JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

How Sotolon Can Impart a Madeira Off-Flavor to Aged Beers

Caroline Scholtes, Sabrina Nizet, and Sonia Collin*

Unité de Brasserie et des Industries Alimentaires, Earth and Life Institute ELIM, Faculté d'Ingénierie biologique, agronomique et environnementale, Université catholique de Louvain, Croix du Sud, 2 box L7.05.07, B-1348 Louvain-la-Neuve, Belgium

ABSTRACT: 4,5-Dimethyl-3-hydroxy-2(5*H*)-furanone or sotolon is known to impart powerful Madeira-oxidized—curry—walnut notes to various alcoholic beverages. It has been much studied in oxidized Jura flor-sherry wines, aged Roussillon sweet wines, and old Port wines, in which it contributes to the characteristic "Madeira-oxidized" aroma of these beverages. No scientific paper describes how sotolon might be involved in the Madeira off-flavor found in aged beers. The specific extraction procedure applied here allowed us to quantify this lactone in 7 special beers, at levels sometimes well above its threshold (from 5 to 42 μg/L after 6, 12, 18, and 24 months of natural aging, while unquantifiable in fresh beer). Investigation of spiked beers led us to highlight the key role of pro-oxidants and acetaldehyde. Addition of ascorbic acid without sulfites should be avoided by brewers, as the former would intensify sotolon synthesis. Acetoin, a beer fermentation byproduct, also emerged as possible precursor in beer when combined with serine.

KEYWORDS: *beer aroma, aging, sotolon, oxidation*

■ INTRODUCTION

The flavor characteristics of beer appear to alter greatly with time at a rate depending on the composition of the medium and its storage conditions. Faced with increasing export of their production, brewers are particularly concerned about aromatic and colloidal instability. Despite decades of research, deterioration of flavor, including loss of fresh aromas and the appearance of off-flavors, is still probably the greatest challenge for brewers. In addition to *trans*-2-nonenal, which contributes mainly to the well-known cardboard off-flavor,[1,2] numerous other odorants have been shown to appear during beer storage. Among them, dimethyltrisulfide, issued from methional and methionol degradations, is known to be involved in onion-soup defects of some lager beers.[3] The Strecker aldehyde methional (3-methylthiopropionaldehyde) can also contribute to wort/stale descriptors in heat-abused or oxidized beers. Cooked-apple-like β-damascenone,[4] solvent-like furfurylethyl ether,[5] smoky 4-vinylsyringol,[6] burnt guaiacol[7] or empyreumatic 2-sulfanylethyl acetate,[8] and ribes 3-sulfanyl-3-methylbutyl formate[9,10] are other compounds shown to be relevant to the sensory profile of aged beer.

According to the Dalgliesh curve,[11] the sweet/Madeira aroma is another defect which can be perceived after oxidative aging. 4,5-Dimethyl-3-hydroxy-2(5*H*)-furanone or sotolon is known to impart powerful Madeira-oxidized—curry—walnut notes to various alcoholic beverages. It has been much studied in aged sake, oxidized Jura flor-sherry wines, aged Roussillon sweet wines, and old Port wines, in which it contributes to the global aroma at a level generally above 10 μg/L.[12] At a very high level (>600 μg/L) it could be responsible for the typical rancio odor found in some fortified French wines.[13] Its presence has also been mentioned in gueuze beers, of which some production steps are very close to those used for yellow wines (long maturation in wood barrels, accentuated oxidation steps).[14] Sotolon exists in two enantiomeric forms, each characterized by particular olfactory characteristics, the (*S*)-form exhibiting a nice curry and walnut aroma, while the (*R*)-form is described as rancid walnut. The perception threshold of (*S*)-sotolon has been assessed at 0.8 μg/L, while the (*R*)-form requires a concentration of 89 μg/L in model solution to be perceived.[12] Its global olfactive detection threshold has been evaluated at 2 μg/L in hydroalcoholic solution and at 7 μg/L in wine.[15]

Sotolon synthesis pathways have been described in various foods and beverages. In yellow and sherry wines, sotolon is produced by aldol condensation of acetaldehyde (produced through fermentation or through ethanol oxidation in the cask)[16] and α-ketobutyric acid (issued from threonin through biochemical deamination)[17] (Figure 1A). Condensation of two molecules of acetaldehyde under oxidative conditions constitutes the main synthesis pathway of α-ketobutyric acid in Sherry and Madeira wine.[18,19] In ascorbic-acid-containing media, the same aldol condensation can occur between acetaldehyde and α-ketobutyric derived from ascorbic acid through loss of its first two carbon atoms[20] (Figure 1B). Ascorbic acid can also be oxidized to another C_4 moiety, generating sotolon through a reaction with one molecule of ethanol (Figure 1C). According to König et al.,[20] yet another pathway, evolving two molecules of ethanol, can lead to integration of the second and third carbon atoms of ascorbic acid into sotolon (Figure 1D). This requires formation of hydroxyacetic acid as an intermediate. In heat-treated media, hydroxyacetic acid can also be formed by Strecker degradation of serine, followed by oxidation.[21] This hydroxyacetic acid could still react with acetoin, a major fermentation metabolite produced by yeast through diacetyl reduction, generating sotolon by aldol condensation and further lactonization (Figure 1E). An alternative which probably requires quite harsh conditions is a direct reaction between hydroxyacetaldehyde and diacetyl[22] (Figure 1F).

