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Investigation of the β-Damascenone Level in Fresh and Aged Commercial Beers

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This study investigated the increase of β-damascenone content during aging in a variety of commercial Belgian beers. Quantities detected in fresh beers were generally low (from 6 ng/g to 25 ng/g). After 5 days at 40 °C, the level increased (to as much as 210 ng/g) in most of the beers studied, according to the type of beer. Further experiments showed that wort initially contains large quantities of β-damascenone (450 ng/g), but that degradation of the compound during fermentation accounts for the low concentrations observed in fresh beers. Production during beer aging can be partially explained by acid hydrolysis of glycosides.

KEYWORDS: ***β*-Damascenone; flavor; beer aging; glycosides; fermentation**

INTRODUCTION

The flavor stability of beer during aging has been the subject of numerous scientific studies over the past decade. Attention has been paid particularly to the mechanisms leading to the release of the well-known compound *trans*-2-nonenal, responsible for the characteristic cardboard flavor of aged beers (*1*−*3*). *Trans*-2-nonenal protein adducts present in fresh beer could release free nonenal during aging, especially at low pH, but clearly other key flavor molecules also contribute to staling. Examples are the less-studied compounds methional and dimethyltrisulfide, whose concentrations rise during storage (*4*, *5*).

An increase in β-damascenone (8*E*-megastigma-3,5,8-trien-7-one) concentration during aging has been reported for tobacco (*6*), hops (*7*, *8*), and wine (*9*), but the contribution of this carotenoid-derived compound to the flavor of aged beer is not clear. Linked by Theddy et al. (*10*) to beer staling in an inconclusive report, β-damascenone has been identified as a key odor in a variety of fruits (peach, lychee, and grape) and beverages (coffee, wine, and beer) (*11*−*16*). It is characterized by a very low odor threshold in water (0.02−0.09 ng/g) (*17*), and examples of its odor description (by gas chromatography−olfactometry) include "fruity-flowery" (*12, 16, 18*), "honey" (*19*, *20*), and "apple" (*11*, *13*, *21*).

β-damascenone can be formed by acid-catalyzed conversion of polyols (enyne diols or allene triols) derived from enzymatic transformations of the carotenoid neoxanthin (*22*, *23*). The polyol precursors of β-damascenone often accumulate as glycosides in the skin of fruits and plants, to be released later under acidic conditions (*24*). A clearer understanding of the factors affecting the release and accumulation of flavor-related norisoprenoids during aging could contribute to better control of beer flavor development.

This study shows how the concentration of β-damascenone increases during artificial aging in a variety of commercial Belgian beers. Possible mechanisms of accumulation of this compound during aging are proposed.

MATERIALS AND METHODS

Chemicals. The chemicals used were β-damascenone (>95%, a kind gift of Haarmann and Reimer GmbH, Puteau, France), dodecane (99%, Sigma-Aldrich, Bornem, Belgium), β-glucosidase (Sigma-Aldrich), and the pure analytical grade solvents dichloromethane (redistilled twice), methanol (purity > 99.8%) (Romil, Gent, Belgium), and ultrapure water (Milli-Q water purification system, Millipore, Bedford, MA).

Beers. Eight Belgian beers were purchased from local supermarkets: 1 white brewed with 40% unmalted wheat (WH), 1 amber (AM), 3 dark ales (D1, D2, D3), 2 fruit (FP (peach) and FR (raspberry)), and 1 lager (LG). Each beer was subjected to artificial aging (40 °C for 5 days in a dark room) and the β-damascenone content was measured in sets of fresh and artificially aged samples.

Extraction of β-Damascenone. β-Damascenone was extracted from beer by the method of Guyot-Declerck et al. (*25*). The resin (XAD-2, Supelco, Bellefonte, PA), pre-washed with dichloromethane and then methanol (4 h each) in a Soxhlet apparatus, was stored in methanol at 4 °C until used.

