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# Flavor Defects of Fresh and Aged NABLABs: New Challenges Against Oxidation

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**ABSTRACT**

At present, non-alcoholic (NAB) and low-alcoholic beers (LAB) exhibit major staling defects even when fresh, partly due to absence of ethanol as an antioxidant. In the present work, the aroma stability of eleven commercial NABLABs available on the Belgian market, issued from different technological processes, was assessed. NABLABs were investigated, both when fresh and after one year of storage at 20 °C in the dark. Six stale-odorant compounds were found above their perception threshold in aged NABLABs: sotolon, abhexon, methional, phenylacetaldehyde, dimethyltrisulfide and β-damascenone. Based on the chemical structure of the first four, it can be concluded that oxidation is the main issue for NABLABs aging. Yet, five of these usual staling defects of a six-month lager beer were already key-odorants in fresh NABLABs: dimethyltrisulfide, methional, and β-damascenone were above the thresholds in all samples, phenylacetaldehyde in 10 out of 11, and sotolon in 7. In conclusion, development of efficient antioxidants is needed to improve NABLABs acceptability.

**Abbreviations:** NABs: non-alcoholic beers; LABs: low-alcoholic beers; SAFE: Solvent Assisted Flavor Evaporation; SPE: Solid Phase Extraction; IST: internal standard; EST: external standard; HPLC-ELSD: High Pressure Liquid Chromatography with Evaporative Light Scattering Detector; GC-MS: Gas Chromatography with Electronic Impact Mass Spectrometry; SIM: Single Ion Monitoring; ABV: alcohol by volume

**KEYWORDS**

Beer aging; methional; NABLABs; oxidation; sotolon; stale odorants

## Introduction

The growing interest for non-alcoholic (NAB) and low-alcoholic beers (LAB) reflects the desire of consumers to adopt healthier and more civic-minded lifestyles.[1,2] At the same time, NABLABs meet the restrictions in terms of road safety or work rules, or even religious grounds.[1–3] With a low calorie content (30–40% less than standard beer), and antioxidant or isotonic properties, NABLABs could also become alternative drinks for sportsmen, pregnant women or people under medication.[2]

In most European countries, NABs have an alcohol content of 0.5% or less (ABV) while LABs are characterized by a limit of no more than 1.2% (ABV).[1] In the United States, NAB is known as "near beer" and the term "alcohol-free" is rather given to beer that contains absolutely no alcohol (at least, below the detection level of 0.05% (ABV)).[1] In Islamic countries, any trace of alcohol in beer is totally prohibited.[2,4]

Two main types of processes are currently used to produce NABLABs: physical and biological methods.[1–3] Physical methods, such as vacuum rectification and evaporation (thermal systems), dialysis, and reverse osmosis (membrane systems) are based on the removal of alcohol (dealcoholization) from conventional beer. On the other hand, biological approaches consist in restricting ethanol formation during beer fermentation,[1–3] by cold contact fermentation (short fermentation time of 24–48 h at low temperature, just above 0 °C)[2,5] or the use of special microorganisms (*Saccharomycodes ludwigii*, *Saccharomyces cerevisiae* var. *chevalieri*, lactic acid bacteria,…).[4] Unfortunately, regardless of the technology adopted, most NABLABs exhibit major staling defects already when fresh.

In regular beer, ethanol is a good radical scavenger of hydroxyl radicals (HO°) and other reactive oxygen species ROS (e.g., $O_2^{\circ-}$, HOO° issued from metal-induced Fenton and Haber-Weiss reactions[6–10]). Hence, it can prevent volatile and non-volatile compounds to be oxidized during storage. Bitter compounds and polyphenols are recognized to be particularly sensitive to deterioration during beer storage, especially in dry hopped beers.[11–14] In NABLABs, we can suspect that they will be still less protected in the absence of radical scavengers.

NABLABs also suffer from a lack of fruity fermentation aromas, and a persistent worty taste attributable to methional (3-methylthiopropionaldehyde, perception threshold of 0.47 µg/L), and to a less extent, 2- and 3-methylbutanal.[15–18] These Strecker aldehydes are insufficiently reduced to

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alcohol in most biological processes and can be regenerated in thermal ones. Recent literature also shows that other compounds could eventually participate in this worty off-flavor: 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (abhexon; curry, madeira), (E)-β-damascenone (cooked apple), and phenylacetaldehyde (floral, honey).[18,19]

The aim of the present work was to assess the aroma stability of a large panel of commercial NABLABs (lager, amber, white, acidic, and dry hopped beers) issued from different technological processes. Eleven NABLABs available on the Belgian market were investigated both when fresh and after six months and one year of storage at 20 °C in the dark. Standard global methods were first applied. Then, the key-odorant compounds contributing to NABLABs flavor were extracted by Solvent Assisted Flavor Evaporation (SAFE) and analyzed by Gas Chromatography (GC) with MS detection. As sotolon and abhexon are not well recovered by the SAFE methodology, a more specific extraction procedure developed in our laboratory (SPE on XAD-2 resin at a fixed pH)[20] was applied for them.

## Experimental

### Chemicals

Acetonitrile, anhydrous sodium sulfate, dichloromethane, ethanol absolute (99%), hydrochloric acid 37%, isooctane, methanol and sodium hydroxide were purchased from VWR International (Leuven, Belgium). D-(−)-fructose, maltose monohydrate, and saccharose were purchased from Merck (Darmstadt, Germany). D-(+)-glucose, L-rhamnose, and maltotriose were purchased from Sigma-Aldrich (St-Louis, U.S.A.). Amberlite XAD-2 resin (used as adsorbent) was from Sigma-Aldrich (Overijse, Belgium). Standards of 2-acetylthiophene, β-damascenone, decane, dimethyltrisulfide, dodecane, 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (abhexon), geraniol, 3-hydroxy-4,5-dimethylfuran-2(5H)-one (sotolon), isoamyl acetate, linalool, methional, γ-nonalactone, nonanal, phenylacetaldehyde, and 4-vinylguaiacol were purchased from Sigma-Aldrich (Overijse, Belgium). Milli-Q water was used (Millipore, Bedford, U.S.A.).

### Beer samples

Eleven commercial NABLABs were investigated: Star Light (A), Energibajer (B), Pico Bello (C), Leopold 7 Road Trip (D), Palm N.A. (E), Maes 0.0% (F), Hoegaarden rosée 0.0% (G), Carlsberg 0.0% (H), Jupiler 0.0% (I), Leffe Blonde 0.0% (J), and Brugse Sport Zot alcoholvrij (K). Beers were received from brewers or bought in the market (freshly released). All beers, from the same batch, were stored for one year at 20 °C in the dark and analyzed in duplicate (before and after 6 or 12 months of natural aging).

### Standard analyses

Prior to analysis, beers were degassed by shaking and filtered through paper filters (MN 614 $^1/_4$ Macherey-Nagel, Düren, Germany) except for haze measurement. Alcohol content, real and original extracts were determined with the DM4500 apparatus (Anton Paar GmbH, Graz, Austria), pH and color were analyzed by means of Analytica-EBC methods 9.2.6, 9.35 and 9.6. Permanent haze was determined according to an Analytica-EBC method 9.29 by using a Ratio2000 Turbidimeter (HACH, Loveland, U.S.A.).[21] Bitterness was measured by means of Analytica-EBC method 9.8.[21]

### Analyses of fermentable sugars by HPLC-ELSD

Fructose, glucose, saccharose, maltose, and maltotriose were quantitated by HPLC-ELSD according to a method adapted from Buckee and Long.[22] Beer sugars (containing of IST (L-rhamnose)) were recovered through a SPE cartridge (Sep-Pak® C18, Waters, Milford, U.S.A.). Separation was performed on Prevail Carbohydrate ES 250 × 4.6 mm, 5 µm column (Grace, Deerfield, U.S.A.) using a linear gradient of acetonitrile: water (75:25, v/v) at a flow rate of 1.0 mL/min. The column temperature was kept at 25 °C and the injection volume was 10 µL. Chromatograms were acquired with an Evaporative Light Scattering Detector. Compound identification was performed by injection of commercial standards and quantitation was achieved using the calibration curves.