Received: December 9, 2014
Revised: February 24, 2015
Accepted: February 28, 2015
Published: February 28, 2015

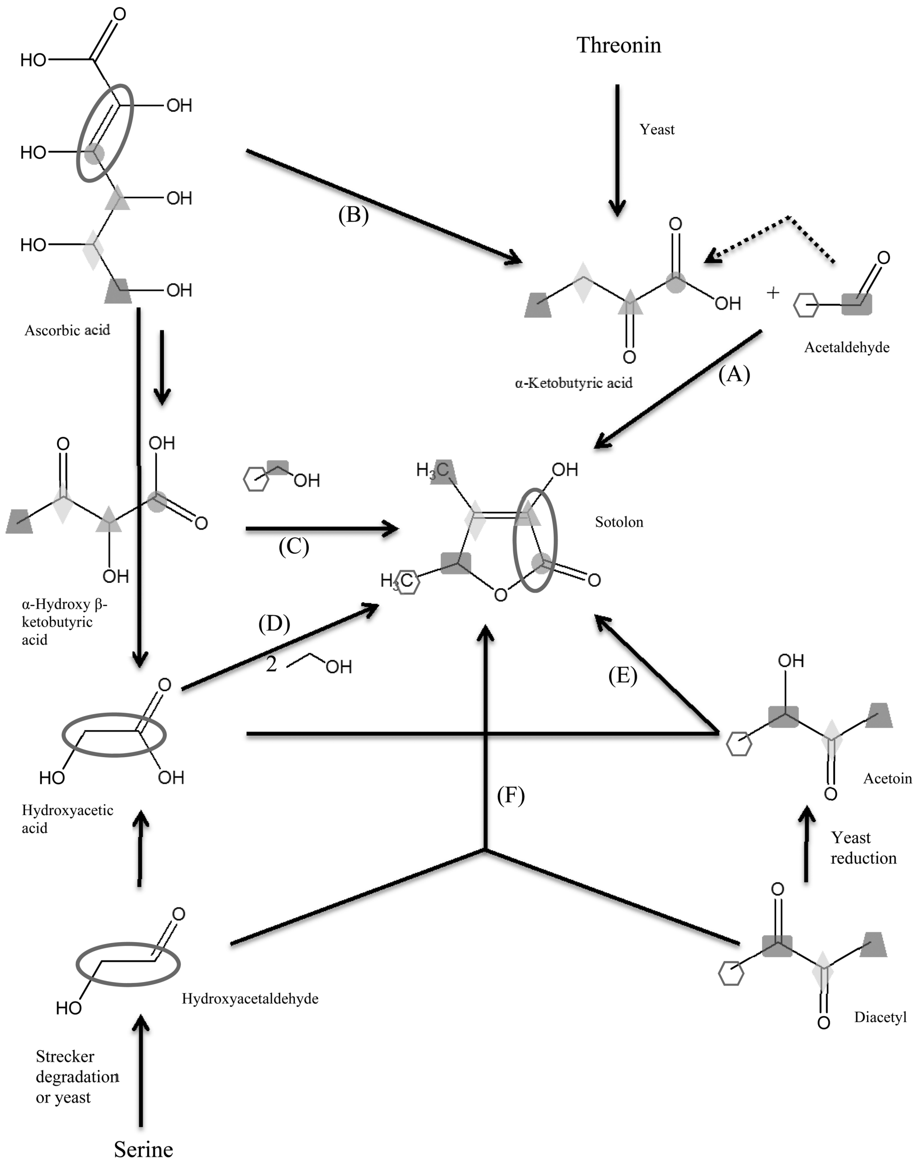

Figure 1. Potential synthesis pathways for sotolon in beverages.

Table 1. Chemical and Sensorial Characteristics of the Selected Special Beers

type	beer	alcohol (% vol)	original extract (g/100g)	real extract (g/100g)	pH	bitterness (BU)	color (EBC)	sensorial characteristics	particularities
blond	BL1	8.5	16.3	4.2	4.4	29	14.5	lemon, banana, apple, spicy, phenols	unfiltrated, bottle refermentation
	BL2	7.5	14.9	3.7	4.5	24	15.5	orange, pineapple, spicy, phenols	organic beer (organic version of BL1), unfiltrated, bottle refermentation
	BL3	6.3	14.4	4.6	4.2	15	12.5	butter, apple, hop, green	centrifuged, flash pasteurization, ascorbic acid addition
	BL4	7.5	16.8	5.1	4.5	21	16.5	butter, sulfur, hop	high gravity brewing, unfiltrated, bottle refermentation, flash pasteurization, ascorbic acid addition
	BL5	6.3	14.4	4.6	4.2	15	12.5	butter, apple, hop, green	BL3 without ascorbic acid
dry hopped trappist	TR1	6.2	13.3	3.2	4.1	29	26	hop, phenols, citrus, cheese	dry hopping, unfiltrated, bottle refermentation (with *Brettanomyces*)
amber	AM1	12.0	20.8	5.5	4.4	18	11	alcohol, banana, cheese, phenols	caramel malt, filtrated, ascorbic acid and sulfites addition
brown	BR1	8.0	19.8	6.2	4.7	16	11	malt, sulfur, green	chocolate malt (7%), light filtration, bottle refermentation

