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Influence of pH and ageing on beer organoleptic properties. A sensory analysis based on AEDA data

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Abstract

Brewers need to know how ageing affects the organoleptic properties of beer. This paper describes sensory assessments of aroma changes in lager beer subjected to accelerated staling (5 days at 40 °C) at different pH values (4.2 and 4.6), according to a new method for training panellists, based on AEDA results. It shows, for 10 key compounds whose concentrations are known to increase during staling, how modifications in pH affect ageing and perception thereof.

In agreement with the AEDA results, "dimethyltrisulphide"-, "*trans*-2-nonenal"- and "methional"-like perceptions increased significantly as a result of ageing in regular pH 4.2 lager beers. A higher pH led to a smaller age-related intensity increase in the "cardboard" note ("*trans*-2-nonenal"-like). It also enhanced the "coconut" character ("γ-nonalactone"-like) but did not change the age-related intensification of the "cabbage" note ("dimethyltrisulphide"-like). Perception of the remaining six compounds ("furaneol"-, "ethyl butyrate"-, "2-methoxypyrazine"-, "acetylpyrazine"-, "β-damascenone"- and "ethyl cinnamate"-like) did not evolve with ageing or pH.

Keywords: Beer; pH; Ageing; Sensory analysis

1. Introduction

One of the main concerns of brewers is to preserve the organoleptic stability of beer during ageing. A key factor influencing beer ageing and its stability is pH (Bamforth, 2001). From a sensory standpoint, if the pH of fresh beer decreases below 4.0, sharp, acid, bitter, and drying effects increase rapidly in intensity, with a markedly enhanced metallic after-palate for pH values below 3.7. On the other hand, above 4.0, palate effects relate to increased mouth-coating, with higher scores for biscuity and toasted characters, and even soapy and caustic notes if the pH rises above 4.4 (Taylor, 1990).

Increasing the pH proved to be an interesting way to reduce formation of *trans*-2-nonenal, responsible for an unpleasant cardboard note in aged beer. Retention of *trans*-2-nonenal by amino acids and proteins during ageing is enhanced at higher pH, explaining why a lesser cardboard flavour is perceived (Lermusieau, Noël, Liégeois, & Collin, 1999; Noël et al., 1999).

A higher pH also makes it possible to control the apparition of β-damascenone (cooked apple-like) (Chevance, Guyot-Declerck, Dupont, & Collin, 2002) and dimethyltrisulphide (cabbage-like) through ageing. Unfortunately, methional (cooked potato-like) production is enhanced under these conditions (Gijs, Chevance, Jerkovic, & Collin, 2002).

To highlight the overall flavour consequences of pH modification on beer ageing, sensory analysis (and especially Quantitative Descriptive Analysis) appears as a prime technique based on a comparative description of the organoleptic properties of several products (Stone, Sidel, Oliver, Woolsey, & Singleton, 1974). This approach requires two steps: first a vocabulary generation step, which involves obtaining a consensual descriptor list from the panel members, and then a quantitative intensity assessment of each selected term

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for all products studied. With this methodology, training of the sensory panel is essential. Yet it appears tedious because of the time required (i) to define vocabulary and (ii) to coach the subjects for quantitative assessments. Aroma Extract Dilution Analysis (AEDA), as proposed by Ullrich and Grosch (1987), makes it possible to select key aroma compounds likely to act on overall food flavour. The principle is to dilute a food extract until no odour is perceived. The higher the dilution factor, the more important the odour impact. Changes in key aroma compounds during natural beer ageing (Schieberle, 1991; Schieberle & Komarek, 2003), or accelerated ageing under different pH conditions (Gijs et al., 2002) have been already investigated by this technique.

If molecules evidenced by AEDA are key compounds of beer flavour, they could be useful as references to train a sensory panel to assess changes due to pH during ageing. This new approach could result in a gain of time in panel training.

In this framework, 10 compounds were selected according to the results obtained by Gijs et al. (2002): methional, *trans*-2-nonenal, 2-methoxypyrazine, acetylpyrazine, γ-nonalactone, furaneol, β-damascenone, ethyl butyrate, ethyl cinnamate, and dimethyltrisulphide. Added to fresh beer, these compounds constituted odour references and enabled the panellists to quantitatively assess beer changes due to pH during ageing.

