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Further Insights into the Role of Methional and Phenylacetaldehyde in Lager Beer Flavor Stability

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This work attempts to measure the importance of methional and phenylacetaldehyde on the flavor stability of beer, as direct participants or as indicators of aroma deterioration. A trained sensory panel identified the most important descriptors related to the typical aroma of aged beer: "malty", "honey-like", "cooked potato", and "metallic". By GC−olfactometry analysis, six aromatic zones related to the selected descriptors were highlighted, and by using GC-MS techniques it was possible to identify methional and phenylacetaldehyde as being responsible for two odor zones. The quantification of these molecules in samples submitted to forced aging treatments showed that the levels of methional and phenylacetaldehyde are dependent on the temperature of storage. Normal aged beers were also analyzed, and it was observed that these compounds accumulate with time of storage. Furthermore, these molecules were negatively correlated with the aroma quality of beer as evaluated by a sensorial panel. To validate the sensory impact of these substances, a fresh beer was spiked with these molecules and also with *trans*-2-nonenal, singly and in combination, and the similarity value between samples and the aged beer was then determined. The highest value from the similarity tests was 72% when the three compounds were added simultaneously. The combination of the two Strecker aldehydes increases by 54% the degree of similarity, indicating the key role played by these molecules in the aroma deterioration of beer. Finally, the kinetic parameters, E_a and k, were calculated, and it was observed that the Arrhenius equation described well the temperature dependence of the reaction rate constant. Measuring the concentration of methional and phenylacetaldehyde may provide information about the key steps along the process that most affect the flavor stability of beer, which may be useful in establishing the best storage conditions.

KEYWORDS: Beer flavor stability; methional; phenylacetaldehyde; beer aging

INTRODUCTION

Flavor stability has become one of the most important subjects in brewery research, as demonstrated by the large number of studies available in the literature (*1*−*21*). Several mechanisms are recognized as contributors to the undesirable "stale character" typical of flavor deterioration (*18*). Two are widely accepted as the most relevant: lipid oxidation and Maillard reaction (*13*). The first pathway is responsible for the presence of *trans*-2-nonenal, involving the chemical or enzymatic oxidation of polyunsaturated fatty acids, linoleic acid isomers. This aldehyde, which has the unpleasant smell of cardboard with an extremely low odor threshold of 0.035 μg/L (*2*), is responsible for one of the most undesirable aromas in beer. More recently it has also been suggested that it can be released from proteins of wort during beer storage (*8*).

The Maillard reaction involves sugars and amino compounds in a very complex network of reactions (*22*). Strecker degradation is a minor pathway of this wide mechanism, but it is very important in the formation of many volatile organic compounds, the "Strecker aldehydes".

In beer, the degradation of amino acids such as leucine, isoleucine, and valine into the Strecker aldehydes 3-methylbutanal, 2-methylbutanal, and 2-methylpropanal, respectively, are positive contributors to aroma quality, considered to be responsible for the malty character (*9*, *10*, *13*). However, Strecker aldehydes derived from methionine and phenylalanine degradation, respectively, methional and phenylacetaldehyde, were related to the typical aromas of spoiled white wines (*23*, *24*). In beer, methional was also related to the flavor of aged beer (*14*, *15*).

Carbonyl compounds are known constituents of beer raw materials and can also be formed during the prefermentation stages of the production process (*18*). Nevertheless, many of these molecules are reduced to alcohols during fermentation,

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to levels below the analytical detection limit, by yeast activity, even in cold conditions (*25*−*27*).

The sulfur dioxide formed by the yeast metabolism can bind these molecules, thus contributing to a reduction in the perceived "aged character". Nevertheless, the possible release of aldehydes from the sulfite adducts during bottle storage may also contribute to the accumulation of these molecules in beer.

The aim of this work was to select suitable chemical substances, responsible for aroma deterioration, to be monitored during beer production, to provide information concerning the key steps along the process that most affect the flavor stability and thus the beer's shelf life.

The aim was selection of the most relevant descriptors related with the typical aged aroma of a lager-type beer. The correlation between sensorial data and the presence of chemical substances was carried out by gas chromatography−olfactometry (GC-O) measurements. Beer samples, lager type, were provided from an industrial brewery. To promote aroma degradation, some samples were submitted to forced aging experiments, in which temperature was the only technological parameter changed. The kinetic parameters, E_a and k, of the Strecker aldehydes were also calculated.