DOI: 10.1021/jf505953u
J. Agric. Food Chem. 2015, 63, 2886−2892

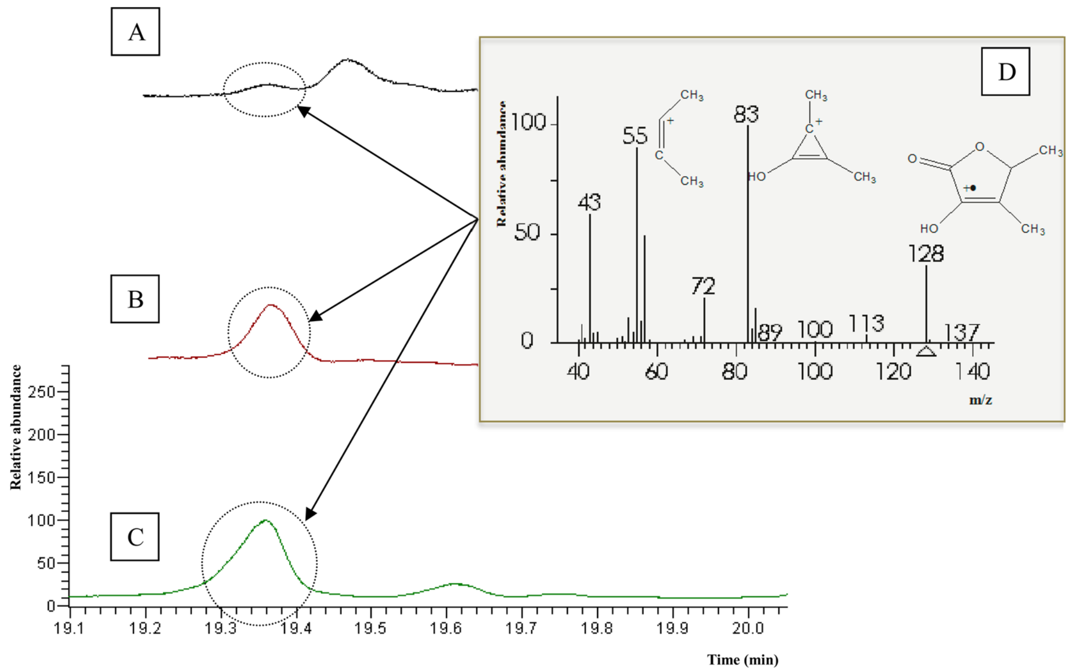

Figure 2. MS (SIM) chromatogram of fresh (A), 18-month-aged (B), and 24-month-aged (C) BL3 beer, and TIC mass spectrum of sotolon after 24 months (D).

No scientific paper describes how sotolon might be involved in the Madeira off-flavor found in aged beers, especially when strongly oxidized. Callemien et al.[6] detected the presence of sotolon in an aged lager beer. The aim of the present work was to compare the concentration of sotolon in fresh and aged samples of different commercial specialty beers. A specific lactone extraction procedure was applied, and the extracts were analyzed by GC-MS (gas chromatography-mass spectrometry). Beers or model media spiked with different hypothetical precursors and subjected to different beer storage conditions were compared in order to evidence which key factors should be controlled by brewers.

■ MATERIALS AND METHODS

Chemicals. Diethyl ether (99.9%), nonadecane (99.9%), hydrogen peroxide (30%), iron(II) chloride (98%), sotolon (99%), L-ascorbic acid (>99%), acetoin (>96%), diacetyl (97%), acetaldehyde (≥98%), and glycolaldehyde (hydroxyacetaldehyde dimer) were purchased from Sigma-Aldrich (Bornem, Belgium). Methanol (99.9%), acetone, and absolute ethanol were obtained from Analar Normapur (Fontenay-sous-bois, France). Anhydrous sodium sulfate (99%) was obtained from Merck (Darmstadt, Germany). Hydrochloric acid (36%) was purchased from Fisher Scientific (Tournai, Belgium). Sodium hydroxide was purchased from VWR international (Radnor, PA). Dichloromethane (99.9%) from Romil (Cambridge, U.K.) was distilled before use. Milli-Q water was used (Millipore, Bedford, MA). Amberlite XAD 2 resin (Supelco, Bellefonte, PA) (with a pore size of 9 nm and a specific area of 330 m^2/g) was sequentially washed with methanol and diethyl ether (each for 4 h) in a Soxhlet and stored in methanol at 4 °C.

Beer Samples. Seven commercial top-fermented beers (BL1, BL2, BL3, BL4, AM1, BR1, and TR1) were kindly supplied by Belgian brewers and stored in the dark at 20 °C for 2 years (Table 1) for natural aging. An analog of BL3 without ascorbic acid (BL5) was produced by a brewer for some spiking and accelerated aging experiments.

