Beer astringency assessed by time–intensity and quantitative descriptive analysis: Influence of pH and accelerated aging

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Abstract

pH and oxygen are usually considered as the main factors influencing the organoleptic stability of beer. Polyphenols from malt and hop contribute to several characteristics of beer flavor, but their incidence on astringency is rarely mentioned. In this work, effects of pH (3.0 and 5.0) and accelerated aging (0 or 5 days at 40 °C with low or high level of oxygen in the bottle) on astringency were measured by two sensory analysis methods: quantitative descriptive analysis and time–intensity analysis. In addition, the polymerization degree of polyphenols was determined for the different samples in this study. Whatever the sensory technique used, a trained panel showing high repeatability identified pH as the only factor having a significant effect on astringency. Accelerated aging, even with high level of oxygen in the bottle, does not significantly modify perception of this sensation compared with the 4 °C-stored beer, probably because the variation of polymerization degree of polyphenols was not sufficient to be detected by the panelists.

Keywords: Beer; Astringency; Sensory analysis; Time–intensity analysis; Quantitative descriptive analysis

1. Introduction

Astringency is a complex sensory property, characterized by drying, roughing, and puckering of the skin or mucosal surface in the mouth. It is not always perceived immediately, but evolves continually in the mouth after swallowing (Guinard, Pangborn, & Lewis, 1986), and increases upon repeated ingestion (Courregelongue, Schlich, & Noble, 1999). A wide range of compounds, forming four groups, elicit this sensation: salts of multivalent metallic cations (particularly aluminum salts such as alum), dehydrating agents (ethanol and acetone), mineral and organic acids, and plant tannins (polyphenols) (Siebert & Chassy, 2003). Consequently, the sensation of astringency includes many nuances. For this reason Gawel, Iland, and Francis (2001) have established a list of twenty or so descriptors of this sensory attribute in wine.

Astringency produced by foods rich in polyphenols is fairly well understood: saliva contains a considerable quantity of proteins that lubricate the mouth. These proteins, especially those rich in proline, bind preferentially to polyphenols derived from foods. This leads (i) to formation of insoluble complexes, (ii) to a decrease in salivary lubrication properties, and (iii) to the perception of astringency (Corrigan Thomas & Lawless, 1995; Gawel et al., 2001; Siebert & Chassy, 2003).
Oxygen and pH are two of the main factors likely to influence the astringency perception. Oxidation enhances the polymerization degree of polyphenols, and consequently increases their astringent properties (Noble, 2002). Concerning pH, it is accepted that astringency is intensified at low pH, at least in model solutions and wine (Guinard et al., 1986), although pH 4.4 is the optimum for beer colloidal instability (Siebert & Chassy, 2003).

In beer, polyphenols from malt and hop are known to contribute to several characteristics of beer flavor, notably astringency but also bitterness, body, and fullness (Dalgliesh, 1977). Reduced polyphenols impart to beer a note of freshness, while their oxidized counterparts give it an “aged flavor” (Dadic & Belleau, 1973). The impact of polyphenols on flavor during aging has been especially highlighted because of their susceptibility to oxygen in the package (Lermusieau, Noël, Liégeois, & Chassy, 2003). Grapeseed extracts were obtained according the method developed by Counet and Collin (2003). Grapeseed extracts were prepared in ultrapure water (Reagent Water Sigma–Aldrich (Bornem, Belgium), and Romil Chemicals (Merelbeke, Belgium). Total polyphenols quantification: carboxymethylcellulose was purchased from Sigma–Aldrich (Bornem, Belgium). EDTA and ammonia (solution min. 25%) were obtained from VWR (Leuven, Belgium).

2.2. Beer preparation

The chosen reference beer was as a commercial lager beer (pH 4.4) stored at 4 °C to limit any sensory change. Eight beers were then prepared by modifying this commercial reference beer. The variable parameters were the oxygen level (high or low), the pH (3.0 or 5.0), and aging (0 or 5 days at 40 °C) (Fig. 1). To this end, bottles were opened, and if oxygen was not desired, the bottom of the bottle was struck to produce foam. When the foam reached the top, we sealed the bottle with a silicone derivative (especially during natural aging).