### Aroma extraction by Solvent Assisted Flavor Evaporation

Most odorant compounds were extracted with the Solvent Assisted Flavor Evaporation (SAFE) system adapted from Engel et al.[23] The conditions for the SAFE analysis were as follows: the water bath temperature was set at 40 °C, the pressure was kept below $10^{-3}$ Pa, and the apparatus body (Glasblaeserei Bahr, Manching, Germany) was at 30 °C. Degassed samples (50 mL) were spiked with 150 µL of 2-acetylthiophene solution (8 mg/L; final beer concentration = 24 µg/L) as internal standard (IST). Samples were then extracted with bidistilled dichloromethane (1 × 75 mL) for 20 min. After centrifugation (20 min at 45,000 rpm) of the resulting emulsion, the aqueous phase was discarded, and the remaining organic phase was dried over anhydrous sodium sulfate. Non-volatile compounds were then separated by high-vacuum distillation using the SAFE system. The distillate was continuously recovered in a SAFE flask cooled with liquid nitrogen for 15–20 min distillation. The extract was dried over anhydrous sodium sulfate. Decane solution (25 µL) (250 mg/L) was spiked as an external standard (EST) before concentration to 500 µL in a Kuderna-Danish apparatus at 45 °C. Extracts were stored at −80 °C until analysis by Gas Chromatography—Electronic Impact Mass Spectrometry.

### Specific extraction procedure for sotolon and abhexon

Since sotolon and abhexon are poorly extracted by the SAFE method, a specific extraction procedure, derived from Blank

et al.,[24] and Bailly et al.[20] was used. Firstly, 2 g of XAD-2 resin was added to a degassed beer sample (50 mL) in a flask. Before the beer was placed in contact (2 h, 200 rpm) with the resin, its pH was adjusted at 11.5 with sodium hydroxide to deprotonate interest compounds (thus avoiding adsorption onto the resin). The eluate from the XAD-2 resin and the first 50 mL of resin washing water were mixed before bringing the pH to 3.0 with hydrochloric acid (37%). This aqueous phase was extracted three times with 40 mL bidistilled dichloromethane (10 min, 2500 rpm). Extracts were then dried with anhydrous sodium sulfate and 250 µL of dodecane (5 mg/L) was added as EST before concentration until 500 µL in a Kuderna-Danish at 45 °C (total concentration factor = 100, final EST concentration = 2.5 mg/L). Extracts were stored at −80 °C until analysis by Gas Chromatography—Electronic Impact Mass Spectrometry.

### Gas Chromatography—Electronic Impact Mass Spectrometry (GC-MS) of SAFE extracts

One microliter of each SAFE extract was analyzed with an Agilent Technologies 7890 NB Gas Chromatograph System equipped with a splitless injector maintained at 250 °C. The split vent was opened after 0.5 min. Compounds were separated with a wall-coated open tubular apolar WCOT capillary column (CP-Sil 5 CB, 50 m × 0.32 mm, 1.2 µm film thickness). The carrier gas was helium, and the pressure was set at 65 kPa. The oven temperature was programmed to rise from 36 °C to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, at last to 220 °C at 3 °C/min, and then held at this temperature for 30 min. The column was connected to a single quadrupole mass spectrometer (Agilent 5977B MSD) operating in total ion (full scan) or single ion monitoring (SIM) mode with electron ionization (EI) at 70 eV. The following *m/z* values were monitored: 111 and 126 for 2-acetylthiophene (IST), 71 and 85 for decane (EST), 85 and 100 for γ-nonalactone, 150 and 135 for 4-vinylguaiacol, 69 and 93 for geraniol, 70 and 87 for isoamyl acetate, 91 and 120 for phenylacetaldehyde, 69 and 121 for β-damascenone, 57 and 98 for nonanal, 104 and 76 for methional, 126 and 79 for dimethyltrisulfide, and 71 and 121 for linalool. Chromatograms were recorded throughout elution. Agilent OpenLab software was used to record the resulting data. Calibration curves (with areas relative to IST) were constructed for each compound, and the following equation was used for quantitation of compound A: concentration of A (in µg/L) = IST concentration (in µg/L) × (A area/IST area) × (IST response coefficient/A response coefficient) × (IST recovery factor/A recovery factor). The IST relative recovery factor was set at 1 for all compounds.

### Gas Chromatography—Electronic Impact Mass Spectrometry (GC-MS) of sotolon and abhexon extracts

One microliter of each sotolon/abhexon extract was analyzed with the here-above described GC system (same conditions for injection, and MS acquisition). The compounds were here separated with a WCOT polar capillary column (FFAP CB, 25 m x 0.32 mm, 0.3 µm film thickness). The carrier gas was helium at a pressure of 35 kPa. The oven temperature was programmed to rise from 36 °C to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, then to 160 °C at 3 °C/min, at last to 230 °C at 3 °C/min, and then held at this temperature for 30 min. In addition to the EST (*m/z*: 71 and 85), the following *m/z* values were monitored: 83 and 128 for sotolon, and 97 and 142 for abhexon.

### Statistical analyses

All analytical measurements were carried out in duplicate. Multiple comparisons of means were performed with the Student-Newman-Keuls test with SAS software. Values not sharing any common letter in the same row of a table are significantly different ($p < 0.05$).

## Results and discussion

### Standard analyses of fresh and aged commercial NABLABs

Firstly, basic properties of 11 fresh commercial NABLABs (A-K) were determined by standard global methods (Table 1). Our panel of lager (A, F, H and I), amber (E and K), white (G) and dry hopped (B, C and D) NABLABs explains the large distribution obtained for color (5–20°EBC) and bitterness (7–37 BU). Most beers had a slight permanent haze (between 0.4 (E) and 4.7°EBC (B)), except beers D (15.8°EBC; can refermentation) and G (14.8°EBC; non-filtered white beer). NABLABs exhibited pH values between 4.0 and 4.6, except three samples for which lower pH could be attributed to the use of lactic acid bacteria (C and D) or the fruit acidity (G). In contrast, the highest pH value (B) was reported as a possible consequence of the dry hopping process.[25] As shown in Table 1, the alcohol content (% ABV) of commercial NABLABs ranged from less than 0.1 for dealcoholized beers (below the detection limit of the device) to 0.5 (A) for those produced by a biological process (especially with special yeasts). Beer D, a dry hopped and refermented beer, displayed the highest alcohol content (0.8% ABV). The production of more ethanol might be associated with the residual yeast activity resulting from the presence of hop dextrin hydrolases.[26]

NABLABs exhibited globally lower original extracts (between 4 (H) and 9°P (J)) compared to lagers (12°P on average) and traditional dry hopped beers. Sugar content in fresh commercial NABLABs was determined by HPLC-ELSD. The sugar composition allowed us to distinguish the dealcoholized beers from those fermented with special yeasts. As expected, dealcoholized beers F, H, I, J, and K globally contained less than 2 g/100 mL while beers A, B, C, and E showed values ranging from 3 to 5 g/100 mL, with a dextrin content from 0.60 to 1.90 g/100 mL. Yet, the can refermented beer D and the fruity beer G showed a low and a high fermentable sugar concentration respectively, despite their respective production process.