2. Materials and methods

2.1. Chemicals

Absolute ethanol (>99.8%), methional (3-(methylthio)propionaldehyde), *trans*-2-nonenal (97%), 2-methoxypyrazine (95%), acetylpyrazine (97%), γ-nonalactone (γ-nonanoic lactone) (97%), ethyl cinnamate (98%) and furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) (95%) were purchased from Aldrich Chemicals (Belgium). Ethyl butyrate (99%) was obtained from Janssen (Belgium). Dimethyltrisulphide (>98%) was purchased from Acros Organics (Belgium). β-Damascenone (8E-megastigma-3,5,8-trien-7-one) (>95%) was a kind gift from Haarman and Reimer GmbH (Nanterre, France).

2.2. Accelerated ageing of bottled beer at various pH values

Bottles of a commercial lager beer (pH = 4.4) were opened and struck to produce foam. When foam reached the top of the bottle, the bottle was sealed with a silicone top (Vel no. 5). Beer pH was adjusted to 4.2 or 4.6 by injection of 10 mol/l HCl or NaOH with a glass syringe into bottles through the silicon top. The bottles were then crown-sealed and the beers aged at 40 °C for 5 days in a dark room. After ageing, the beers were stored at 4 °C until used. As far as the beer off-flavours we have considered are concerned, our experiment indicates that this accelerated ageing mimics natural storage at 20 °C very well. "Fresh" (i.e. not aged) beers were also stored at 4 °C.

2.3. Sensory analysis

2.3.1. Panel

Scientists and students of the "Université catholique de Louvain" were selected as judges on the basis of their ability to describe the perceived sensations and to distinguish flavours in beer both qualitatively and quantitatively. Selection tests proposed by Issanchou, Lesschaeve, and Köster (1995) were adapted to beer and used to recruit 10 unpaid volunteers, 3 males and 7 females, aged 25–44 years.

2.3.2. Vocabulary generation and panel training

Stock solutions of each compound were prepared in absolute ethanol. The 10 odour references were finally obtained by adding each stock solution (less than 0.2% vol.) to fresh pH 4.2 beer (Table 1). In all cases, the final concentrations were close to or above the odour thresholds. As far as methional, dimethyltrisulphide, *trans*-2-nonenal, γ-nonalactone, furaneol and ethyl butyrate are concerned, the final concentrations were similar to those usually quantified in aged lager beers. During the first week, two sessions were devoted to vocabulary generation: panellists were invited to compare odours and aromas perceived in mouth of the fresh pH 4.2 beer (without aroma addition) with the 10 references (two tests per session: one concerning odour and the other, aroma perceived in mouth; five references in each test; all references tested). The next week it was possible to reach a panellists consensus on the use of generated descriptors with the help of food or chemical references. The third week was a replicate of the first one: all the references were tested again in order definitely to assign appropriate terms concerning odour and aroma perceived in mouth for each compound.

2.3.3. Sensory assessments

At each session, one fresh pH 4.2 beer, two references, and four coded beer samples (fresh and aged beer, each of them at pH 4.2 and 4.6) were presented in "Breughel" 500 ml glasses (Durobor, Belgium) covered with a glass top. The coded beer samples were given in an order balanced for position and carry-over effects using William's Latin square (Macfie, Bratchell, Greenhoff, & Vallis, 1989). Subjects were instructed to smell the fresh pH 4.2 beer and memorize the difference with respect to the first reference. Then they had to assess in mouth the intensity of this perceived difference in

Table 1
Odour references added to fresh beer: concentration, related odour descriptors and thresholds

Compound	Concentration added in beer ($ng\,g^{-1}$)	Descriptor	Odour threshold in beer[a,b,c,d] or in water[e,f,g,h] ($ng\,g^{-1}$)
Dimethyltrisulphide	0.325	"Cabbage"	0.00033[a]–0.01[b]
Methional	50	"Potato"	40–250[b]; 250[c]
Trans-2-Nonenal	0.35	"Cardboard, leather, shoe box"	0.11[b,c]
Acetylpyrazine	100	"Roasted hazel nut, containing ground praline"	62[e]
γ-Nonalactone	100	"Coconut"	(in mouth) 65[f]
Ethyl cinnamate	5000	"Acid red fruits, banana"	–
β-Damascenone	200	"Red fruits, stewed apples"	150[b]
Furaneol	1000	"Reference 1"	700[d]
2-Methoxypyrazine	700	"Reference 2"	400[g]–700[h]
Ethyl butyrate	400	"Reference 3"	400[b,c]