The resin (2 g) was thoroughly rinsed with ultrapure water (100 mL) and poured into a 100-mL Schott flask (Vel, Leuven, Belgium) containing 50 mL of beer, and the flask was shaken at 200 rpm for 2 h at 20 °C. The content of the flask was transferred to a glass column (60 × 1 cm i.d.), and the resin was rinsed with 100 mL ultrapure water. Molecules bound to the resin were eluted with dichloromethane (40 mL, flow rate 0.75 mL/min). The extract was dried over anhydrous sodium sulfate (99%, Janssen, Geel, Belgium) and concentrated to 0.5

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mL in a Snyder-Kuderna column at 45 °C with 500 μL of standard solution (dodecane, 15 μg/g in dichloromethane) prior to GC analysis. The β-damascenone concentration of each beer type was calculated from standard curves obtained by spiking beer samples with β-damascenone (0, 10, 50, 100, and 250 ng/g in beer).

Gas Chromatography−FID Analytical Conditions. GC analyses (1-μL samples) were conducted on a Hewlett-Packard 5890 gas chromatograph with a 7673 automatic sampler, a cold on-column injector, a flame ionization detector, and a Shimadzu CR4A integrator. Volatile compounds were separated with helium as a carrier gas (1.5 mL/min) in a 50 m × 0.32 mm i.d., wall-coated, open tubular (WCOT) CP-SIL5 CB (Chrompack, Antwerp, Belgium) capillary column (film thickness, 1.2 μm), preceded by a 1 m × 0.53 mm i.d. capillary column coated with a thin film of methyl silicone phase (Hewlett-Packard, Brussels, Belgium). The oven temperature was raised from 36 to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and to 250 °C at 3 °C/min. The injector was maintained at 3 °C above the oven temperature and the detector was at 260 °C. The minimum peak area for data acquisition was set at 500 μV/sec.

Quantification of β-Damascenone. In each beer, the concentration of β-damascenone was determined according to eq 1:

$$\frac{A_{\beta-\text{damascenone tot}}}{A_{\text{dodecane}}} = S\left(\frac{C_{\beta-\text{damascenone}}}{C_{\text{dodecane}}}\right) + S\left(\frac{C_{\beta-\text{damascenone added}}}{C_{\text{dodecane}}}\right) \quad (1)$$

$A_{\beta-\text{damascenone tot}}$ and A_{dodecane} refer to the areas obtained in GC−FID for total β-damascenone and dodecane (external standard), respectively; $C_{\beta-\text{damascenone}}$ and $C_{\beta-\text{damascenone added}}$ correspond to the initial and added β-damascenone concentrations in beer, respectively; and C_{dodecane} is the dodecane concentration in the dichloromethane extract equal to 15 μg/g. The slope of the regression line, S, can be defined by eq 2:

$$S = \left(\frac{\text{RC}_{\beta-\text{damascenone}}}{\text{RC}_{\text{dodecane}}}\right) \times \text{RF}_{\beta-\text{damascenone}} \times 100 \quad (2)$$

with $\text{RC}_{\beta-\text{damascenone}}$ and $\text{RC}_{\text{dodecane}}$ = FID response coefficients of β-damascenone and dodecane, respectively; $\text{RF}_{\beta-\text{damascenone}}$ = recovery factor of β-damascenone; and 100 = concentration factor.

Determination of the initial β-damascenone concentration was performed by graphic extrapolation on the X axis of the regression line ($A_{\beta-\text{damascenone tot}}/A_{\text{dodecane}}$ versus $C_{\beta-\text{damascenone added}}/C_{\text{dodecane}}$).

Gas Chromatography−Olfactometry. Samples (2-μL) were analyzed on a Chrompack CP9001 gas chromatograph equipped with a splitless injector at 250 °C, an FID detector (280 °C), and a GC-odor port (250 °C). The separation conditions were as described in the previous paragraph. The eluent was equally split between an FID detector and an odor port (humidified air at 15 mL/min).