MATERIALS AND METHODS

Samples were produced in Portugal at UNICER Brewery facilities, following the standard production parameters corresponding to the commercial brand lager-type beer. The pH, ethanol, CO_2, and total SO_2 values ranged from 4.3 to 4.5, from 5.4 to 5.6 (% v/v), from 5.1 to 5.5 g/L, and from 15 to 24 mg/L, respectively. The dissolved oxygen was <0.2 mg/L.

Beer Group 1: Forced Aging Experiment. To promote the aroma degradation of beer, a forced aging experiment was implemented. Thirty-four lager beer samples (330 mL bottles, crown cork sealing cap) were divided into two groups: (i) one was stored at a temperature of 37 °C, for 7 days (standard procedure in the brewery in order to evaluate flavor stability); (ii) the other was kept at 4 °C for 180 days. Samples were analyzed immediately after the storage period.

Beer Group 2: Normal Aged Beers. Eight beer samples were analyzed after the 6 months corresponding to the shelf life period of the product. Two were kept at 4 °C at the brewery, and the other six, kept under commercial storage conditions, were returned to the point of production after the shelf-life period had expired ("boomerang beers").

Two samples of 25- and 40-year-old lager beers, belonging to the Carlsberg collection, were graciously furnished by the company (Carlsberg, Denmark). Despite being individual samples, and the unrealistically long period of storage, it was chosen to include these samples in the study. These samples could be of interest as "indicators" of chemical changes occurring after several years of bottle aging, considering that the aroma degradation of the product results from a progressive accumulation of molecules responsible for off-flavors during storage.

Beer Group 3: Kinetics Studies. Thirty bottles of 330 mL (five groups of six bottles), crown cork sealing cap, of the same beer were stored at different temperatures: 22, 30, 37, 44, and 58.5 °C. Samples were analyzed each 24 h for a total of nine sampling times. Kinetics of formation of methional and phenylacetaldehyde were determined as a function of time and temperature.

Sensory Studies. *Sensorial Panel.* Two sensorial panels were used: (i) one composed of 7 trained assessors, brewery workers, and (ii) a second panel composed of 10 persons, university students and laboratory personnel. The latter panel was trained weekly for 2 months. Tests were performed individually, using tulip glasses containing 30 mL of beer in a room at a controlled temperature of 20 °C. Samples for sensorial analysis were kept in a refrigerator at 4 °C and presented to the panel immediately after opening.

Descriptor Selection. The AFNOR NFV-09-021 (*28*) procedure was used to select the most important descriptors related to the typical aroma of aged beer. In a first set of sessions, every member of the panel was asked to freely describe the aroma of the beer. The hedonic and redundant terms, as well as the nonpertinent terms, were then disregarded and a first group of descriptors was obtained in this manner. Then, the panel was asked to determine if the first series of descriptors were present or absent. Those descriptors considered to be absent by 50% of the panel were eliminated, and a second group of descriptors was obtained. The panel was then asked to rank each of these descriptors on a scale of 0−10.

Similarity Testing. The aroma-active compounds methional, phenylacetaldehyde, and *trans*-2-nonenal were added singly and in combination to a fresh beer in the following concentrations: 3, 4, and 0.25 μg/L. The similarity value (SV) was determined by a comparison test. Each supplemented sample (coded) was presented to the panel together with an aged beer, and the panel was asked to rate the similarity, on a discontinuous scale from 0 (no similarity) to 10 (indistinguishable), of each sample with the aged beer. The data obtained were treated according to the ANOVA procedure (*29*).

Quality Scoring. The impact of methional and phenylacetaldehyde on the typical aroma of aged beer was evaluated using a continuous "quality scale" from +1 (no defect) to −3 (major defect), as normally used at the brewery. The lower acceptance level is considered to be −1.4. Tests were performed individually. The results were collected after two tasting sessions by the trained industry sensorial panel using the same sample preparation for each session. The average scores rated by the panel were then calculated.