XAD 2 Procedure for Sotolon Extraction.[14] Amberlite XAD 2 resin (2 g) was thoroughly rinsed with Milli-Q water (approximately 400 mL), and the mixture was poured into a 250 mL Schott flask containing 50 mL of degassed beer adjusted at pH 11.5 by addition of 4 M NaOH. Beer alkalinization was done to increase lactone polarity, thus avoiding its adsorption onto the resin. Samples were shaken in the dark for 2 h and then poured onto a glass XAD column for separation. Beer percolating from the XAD 2 resin was recovered. Then, resin was washed with 50 mL of water, which was collected and mixed to the first eluate. This mixture was extracted three times with 40 mL of dichloromethane (10 min, 1000 rpm) after adjusting the pH to 3 to allow lactone cyclization. The organic phases were finally pooled and dried with anhydrous sodium sulfate. Then 0.5 mL of nonadecane stock solution (20 mg/L) was added as EST (external standard) before concentration to 0.5 mL in a Kuderna−Danish apparatus at 45 °C (total concentration factor = 100, final EST concentration = 20 mg/L). The final extract was stored at −80 °C for further analysis. A recovery factor of 89% was calculated for sotolon by the standard addition method in BL3.

Gas Chromatography Connected to Mass Spectrometry (GC-MS). Electronic impact mass spectra were recorded at 70 eV on a ThermoFinnigan (Waltham, MA) Trace MS simple quadrupole mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a split/splitless injector (splitless time = 0.5 min) and a polar FFAP-CB column (Varian, CP7485, 25 m × 0.32 mm i.d., 0.3 μm film thickness). The carrier gas was helium, and the pressure was set at 50 kPa (100 kPa were applied on the CP-Sil5-CB MS column, 50 m × 0.32 mm, 1.2 μm film thickness, that we used for RI (retention index) checking on a second chromatographic phase). The injection volume was 1 μL. The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and finally to 250 °C at 3 °C/min. Sotolon was quantified by standard addition with an EST (spiking to beer of 0.5, 1, 7, 15, and 20 μg/L), as recently described by Collin et al.[23] The SIM mode (single-ion monitoring mode) was used with m/z = 83 and 128. Spectral recording was automatic throughout separation (Xcalibur software, NIST database).

Gas Chromatography Connected to Olfactometric Detection (GC-O). A 1 μL amount of each extract was analyzed with a Chrompack CP9001 (Santa Clara, CA) gas chromatograph equipped with a split/splitless injector maintained at 250 °C (the split vent was opened 0.5 min postinjection) and connected to a GC-O port (Chrompack), also at 250 °C. The sotolon odor was checked with a polar FFAP-CB capillary column (Varian, CP7485, 25 m × 0.32 mm

DOI: 10.1021/jf505953u
J. Agric. Food Chem. 2015, 63, 2886−2892

i.d., 0.3 μm film thickness) and the CP-Sil5-CB MS column (50 m × 0.32 mm, 1.2 μm film thickness). The carrier gas was nitrogen, and the pressure was set at 30 (FFAP-CB) or 60 kPa (CP-Sil5-CB). The oven temperature program was the same as that described for GC-MS. The effluent was diluted with a large volume of air (20 mL/min) prehumidified with an aqueous copper(II) sulfate solution.

Beer Standard Analysis. Alcohol and extract determination were done with an Anton Paar (Graz, Austria) density meter DMA 4500M. Color, pH, and bitterness analyses were performed according to EBC analytical procedures.[24]

Sensory Analyses. Ten trained panelists scored 6 aging attributes on a scale of 1−5: cardboard, Madeira, nutty, bread, cooked fruit, and honey. A score of 0 meant the panelist did not detect the aroma, whereas a score of 5 meant the aroma was strongly perceived.

Determination of Sotolon Flavor Threshold in Beer. Determination of the flavor threshold of sotolon was performed with the ASTM method (Ascending Concentration Series Method of Limits), as recommended by the European Brewery Convention (method 13.9).[24] Sotolon was added to fresh beer (reference beer = fresh BL3) at six different levels of concentrations (from 0.5 to 20 μg/L). The panel was composed of 10 nontrained university students and 10 trained experts. Each assessor received 6 sequences of 3-Alternate Forced Choice (3-AFC) tests. For each sequence, sotolon was spiked into one test sample, the other two containing reference beer without any addition. The "best estimated threshold" (BET) of a panelist corresponds to the geometric mean of the highest concentration missed and the next highest adjacent concentration tested. The group best estimated threshold (group BET) was calculated as the geometric mean of the individual best estimated thresholds.

Investigation of Sotolon Pathways. *Pathway A.* BL3 bottles were spiked with 100 mg/L acetaldehyde and aged in the dark at 40 °C for 10 or 30 days. Pathways E/F: diacetyl 40 μg/L, acetoin 400 μg/L, serine 10 mg/L, and/or glycolaldehyde 40 μg/L were added to a sterilized phosphate buffer (pH = 4.5) containing 5% v/v ethanol. Samples were stored in the dark at 40 or 60 °C for 30 days. Pathways B/C/D: Oxygenated and control samples consisted in fresh BL5 exposed or not to sterile oxygen (final concentration = 6 mg/L in the "oxygen" sample) without any other spiking. Ascorbic acid was added at 880 mg/L final concentration, with or without 555 mg/L SO_2, to 200 mL of fresh BL5 in a glass flask exposed to oxygen by bubbling 6 mg/L sterile oxygen. A combination of iron at 1 mg/L and hydrogen peroxide at 1 g/L was also spiked as pro-oxidants to a BL5 sample exposed in the same way to 6 mg/L oxygen. Samples were stored for 30 days in the dark at 40 °C.