[a] Gijs (2003).
[b] Moll (1991).
[c] Meilgaard (1975).
[d] Sakuma, Kobayashi, Tayama, and Yokoyama (1996).
[e] Silwar (1988).
[f] Siek, Albin, Sather, and Lindsay (1971).
[g] Masuda and Mihara (1986).
[h] Seifert, Buttery, Guadagni, Black, and Harris (1970).

each of the four samples and rank them by increasing intensity. Lastly they had to put the sample exhibiting the maximum intensity on the highest anchor of the 10-cm structured scale and to rate the other samples with respect to this maximum. Assessment in mouth of the aroma relative to the second reference was done in the same way. Evaluations of all the references were done in triplicate in the course of 15 different sessions. Samples were prepared half an hour before testing and assessed at room temperature in individual booths illuminated with red light.

2.4. Statistical inference

For each reference, the intensities of the four beers were assessed, scored from 0 to 10 (most intense), and ranked (rank 1 for the least intense and rank 4 for the most intense). In principle, since both the score and the rank were determined by trained subjects, differences between panellists should be minimal. Moreover, scores should permit a more refined assessment of the respective sensory intensities. However, inspection of the scores revealed some judge-related inconsistencies, so the statistical analysis of the results was limited to the ranks. The findings obtained on the ranks were validated against those which could have been obtained from the scores; no general disagreement between the two evaluations was observed.

The objective of the statistical analysis was to check whether pH and ageing affected the perceived intensity of each reference in a statistically significant way. This test was carried out by simple two-way analysis of variance on the ranks (this was done using the ANOVA procedure of the statistical package SAS 8.2, SAS Institute Inc., Cary, NC, USA). Although residual normality of the ranks cannot be assumed, simple analysis of variance of the ranks is justifiable on the grounds that the F-test approximates the corresponding distribution-free permutation test and is therefore quite robust against departures from residual normality (see Box & Anderson, 1955 or Scheffé, 1959 for detailed discussions of this subject).

For references for which the F-test indicated significant effects of pH and/or ageing at the 5% level, the four treatments (4.2 pH/fresh beer, 4.2 pH/aged beer, 4.6 pH/fresh beer, 4.6 pH/aged beer) were compared pairwise using multiple comparisons based on a Bonferroni adjustment.

3. Results and discussion

After training, panellists defined by mutual agreement the references as described in Table 1. The results are displayed according to the effect type. We first present results with a pH effect, then those where no significant pH effect was found.

3.1. pH effect

3.1.1. Trans-2-nonenal (Fig. 1A)

The cardboard perception associated with *trans*-2-nonenal increased significantly during ageing at pH 4.2 (p value is < 0.0001), in agreement with results reported by Gijs et al. (2002).