Organic Extract Selection: Aroma Representivity. Aromas were extracted from a beer sample using different organic solvents: hexane, ether, ethyl acetate, and dichloromethane. The same volume of beer (50 mL) was processed twice with 10 and 5 mL of each solvent. Similarity tests were performed between the aroma of the obtained extracts and the beer (*30*). Two 2-mL aliquots of each organic extract were concentrated under nitrogen stream to 0.5 mL. A drop was then put on a "perfumer's blotter" sampling paper, and the aroma was compared with the original beer as a pair. The panel was asked to rate the similarity on a discontinuous scale from 0 (no similarity) to 10 (indistinguishable) of each sample with the aged beer. The data obtained were processed according to the ANOVA procedure, and the Tukey test was used to establish differences among organic solvents (*31*).

Gas Chromatography−Olfactometry. To identify the substances responsible for the aromatic notes associated with the selected descriptors of the aged beer, GC-O analysis was employed. Several dichloromethane extracts from beers with different ages were submitted to GC-O. Two microliters of the extract was injected into the GC equipped with an olfactometric detector. Chromatographic conditions were the following: Hewlett-Packard HP 5890 gas chromatograph; column, BP-21 (50 m × 0.25 mm × 0.25 μm) fused silica (SGE); hydrogen (5.0, Air−liquid); flow, 1.2 mL/min; injector temperature, 220 °C; oven temperature, 40 °C for 1 min programmed at a rate of 2 °C/min to 220 °C, maintained during 30 min; splitless time, 0.5 min; split flow, 30 mL/min. The makeup gas employed on the olfactometric device (SGE) was air (80% N_2; 20% O_2) (Air−liquid). Two streams were used: one was bubbled through water to humidify the air and avoid drying out the assessor's mucus membranes; the other was applied at the exit of the GC column to lower the temperature of the effluent. A panel of four individuals, using the same operational conditions and on the same chromatograph, repeated this procedure. The odor zones reported by each panel member were compared with each retention index. The descriptors were selected according to their frequency of citations. Hedonic terms were not considered (good/bad), and those considered to be analogous were replaced by the most cited.

Chemical Studies. *Standards Preparation.* The following molecules were purchased from Sigma-Aldrich: 3-(methylthio)propionaldehyde (27,746-0) (100% purity); 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (W36,340-5) (97%); phenylacetaldehyde (10,739-5); *trans*-2-nonenal (18829-56-6) (97%); isoamyl acetate (30,696-7). β-Damascenone was graciously furnished by Firmenich.

Quantification Methods: Gas Chromatography−Mass Spectrometry (GC-MS/MS). To quantify methional, phenylacetaldehyde, and sotolon an extraction was performed according to the previous works (*23*). To 50 mL of beer were added 50 μL of octan-3-ol at 466 mg/L as internal

standard and 5 g of anhydrous sodium sulfate. The beer was extracted twice with 5 mL of CH_2Cl_2 (Merck). The two organic phases obtained were combined and dried over anhydrous sodium sulfate. Four milliliters of this organic extract was concentrated until 1/10 under a nitrogen stream with a 20 mL/min gas flow, and 2 μL was injected on the chromatograph.

A gas chromatograph equipped with a GCQ mass detector, from Finnigan Mat (San Jose, CA), was used. The carrier gas was helium C-60 (Gasin), at a constant volumetric flow rate of 1 mL/min. The transfer line and source temperatures were 200 and 190 °C, respectively. The emission current was 70 mV, and the electron multiplier was set according to the autotune procedure. The split valve was opened 1 min after injection with a flow of 60 mL/min. Volatile compounds were separated in a FFAP (CP-Wax 58) column (50 m × 0.25 mm × 0.20 μm) Varian-Chrompack (Walnut Creek CA) at a constant flow of 1 mL/min. The oven temperature was held at 60 °C for 1 min and then increased at 5 °C/min to a final temperature of 230° C, which was held for 20 min. Mass spectra were acquired in MS/MS mode in four segments: segment I (internal standard, 3-octanol), time 15−17 min, precursor ion 83, width 1.0, time 16 μs, excitation voltage 0.5 V, time 30 ms, $q = 0.300$, quantification current 55 *m/z*; segment II (methional), time 17−19 min, precursor ion 104, width 1.0, time 16 μs, excitation voltage 0.3 V, time 10 ms, $q = 0.225$, quantification current 48 *m/z*; segment III (phenylacetaldehyde), time 19−21 min, precursor ion 120, width 1.0, time 16 μs, excitation voltage 2.0 V, time 30 ms, $q = 0.225$, quantification current 92 *m/z*; segment IV (sotolon), time 21−29 min, precursor ion 128, width 1.0, time 16 μs, excitation voltage 1.2 V, time 30 ms, $q = 0.300$, quantification current 83 *m/z*. All segments were acquired at 20 μScans.