In the present paper we report experiments designed to establish the time-course of oral astringency of lager beer, during accelerated aging (5 days at 40 °C) at different pH values (3.0 and 5.0) and low or high level of oxygen in the bottle. Attention is paid to the astringency responses of individual panelists in both quantitative descriptive analysis (QDA) and time–intensity (TI) analysis. Total polyphenols and flavanoids were also quantified in order to check their relationship with the sensory analyses.

2. Materials and methods

2.1. Chemicals

Sensory analysis: (+)-catechin, (−)-epicatechin, and potassium aluminum sulfate (>98%) were purchased from Sigma–Aldrich (Bornem, Belgium). Chocolate extracts were obtained according the method developed by Counet and Collin (2003). Grapeseed extracts were a kind gift of the “Société Française de Distillerie” (ref. SFD, PPB, Vallon-Pont-d’Arc, France). All solutions were prepared in ultrapure water (Reagent Water System, Millipore Corporation, Bedford, USA). Flavanoids quantification: p-dimethylnaminocinnamaldehyde (>98%), hydrochloric acid (37%), and methanol were purchased, respectively, from Fluka (Bornem, Belgium), Sigma–Aldrich (Bornem, Belgium), and Romil Chemicals (Merelbeke, Belgium). Total polyphenols quantification: carboxymethylcellulose was purchased from Sigma–Aldrich (Bornem, Belgium). EDTA and ammonia (solution min. 25%) were obtained from VWR (Leuven, Belgium).
ing different concentrations of (+)-catechin in ultrapure water or sparkling water (Bru®, Belgium); (iv) quantitatively assessing astringency of the modified beers in this study by comparison with the reference beer. This last step was carried out to make panelists aware of minimal and maximal intensities and to reach agreement on the quantification notation. Other sessions were then organized to familiarize the panelists with the time–intensity method and data acquisition software (Fizz®, Biosystèmes, Couternon, France). The reference beer and four modified beers (pH3O₂0, pH3O₂5, pH5O₂0, and pH5O₂5) (see Fig. 1) were used for this purpose in a well-balanced experimental design for presentation. These sessions were organized until panelists showed at least two reasonably overlapping replicate TI curves out of three (Peyvieux & Dijksterhuis, 2001). After three replicates of each beer, the panel’s repeatability was judged sufficient.

2.3.3. Quantitative descriptive analysis (QDA)

At each session, the reference pH 4.4 beer and three digit-coded beer samples among the eight types of modified beer (see Fig. 1) were presented to the panelists. The subjects were instructed to taste the reference beer (identified as “Reference”) and to memorize its astringency intensity. By definition, this intensity corresponds to the point 5 of a 0-to-10 structured scale. Then panelists had to taste the first coded beer sample and to assess the perceived astringency by comparison with the reference sample. After mouth rinsing with a piece of unsalted rusk (Cracotte®, Danone, France) and spring mineral water (Louise®, Belgium), they had to appraise the astringency of the next coded samples in the same way. Each panelist assessed all modified beers in triplicate in the course of eight different sessions. The coded beer samples were presented according to an incomplete block design balanced for position effects within the eight sessions of each judge and nearly balanced for carry-over effects in the whole experiment.

2.3.4. Time–intensity analysis (TI)

As for the quantitative descriptive analysis, the panelists had to assess four coded samples at each session: the reference pH 4.4 beer and three of the eight modified beer samples (see Fig. 1). The Fizz data acquisition system is designed to assess the time evolution of a response (here the astringency) on an unstructured scale defined on the left side by “0” corresponding to “no perception” and on the right side by “Max” corresponding to the highest intensity perceived by the panelist. After taking the sample in the mouth, the panelist has to click on the computer mouse to activate the astringency record. The intensity assessment of this perception is then performed on line by moving the cursor on the scale with the help of the mouse. After 5 s, a message appears on the screen, indicating to the panelist to swallow the sample and to continue to assess astringency until the message “end of record” is displayed. Data were collected every second for 2 min.