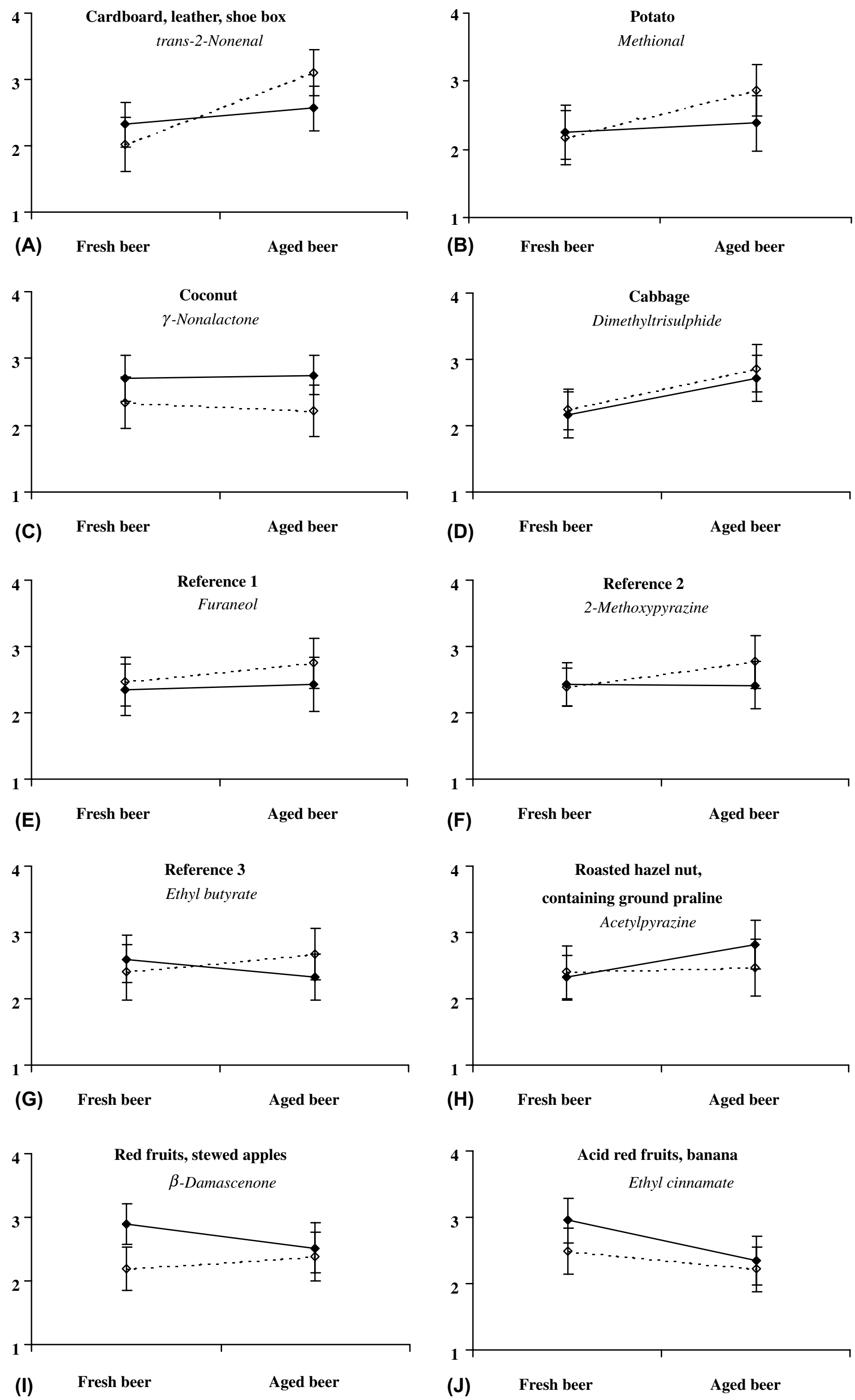

Fig. 1. Mean ranks of each reference compound intensity in fresh and aged beer at pH 4.2 (---◇---) and pH 4.6 (—◆—). For each mean value, 95% confidence intervals are represented.

It was found that pH variation did not significantly modify the aroma perception on fresh beer, whilst a pH increase during ageing reduced the appearance of "cardboard" off-flavours by stabilizing the initial aroma intensity ($p < 0.05$).

3.1.2. Methional (Fig. 1B)

When ageing was carried out at pH 4.2, the sensory panel perceived a significant increase ($p < 0.01$) in the "cooked potato" character. These results agree with those reported by Gijs et al. (2002), showing a

4-fold increase in the methional concentration during ageing.

As compared to beer aged at pH 4.2, the same authors measured a slight concentration increase (5 ppb) in methional in beer aged at pH 4.6. Yet from a sensory standpoint, the pH change tended to reduce perception of the "cooked potato" character ("methional"-like perception) in aged beer, even when that was very close to being significant for perception ($p = 0.065$). In this case, ageing at pH 4.2 may have enhanced perception of other aroma compounds with odours close to the potato-like odour, in a such way that these may have disguised the methional and introduced a bias in the sensory panellists' assessment.