The quantification limits (*30*) in beer samples for the molecules under investigation were calculated (LQ = [mean × 10 × standard deviation]) as well as the respective variation coefficients [CV% = (standard deviation/mean) × 100]. They were, respectively, for 3-(methylthio)-propionaldehyde, 0.1 μg/L and 6.7%, for 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone, 0.4 μg/L and 3.2%, and for phenylacetaldehyde, 0.4 μg/L and 7.1%.

To determine *trans*-2-nonenal, a solid-phase microextraction was used as described in ref (*31*).

Statistical Treatments. An analysis of variance (ANOVA), using the Excel software Windows 98 v. 7.0, was applied to the experimental data; the results were considered to be significant if the associated p value was <0.05. To determine which samples were significantly different from another, the Tukey test was used. Samples were arranged according to magnitude, and the least significant difference at 95% (LSD) was determined. Any two samples that differed by a value ≥LSD were regarded as significantly different (*32*).

Kinetic Analysis. Statistical data analysis was performed using the Statistica program version 6.0 (*33*). The most usual kinetic models reported in the literature to describe kinetic of compound formation are zero-order ($c = c_0 + kt$), first-order [$c = c_0 \exp(kt)$], or second-order ($1/c = 1/c_0 + kt$) reaction models. The Arrhenius equation, $k = k_{ref} \exp(-E_a/R \times (1/T - 1/T_{ref}))$], is usually applied to evaluate the effect of temperature on the reaction rate constant (*31*), where T_{ref}, k, k_{ref}, and E_a are, respectively, the reference temperature, the apparent reaction rate constant, the Arrhenius equation constant, and the activation energy.

To obtain meaningful narrower confidence limits for Arrhenius parameters, the one-step nonlinear regression method was employed. This statistical technique takes into account all experimental data, attempting to fit a model to all concentration versus time data for all tested temperatures (*35*).

Other Analytical Measurements. The concentration of dissolved oxygen was measured using a WTW 340 oxygen probe. Kovats index is calculated according to the literature (*36*).

RESULTS AND DISCUSSION

Descriptor Selection. The selection of the most relevant descriptors of the characterization of the typical aroma of aged beer was carried out using the AFNOR NFV-09-021 (*25*) procedure. Samples from the group 1 forced aging experiment were employed. From an initial large number of descriptors, four were selected with the highest ratings: "malty", "honey-like", "cooked potato", and "metallic".

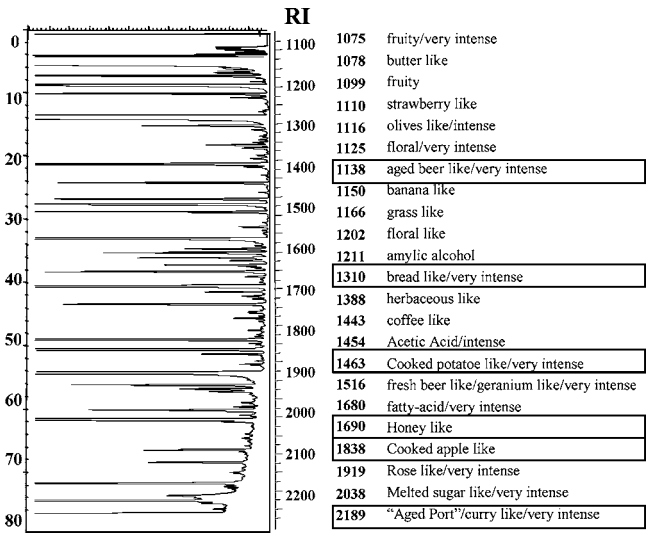

Figure 1. Chromatogram-FID and main descriptors detected by GC-O of a dichloromethane extract of a 40-year-old beer.

GC-O Results. The similarity tests performed by the panel, among samples that were in several sessions, unanimously considered as presenting the typical aroma of aged beer and the respective organic extracts, showed that the typical aroma was better represented in the dichloromethane extract, 71% (standard deviation = 17.6). Hence, this solvent was chosen to perform the GC−O using samples from group 1, forced aging, and group 2, normal aged, including the 25- and 40-year-old lager beers.