**Table 1.** Standard analyses of the 11 fresh NABLABs.

| Beer | Alcohol content (% ABV) | Original extract (°P) | Real extract (°P) | Fermentable sugars (g/100 mL)♠ | Dextrin (Real extract-Fermentable sugars♠) (g/100 mL) | pH | Color (°EBC) | Bitterness (BU) | Permanent haze (°EBC) |
|---|---|---|---|---|---|---|---|---|---|
| *Biological processes* | | | | | | | | | |
| **Special yeast** | | | | | | | | | |
| A | 0.5 (+0.0) | 4.52 (−0.06) | 3.77 (−0.03) | 3.15 | 0.62 | 4.2 (−0.5) | 6.9 (+0.6) | 12.8 (−3.6) | 0.7 (+1.6) |
| B♣ | 0.3 (+0.0) | 7.26 (−0.05) | 6.80 (−0.09) | 4.94 | 1.86 | 4.6 (−0.7) | 9.3 (+2.4) | 30.7 (−15.2) | 4.7 (+1.8) |
| C♣ | 0.2 (+0.0) | 7.25 (−0.07) | 6.96 (−0.06) | 5.08 | 1.88 | 3.0 (−0.1) | 4.7 (+2.1) | 11.0 (−5.5) | 1.1 (+1.2) |
| **Mixed fermentation** | | | | | | | | | |
| D*♣ | 0.8 (−0.1) | 5.32 (−0.26) | 4.13 (−0.18) | 0.39 | 3.74 | 3.5 (−0.5) | 5.6 (+2.2) | 6.8 (−3.2) | 15.8 (+6.5) |
| **Cold Contact** | | | | | | | | | |
| E | 0.1 (−0.1) | 6.41 (−0.18) | 6.27 (−0.04) | 5.01 | 1.26 | 4.3 (−0.2) | 19.7 (+2.3) | 19.2 (−5.5) | 0.4 (+0.2) |
| *Physical processes* | | | | | | | | | |
| **Distillation** | | | | | | | | | |
| F | < 0.1 (+0.0) | 4.94 (+0.01) | 4.83 (+0.05) | 1.79 | 3.04 | 4.3 (−0.4) | 8.0 (+3.9) | 15.2 (−6.5) | 1.3 (+3.7) |
| G | < 0.1 (+0.0) | 6.98 (+0.51) | 6.91 (+0.56) | 3.76 | 3.15 | 3.7 (−0.5) | 17.8 (+1.9) | 10.2 (−6.2) | 14.8 (+2.3) |
| H | 0.1 (+0.0) | 3.99 (+0.13) | 3.83 (+0.20) | 0.51 | 3.32 | 4.2 (+0.4) | 7.8 (+4.2) | 12.6 (−3.0) | 0.8 (+0.7) |
| I | < 0.1 (+0.0) | 8.14 (+0.66) | 8.13 (+0.64) | 1.14 | 6.99 | 3.9 (−0.2) | 7.0 (+1.6) | 19.9 (−11.4) | 0.7 (+0.7) |
| J | < 0.1 (+0.0) | 9.14 (+0.52) | 9.14 (+0.54) | 1.76 | 7.38 | 4.2 (−0.5) | 10.9 (+2.7) | 20.6 (−9.8) | 1.7 (+0.8) |
| **Membrane filtration** | | | | | | | | | |
| K | 0.5 (−0.1) | 7.88 (+1.31) | 7.12 (+1.42) | 1.35 | 5.77 | 4.4 (−0.3) | 13.9 (+8.6) | 37.3 (−15.2) | 1.9 (+4.0) |

Values in parentheses indicate differences observed after one year of storage at 20 °C in the dark.
*:with can refermentation; ♣: with dry hopping; ♠: sum of fructose, glucose, saccharose, maltose, and maltotriose by HPLC method.

**Table 2.** Flavor concentrations (µg/L) in eleven NABLABs, fresh (0), after six months (6 M) or after one year (12 M) at 20 °C in the dark.

| | | | | Biological processes | | | | | Physical processes | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Special yeast* | | | *Mixed fermentation* | *Cold Contact* | *Distillation* | | | | | *Membrane filtration* | Perception threshold |
| Compound | Odor | RI (CPSil-5) or (FFAP)♦ | Months at 20 °C | A | B♣ | C♣ | D*♣ | E | F | G | H | I | J | K | |
| Sotolon | Curry, spicy | 2196♦ | 0 | $0.5^{a}$ | $1.3^{a}$ | $0.4^{a}$ | $0.9^{a}$ | $0.6^{a}$ | $1.4^{a}$ | $0.8^{a}$ | $0.2^{a}$ | $2.4^{a}$ | $2.3^{a}$ | $1.1^{a}$ | 0.8[27] |
| | | | 6M | $0.2^{a}$ | $6.2^{a}$ | $1.5^{a}$ | $2.5^{a}$ | $1.5^{a}$ | $2.1^{a}$ | $0.3^{a}$ | $0.7^{a}$ | $1.3^{a}$ | $2.5^{a}$ | $1.0^{a}$ | |
| | | | 12M | $0.4^{a}$ | $6.5^{a}$ | $1.7^{a}$ | $3.1^{a}$ | $2.3^{a}$ | $5.7^{a}$ | $1.0^{a}$ | $1.4^{a}$ | $1.2^{a}$ | $3.0^{a}$ | $3.7^{a}$ | |
| Abhexon | Curry, honey | 2260♦ | 0 | $0.3^{a}$ | $0.1^{a}$ | $0.1^{a}$ | $0.3^{a}$ | $0.5^{a}$ | $0.1^{a}$ | $0.1^{a}$ | $0.0^{a}$ | $0.2^{a}$ | $0.1^{a}$ | $0.4^{a}$ | 1.2[19] |
| | | | 6M | $0.2^{a}$ | $2.9^{a}$ | $0.1^{a}$ | $1.0^{a}$ | $0.1^{a}$ | $0.1^{a}$ | $0.1^{a}$ | $0.0^{a}$ | $0.2^{a}$ | $0.6^{a}$ | $0.2^{a}$ | |
| | | | 12M | $0.5^{a}$ | $4.4^{a}$ | $2.3^{a}$ | $1.7^{a}$ | $1.1^{a}$ | $0.3^{a}$ | $0.4^{a}$ | $0.8^{a}$ | $0.5^{a}$ | $1.0^{a}$ | $0.2^{a}$ | |
| Methional | Boiled potato | 872 | 0 | $7.1^{b,c}$ | $3.0^{b,c}$ | $1.8^{c}$ | $4.1^{b,c}$ | $17.7^{a}$ | $14.5^{a}$ | $3.0^{b,c}$ | $2.1^{c}$ | $2.4^{c}$ | $4.1^{b,c}$ | $12.2^{a,b}$ | 0.5[19] |
| | | | 6M | $11.8^{b}$ | $4.0^{b}$ | $3.1^{b}$ | $5.3^{b}$ | $27.2^{a}$ | $28.3^{b}$ | $9.0^{b}$ | $4.8^{b}$ | $5.2^{b}$ | $7.4^{b}$ | $13.3^{b}$ | |
| | | | 12M | $13.1^{b}$ | $8.0^{b}$ | $4.2^{b}$ | $6.7^{b}$ | $48.1^{a}$ | $32.5^{b}$ | $13.1^{b}$ | $12.9^{b}$ | $22.5^{b}$ | $8.2^{b}$ | $14.4^{b}$ | |
| Phenylacetaldehyde | Floral, honey | 853 | 0 | $19.5^{a,b}$ | $6.8^{a,b}$ | $5.1^{b}$ | $9.0^{a,b}$ | $27.2^{a,b}$ | $20.8^{a,b}$ | $8.0^{a,b}$ | $7.1^{a,b}$ | $30.5^{a,b}$ | $9.7^{a,b}$ | $33.1^{a}$ | 5.4[19] |
| | | | 6M | $22.5^{a}$ | $9.1^{a}$ | $10.2^{a}$ | $15.9^{a}$ | $41.4^{a}$ | $27.8^{a}$ | $11.2^{a}$ | $12.0^{a}$ | $40.8^{a}$ | $18.5^{a}$ | $30.7^{a}$ | |
| | | | 12M | $28.2^{a}$ | $14.1^{a}$ | $36.0^{a}$ | $24.7^{a}$ | $61.6^{a}$ | $37.3^{a}$ | $25.0^{a}$ | $19.9^{a}$ | $66.1^{a}$ | $26.2^{a}$ | $19.1^{a}$ | |
| β-Damascenone | Apple, jam | 1376 | 0 | $1.3^{b}$ | $0.3^{b}$ | $1.5^{b}$ | $0.5^{b}$ | $0.7^{b}$ | $5.6^{b}$ | $2.0^{b}$ | $5.3^{b}$ | $10.3^{b}$ | $93.5^{a}$ | $3.8^{b}$ | 0.2[19] |
| | | | 6M | $1.1^{b}$ | $0.6^{b}$ | $1.8^{b}$ | $0.7^{b}$ | $0.8^{b}$ | $3.3^{b}$ | $1.8^{b}$ | $0.6^{b}$ | $6.3^{b}$ | $88.4^{a}$ | $3.0^{b}$ | |
| | | | 12M | $1.1^{b}$ | $1.6^{b}$ | $2.6^{b}$ | $1.9^{b}$ | $1.0^{b}$ | $2.2^{b}$ | $1.1^{b}$ | $0.1^{b}$ | $3.5^{b}$ | $89.3^{a}$ | $1.8^{b}$ | |
| Dimethyltrisulfide | Onion, garlic | 959 | 0 | $0.7^{b}$ | $0.1^{b}$ | $0.6^{b}$ | $0.2^{b}$ | $1.9^{b}$ | $0.2^{b}$ | $2.4^{a}$ | $2.0^{b}$ | $1.8^{b}$ | $1.6^{b}$ | $0.2^{b}$ | 0.1[29] |
| | | | 6M | $0.2^{f}$ | $0.3^{d,e,f}$ | $0.7^{d}$ | $0.6^{d,e}$ | $0.7^{d,e}$ | $nd^{e,f}$ | $1.3^{f}$ | $0.6^{f}$ | $0.7^{b}$ | $0.9^{a}$ | $0.3^{c}$ | |
| | | | 12M | $nd^{f}$ | $0.5^{d,e,f}$ | $1.2^{d}$ | $1.5^{d,e}$ | $0.3^{d,e}$ | $nd^{e,f}$ | $0.3^{f}$ | $0.1^{f}$ | $0.3^{b}$ | $nd^{a}$ | $0.3^{c}$ | |