Statistical Analyses. Analyses were carried out in duplicate, and comparisons of means were performed with the Student−Newman−Keuls test with SAS software version 9.2 (SAS Institute, Inc., Carry, NC). Values sharing no common letter are significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Identification and Quantitation of Sotolon in Aged Beers. As depicted in Figure 2 and Table 2, only traces of sotolon (<1 μg/L) were found in fresh beer samples. In contrast, the lactone-specific extraction enabled us to quantify it in all special beers after 6, 12, 18, and 24 months of natural aging, whatever the type of beer (blond, amber, trappist, or brown, with or without bottle refermentation or ascorbic acid addition; Table 1). Identification was confirmed by mass spectrometry (coincidence of the main *m/z* with the commercial reference and the NIST library entry), the retention indices on two capillary columns (RI = 2213 on FFAP-CB and 1068 on CP-Sil5-CB), and odors (curry, walnut). For the 7 beers analyzed, the amount of sotolon already reached 4.6−14.2 μg/L after 6 months and continued to increase thereafter up to 42.1 μg/L in the ascorbic-acid-protected beer BL3 after 2 years. These values are well above the 2 μg/L sensory threshold previously assessed in hydro-alcoholic solution.[15] They also exceed after 12 months the 8 μg/L sensory threshold that a jury of 20 panelists here determined in BL3 (already after 6 months for BL2, BL3, and BL4). The Madeira attribute was also scored (together with five other aging descriptors) in all beers over the first year of storage by a jury of trained panelists (data given for BL3 in Figure 3). The Madeira character was set above 2 in all 6- and 12-months-aged beers and under 1 in fresh beers.

Table 2. Sotolon Concentration (μg/L) in Top-Fermentation Beers Stored at 20 °C[a]

	BL1	BL2	BL3	BL4	TR1	AM1	BR1
fresh	nq	nq	nq	nq	nq	nq	nq
6 months	6.4	14.2	9.5	11.6	7.8	4.6	6.8
12 months	8.9	17.0	9.0	10.1	12.0	9.4	13.7
18 months	10.9	36.2	27.1	20.8	16.2	14.7	13.0
24 months	8.9	24.3	42.1	37.5	29.1	12.1	16.5

[a]GC-MS (SIM mode) Data. nq = detected but not quantifiable (<1μg/L). Means of duplicates (CV < 5%).

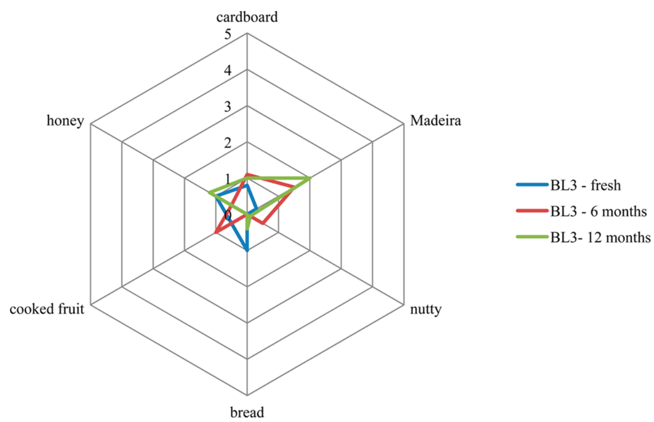

Figure 3. Aging off-flavors in BL3 through natural aging.

As depicted in Figure 4 for BL3, 30 days at 40 °C treatment revealed to produce sotolon (9 μg/L) in a range close to what we observed after 6 months of natural aging. Such accelerated aging was therefore applied to assess in a short time which synthesis pathways could occur in beer. As BL3 contained

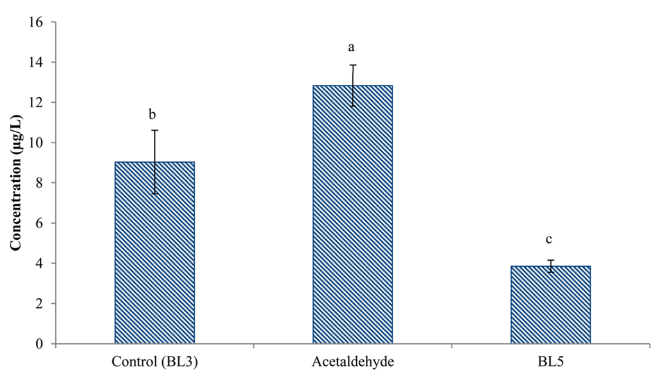

Figure 4. Sotolon concentration (μg/L) in a control beer (BL3, containing ascorbic acid), in the same beer spiked with 100 mg/L acetaldehyde, and in BL5 (devoided of ascorbic acid) after 30 days at 40 °C. Values sharing no common letters are significantly different ($p < 0.05$).