After each sample, a 3-min pause was proposed to panelists to allow them to rinse the mouth with a piece of unsalted rusk (Cracotte®, Danone, France) and spring...
spring mineral water (Louise®, Belgium). In each session, the reference beer was always presented first in order (i) to provide a palate cleanser (King & Duineveld, 1999), (ii) to suppress the “first product” effect and create the same conditions as in the QDA, and (iii) to monitor the panelists’ repeatability. Assessments of all modified beers were performed in triplicate in the course of eight different sessions. At each session the modified beer samples were presented to the panelists according to a well-balanced design similar to that used in the QDA.

2.4. Determination of the polymerization degree

Flavanoids were quantified by means of a colorimetric assay based on the reaction of their A-rings with p-dimethylaminocinnamaldehyde (Delcourt & Janssens de Varebeke, 1985). As for total polyphenols, their concentration was determined according to Bishop (1972). All quantifications were performed in duplicate. The ratio of total polyphenols to total flavanoids provides a reasonable estimate of the degree of polymerization.

2.5. Statistical data analysis

For statistical analysis of the QDA data, the recorded astringencies were used directly. In the case of the TI data, four parameters were extracted from the individual curves: $I_{\text{max}}$ (maximum intensity at peak/plateau), $T_{\text{max}}$ (minimum time where intensity reaches a maximum peak/plateau), area under the curve (AUC) and $D_{\text{tot}}$ (total duration). For statistical assessment of the judges’ performances (repeatability and reproducibility) the method proposed by Rossi (2001) was applied to these five responses (intensity for QDA and four extracted parameters for TI). The homogeneity of judges’ variability was tested by Levene’s (1960) classical equality of variance test.

A four-way mixed-linear-model was then chosen to analyze the effect of pH, oxygen, and aging on the different responses, the judges being defined as a random effect in the model (procedure MIXED in SAS 8.2, SAS Institute Inc., Cary, NC, USA).

Linear relationships between the four time–intensity parameters were measured on the basis of classical Pearson correlation coefficients.

Finally, a hierarchical classification was also done (SPAD 4.02, Décsia, Levallois, France) on the four TI responses in order to classify the eight types of beers and to confirm the results obtained by the mixed-linear-model approach. For this classification, we coarsely averaged the four extracted parameters over replicates and judges, such that we got the four responses for eight beers (8 “individuals”, 4 variables). Then, we performed a hierarchical classification with the Ward’s aggregation criterion.

3. Results and discussion

3.1. Repeatability and reproducibility of panelists’ performance

The respective performances of the eight judges were compared with each other as regards repeatability. This was done on the basis of the ratio of the judge variability to the average of these variances for all judges (Rossi, 2001). These ratios, averaged over the eight beers, are represented for each judge and each response in the left part of Fig. 2. They show that no judge showed much higher or much lower repeatability than the others. Levene’s test, however, showed for all responses a significant difference between “within-judge” variances. This had to be taken into account in later mixed modeling (see below).

Reproducibility (or homogeneity) analysis aims to detect whether some judges give systematically higher or lower responses than the others. It is always good to highlight such effects but they can be taken into account in the statistical analysis by introducing a judge effect into the mixed-linear-model. The reproducibility measurements presented on the right of Fig. 2 compare, for each beer, the mean response of one judge with the mean response of all judges, concerning astringency. This difference is standardized and averaged over the beers. The graphics show that no judge is a systematic outlier and no judge gives systematically higher or lower scores for all responses.