Mean of duplicates, coefficient of variation <10% for SAFE extracts and for sotolon and abhexon.
Fresh and aged beer values within a row with different letters are significantly different ($p < 0.05$) according to the Student-Newman-Keuls test.
*:with can refermentation; ♣: with dry hopping; nd: not detected.

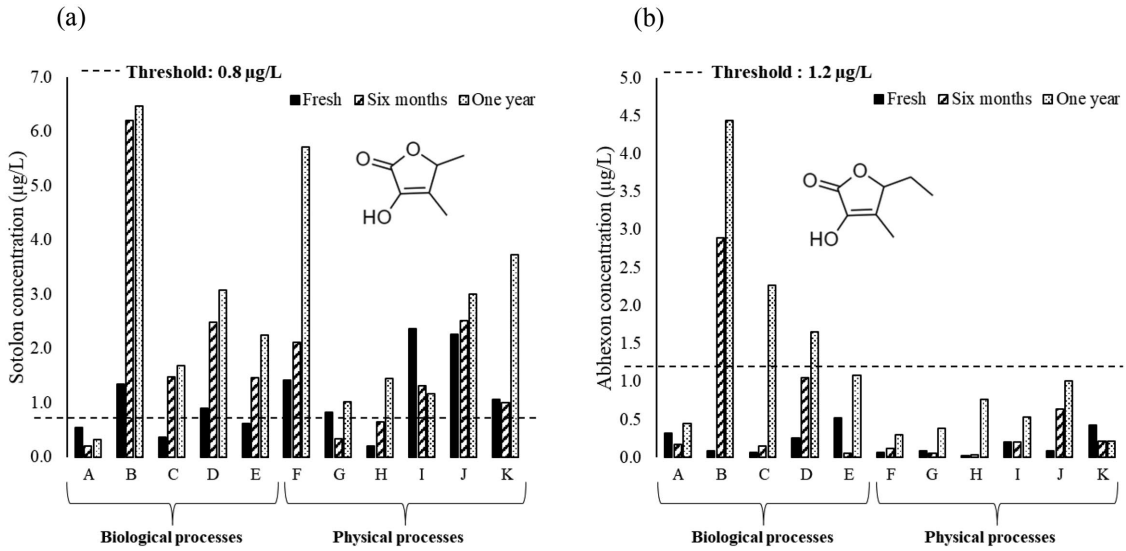


**Figure 1.** Concentration (µg/L) of (a) sotolon and (b) abhexon in NABLABs, fresh and after six months or one year of storage at 20 °C in the dark.

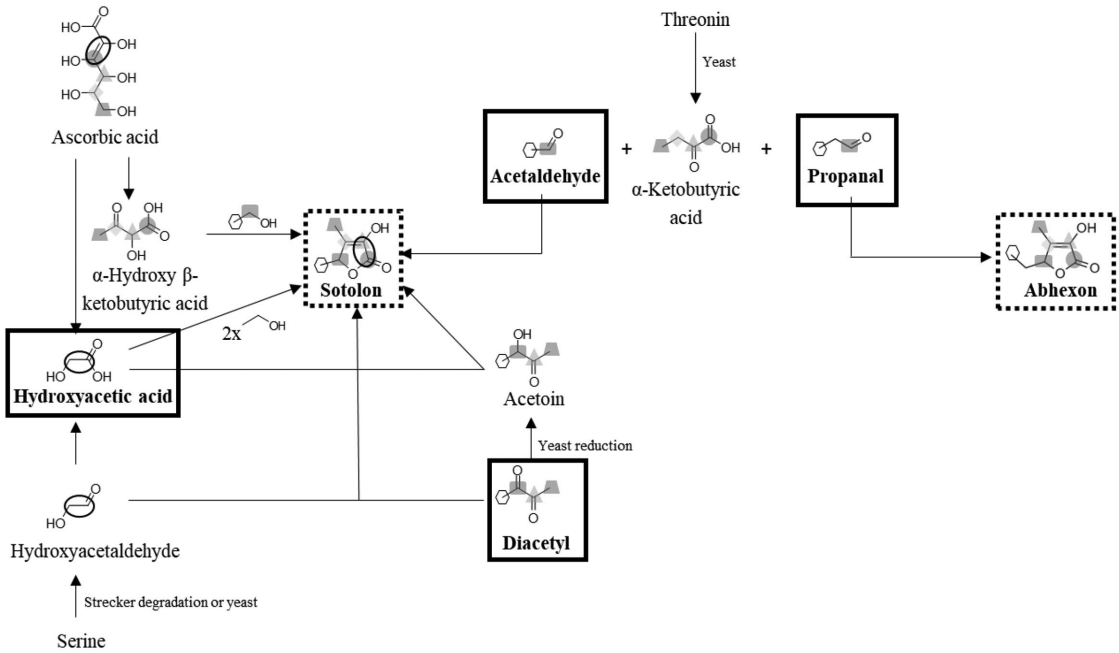


**Figure 2.** Synthesis pathways for sotolon and abhexon in NABLABs.

After one year of aging at 20 °C in the dark, the 11 NABLABs were subjected to the same standard analyses (Table 1, in parentheses). Alcohol content, original extract, and real extract remained fairly constant for most beers. The pH decreased (0.4 on average) in most samples except for beer H. On the other hand, color increased for all NABLABs, by 3°EBC on average. The BU values significantly dropped during storage (up to 61% in beer G) while permanent haze increased from 0.2 (E) to 6.5°EBC (D). Such evolution of color, BU values, and haze is a strong indicator of oxidative chemical reactions.[12,27]