DOI: 10.1021/jf505953u
J. Agric. Food Chem. 2015, 63, 2886−2892

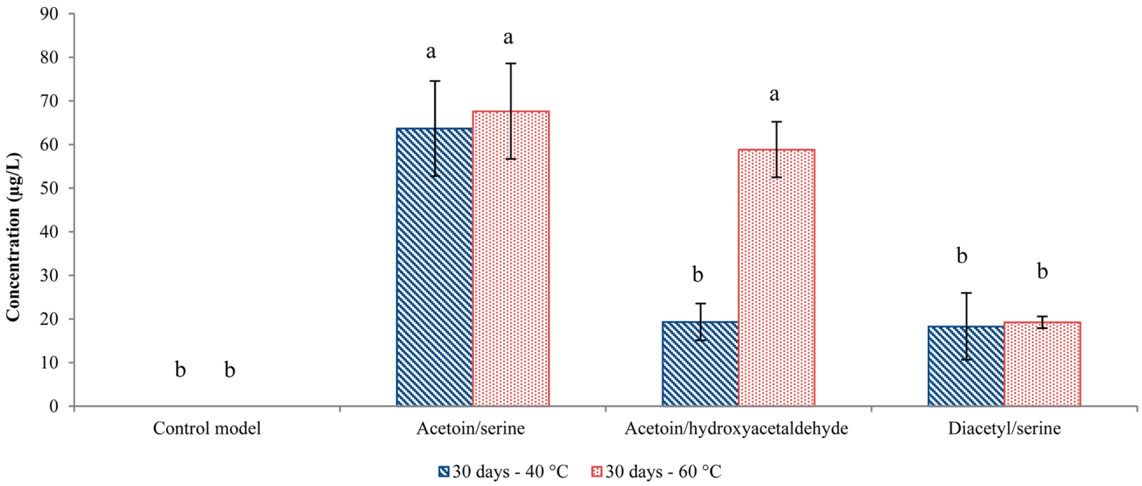

Figure 5. Sotolon concentration (μg/L) in a model beer medium (control model), and the same solution spiked with acetoin, serine, diacetyl, and/or hydroxyacetaldehyde after 30 days at 40 or 60 °C. Values sharing no common letters are significantly different ($p < 0.05$).

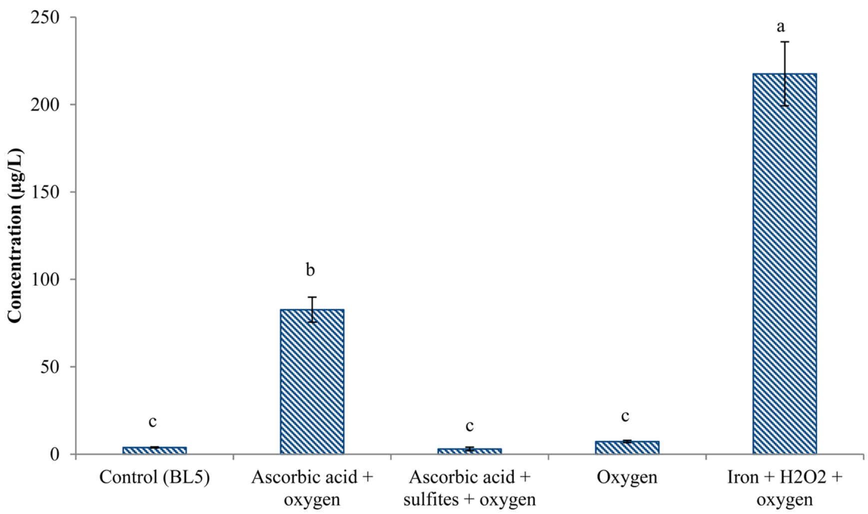

Figure 6. Sotolon concentration (μg/L) in a control beer (BL5, devoided of ascorbic acid) and the same BL5 beer spiked with 8 mg/L oxygen, with 880 mg/L ascorbic acid and 8 mg/L oxygen, a combination of 880 mg/L ascorbic acid, 6 mg/L oxygen, and 555 mg/L sulfites, or pro-oxidant species (Fe^{2+} (1 mg/L) + H_2O_2 (1 g/L)) after 30 days at 40 °C. Values sharing no common letters are significantly different ($p < 0.05$).

ascorbic acid, a pilot version without ascorbic acid addition at bottling (BL5) was requested of the manufacturer. This beer was used for all experiments where oxidation could play a key role. As shown by its accelerated aging (30 days at 40 °C), the absence of ascorbic acid already allowed minimizing the content of sotolon (4 μg/L in BL5 instead of 9 in BL3).

Potential Pathways for Sotolon Formation in Beer. *Pathway A.* Many researchers have demonstrated the key role of acetaldehyde in the appearance of sotolon in beverages.[19−25] Therefore, fresh BL3 was spiked with 100 mg/L acetaldehyde (usual concentration in fresh ales = 3.8−33.8 mg/L[26]) in the absence of oxygen, and samples were aged for 30 days at 40 °C. As depicted in Figure 4, sotolon was found to be significantly produced from added acetaldehyde (12.8 μg/L versus 8 in the control).

