Effect of malt kilning temperature on the concentration of (E)-β-damascenone in malt, mashing and wort boiling in the brewing process

José Antonio Piornos Martinez, Jean-Philippe Kanter, Dimitrios P Balagiannis, Elisabeth Koussissi, August Bekkers, Jane K Parker, Johan Vissenaekens, Bert-Jan Grootes, Eric Brouwer

Effect of malt kilning temperature on the concentration of (*E*)-β-damascenone in malt, mashing and wort boiling in the brewing process

JOSÉ A. PIORNOS[1], Jean-Philippe Kanter[1], Dimitrios P. Balagiannis[1], Elisabeth Koussissi[2,†], August Bekkers[2], Johan Vissenaekens[3], Bert-Jan Grootes[3], Eric Brouwer[2] and Jane K. Parker[1]

[1] *Department of Food and Nutritional Sciences, University of Reading, RG6 6DZ, UK. j.piornos@reading.ac.uk*

[2] *Heineken Supply Chain BV, Burgemeester Smeetsweg, 1, 2382 PH Zoeterwoude, The Netherlands.*

[3] *Mouterij Albert, Kanaaldijk, 2870 Ruisbroek-Sint-Amands, Belgium.*

[†] *Current address: Department of Wine, Vine and Beverage Sciences, University of West Attica, Ag. Spyridona Str., 12,210 Athens, Greece.*

Abstract

(*E*)-β-Damascenone (bDam) is one of the most important aroma compounds in foods and in both regular and alcohol-free beers. In the present study, the effect of the curing temperature during kilning on the concentration of bDam in malt was monitored, as well as during mashing and wort boiling. Two different varieties of malt (spring and winter) were compared. The results showed different trends during malt kilning, with an increase in the levels of the aroma compound over time at 78 and 90 °C. During mashing and wort boiling, bDam was formed following different trends, this attributed to a balance between formation and evaporation. Moreover, malts and worts from winter barley contained more bDam than spring barley. Further research is required in order to identify and monitor the precursors of this potent aroma compound.

Keywords: damascenone, malting, wort boiling, brewing.

Introduction

(*E*)-β-Damascenone (bDam) is widely known in flavour science because of its extremely low orthonasal detection threshold of 0.004 µg/L in water [1] or even 0.00075 µg/L in water [2]. Despite being present at very low concentrations, it has been demonstrated that it plays a key role in the aroma of several foods as varied as rape honey [3], Syrah wine [4] and blackberries [5], where this compound imparts their characteristic pleasant, fruity aroma. In beers, fruity aroma has been traditionally related to the presence of esters and alcohols. However, the role of bDam must not be underestimated. In Bavarian wheat beers, this compound was reported to have the highest OAV besides ethanol [1]. In pale lager and Pilsner, its concentration was 1.6 µg/L [6] and 2.3 µg/L [7], respectively and bDam has been previously reported in unhopped wort [8]. However, hops have also been reported as one possible source of bDam, where the presence of its precursor, the β-D-glucoside of 3-hydroxy-β-damascone, has been previously identified [9]. However, bDam has also been found in barley malt [10].

bDam in wort was detected at extremely high level (450 µg/kg wort) [11]. Nonetheless, its concentration decreased remarkably after fermentation (7 days at 20 °C), thus leading to a non-detectable final concentration in fresh beer in the cited study. However, in the case of alcohol-free beers produced following a cold contact fermentation (CCF) procedure, the low fermentation temperature (close to 0 °C) not only limited the formation of ethanol, but also limited the reduction of the level of bDam. In our previous study, the concentration of bDam in an alcohol-free beer brewed by CCF was 10.4 µg/L, well above its orthonasal detection threshold 0.23 µg/L [12].

Because of the high contribution of this compound to the overall aroma of beer, it is essential to understand the factors affecting its formation throughout the brewing process. Since it was demonstrated that bDam was formed during thermal processes [13], the hypothesis of this study was that the concentration of bDam in malt is affected by the kilning temperature, and that kilning at different temperature has an impact on the worts prepared from these malts. Consequently, the objectives of the present research were to study the formation of this compound during the curing stage of malt kilning from two varieties of barley, as well as to determine its levels at different stages of mashing and wort boiling prepared with malts cured at different temperatures.