3.1.3. γ-Nonalactone (Fig. 1C)

γ-Nonalactone has previously been proposed as an indicator of beer staling by several authors (Foster, Samp, & Patino, 2001; Gijs et al., 2002; Miedaner, Narziss, & Eichhorn, 1991). In our study, however, the intensity of the coconut note ("γ-nonalactone"-like) was perceived as stationary by the panellists before and after storage, whatever the pH applied.

On the other hand, whatever the freshness of the beer, a pH increase led to perception of a more intense coconut note, only significant ($p < 0.05$) in aged beers.

3.2. No pH effect

3.2.1. Dimethyltrisulphide (Fig. 1D)

As expected, ageing was found to enhance the cabbage intensity. Whilst Gijs et al. (2002) evidenced in aged beer a dimethyltrisulphide concentration difference equivalent to about 0.13 ng g^{-1} between pH 4.2 and 4.6, the pH change did not appear to affect perception of the cabbage note by the panellists. Thus, increasing the pH does not significantly decrease perception of the cabbage ("dimethyltrisulphide"-like) character.

3.2.2. Furaneol, 2-methoxypyrazine, acetylpyrazine and ethyl butyrate (Fig. 1E–H)

Sakuma et al. (1996) evidenced that furaneol contributes to the sweet flavour of fresh beer at concentrations ranging from 0.1 to 0.5 mg/l, although its flavour threshold has been estimated to be 0.7 mg/l in beer. In our study, added furaneol (1 mg/l) was qualified as "reference 1" in the absence of a consensus among the sensory panel members concerning the odour and aroma in mouth related to this compound (Table 1). This difficulty of specifying the descriptor, and thus of perceiving an aroma change related to pH variation and ageing, was confirmed by our results: no significant pH or ageing effect concerning furaneol was observed. Similar conclusions were reached with reference 2 ("2-methoxypyrazine"-like perception) and reference 3 ("ethyl butyrate"-like perception).

Although the odour of acetylpyrazine in beer was well defined by the sensory panel as "roasted hazel nut, containing ground praline", no significant effect of pH and ageing on its intensity was observed. Taking into account that our fresh beer was at least one week old from the date of bottling, these results are in agreement with those reported by Qureshi, Burger, and Prentice (1979), who observed relatively little change in pyrazines during prolonged storage, most of the main variations occurring within a few days of packaging.

3.2.3. β-Damascenone and ethyl cinnamate (Fig. 1I and J)

The quantification and AEDA results of Gijs et al. (2002) showed a slight increase in β-damascenone and ethyl cinnamate during ageing at pH 4.2, but the "red fruits, stewed apple", and "acid red fruits, banana-like" notes related to these compounds did not change significantly, from a sensory point of view, during accelerated staling.

Increasing the pH intensified the "β-damascenone"-like ($p < 0.01$) and "ethyl cinnamate"-like ($p = 0.07$) notes in fresh beer, but during ageing the fruity character tended to diminish, becoming equivalent to that observed in beers aged at pH 4.2. This decrease was significant for the "acid red fruits, banana-like" note related to ethyl cinnamate ($p < 0.05$), but not for the "red fruits, stewed apple" character associated with β-damascenone ($p = 0.11$).

4. Conclusion

This study has enabled us to pinpoint which of the 10 compounds identified in the AEDA process play a significant role in the sensory perception of beer ageing, and which do not. In agreement with the AEDA results, "dimethyltrisulphide"-, "*trans*-2-nonenal"- and "methional"-like perceptions increases significantly upon ageing in regular pH 4.2 lager beers. Neither increasing the pH nor ageing leads to significant variations in the perception of the "furaneol"-, "ethyl butyrate"-, "2-methoxypyrazine"-, "acetylpyrazine"-, "β-damascenone"- or "ethyl cinnamate"-like characters.

Increasing the pH during ageing has no influence on the "dimethyltrisulphide"-like character, but enhances the "coconut" note and reduces the age-related increase in two main off-flavours, i.e. the "*trans*-2-nonenal"- and "methional"-like perceptions. Our finding concerning "methional" is surprising, since the concentration of this compound increases with ageing regardless of the pH. This might be due to confusion of the perceived character with other molecules present at high concentration when the pH is low.

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