Pathways E/F. Diacetyl has been mentioned in the literature as a possible precursor of sotolon when combined with hydroxyacetaldehyde under quite harsh conditions[22] (usual diacetyl concentration in ale = 60−300 mg/L[27]). Its reduced form, acetoin, is another well-known constituent of beer (usual acetoin concentration in ale = 10−200 mg/L[27]) which could react with serine-derived compounds such as hydroxyacetic acid by aldol condensation (usual serine concentration in beer = 2−12 mg/L[28]). The compounds diacetyl, acetoin, glycolaldehyde (the commercial dimer of hydroxyacetaldehyde), and serine were mixed in different combinations in a beer model solution before aging for 30 days at 40 or 60 °C. As depicted in Figure 5, only acetoin proved able to generate large amounts of sotolon. The highest concentrations (63−67 μg/L) were obtained after addition of acetoin in the presence of serine. Hydroxyacetaldehyde together with acetoin led to lower amounts of sotolon at 40 °C (19.3 μg/L after 30 days) but reached 59 μg/L at 60 °C, probably because glycolaldehyde must dissociate to hydroxyacetaldehyde before it can react. In the case of diacetyl together with serine, both temperatures led to only 12−14 μg/L, suggesting that it is probably a minor pathway in natural beer aging. Transferred to beer, these results could mean that the efficiency of the reduction of diacetyl to acetoin by yeast could impact the Madeira off-flavor.

Pathways B/C/D. In the brewing industry, ascorbic acid is sometimes used as antioxidant at the bottling step in association (e.g., AM1) or not (e.g., BL3) with sulfites. As

DOI: 10.1021/jf505953u
J. Agric. Food Chem. 2015, 63, 2886−2892

illustrated in Figure 1 from the literature (pathways B–D), degradation of ascorbic acid can generate different species, possibly involved in sotolon synthesis. Ascorbic acid can also act as a pro-oxidant in beer.[28] Spiking of BL5 with 880 mg/L ascorbic acid (much higher amount than the industrial spiking of BL3 = 30 mg/L) and 6 mg/L oxygen led to sotolon concentrations up to 82.7 μg/L after 30 days at 40 °C, whereas the same beer, when untreated (control = no ascorbic acid and <0.05 mg/L oxygen), did not even reach the 8 μg/L flavor threshold of the lactone in beer (Figure 6). As expected, mixing 555 mg/L sulfites with ascorbic acid annihilated the latter's negative effect. In beer, sulfites arise either through the action of yeast or through exogenous addition at the bottling step.

BL5 was also aged for 30 days at 40 °C in absence of ascorbic acid, under a bottleneck saturated with 6 mg/L oxygen. Only 7.3 μg/L sotolon was measured here, suggesting that beer polyphenols are able to minimize its effect. In comparison, spiking of hydrogen peroxide and iron (well known for the Fenton reaction) dramatically increased sotolon concentration (up to 217 μg/L). Ethanol most probably acts as a quencher of hydroxyl radicals, as mentioned by Andersen et al.,[29] leading to the formation of acetaldehyde involved himself in sotolon synthesis (Pathway A).

In conclusion, prevention of oxidation is required to decrease the Madeira off-flavor brought to the beer by sotolon. Yet addition of ascorbic acid at bottling should be avoided, at least if not combined with sulfites, because of the role of ascorbic acid either as a reagent (precursor of α-ketobutyric acid, α-hydroxy-β-ketobutyric acid, or hydroxyacetic acid) or as a pro-oxidant (for acetaldehyde production). Despite a very low oxygen content in such beers, refermented beers seem not to be fully protected against the Madeira off-flavor, probably because of additional pathways, including the biochemical deamination of threonine and serine by yeast, followed by combination with either acetaldehyde or acetoin.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +32 10 47 29 13. Fax: +32 10 47 21 78. E-mail: sonia.collin@uclouvain.be.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the CONSALIM project for kind collaboration.

■ REFERENCES

(1) Noël, S.; Liégeois, C.; Lermusieau, G.; Bodart, E.; Badot, C.; Collin, S. Release of deuterated nonenal during beer ageing from labeled precursors synthetized in the boiling kettle. *J. Agric. Food Chem.* **1999**, *47*, 4323–4326.

(2) Liégeois, C.; Meurens, N.; Badot, C.; Collin, S. Release of deuterated (E)-2-nonenal during beer ageing from labeled precursors synthetized before boiling. *J. Agric. Food Chem.* **2002**, *50*, 7634–7638.

(3) Gijs, L.; Chevance, F.; Jerkovic, V.; Collin, S. How low pH can intensify beta-damascenone and dimethyl trisulfide production through beer aging. *J. Agric. Food Chem.* **2002**, *50*, 5612–5616.

(4) Chevance, F.; Guyot-Declerck, C.; Dupont, J.; Collin, S. Investigation of the beta-damascenone level in fresh and aged commercial beers. *J. Agric. Food Chem.* **2002**, *50*, 3818–3821.

(5) Vanderhaegen, B.; Neven, H.; Daenen, L.; Verstrepen, K. J.; Verachtert, H.; Derdelinckx, G. Furfuryl ethyl ether: important aging flavor and a new marker for the storage conditions of beer. *J. Agric. Food Chem.* **2004**, *52*, 1661–8.

(6) Callemien, D.; Dasnoy, S.; Collin, S. Identification of a stale-beer-like odorant in extracts of naturally aged beer. *J. Agric. Food Chem.* **2006**, *54*, 1409–1413.

(7) Scholtes, C.; Nizet, S.; Collin, S. Guaiacol and 4-methylphenol as specific markers of torrefied malts. Fate of volatile phenols in special beers through aging. *J. Agric. Food Chem.* **2014**, *62*, 9522–9528.