Experimental

Briefly, the green malt from two different varieties (spring variety “Planet” and winter “Etincel”) were kilned in a pilot scale micro-malting equipment from Nordon & Cie. (Nancy, France). The samples (650 g) were kilned following a temperature gradient: initial temperature of 25 °C, raised to 55 °C over 10 min, then increased to 64 °C over 45 min, kept for 4 hours and 50 min and then increased to 65 °C over 3 hours and 15 min. After this stage (drying stage), the malt was cured isothermally at 65, 78 or 90 °C for 8.4 h. Samples were collected at the beginning

DOI: 10.5281/zenodo.5574966

of the curing stage, after 4.8 h and at the end of curing. After collection from the micro-malting equipment, the rootlets of the grains were removed by manual rubbing, sieved off and the malt stored at -30 °C.

For the preparation of mash and wort, the final malt samples, i.e., those cured for 8.4 h, were coarsely ground using a coffee grinder and sieved through a 355-µm mesh size sieve in order to remove undesired fine particles. The ground malt (40 g) was added to 120 mL deionised water at 55 °C (mash). The mash was kept at this temperature for 15 min with constant magnetic stirring. Then, the temperature was increased to 63 °C, kept for 40 min, then raised to 72 °C and kept for another 15 min, and a final step of 78 °C for 20 min. The wort obtained this way was filtered through a 1-mm mesh sieve and then boiled for 30 min in an open beaker. Samples were taken at the beginning of the 63 °C-step, at the end of the 72 °C-step and after wort boiling.

Quantification of (E)-β-damascenone

Malt samples (5 g) were extracted with 25 mL of deionised water at room temperature for 60 min under stirring. The malt extracts, as well as the mash and wort, were centrifuged at 5500×g for 15 min at 4 °C. The pH was adjusted to 3.0 using 1 M HCl. Aliquots (5 mL) were poured into 20-ml SPME vials containing 1.3 g NaCl. α-Ionone (5 µL at 5 mg/L in ethanol) was used as internal standard. The vials were incubated at 60 °C for 10 min, and then a DVB/Carboxen®/PDMS SPME fibre (65 µm, 2 cm) from Supelco (Bellefonte, PA, USA) was exposed for 45 min. The fibre was desorbed in the injection port of the GC in splitless mode at 250 °C for 20 min. The instrument used was a 7890A gas chromatograph coupled to a 5975C inert XL EI/CI MSD triple axis mass spectroscopy detector from Agilent Technologies (Santa Clara, CA, USA). The carrier gas was helium at 3 mL/min. A non-polar ZB-5MSi column (30 m, 0.25 mm i.d., 1.0 µm df) from Phenomenex (Torrance, CA, USA) was used. Data acquisition was performed in SIM mode, using the ions (first ion for quantification) 121 and 190 for bDam, and 121 and 192 for α-ionone. Mass spectra were recorded in the EI mode at an ionisation voltage of 70 eV and source temperature of 230 °C. The experiments were performed in duplicate, and the results were expressed in relation to the concentration of the internal standard.

Statistical analysis

Duncan's test for multiple comparisons was applied at a significance level α=0.95 by using the software InfoStat 2017, developed by the National University of Córdoba (Córdoba, Argentina).

Results and discussion

The effect of curing time on the level of bDam in barley malt at different kilning temperatures (isothermally) was studied. Figure 1 shows the concentration of this compound in terms of the ratio of the peak areas of bDam and the internal standard. The results show an increase in the amount of bDam in malts kilned at 78 and 90 °C over time. At 78 °C the maximum level was reached after 8.4 h curing, whereas at 90 °C the levels stabilised at the maximum after 4.8 h, this being not significantly different to the samples kilned for longer time (8.4 h).