Twenty-three odor-active zones were selected as the most frequently cited by the four members of the GC-O panel. Among them, six showed aromas close to the descriptors selected by the AFNOR procedure as characteristic of oxidative aged beer. They were described as "aged beer like", "bread", "cooked potato" for a retention index (RI) = 1463), "honey" (RI = 1690), "melted sugar" (RI = 2038), and "aged Port" (RI = 2189) (**Figure 1**).

It was possible to identify, using GC-MS and chemical standards, the corresponding molecules for the retention indices: 3-(methylthio)propionaldehyde (methional) (RI = 1463), phenylacetaldehyde (RI = 1690), β-damascenone (RI = 1838), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (RI = 2189). It is interesting to note the absence of the "cardboard-like" descriptor related to the presence of *trans*-2-nonenal on the very old samples, whereas the Strecker aldehydes, methional and phenylacetaldehyde, were identified.

Strecker Aldehyde Levels in Samples. To correlate the quantities of methional and phenylacetaldehyde with the flavor quality of samples, a quantification method was developed using GC-MS/MS.

Beer from group 1 was analyzed after a forced aging experiment to promote aroma deterioration. Strecker aldehydes were quantified after 7 days of storage at 37 °C and also on a second group (control) kept at 4 °C for 180 days. The results are shown in **Figure 2**.

As shown in **Figure 2**, the quantities of these two aldehydes are significantly higher in samples stored at the higher temperature. Using ANOVA processes significant differences were not observed regarding these Strecker aldehydes among samples (p value = 2.38E−8 and p value = 4.46E−6). Conversely,

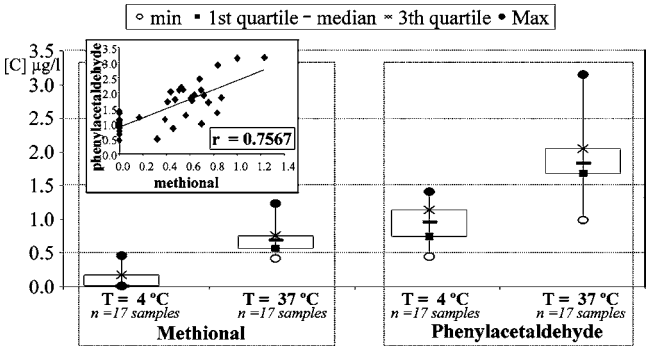

Figure 2. Levels of methional and phenylacetaldehyde in samples stored at 4 and 37 °C for 180 and 7 days, respectively (μg/L).

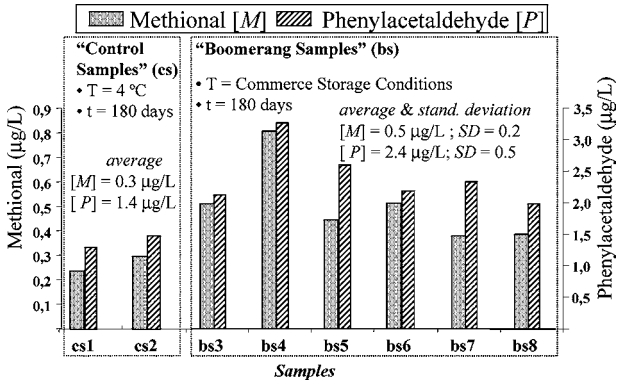

Figure 3. Levels of methional and phenylacetaldehyde observed in beers kept under different storage conditions: cs (control samples); **bs** (boomerang samples) (μg/L).

differences were observed (95% level) for methional and phenylacetaldehyde between samples stored at 4 and 37 °C respectively, p value = 0.27 and p value = 0.33. As shown in the inset (**Figure 2**) these two molecules have a high correlation value (r = 0.7567), indicating a concomitant formation during Strecker degradation. These results are in agreement with previous works (*23*, *24*).

Considering the extreme conditions applied to forced aged samples and knowing that the reactions due to high temperatures are different from those produced during regular aging (*37*), methional and phenylacetaldehyde were analyzed in beers submitted to normal aging conditions (group 2 samples), after the 6-month shelf-life period. Results are shown in **Figure 3**.