Biotransformation of β-Damascenone by Yeast. Worts without (control) or with added β-damascenone (524 and 1045 ng/g) were fermented in duplicate. The green wort, purchased from a local brewery (12 °Plato; 90% malt; 10% corn), was boiled without hops for 75 min and further clarified for 20 min. The hot trub was removed and the wort was cooled. After spiking with β-damascenone, a top fermentation brewing yeast was pitched in 1-L EBC tubes (*Saccharomyces cerevisiae* BRAS 212, pitching rate 7.5 ×10^6 cells/mL). Samples were taken just after pitching and after 7 days at 20 °C, centrifuged, and their β-damascenone content determined.

Glycoside Hydrolysis by β-Glucosidase. β-damascenone formation via enzymatic hydrolysis was studied in white (WH) and dark (D3) beers. The latter was chosen for its high β-damascenone content after artificial aging. β-Glucosidase (10 mg) was suspended in 50 mL of beer, flushed with N_2, and incubated at 37 °C for 1 h before analysis. For each beer, a control without added enzyme was also prepared.

RESULTS AND DISCUSSION

Recovery Factors and Reproducibility. These were determined by analyzing 10 replicates of a spiked beer sample (50 μg/g β-damascenone added to fresh beer with an initial level close to 0.01 μg/g). The coefficient of variation was 8% with a recovery factor of 82%.

Table 1. Comparison of the Dilution Factors Obtained for Isoamyl Acetate and β-Damascenone in Fresh and Artificially Aged Beers (lager pH 4.2) by Aroma Extract Dilution Analysis (AEDA)

compound	RI[a]	dilution factor (3^{n-1})[b] fresh beer	aged beer	description of the odor
isoamyl acetate	853	1	9	fruity
β-damascenone	1372	12	243	fruity

[a] RI = retention index on CPSil5CB determined by interpolation of the retention times of an *n*-alkane mixture (C_6–C_{19}) analyzed under identical conditions. [b] Dilution factor: n = number of 3-fold dilutions until no odor was perceived.

Table 2. Concentrations (ng/g) of β-Damascenone in Fresh and Artificially Aged Belgian Beers

beer[a]	fresh conc.	slope[b]	R^2 [c]	aged conc.	slope[b]	R^2 [c]
LG	7	0.57	0.999	23	0.35	0.984
WH	7	0.39	0.992	14	0.39	0.999
AM	10	1.91	0.997	16	1.59	0.996
FP	25	0.53	0.996	160	0.39	0.905
FR	12	0.66	0.985	38	0.41	0.999
D1	23	0.83	0.976	23	0.41	0.997
D2	6	0.19	0.999	56	0.23	0.959
D3	8	0.30	0.991	210	0.33	0.999

[a] Beers: LG = lager; WH = white; AM = amber; FP = peach flavored; FR = raspberry flavored; and D1, D2, D3 = dark type ales. [b, c] Slope (S) and correlation coefficient (R^2) of the calibration curves obtained by standard addition: $A_{\beta-\text{damascenone tot}}/A_{\text{dodecane}} = S(C_{\beta-\text{damascenone added}}/C_{\text{dodecane}})$.

Comparison of β-Damascenone Concentrations in Fresh and Artificially Aged Beers. First, Grosch's aroma extract dilution analysis (AEDA) method (*26*) was used to assess the impact of β-damascenone in fresh and artificially aged lager beers. Compared to isoamyl acetate, which is known to be flavor-active in beers with a flavor threshold equal to 1.6 μg/g (*27*), β-damascenone was characterized by an exceptionally high FD value (**Table 1**). With its very low odor threshold (0.02 to 0.09 ng/g in water (*17*)), this fruity aroma was perceived by GC−olfactometry more intensely in artificially aged than in fresh beer extracts, thus suggesting an increase in the concentration of β-damascenone during aging.

To see whether a similar increase could be observed in other types of beers, the β-damascenone concentration was measured in several commercial Belgian beers (**Table 2** and **Figure 1**): 1 lager (LG), 1 white (WH), 1 amber (AM), 2 fruit (FP (peach) and FR (raspberry)), and 3 dark ales (D1, D2, and D3). In all cases, the fresh beers were found to contain very low levels of β-damascenone (below or near 25 ng/g).