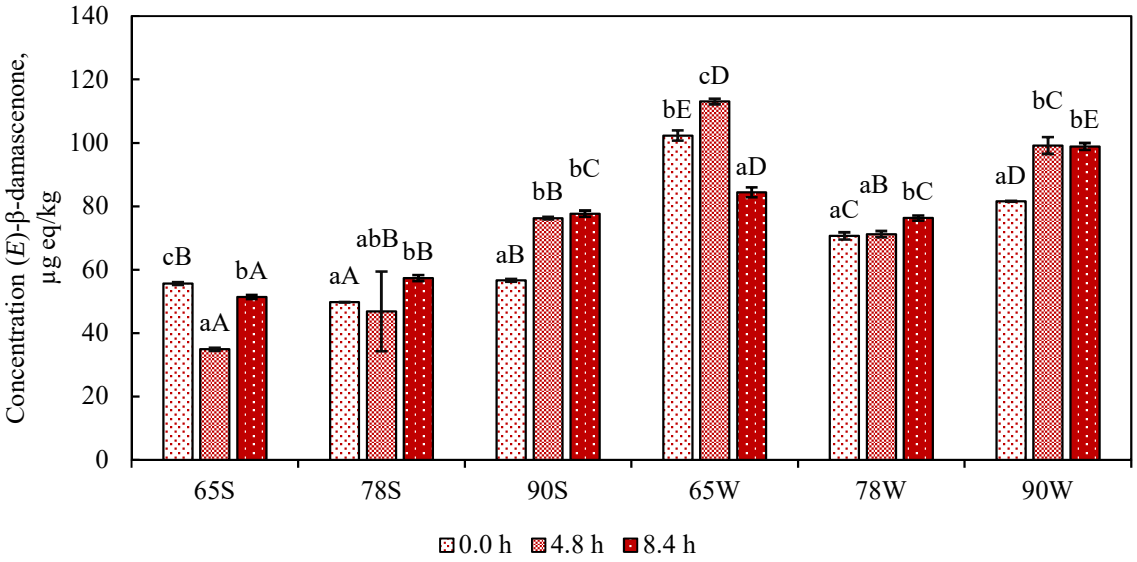

Figure 1: *Effect of curing time and temperature (65, 78 and 90 °C) on the formation of (E)-β-damascenone during malt kilning for spring (S) and winter (W) barley. Results expressed as µg equivalent of α-ionone per kg malt. Significant differences ($p<0.05$) between curing time within experiments and between experiments at the same curing time are represented by lower and uppercase letters, respectively.*

The results showed a certain degree of variability, especially those samples kilned at 65 °C. The reason behind this might be the variability associated with real food matrices. The green malt samples were collected from industrial germination boxes and kilned using a pilot-scale oven. Heating in this kind of pilot-scale ovens is not completely homogenous and also the hot air employed to process the malt may not be spread equally across the samples. The processed malts were placed in aeriated boxes in the oven in different positions, this possibly leading to uneven air flow.

The malts prepared for the first part of this study were used for mashing and the preparation of wort. The mixture of malt grains steeped in water is known as mash, whereas the wort is the liquid extract after filtration. In order to understand the behaviour of bDam during mashing and wort boiling, different samples were collected at the beginning of the mashing process, i.e., before the step at 63 °C, at the end of mashing and at the end of wort boiling. Figure 2 shows the levels of bDam throughout the mashing and wort boiling processes. The data showed different trends dependent on the variety of barley used. Spring barley malts kilned at 65 and 78 °C showed an increasing trend during mashing and wort boiling. However, bDam decreased slightly during mashing and increased after wort boiling when using malts kilned at 90 °C. Similar behaviour was observed in malts from winter barley, but the differences were larger. For these malts, the concentration after mashing decreased significantly and increased to their highest values after wort boiling. These results suggested a balance of loss and formation of bDam: this compound was lost during mashing due to evaporation and its formation was not enough to result in an overall accumulation. However, the higher temperature applied during wort boiling might lead to a formation rate higher than the loss, and also the evaporation of water during boiling could have contributed to an increase in the final concentration of bDam.

With respect to the varieties of barley used in this study, a higher average amount of bDam was found in winter barley than in the spring variety. The differences were significant ($p<0.05$) for both the malts, as well as in the worts prepared from those malts. Malts from barley winter contained 57% more bDam than spring barley, on average. In the case of the wort, the difference was smaller, around 33% higher.

bDam is an aroma compound present in a huge variety of foods, mainly of plant origin. It is widely accepted that this compound can be formed from the hydrolytic degradation of the carotenoid neoxanthin [14, 15]. These authors proposed a formation mechanism via the so-called "grasshopper ketone". However, this formation route in barley has not been confirmed yet, despite neoxanthin being found in barley grains [16, 17]. Little is known regarding the role of cultivar on the content of carotenoids in barley grain. Carotenoid content in wheat was found to be lower in spring varieties, but this was not proven in barley [18]. Other studies proved that this compound can be formed from the enzymatic hydrolysis of the β-D-glucosides of 5-megastigmen-7-yne-3,9-diol and 3-hydroxy-β-damascone [19]. These precursors have been identified in fruit juice but not in barley. Nonetheless, the theory of a glycoside precursor has been supported by results showing an increase in the concentration of bDam in beers after the addition of β-glucosidase [11].