The samples exposed to commercial storage conditions presented higher levels of both aldehydes, indicating that these molecules are formed in normal aging conditions, with average values of 0.5 μg/L (SD = 0.2) and 2.4 μg/L (SD = 0.5) for methional and phenylacetaldehyde, respectively, whereas the levels observed in samples kept at 4 °C were, for the same molecules, 0.3 and 1.4 μg/L.

Strecker aldehyde formation is greatly affected by two parameters: (i) the temperature and (ii) the levels of dissolved oxygen, mainly when close to saturation (*24*). Because the values of oxygen in these samples were <0.2 mg/L, the observed variations within the commercial storage samples most likely related to temperature, that is, the storage conditions.

The two old lager samples, 25 and 40 years old, were also submitted to chemical analysis after it was confirmed that they presented the typical aromas of aged beer by a previous sensorial evaluation.

The levels of methional and phenylacetaldehyde found in the 25- and 40-year-old samples were 4.8 and 7.7 μg/L for the 25-year-old beer and 2.4 and 6.5 μg/L for the 40-year-old one, respectively. It is interesting to note the presence of sotolon in the 40-year-old beer (1.0 μg/L), which is in accordance with the "Port-like" aromas reported by the sensory panel. Finally, the levels of *trans*-2-nonenal were close to the limit of detection of the method (0.01 μg/L) on the oldest beer and above this limit on the 25-year-old beer (0.10 μg/L), which is consistent with the "more oxidized character" reported by the panel for this sample. It is important to emphasize that Strecker aldehydes were always present in high levels.

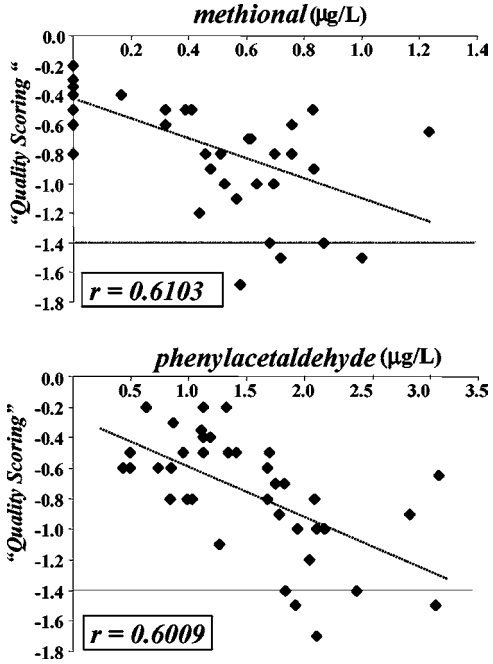

Figure 4. Relationship between quality means scores and methional and phenylacetaldehyde contents (μg/L) for group 1 beer samples.

All of these observations point toward a progressive accumulation of Strecker aldehydes during storage; thus, special attention was taken regarding methional and phenylacetaldehyde as possible "indicators" of aroma deterioration.

Correlation between Sensorial and Chemical Data. To estimate to what extent methional and phenylacetaldehyde levels were related with flavour quality, the samples were submitted to sensorial evaluation. Sensorial data were collected by the brewery panel. The global quality of the samples was rated using a continuous scale ranging from +1 to −3 using group 1 beers. Average scores for each beer were calculated (quality means scores), and results are shown in **Figure 4**.

The quality means scores are correlated with methional and phenylacetaldehyde concentrations with r = 0.6103 and r = 0.6009, respectively. Higher values could be reached if chromatographic procedures that had low detection limits or "outliers" of sensorial data were rejected. These outliers can correspond to beers with a higher aromatic complexity (matrix effect), for instance, with a high concentration of β-damascenone or isoamyl acetate. In fact, previous works reported that these two compounds are key odorants in beers, responsible for "sweet notes" (*9*). For each pair stored at 4 and 37 °C the levels of β-damascenone and isoamyl acetate were quantified. The typical concentrations observed in the samples stored at 4 °C for the β-damascenone and the ester ranged, respectively, from 3 to 5 μg/L and from 200 to 400 μg/L. These levels were increased by factors of 35 and 12% on samples kept at 37 °C, respectively, for β-damascenone and isoamyl acetate. The same behavior was explained in wines by the hydrolysis of the norisoprenoid

Table 1. Sample Evaluation Results of the Multiple-Comparison Test (Level = 95%)[a]