### *Key- and stale-odorant compounds in fresh NABLABs*

Surprisingly, sotolon was here evidenced in most fresh NABLABs above its perception threshold (0.8 µg/L[28]), up to 2.4 µg/L (beer I), except in beers A, C, E and H (0.2–0.6 µg/L) (Table 2 and Figure 1a). No trace was reported in the literature for fresh conventional beers,[30] except for Gueuze beers.[31] On the other hand, sotolon is known as an oxidation indicator in aged regular beers.[28,31] Different potential synthesis pathways were proposed for traditional beers[28]: aldol condensation of acetaldehyde and α-ketobutyric

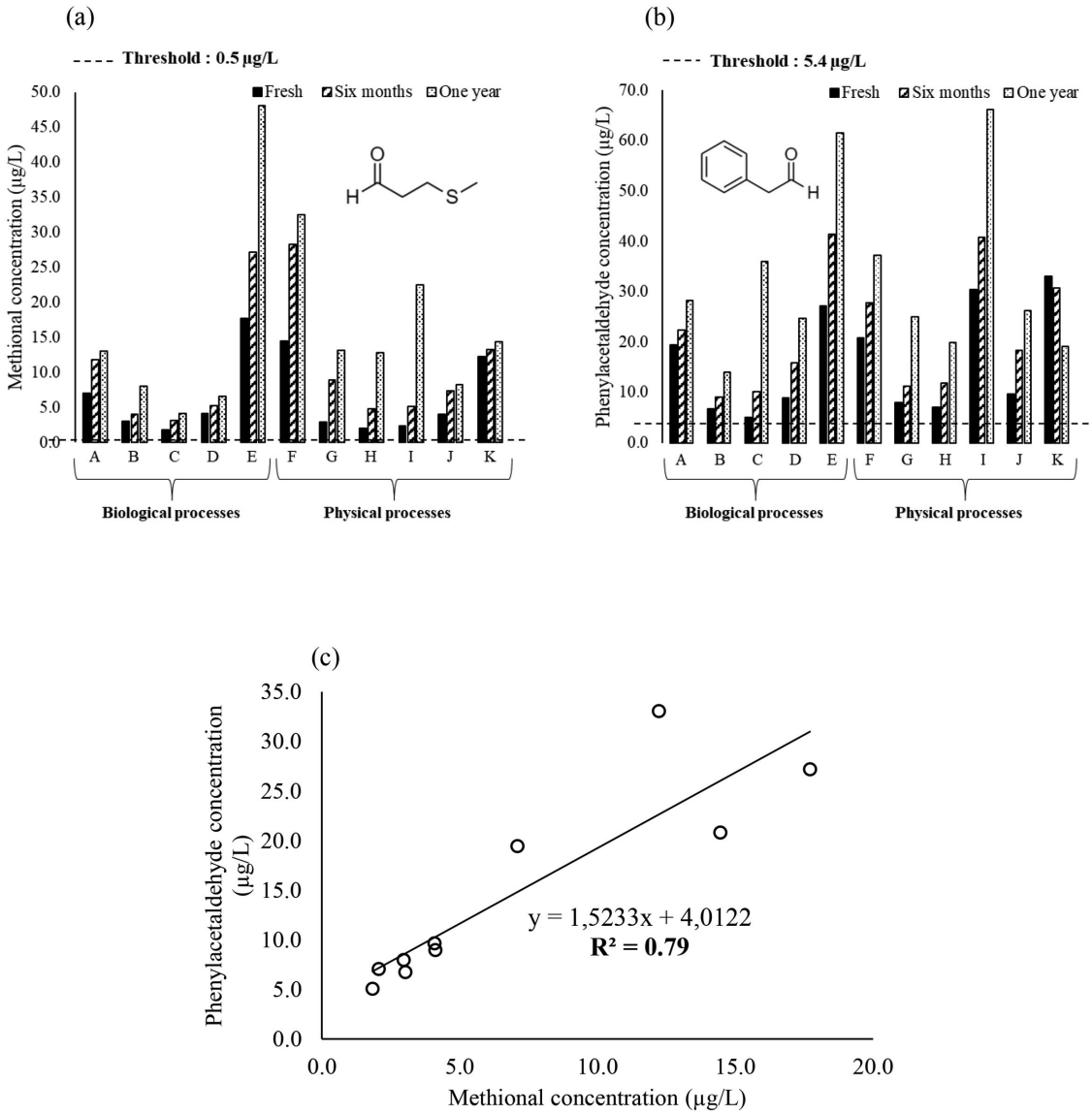


**Figure 3.** Concentration (µg/L) of (a) methional and (b) phenylacetaldehyde in NABLABs, fresh and after six months or one year of storage at 20 °C in the dark. (c) Correlation between them in fresh samples.

acid,[32] reaction between hydroxyacetic acid and acetoin,[33] reaction of hydroxyacetaldehyde with diacetyl, and oxidation of ascorbic acid into hydroxyacetic acid (Figure 2). In NABLABs, a higher occurrence of several of these precursors is suspected: acetaldehyde, hydroxyacetic acid, and diacetyl, all issued from oxidation.[7,33,34]

In contrast, its ethyl analog, abhexon (here requiring propanal instead of acetaldehyde, Figure 2) was found below its perception threshold in all fresh NABLABs (1.2 µg/L[19]), with values ranging from 0.03 (beer H) to 0.5 (beer E) µg/L (Table 2 and Figure 1b). These results contradict a recent paper suggesting that abhexon could be a main contributor to the worty off-flavor of fresh NABLABs (42.3 µg/L detected in one beer[18]).

In all investigated fresh NABLABs, methional was detected at concentrations from 1.8 (beer C) to 17.7 (beer E) µg/L, close to aged regular beers[35,36] and well above its perception threshold (0.5 µg/L[19]) (Table 2 and Figure 3a). As a result, this methionine-derived Strecker aldehyde most probably imparts their worty off-flavor. In most biological procedures, methional is probably insufficiently reduced to alcohol, while it is regenerated in thermal dealcoholization. Interestingly, beer E showed the highest level, although issued from a Cold Contact Process (usually described with a lower aldehyde reduction capacity[5]) on colored malts (richer in Strecker aldehydes).

Phenylacetaldehyde, the phenylalanine-derived Strecker aldehyde, was found at concentrations between 5.1 (beer C) to 33.1 (beer K, produced with special malts −14°EBC) (Table 2 and Figure 3b). These values also were close to aged regular beers[35,36] and very often above its perception threshold (5.4 µg/L[19]).