During aging, the β-damascenone content showed a tendency to rise in most of the tested beers. The values for the lager beer (LG) reflected well the results obtained by GC−olfactometry (**Table 1**), being 3- to 4-fold higher in the artificially aged beer (**Table 2**). In most of the beers studied, the concentration rise rarely exceeded 15 ng/g (**Table 2**). Only three beers, the peach beer (FP) and two of the dark ales (D2 and D3), showed a higher increase (50 to 200 ng/g). This is probably due to the special ingredients used in these cases, such as spices, peach flavor (*11*), etc.

Effect of Fermentation on the β-Damascenone Level in Fresh Beer. It is generally accepted that yeast enzymes catalyze the reduction of off-flavor aldehydes to more neutral alcohols (*4*, *28*−*30*). Among these enzymes, alcohol dehydrogenase and

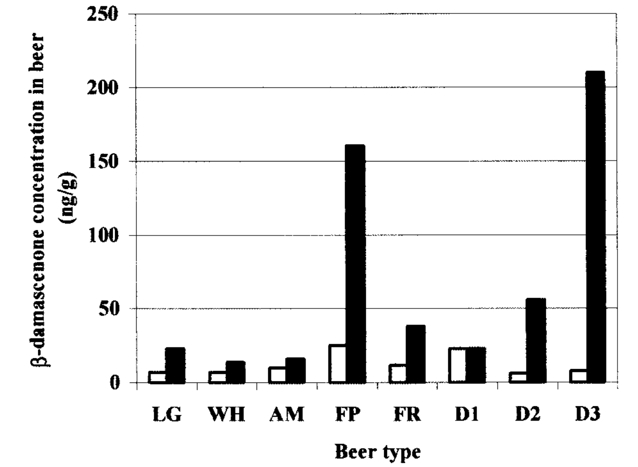

Figure 1. Evolution of β-damascenone concentrations in some commercial Belgian beers during aging. □ Fresh beer; ■ aged beer. LG = lager; WH = white; AM = amber; FP = peach flavored; FR = raspberry flavored; D1, D2, D3 = dark type ales.

Table 3. Removal of β-Damascenone during Fermentation

	β-damascenone concentration(ng/g)			density[a] (° Plato)
initial wort	450	450	450	11.4
level added to wort	0	500	1000	
beginning of fermentation	-	486-590	746	-
after 7 days at 20 °c	0–0	109–114	149–172	1.7

[a] Density of the corresponding worts and beers.

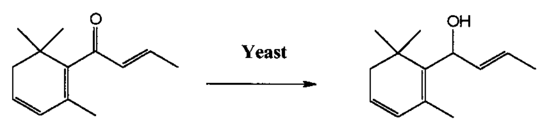

Figure 2. Reduction of β-damascenone during beer fermentation.

aldoketoreductase use either NADH or NADPH as a cofactor. The former enzyme reduces linear aldehydes particularly efficiently, whereas the latter may be responsible for reduction of the Strecker aldehydes isobutanal, 2- and 3-methylbutanal, and methional. An 11.4 °Plato wort enhanced with 0, 500, or 1000 ng/g β-damascenone was pitched with a *Saccharomyces cerevisiae* strain (7.5 × 10^6 cells/mL). As depicted in **Table 3**, β-damascenone was already present at a high level in the initial wort (450 ng/g). Up to now, this compound was frequently evidenced in hops (*31*), but since wort was boiled without this ingredient, our results suggest that β-damascenone in beer does not originate exclusively from hops. This supports the findings of Lermusieau et al. (*16*) who, by GC−olfactometry, also detected this compound in a nonhopped beer.

In all six cases, β-damascenone was extensively removed from the wort, leading to 0−172 ng/g after 7 days at 20 °C. Reduction and/or adsorption was very rapid, as shown by the amounts detected at the beginning of fermentation (which were lower than the initial concentration plus the spiking amount). This experiment strongly supports the hypothesis that fermentation is responsible for there being so little β-damascenone in fresh commercial beers (**Table 1**), but complementary data are necessary to confirm whether biotransformation to alcohol (**Figure 2**) is really involved.