Tukey's Test (0.5%)	M&T&P	T&P	M&T	M&P	T	M	P	No Add	Samples Scores Average
M&T&P									7,2
T&P	-								6,0
M&T	-	-							5,7
M&P	-	-	-						5,4
T	X	-	-	-					5,0
M	X	X	-	-	-				3,7
P	X	X	X	X	-	-			3,1
No Add	X	X	X	X	X	-	-		2,2

Least Significant Difference (LSD) : 2,20
Standard Error (SE) : 0,49

[a] Samples marked with an "X" are significantly different.

glycoside precursor (*38*, *39*), as well as the esterification rate increment to attain the equilibrium, both facts promoted by temperature. The odor perception of methional or phenylacetaldehyde is certainly disturbed by the presence of other substances in the beer, which could mask their aroma. Nevertheless, methional and phenylacetaldehyde seem to be associated with beer quality.

Sensorial Impact Validation. To investigate further the contribution of these two molecules (methional and phenylacetaldehyde) and validate the observed correlation between concentrations and flavour quality on the beer aroma, these molecules were added separately or in combination to a fresh beer, in concentrations close to those found in the aged beers: methional at 3.0 μg/L and phenylacetaldehyde at 4.0 μg/L. Considering the high impact of *trans*-2-nonenal on the beer aroma degradation, this compound was also included in the test, at a 0.25 μg/L level. Although threshold limits in beer were not performed by our panel, triangular tests with spiked 1 μg/L phenylacetaldehyde and 0.5 μg/L methional were carried out by 10 assessors. All assessors recognized spiked samples as being different from the control. These results imply that the threshold limits for these two compounds are lower than those concentrations.

Despite the high concentration of *trans*-2-nonenal added, ~7 times the published threshold (*2*), supplemented samples were not considered by the panel as "aberrant"; the samples were accepted by the assessors, and this high level was kept.

Panelists were instructed to compare aged beer with spiked new beer samples. A simple comparison pair test was carried out to rate the degree of similarity between each of the supplemented samples and the aged beer. To determine which samples were significantly different from another, the Tukey test was used. Mean rating scores (MRS) were arranged according to magnitude, and the LSD at 95% was determined. If, for two samples i and j, $MSR_i - MSR_j > LSD$, then the samples are regarded as significantly different (*32*). The average of the similarity values and the standard deviation (SD) calculated for each pair, as well as the Tukey test, are given in **Table 1**.

The ANOVA calculations for the data showed differences between samples (p value = 8.19E−9) at the 95% level and no significant differences between assessors. The highest similarity value found was observed when the three molecules were added simultaneously to the beer (SV = 7.2). The panel rated all supplemented samples with higher similarity values than the nonsupplemented beer. The single major contributor was *trans*-2-nonenal (SV = 5.0). Furthermore, this molecule was the only one among the three single additions that produced a statistically significant difference with a nonsupplemented sample (difference = 2.8 and LSD = 2.2) (**Table 1**).

All pair additions contributed in a high degree to "aroma spoilage" perception, SV ranging from 5.44 to 6.0 (**Table 1**). It is interesting to note that the SV values for both Strecker aldehyde additions (5.4) were close to that of *trans*-2-nonenal (SV = 5.0). Hence, these results suggest that *trans*-2-nonenal contributes more to aroma degradation than methional. Nevertheless, the combined effect of the two Strecker aldehydes, formed by Maillard mechanisms, has a higher impact on the perceived aroma associated with the aged beer.

Because of this, these key odorants, due to the cumulative behavior observed during aging, could be useful as indicators in shelf-life estimations. In fact, the quantities found after the shelf-life period (6 months), on the boomerang beers, are in agreement with what would be expected, that is, above the aroma threshold. These findings look promising in view of the possible applications in shelf-life control by minimizing the Strecker aldehyde formation.

Nevertheless, this last application needs to be validated with more experimental data, as well as the comparative study of the evolution of the quantities of Strecker aldehydes versus *trans*-2-nonenal during storage.

Kinetics Results. To gather more information concerning the relationship between the quantities of Strecker aldehydes, storage temperature, and the shelf-life period, a kinetic study was performed. These models are very important to understand the extent of a specific chemical reaction and the rate at which the changes occur and also to be able to optimize the food-processing or storage conditions. In addition, the knowledge and control of the kinetic parameters can greatly enhance the thermal processing conditions in terms of maximizing the rate of generation of the desired flavor compounds or suppressing the rate of formation of off-flavor or undesired products (*40*).