The formation of bDam in malt and wort was favoured at higher temperatures. This has been observed in other foods after heat treatment. For instance, a considerable increase in the concentration of bDam (13.5 times higher) has been reported in black tea infusions after sterilisation by heat treatment (121 °C for 10 min) [13]. It is of great

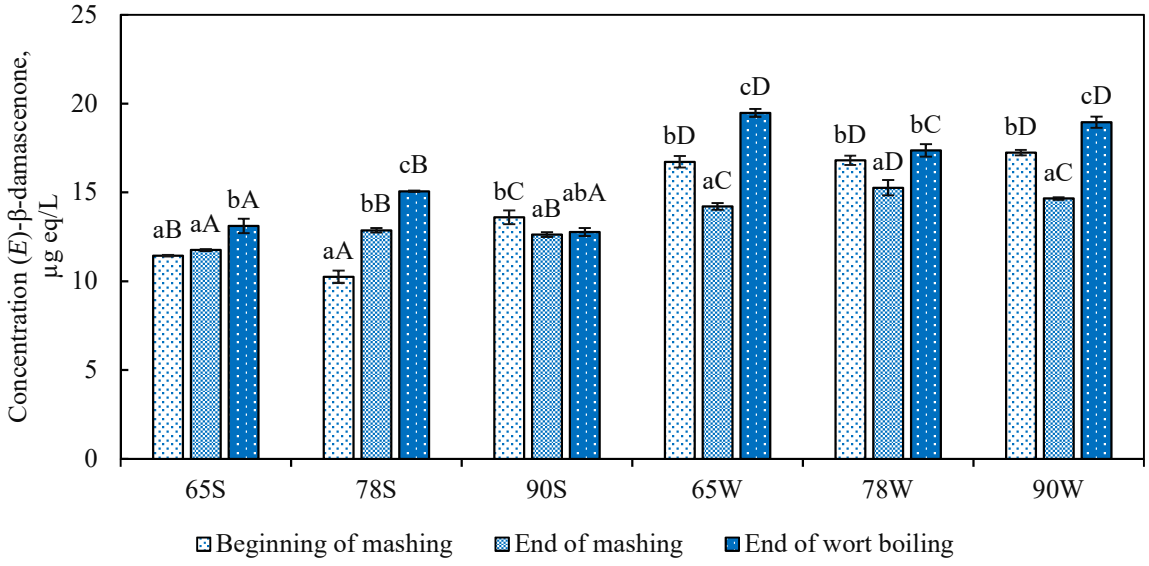

Figure 2*: Levels of (E)-β-damascenone during mashing and wort boiling for spring (S) and winter (W) malts kilned at different temperatures (65, 78 and 90 °C). Results expressed as µg equivalent of α-ionone per L wort. Significant differences ($p<0.05$) between samples within experiments and between experiments at the same sampling stage are represented by lower and uppercase letters, respectively.*

importance to understand how process conditions, like temperature, affect the formation of bDam in malt and wort. This is even more critical when brewing alcohol-free beers by fermentation at very low temperature (such is the case of CCF). The formation of bDam was proven to occur during the thermal degradation of 9'-*cis*-neoxanthin in a model system containing peroxyacetic acid at temperatures from 60 to 90 °C [20]. Moreover, identification of precursors and monitoring of their behaviour throughout processing are required in order to elucidate the chemical mechanism and the factors that affect its rate of formation. This would help control the amount of bDam in malts and beers by monitoring and possibly manipulation of the concentrations of the precursors in the raw materials.

Conclusion

bDam was monitored during the curing stage of malt kilning as well as during mashing and wort boiling. During curing in kilning, the level of bDam increased over time at the highest curing temperatures (78 and 90 °C), with maxima reached after 8.4 h curing at 90 °C. These results demonstrated the great importance of temperature and time during malt kilning. The temperature of the malt curing did not have a significant impact on the levels of bDam formed in the subsequent mashing and boiling steps, with different trends for winter and spring barleys. On average, malts and worts from winter barley presented significantly higher amounts of bDam than those from spring barley ($p<0.05$). Further research could provide better understanding of the differences related to variety, and thus be able to better control the concentration of this powerful aroma compound in the beer.