(8) Tran, T. T. H.; Gros, J.; Bailly, S.; Nizet, S.; Collin, S. Fate of 2-sulphanylethyl acetate and 3-sulphanylpropyl acetate through beer aging. *J. Inst. Brew.* **2012**, *118*, 198–204.

(9) Clapperton, J. F. Ribes Flavour in Beer. *J. Inst. Brew.* **1976**, *82*, 175–176.

(10) Tran, T. T. H.; Nizet, S.; Gros, J.; Collin, S. Occurence of the ribes odorant 3-sulphanyl-3-methylbutyl formate and its analogues in beer. *Flavours Fragrances J.* **2013**, *28*, 174–179.

(11) Dalgliesh, C. E. Flavour stability. *Proc. Congr.-Eur. Brew. Conv* **1977**, 623–659.

(12) Pons, A.; Lavigne, V.; Landais, Y.; Darriet, P.; Dubourdieu, D. Distribution and organoleptic impact of sotolon enantiomers in dry white wines. *J. Agric. Food Chem.* **2008**, *56*, 1606–1610.

(13) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Rôle du sotolon dans l'arôme des vins doux naturels, influence des conditions d'élevage et de vieillissement. *J. Int. Sci. Vigne Vin* **1998**, *32*, 223–233.

(14) Scholtes, C.; Nizet, S.; Collin, S. Occurrence of sotolon, abhexon and theaspirane-derived molecules in gueuze beers. Chemical similarities with "Yellow wines". *J. Inst. Brew.* **2012**, *118*, 223–229.

(15) Lavigne-Cruège, V.; Dubourdieu, D. Role of glutathione on development of aroma defects in dry white wines. *13th International Enology Symposium*, Montpellier, 2002.

(16) Cantarelli, C. Etude de la madérisation et de sa prévention dans les vins blancs. *Deuxième Symposium*; Institute d'Œnologie, Bordeaux Cognac, 1967; Vol. 2, pp392– 407.

(17) Sulser, H.; Depizzol, J.; Büchi, W. A probable flavoring principle in vegetable-protein hydrolysates. *J. Food Sci.* **1967**, *32*, 611–615.

(18) Takahashi, K.; Tadenuma, M.; Sato, S. 3-Hydroxy-4,5-dimethyl-2(5H)-furanone, a burnt flavoring compound from aged sake. *Agric. Biol. Chem.* **1976**, *40*, 325–330.

(19) Pisarnitsky, A. K.; Bezzubov, A. A.; Egorov, I. A. Nonenzymatic formation of 4, 5-dimethyl-3-hydroxy-2(5H)-furanone in foodstuffs. *Prikl. Biokhim. Mikrobiol.* **1987**, 642–646.

(20) König, T.; Gutsche, B.; Hartl, M.; Hübscher, R.; Schreier, P.; Schwab, W. 3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) causing an off-flavor: elucidation of its formation pathways during storage of citrus soft drinks. *J. Agric. Food Chem.* **1999**, *47*, 3288–3291.

(21) Silva Ferreira, A. C.; Barbe, J. C.; Bertrand, A. 3-Hydroxy-4,5-dimethyl-2(5H)-furanone: A key odorant of the typical aroma of oxidative aged port wine. *J. Agric. Food Chem.* **2003**, *51*, 4356–4363.

(22) Hofmann, T.; Schieberle, P. Studies on intermediates generating the flavour compounds 2-methyl-3-furanthiol, 2-acetyl-2-thiazoline and sotolon by Maillard-type reactions. *Flavour Science: Recent Developments*; The Royal Society of Chemistry: London, 1996, pp 182–187.

(23) Collin, S.; Nizet, S.; Claeys Bouuaert, T.; Despature, P.-M. Main odorant in Jura flor-sherry wines. Relative contributions of sotolon, abhexon, and theaspirane-derived compounds. *J. Agric. Food Chem.* **2012**, *60*, 380–387.

(24) European Brewery Convention. *Analytica EBC*; Getränke-Fachverlag: Nürnberg, Germany, 2006.

(25) Pons, A.; Lavigne, V.; Landais, Y.; Darriet, P.; Dubourdieu, D. Identification of a sotolon pathway in dry white wines. *J. Agric. Food Chem.* **2010**, *58*, 7273–7279.

(26) Otter, G. E.; Taylor, L. Estimation and occurrence of acetaldehyde in beer. *J. Inst. Brew.* **1971**, *77*, 467–472.

(27) Harrison, G. A. F.; Byrne, W. J.; Collins, E. The determination of diacetyl and 2,3-pentanedione in beer head space by gas chromatography. *J. Inst. Brew.* **1965**, *71*, 336–341.

(28) Moll, M. *Bières & Coolers*; Tech & Doc–Lavoisier: Paris, France, 1991.

DOI: 10.1021/jf505953u
J. Agric. Food Chem. 2015, 63, 2886–2892

(29) Andersen, M. L.; Outtrup, H.; Skibsted, L. H. Potential antioxidants in beer assessed by ESR spin trapping. *J. Agric. Food Chem.* **2000**, *48*, 3106−3111.

DOI: 10.1021/jf505953u
J. Agric. Food Chem. 2015, 63, 2886−2892