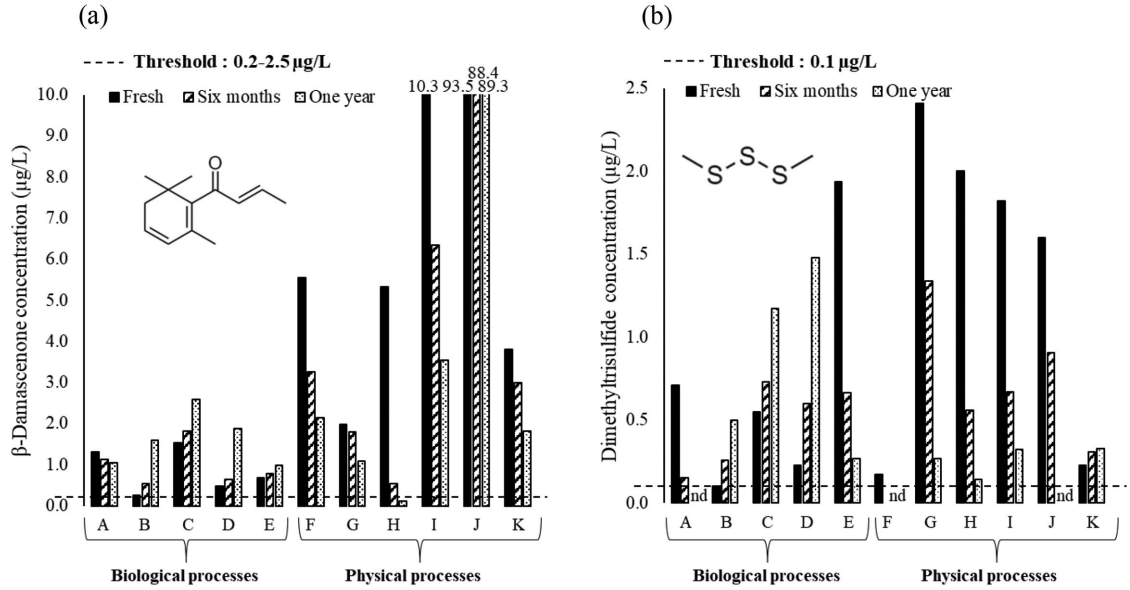


**Figure 4.** Concentration (µg/L) of (a) β-damascenone and (b) dimethyltrisulfide in NABLABs, fresh and after six months or one year of storage at 20 °C in the dark. (nd: not detected).

As depicted in Figure 3(c), a correlation ($R^2$ = 0.79) was observed between levels of phenylacetaldehyde and methional, suggesting a similar pathway for both (oxygen-enhanced Strecker degradation mechanism most probably involved).

The compound β-damascenone was also observed above its perception threshold (0.2 µg/L[19]) in all fresh beers (Table 2 and Figure 4a): from 0.3 in beer B to 93.5 µg/L in beer J, as for other aged regular beers (14–210 µg/L reported[37]). Beer J significantly emerged here from the others, possibly explained by the hop variety used. The release from hop glycosides (such as grasshopper ketone[29]) is known to be promoted by high temperatures. Interestingly, NABLABs processed by physical methods revealed to show 96% more β-damascenone compared to the biological process-derived samples.

Surprisingly, dimethyltrisulfide (Table 2 and Figure 4b) was quantified in all fresh NABLABs above its perception threshold (0.1 µg/L[38]). Its levels are not to be ignored: they were close or higher than in aged regular beers (0.01 µg/L[35]), ranging from 0.1 (beer B) to 2.4 (beer G) µg/L. Methional and methionol are its potential precursors.[38]

A large number of other key-odorant compounds were found in the SAFE extracts (Figure 5). Among them, terpenols, such as linalool (citrus, 13–734 µg/L for a threshold = 8.0 µg/L[39]) and geraniol (geranium, 0.2–355 µg/L for a threshold = 4.0 µg/L[39]) were detected above their perception threshold in almost all fresh NABLABs as for regular beers. On the other hand, fermentation esters such as isoamyl acetate (banana, 48–1912 µg/L for a threshold = 500.0 µg/L[35]) were often below their perception threshold. Moreover, 4-vinylguaiacol (clove, 5–630 µg/L for a threshold = 125.0 µg/L[40]), nonanal (citrus, 0.7–41 µg/L for a threshold = 18.0 µg/L[41]), and γ-nonalactone (coconut, 4–44 µg/L for a threshold = 11.2 µg/L[42]), were found above their threshold in a few beers (all in G, J and K).

## *Fate of key- and stale-odorants through aging*

This part of the paper will be only focused on stale flavors. Globally, as for regular beers,[43] a concentration increase or decrease of hop- and fermentation-derived compounds was product dependent.

In almost all NABLABs, sotolon and abhexon revealed produced through aging (+ 64% and + 76% on average, respectively) (Figure 1). Yet, despite its occurrence in fresh NABLABs, sotolon concentrations were here very close to values published for some aged regular beers (up to 8.7 µg/L[28]). Abhexon was found above its perception threshold only in beers B, C, and D (Table 2). Interestingly, aged beer B was the most concentrated both in sotolon (6.5 µg/L) and abhexon (4.4 µg/L).

Strecker aldehydes are known not only to impart worty off-flavor to fresh NABLABs but also to be continually produced through aging, even in regular beers.[44] The formation of these aldehydes is impacted by the storage temperature and the level of dissolved oxygen.[36] After one year of storage at 20 °C in the dark, methional and phenylacetaldehyde concentrations were increased by 58% on average for both (Table 2 and Figure 3). Only beer K showed a phenylacetaldehyde decrease of 42% (phenylacetaldehyde possibly oxidized into its corresponding acid).

Although β-damascenone was reported to be produced in regular beers through aging (to as much as 210 µg/L, acidic hydrolysis of carotenoid-derived glycosides[37]), its behavior emerged in aged NABLABs as product-dependent (Figure 4a): increased in beers B, C, D, and E issued from biological processes (+57% on average) while decreased in beers F, G, H, I, J, and K (−54% on average) from physical processes (the most concentrated when fresh) (Table 2).

Dimethyltrisulfide content also emerged as product-dependent through aging, but a reduction of