3.2. Panelist signatures

Individual TI curve profiles show extreme diversity (Fig. 3). This figure presents, for each judges, the eight curves for the astringency intensity obtained for the reference beer and, in bold, the average (at each time point) of these eight curves. The main differences concern (i) the ascending slope (subject 3 versus subject 4), (ii) the decreasing phase (sometimes absent because of the limited recording time, as for subject 4), and (iii) the maximal astringency intensity. Physiological features, especially different salivary flows, are frequently invoked to explain such differences (Lawless & Heyman, 1998; Pangborn, Lewis, & Yamashita, 1983). This phenomenon is called the panelist’s signature, always present in time–intensity studies (DijkstraHuis & Piggott, 2001). In our case study, we do observe this phenomenon: for the eight other beers, each judge presents exactly the same pattern (“signature”) as for the reference beer.

3.3. QDA intensity, $I_{\text{max}}$, $T_{\text{max}}$, AUC, $D_{\text{tot}}$

For these five responses we have used a linear-mixed-model:
Fig. 2. Judges comparison with respect to their repeatability and reproducibility for each response of interest.
$Y_{ijk} = \mu + a_{\text{pH},i} + a_{\text{O}_2,i} + a_{\text{Aging},i} + a_{\text{pH} \times \text{O}_2,i} + a_{\text{pH} \times \text{Aging},i}
+ a_{\text{Aging} \times \text{O}_2,i} + a_{\text{pH} \times \text{AgingO}_2,i} + b_{\text{judge},j} + e_{ijk}$

where $Y_{ijk}$ is the response for the $i$th beer (determined by the 3 factors), $j$th judge, $k$th measurement ($k = 1, 2, 3$), $a$'s denote fixed effects, $b$ is the random judge effect $b \sim N(0, \sigma_{\text{judge}}^2)$, and $e_{ijk} \sim N(0, \sigma^2)$.

As no interaction effect was significant, so we were able to reduce our model to

$Y_{ijk} = \mu + a_{\text{pH},i} + a_{\text{O}_2,i} + a_{\text{Aging},i} + b_{\text{judge},j} + e_{ijk}$

Whatever the technique used (QDA or TI), i.e. whether the intensity or the $I_{max}$ was considered, the perceived astringency was significantly higher at pH 3.0 than at pH 5.0 ($p < 0.0001$ and $p < 0.01$, respectively), but neither oxygen nor aging had a significant effect. We also observed a significant pH effect on $I_{max}$: astringency appeared faster at pH 3.0 than at pH 5.0 ($p < 0.05$). Moreover the AUC value is significantly larger at pH 3.0 than at pH 5.0 ($p < 0.05$). When beer at pH 3.0 is mixed with saliva, pH increases to around 4.4. The astringency intensification observed with decreasing pH is consistent with the fact that at pH 4.4 interactions between polyphenols and proline-rich proteins are strengthened (Siebert & Chassy, 2003).

Means and 95% confidence intervals for each response are reported for QDA in Fig. 4 and for TI in Fig. 5. The significant effects do not appear “clearly” because of judge variability, which is not eliminated in these confidence intervals. It is worth mentioning that this random judge effect is highly significant for all responses, as was expected.
The nature of the time–intensity curves often implies strong correlations between extracted parameters (King & Duineveld, 1999). In this study, we do observe such correlations; however there is no systematic correlation between one parameter and all the others, so that each brings its own information (Table 1). Among the highest correlations observed is the positive correlation between $I_{\text{max}}$ and AUC: i.e. intensification of the astringency leads to a longer and/or higher persistence. Inversely, $I_{\text{max}}$ correlates negatively with $T_{\text{max}}$, i.e. the more intense the astringency, the less time it takes for astringency to reach its maximum intensity.

A hierarchical classification based on the four parameters enabled us to confirm some previous observations. As shown in Fig. 6, the beers are grouped only according to their pH. No oxygen or aging effect is evidenced. It is also worth to mention that the first PCA axis reflects mainly the pH.

### 3.4. Polymerization degree

The mean polymerization degree of polyphenols is reported in Fig. 7 as a function of pH (3.0 and 5.0),