References

1. Langos D, Granvogl M, Schieberle P. Characterization of the key aroma compounds in two Bavarian wheat beers by means of the sensomics approach. J Agric Food Chem. 2013;61(47):11303–11.
2. Semmelroch P, Laskawy G, Blank I, Grosch W. Determination of potent odourants in roasted coffee by stable isotope dilution assays. Flavour Fragr J. 1995;10(1):1–7.
3. Ruisinger B, Schieberle P. Characterization of the key aroma compounds in rape honey by means of the molecular sensory science concept. J Agric Food Chem. 2012;60(17):4186–94.
4. Zhao P, Gao J, Qian M, Li H. Characterization of the key aroma compounds in Chinese syrah wine by gas chromatography-Olfactometry-Mass spectrometry and Aroma reconstitution studies. Molecules. 2017;22(7):1045.
5. Klesk K, Qian M. Aroma extract dilution analysis of Cv. Marion (Rubus spp. hyb) and Cv. Evergreen (R. laciniatus L.) blackberries. J Agric Food Chem. 2003;51(11):3436–41.
6. Schieberle P. Primary odorants of pale lager beer - Differences to other beers and changes during storage. Z Lebensm Unters Forsch. 1991;193(6):558–65.
7. Fritsch HT, Schieberle P. Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian Pilsner-type beer. J Agric Food Chem. 2005;53(19):7544–51.
8. De Schutter DP, Saison D, Delvaux F, Derdelinckx G, Rock J-M, Neven H, et al. Characterization of volatiles in unhopped wort. J Agric Food Chem. 2008;56(1):246–54.
9. Kollmannsberger H, Biendl M, Nitz S. Occurence of glycosidically bound flavour compounds in hops, hop products and beer. Monatsschrift für Brauwiss. 2006;59(5–6):83–9.
10. Fickert B, Schieberle P. Identification of the key odorants in barley malt (caramalt) using GC/MS techniques and odour dilution analyses. Nahrung / Food. 1998;42(6):371–5.
11. Chevance F, Guyot-Declerck C, Dupont J, Collin S. Investigation of the β-damascenone level in fresh and aged commercial beers. J Agric Food Chem. 2002;50(13):3818–21.
12. Piornos JA, Balagiannis DP, Methven L, Koussissi E, Brouwer E, Parker JK. Elucidating the odor-active aroma compounds in alcohol-free beer and their contribution to the worty flavor. J Agric Food Chem. 2020;68(37):10088–96.
13. Kumazawa K, Masuda H. Change in the flavor of black tea drink during heat processing. J Agric Food Chem. 2001;49(7):3304–9.
14. Isoe S, Katsumura S, Sakan T. The Synthesis of Damascenone and β-Damascone and The Possible Mechanism of Their Formation from Carotenoids. Helv Chim Acta. 1973;56(5):1514–6.
15. Puglisi CJ, Elsey GM, Prager RH, Skouroumounis GK, Sefton MA. Identification of a precursor to naturally occurring β-damascenone. Tetrahedron Lett. 2001;42:6937–9.
16. Sreenivasulu N, Radchuk V, Alawady A, Borisjuk L, Weier D, Staroske N, et al. De-regulation of abscisic acid contents causes abnormal endosperm development in the barley mutant seg8. Plant J. 2010;64(4):589–603.
17. Jaleel CA, Manivannan P, Wahid A, Farooq M, Al-Juburi HJ, Somasundaram R, et al. Drought Stress in Plants: A Review on Morphological Characteristics and Pigments Composition. Int J Agric Biol. 2009;11(1):100–5.
18. Konopka I, Czaplicki S, Rotkiewicz D. Differences in content and composition of free lipids and carotenoids in flour of spring and winter wheat cultivated in Poland. Food Chem. 2006;95(2):290–300.
19. Skouroumounis GK, Massy-Westropp RA, Sefton MA, Williams PJ. Precursors of damascenone in fruit juices. Tetrahedron Lett. 1992;33(24):3533–6.
20. Bezman Y, Bilkis I, Winterhalter P, Fleischmann P, Rouseff RL, Baldermann S, et al. Thermal oxidation of 9′-cis-neoxanthin in a model system containing peroxyacetic acid leads to the potent odorant β-damascenone. J Agric Food Chem. 2005;53(23):9199–206.