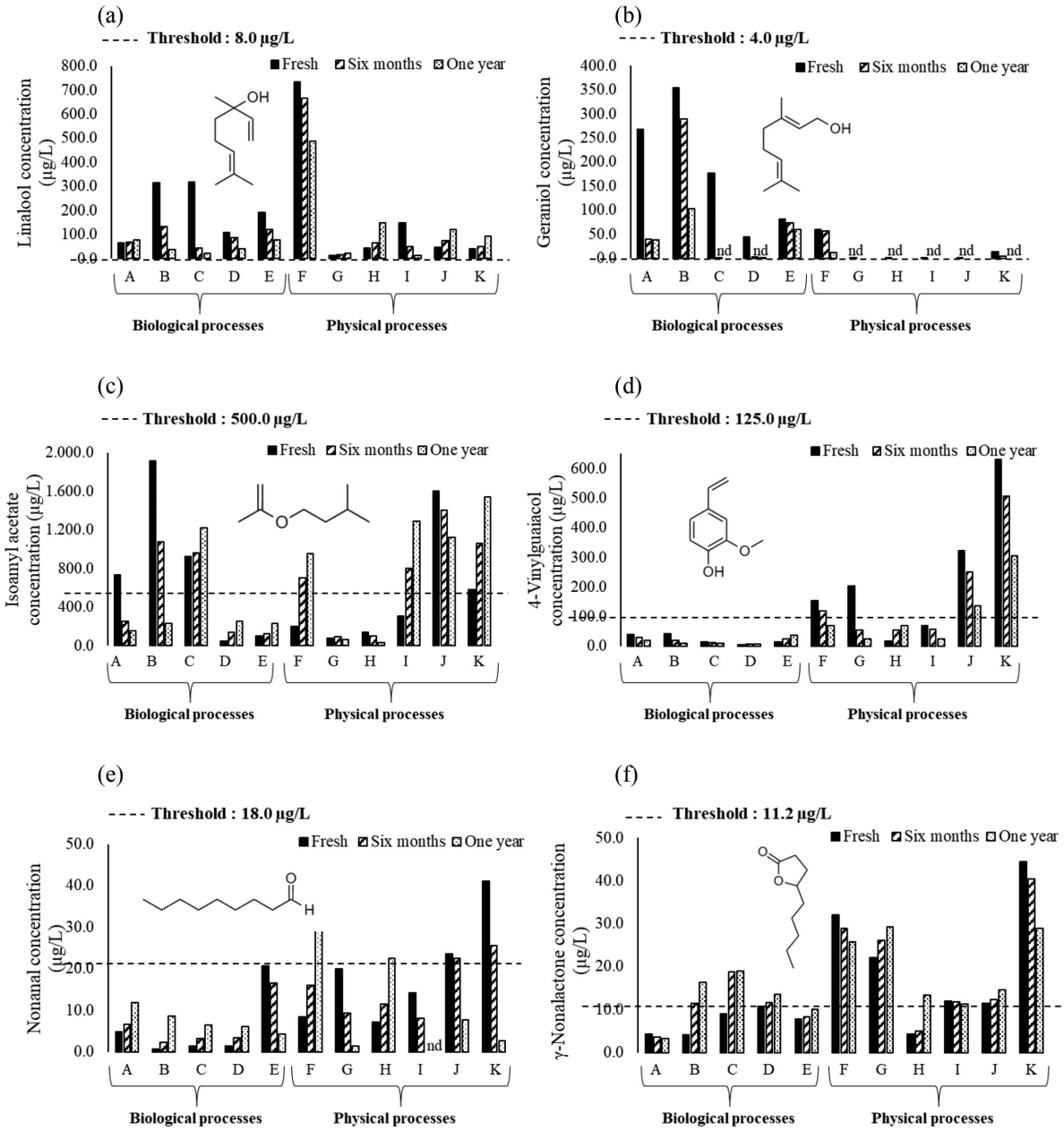


**Figure 5.** Concentration (µg/L) of (a) linalool, (b) geraniol, (c) isoamyl acetate, (d) 4-vinylguaiacol, (e) nonanal and (f) γ-nonalactone in NABLABs, fresh and after six months or one year of storage at 20°C in the dark. (nd: not detected).

concentration was observed in most samples (7 out of 11) (Table 2 and Figure 4b).

## Conclusion

Five stale-odorant compounds were found above their perception threshold in most fresh NABLABs: dimethyltrisulfide (0.1–2.4 µg/L), methional (1.8–17.7 µg/L), and β-damascenone (0.3–93.5 µg/L) in all samples, phenylacetaldehyde (5.1–33.1 µg/L) in 10 out of 11, and sotolon (0.2–2.4 µg/L) in 7. Based on their chemical structure, it can be concluded that oxidation is the main issue for NABLABs. Addition of efficient antioxidants seems to be required to improve NABLABs acceptability, whatever the process used. The follow-up of major beer oxidation-sensitive compounds such as iso-α acids and polyphenols should help to better assess the impact of new technological procedures in NABLABs production.

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## Disclosure Statement

No potential conflict of interest was reported by the authors.

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## Literature cited


[1] Brányik, T.; Silva, D. P.; Baszczyňski, M.; Lehnert, R.; Almeida E Silva, J. B. A Review of Methods of Low Alcohol and Alcohol-Free Beer Production. *J. Food Eng.* **2012**, *108*, 493–506. DOI: 10.1016/j.jfoodeng.2011.09.020.

[2] Sohrabvandi, S.; Mousavi, S. M.; Razavi, S. H.; Mortazavian, A. M.; Rezaei, K. Alcohol-Free Beer: Methods of Production, Sensorial Defects, and Healthful Effects. *Food Rev. Int.* **2010**, *26*, 335–352. DOI: 10.1080/87559129.2010.496022.

[3] Muller, C.; Neves, L. E.; Gomes, L.; Guimarães, M.; Ghesti, G. Processes for Alcohol-Free Beer Production: A Review. *Food Sci. Technol.* **2020**, *40*, 273–281. DOI: 10.1590/fst.32318.

[4] Müller, M.; Bellut, K.; Tippmann, J.; Becker, T. Physical Methods for Dealcoholization of Beverages Matrices and Their Impact on Quality Attributes. *CBEN* **2017**, *4*, 310–326. DOI: 10.1002/cben.201700010.

[5] Perpète, P.; Collin, S. State of the Art in Low-Alcohol Beer Production. *Cerevisia* **1999**, *1*, 27–33.

[6] Vanderhaegen, B.; Neven, H.; Verachtert, H.; Derdelinckx, G. The Chemistry of Beer Aging – A Critical Review. *Food Chem.* **2006**, *95*, 357–381. DOI: 10.1016/j.foodchem.2005.01.006.

[7] Andersen, M. L.; Skibsted, L. H. Electron Spin Resonance Spin Trapping Identification of Radical Formed during Aerobic Forced Aging of Beer. *J. Agric. Food Chem.* **1998**, *46*, 1272–1275. DOI: 10.1021/jf9708608.

[8] 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/jf000354+.

[9] Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Kamada, K. The Role of Free Radicals in Beer Oxidation. *J. Am. Soc. Brew. Chem.* **1989**, *47*, 49–53. DOI: 10.1094/ASBCJ-47-0049.

[10] Bamforth, C. W.; Muller, R. E.; Walker, M. D. Oxygen and Oxygen Radicals in Malting and Brewing: A Review. *J. Am. Soc. Brew. Chem.* **1993**, *51*, 79–88. DOI: 10.1094/ASBCJ-51-0079.

[11] De Cooman, L.; Aerts, G.; Overmeire, H.; De Keukeleire, D. Alterations of the Profiles of Iso-α-Acids during Beer Ageing, Marked Instability of Trans-Iso-α-Acids and Implications for Beer Bitterness Consistency in Relation to Tetrahydroiso-α-Acids. *J. Inst. Brew.* **2000**, *106*, 169–178. DOI: 10.1002/j.2050-0416.2000.tb00054.x.

[12] Ferreira, S.; Collin, C. S. Fate of Bitter Compounds through Dry-Hopped Beer Aging. Why Cis-Humulinones Should be as Feared as Trans-Isohumulones? *J. Am. Soc. Brew. Chem.* **2020**, *78*, 103–113. DOI: 10.1080/03610470.2019.1705037.

[13] McMurrough, I.; Madigan, D.; Kelly, R. J.; Smyth, M. R. The Role of Flavanoid Polyphenols in Beer Stability. *J. Am. Soc. Brew. Chem.* **1996**, *54*, 141–148. DOI: 10.1094/ASBCJ-54-0141.

[14] Aron, P. M.; Shellhammer, T. H. A Discussion of Polyphenols in Beer Physical and Flavour Stability. *J. Inst. Brew.* **2010**, *116*, 369–380. DOI: 10.1002/j.2050-0416.2010.tb00788.x.

[15] Perpète, P.; Collin, S. Contribution of 3-Methylthiopropionaldehyde to the Worty Flavor of Alcohol-Free Beers. *J. Agric. Food Chem.* **1999**, *47*, 2374–2378. DOI: 10.1021/jf9811323.

[16] Perpète, P.; Collin, S. Influence of Beer Ethanol Content on the Wort Flavour Perception. *Food Chem.* **2000**, *71*, 379–385.

[17] Perpète, P.; Collin, S. Evidence of Strecker Aldehyde Excretion by Yeast in Cold Contact Fermentations. *J. Agric. Food Chem.* **2000**, *48*, 2384–2386. DOI: 10.1021/jf000071h.

