonenal/protein adducts, which will be further degraded during mashing. Therefore, selecting pale malt with less nonenal potential does not significantly improve beer stability in terms of cardboard flavour. On the other hand, the increase of (*E*)-2-nonenal/protein adducts brought by 10 % of coloured malt remains significant after boiling, despite the lower lipoxygenase activity during mashing and the lower autoxidation during boiling (higher antioxidant activity).

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