Investigation of the Mechanisms of Formation of β-Damascenone during Beer Aging. As the pH of beer is significantly lower than that of wort, acid hydrolysis of precursors might

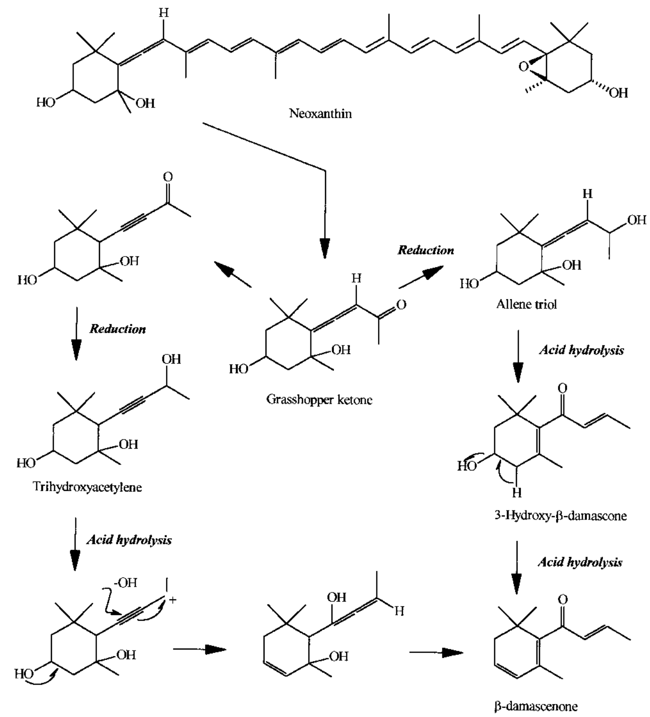

Figure 3. Pathways of formation of β-damascenone from neoxanthin.

Table 4. Effect of β-Glucosidase on the β-Damascenone Concentration (ng/g) in Fresh Beers

β-damascenone concentration	fresh beer	aged beer	fresh beer + β-glucosidase	R^2
WH[a]	7	14	11–11	0.8960
D3	8	210	54–79	0.9994

[a] WH = white beer; D3 = dark ale.

explain the increase in β-damascenone during aging. Potential precursors include the allene triols and acetylene diols arising from the degradation of neoxanthin present in the basic ingredients of beer (**Figure 3**). As such precursors are also known to be linked to sugars, as observed in wines (*32*), β-damascenone might also arise during aging through the chemical hydrolysis of glycosides. To assess the amount of glycosides present in fresh beer, we treated it with β-glucosidase before measuring the β-damascenone content. **Table 4** compares the β-damascenone contents of two beers (WH and D3), fresh and artificially aged, with those obtained after treatment of the fresh beers with β-glucosidase.

In the case of the white beer (WH), quite similar levels of β-damascenone were measured in the β-glucosidase-treated fresh beer and the untreated artificially aged beer. A blank treatment (no addition of β-glucosidase) showed that the temperature applied during incubation with the enzyme (37 °C for 1 h) could account for no more than 2 ng/g of the β-damascenone increase.

In the case of the dark ale (D3), the β-damascenone level was much higher in the fresh beer after β-glucosidase treatment, reaching 54−79 ng/g, but not nearly as high as that in the artificially aged beer (210 ng/g). This suggests, on one hand, that glycosides constitute an important source of off-flavors related to aging. On the other hand, it suggests that either β-glucosidase-catalyzed hydrolysis was incomplete in our experiment or other precursors such as allene triol and trihydroxyacetylene must contribute to β-damascenone during aging.

CONCLUSION

Although β-damascenone is detected at levels as high as 450 ng/g in wort, levels in fresh commercial beers are relatively low (below 25 ng/g). We present preliminary data indicating that the β-damascenone initially present could be degraded and/or adsorbed during the fermentation step. Most beers, depending on the type, show an increase in β-damascenone during artificial aging. This can be explained at least partially by acid hydrolysis of glycosides. Experiments aiming to determine the sensory impact of the β-damascenone increase are in progress.

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