[18] Piornos, J. A.; Balagiannis, D. P.; Methven, L.; Koussissi, E.; Brouwer, E.; Parker, J. K. Elucidating the Odor-Active Aroma Compounds in Alcohol-Free Beer and Their Contribution to the Worty Flavor. *J. Agric. Food Chem.* **2020**, *68*, 10088–10096. DOI: 10.1021/acs.jafc.0c03902.

[19] Piornos, J. A.; Delgado, A.; de La Burgade, R. C. J.; Methven, L.; Balagiannis, D. P.; Koussissi, E.; Brouwer, E.; Parker, J. K. Orthonasal and Retronasal Detection Thresholds of 26 Aroma Compounds in a Model Alcohol-Free Beer: Effect of Threshold Calculation Method. *Int. Food Res. J.* **2019**, *123*, 317–326. DOI: 10.1016/j.foodres.2019.04.034.

[20] Bailly, S.; Jerkovic, V.; Meuree, A.; Timmermans, A.; Collin, S. Fate of Key Odorants in Sauternes Wines through Aging. *J. Agric. Food Chem.* **2009**, *57*, 8557–8563. DOI: 10.1021/jf901429d.

[21] European Brewery Convention. *Analytica-EBC*; Fachverlag Hans Carls: Nürnberg, **2006**.

[22] Buckee, G. K.; Long, D. E. Estimation of Sugars in Worts and Beers Using High Performance Liquid Chromatography with an Improved Column. *J. Am. Soc. Brew. Chem.* **1982**, *40*, 137–140. DOI: 10.1094/ASBCJ-40-0137.

[23] Engel, W.; Bahr, W.; Schieberle, P. Solvent Assisted Flavour Evaporation - A New and Versatile Technique for the Careful and Direct Isolation of Aroma Compounds from Complex Food Matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241. DOI: 10.1007/s002170050486.

[24] Blank, I.; Sen, A.; Grosch, W. Potent Odorants of the Roasted Powder and Brew of Arabica Coffee. *Z. Lebensm. Unters. Forch.* **1992**, *195*, 239–245. DOI: 10.1007/BF01202802.

[25] Maye, J. P.; Smith, R.; Leker, J. Humulinone Formation in Hops and Hop Pellets and Its Implications for Dry Hopped Beers. *Tech. Q. Master Brew. Assoc. Am.* **2016**, *53*, 23–27.

[26] Kirkpatrick, K. R.; Shellhammer, T. H. Evidence of Dextrin Hydrolyzing Enzymes in Cascade Hops (*Humulus lupulus*). *J. Agric. Food Chem.* **2018**, *66*, 9121–9126. DOI: 10.1021/acs.jafc.8b03563.

[27] Silva Ferreira, C.; Simon, M.; Collin, S. Why Catechin and Epicatechin from Early Hopping Impact the Color of Aged Dry-Hopped Beers While Flavan-3-ol Oligomers from Late and Dry Hopping Increase Colloidal Instability. *J. Am. Soc. Brew. Chem.* **2022**. DOI: 10.1080/03610470.2022.2062156.

[28] Scholtes, C.; Nizet, S.; Collin, S. How Sotolon Can Impart a Madeira Off-Flavor to Aged Beers. *J. Agric. Food Chem.* **2015**, *63*, 2886–2892. DOI: 10.1021/jf505953u.

[29] Muzhingi, T.; Yeum, K.-J.; Russell, R. M.; Johnson, E. J.; Qin, J.; Tang, G. Determination of Carotenoids in Yellow Maize, the Effects of Saponification and Food Preparations. *Int. J. Vitam. Nutr. Res.* **2008**, *78*, 112–120. DOI: 10.1024/0300-9831.78.3.112.

[30] Scholtes, C.; Collin, S. How Sotolon and Abhexon Can Impart Madeira off-Flavours to Aged Beers?. 33rd Congress of the European Brewery Convention, Glasgow, Scotland, **2011**.

[31] 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. DOI: 10.1002/jib.34.

[32] Sulser, H.; DePizzol, J.; Bühi, W. A Probable Flavoring Principle in Vegetable-Protein Hydrolysates. *J. Food Sci.* **1967**, *32*, 611–615. DOI: 10.1111/j.1365-2621.1967.tb00846.x.

[33] 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. DOI: 10.1021/jf0342932.

[34] Wainwright, T. Diacetyl—A Review: Part I—Analytical and Biochemical Considerations: Part II—Brewing Experience. *J. Inst. Brew.* **1973**, *79*, 451–470. DOI: 10.1002/j.2050-0416.1973.tb03567.x.

[35] Saison, D.; De Schutter, D.; Uyttenhove, B.; Delvaux, F.; Delvaux, F. Contribution of Staling Compounds to the Aged Flavour of Lager Beer by Studying Their Flavour Thresholds. *Food Chem.* **2009**, *114*, 1206–1215. DOI: 10.1016/j.foodchem.2008.10.078.

[36] Soares da Costa, M.; Gonçalves, C.; Ferreira, A.; Ibsen, C.; Guedes de Pinho, P.; Silva Ferreira, A. C. Further Insights into the Role of Methional and Phenylacetaldehyde in Lager Beer Flavour Stability. *J. Agric. Food Chem.* **2004**, *52*, 7911–7917. DOI: 10.1021/jf049178l.

[37] 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. DOI: 10.1021/jf020085i.

[38] Gijs, L.; Perpète, P.; Timmermans, A.; Collin, S. 3-Methylthiopropionaldehyde as Precursor of Dimethyltrisulfide in Aged Beers. *J. Agric. Food Chem.* **2000**, *48*, 6196–6199. DOI: 10.1021/jf0007380.

[39] Cibaka, M. L. K.; Ferreira, C. S.; Decourrière, L.; Lorenzo-Alonso, C. J.; Bodart, E.; Collin, S. Dry Hopping with the Dual-Purpose Varieties Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace: Minor Contribution of Hop Terpenol Glucosides to Beer Flavors. *J. Am. Soc. Brew. Chem.* **2017**, *75*, 122–129. DOI: 10.1094/ASBCJ-2017-2257-01.

[40] Scholtes, C.; Nizet, S.; Collin, S. Guaiacol and 4-Methylphenol as Specific Markers of the Use of Torrefied Malts. Fate of Volatile Phenols in Special Beers through Aging. *J. Agric. Food Chem.* **2014**, *62*, 9522–9528. DOI: 10.1021/jf5015654.

[41] Ferreira, I. M.; Freitas, F.; Pinheiro, S.; Mourao, M. F.; Guido, L. F.; da Silva, M. G. Impact of Temperature during Beer Storage on Beer Chemical Profile. *LWT* **2022**, *154*, 112688. DOI: 10.1016/j.lwt.2021.112688.

[42] Suzuki, M.; Wanikawa, A.; Kono, K.; Shibata, K. Factors Affecting the Formation of Gamma-Nonalactone and Its Contribution to the Flavor and Aroma of Aging Beer. The Institute of Brewing & Distilling Meeting Asia Pacific Section, **2006**.

[43] Silva Ferreira, C.; Collin, S. Fate of Hop and Fermentation Odorants in Commercial Belgian Dry-Hopped Beers over 2 Years of Bottle Storage: Key-Role of Oxidation and Hop Esterases. *J. Am. Soc. Brew. Chem.* **2021**, *79*, 259–271. DOI: 10.1080/03610470.2020.1843898.

[44] Guedes de Pinho, P.; Ferreira, A. Role of Strecker Aldehydes on Beer Flavour Stability. *Develop. Food Sci.* **2006**, *43*, 529–532.