To determine unambiguous kinetic models for a certain reaction, it is very important to know the reaction's stoichiometry and mechanisms. However, the formations of methional and phenylacetaldehyde are very complex. Therefore, for the global reaction mechanism assumed, only an apparent reaction rate can be calculated and the resultant kinetics determined. The most usual simplified kinetic models, reported in the literature to describe the kinetics of compound formation through Strecker degradation, are zero-order (*41*). Some authors (*42*) reported a first-order model for the formation of the Amadori compound in a phenylalanine−glucose aqueous model system. The formation of the Schiff base complex intermediates followed second-order kinetics. Some other authors (*43*) studied the Maillard browning kinetics at various glucose and glycine ratios. They found that the ratio of the initial reactants affected the reaction kinetic order models.

The temperature dependence of the reaction rate constant was well described by the Arrhenius equation for both aldehydes. In fact, the reaction rate of each compound increased with increasing temperatures, and it was similar for both methional and phenylacetaldehyde (**Figures 5** and **6**).

Statistical analysis using a one-step nonlinear regression method was applied to estimate the Arrhenius kinetic parameters. The experimental data for concentration versus time for all tested temperatures were used, which increases substantially the degrees of freedom and therefore gives much narrower confidence intervals for the estimated parameters. The activation energies (E_a) and rate constants (k_{ref}) estimated at the reference temperature (T_{ref}) of 40 °C and corresponding 95% confidence intervals are reported in **Table 2**.

The activation energy, as well as the rate of reaction observed, was slightly higher for phenylacetaldehyde, indicating a rela-

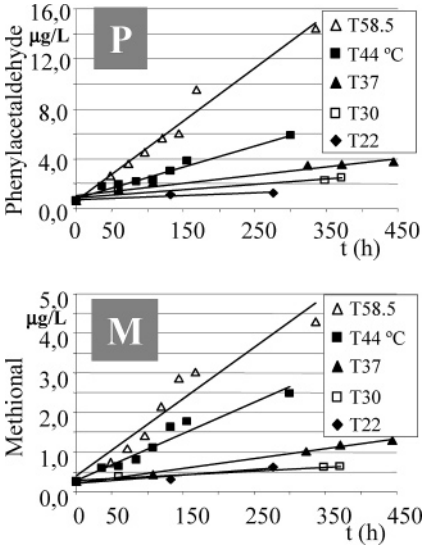

Figure 5. Rate of methional (M) and phenylacetaldehyde (P) formation at five temperatures (22, 30, 37, 44, and 58.5 °C) as a function of time (hours).

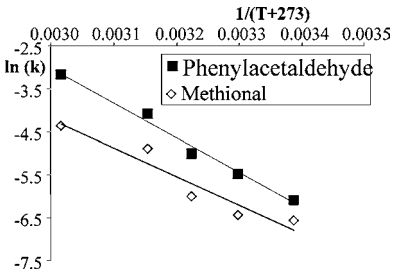

Figure 6. Arrhenius plot for methional and phenylacetaldehyde.

Table 2. One-Step Nonlinear Regression Modeling Parameters, E_a, k_{ref}, and C_0, for Methional and Phenylacetaldehyde

$c = c_0\{tk_{ref} \times \exp^{-(E_a/R)[(1/T)-(1/T_{ref})]}\}$	model order	E_a (kJ/mol)	k_{ref} (μg/L) × h^{-1}	C_0 (μg/L)
methional	zero	62.2 ± 13.6	0.0031 ± 0.0011	0.15 ± 0.11
phenylacetaldehyde	zero	76.7 ± 33.2	0.0051 ± 0.0042	0.73 ± 0.37

tively superior sensitivity to temperature of this molecule. Nevertheless, both constants were of the same order of magnitude, which is in close agreement with the observed correlation reported above. It is important to note that the Strecker aldehyde formation is greatly affected by the levels of dissolved oxygen, mainly when close to saturation (*24*); at the initial time its value was <0.2 mg/L.

Assuming the same matrix is used, these models can be useful tools to determine to what extent temperatures during storage affect the shelf life of the product as well providing information about the key steps along the process that most affect the flavor stability of beer.

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