Relationship of Sensory Staleness in Two Lagers to Headspace Concentrations of *trans*-2-Nonenal and Three Staling Aldehydes

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ABSTRACTS

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Key compounds in lager staling include furfural, hexanal, 5-hydroxymethyl furfural (5-HMF), and *trans*-2-nonenal. Quantitative data of headspace concentration in two lagers – one premium at 5% (abv), the other a standard product at 4% (abv) – were obtained by solid phase microextraction (SPME) followed by gas chromatography using a mass selective detector (GCMS). The concentrations of the aldehydes were used to predict overall *stale* scoring from sensory assessor data, of lagers stored at 4, 12, 30, and 37°C for 7, 14, 21 and 28 days. Concentrations of all four aldehydes increased with time of storage and with higher temperatures. Correlation coefficients for prediction of staleness in the premium lager were similar at 0.81 and 0.84 for partial least square regression (PLS1) and artificial neural network (ANN) modelling respectively, and the latter showed a lower root mean square error (RMS error). For the standard product, the correlation coefficients were 0.72 and 0.86, with ANN showing lower RMS error respectively. In both PLS models, E-2-nonenal had high regression coefficients and 5-HMF lower coefficients. Furfural and hexanal differed in contributions to the lagers.

Key words: Artificial neural network, chemometrics, multivariate modelling, quality control, staling markers.

INTRODUCTION

Flavour stability is a quality issue for lagers and other beers. Staling begins at the end of post-fermentation processing and varies in both nature and rate depending on packaging. Staling sensory characters (*e.g.* catty, cardboard) may be thought desirable by certain consumers, thus differing in the perception of quality loss, from the majority. Additionally an off-flavour in one product may sometimes be essential to the character of another.

Staling changes in lager flavor congeners originate in oxidations and are accelerated by in-package residual oxygen. Oxidation yields carbonyl compounds, notably aldehydes with low off-flavour thresholds, not abundant in fresh lagers[2]. These originate from Strecker degradations of amino acids, melanoidin-mediated oxidations of higher alcohols, lipid breakdown and aldol condensations of short and secondary oxidation of longer aldehydes[2,18].

Trans-2-nonenal is often considered central to staling and reported to confer “cardboard” notes[7,8,11,12,18] but previous multivariate modelling[3] suggested limited contributions. Moreover, 5–hydroxymethylfurfural (5-HMF) is also thought to be important[3,13] and other aldehydes reported to have influence include furfural[2,3,17,18], acetal[3], 2-furfuryl ethyl ether[5,11] and hexanal[18].

Quantifying such aldehydes in lager can be a challenge and solid-phase microextraction (SPME) has advantages in minimising solvent use whilst providing high sensitivity and reproducibility. This strategy has been shown to be suited for the detection and quantification of *trans*-2-nonenal, furfural and hexanal quantifications in aging lagers[18].

Multivariate statistical modelling is suited to exploring the origin of stale characters in lager flavor[3,4]. The purpose of this work was to compare predictions of overall stale character based on measurements of four of aldehydes, *trans*-2-nonenal, furfural, 5-HMF and hexanal in two lagers, namely a 5% (abv) lager with a late hop character and a 4% (abv) product. Partial least square regression (PLS1) and artificial neural network were used for the construction of the models relating sensory data to analytical measurements.

MATERIALS AND METHODS

Lagers

Two commercial canned lager – A (5% ABV, with a late hop character) and B (4% ABV) – were stored at 4, 12, 30, and 37°C for 7, 14, 21, and 28 days with duplicate samples taken and kept until the end of the experiment. The control set was stored at 1°C throughout.

Quantification of aldehydes by SPME gas chromatography

Carbonyl standards contained a mixture in (5%) ethanol of furfural, 5-HMF, hexanal, and *trans*-2-nonenal, each 100 ppb (µg/L). The pH was adjusted to 4.5% with 0.1% phosphoric acid. Reagents were purchased from Sigma-Aldrich (UK).

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A SPME fiber with 65 μm poly (dimethylsiloxane)/divinyl benzene (PDMS/DVB) phase (Supelco, UK) was suspended in the headspace of pre-warmed (30 min) 10 mL lager in a 20 mL glass vial at 50°C for 60 min[18]. The phase was desorbed at 250°C in the injection port of a Varian GC with chromatography on CP-Sil 5 CB (30 m × 0.25 mm × 0.39 μm (Varian, UK)) using helium as carrier at 1.1 mL min^{-1} and a temperature program as follows: 40°C for 2 min; 10°C min^{-1} to 140°C; and 7°C min^{-1} to 250°C held for 10 min; a total run of 38 min. Aldehydes

TABLE I. Aldehyde concentrations (ppb) and overall *stale* score of lager A stored at 7, 12, 30 and 37°C for up to 28 days.

Sample	Furfural	5-HMF	Hexanal	*Trans*-2-nonenal	*Stale* intensity
0 d	37.30	5360	1.66	0.0084	35.36
7 d 4°C	27.45	7544	2.01	0.0101	35.20
7 d 12°C	43.00	8662	1.92	0.0095	25.00
7 d 30°C	54.77	8739	3.01	0.0095	24.60
7 d 37°C	64.52	12736	3.08	0.0110	30.20
14 d 4°C	36.86	11646	1.59	0.0094	24.64
14 d 12°C	38.06	15092	2.39	0.0096	27.79
14 d 30°C	49.30	17517	2.67	0.0101	29.86
14 d 37°C	94.46	17077	2.56	0.0117	31.64
21 d 4°C	43.78	11179	2.81	0.0125	32.43
21 d 12°C	43.72	12491	1.85	0.0146	36.57
21 d 30°C	64.37	15262	2.83	0.0148	32.64
21 d 37°C	104.40	16417	2.62	0.0214	36.93
28 d 4°C	71.93	11688	3.33	0.0154	40.64
28 d 12°C	80.73	11814	2.54	0.0278	46.71
28 d 30°C	102.96	21728	3.18	0.0291	47.93
28 d 37°C	132.27	19626	2.84	0.0309	52.21
P values	0.000	0.000	0.000	0.000	0.054

TABLE II. Aldehyde concentrations (ppb) and overall *stale* score of lager B stored at 7, 12, 30 and 37°C for up to 28 days.

Sample	Furfural	5-HMF	Hexanal	*Trans*-2-nonenal	*Stale* intensity
0 d	12.96	14732	1.60	0.0022	28.43
7 d 4°C	13.89	27897	1.56	0.0021	24.70
7 d 12°C	19.06	14723	1.78	0.0023	27.80
7 d 30°C	22.68	15795	1.62	0.0025	28.70
7 d 37°C	28.23	22492	1.64	0.0032	24.30
14 d 4°C	16.75	15056	2.63	0.0023	40.75
14 d 12°C	22.04	23031	2.42	0.0092	44.25
14 d 30°C	30.24	22833	2.66	0.0071	52.92
14 d 37°C	38.57	30278	2.71	0.0071	40.67
21 d 4°C	17.30	31297	2.44	0.0024	25.36
21 d 12°C	20.02	24166	2.75	0.0029	30.86
21 d 30°C	33.06	21295	2.89	0.0036	27.86
21 d 37°C	59.28	32685	2.87	0.0038	29.57
28 d 4°C	14.89	29028	3.01	0.0032	33.71
28 d 12°C	19.34	30132	3.16	0.0037	44.43
28 d 30°C	31.27	30237	2.97	0.0043	38.07
28 d 37°C	56.10	36603	3.34	0.0054	37.00
P values	0.000	0.036	0.000	0.000	0.077

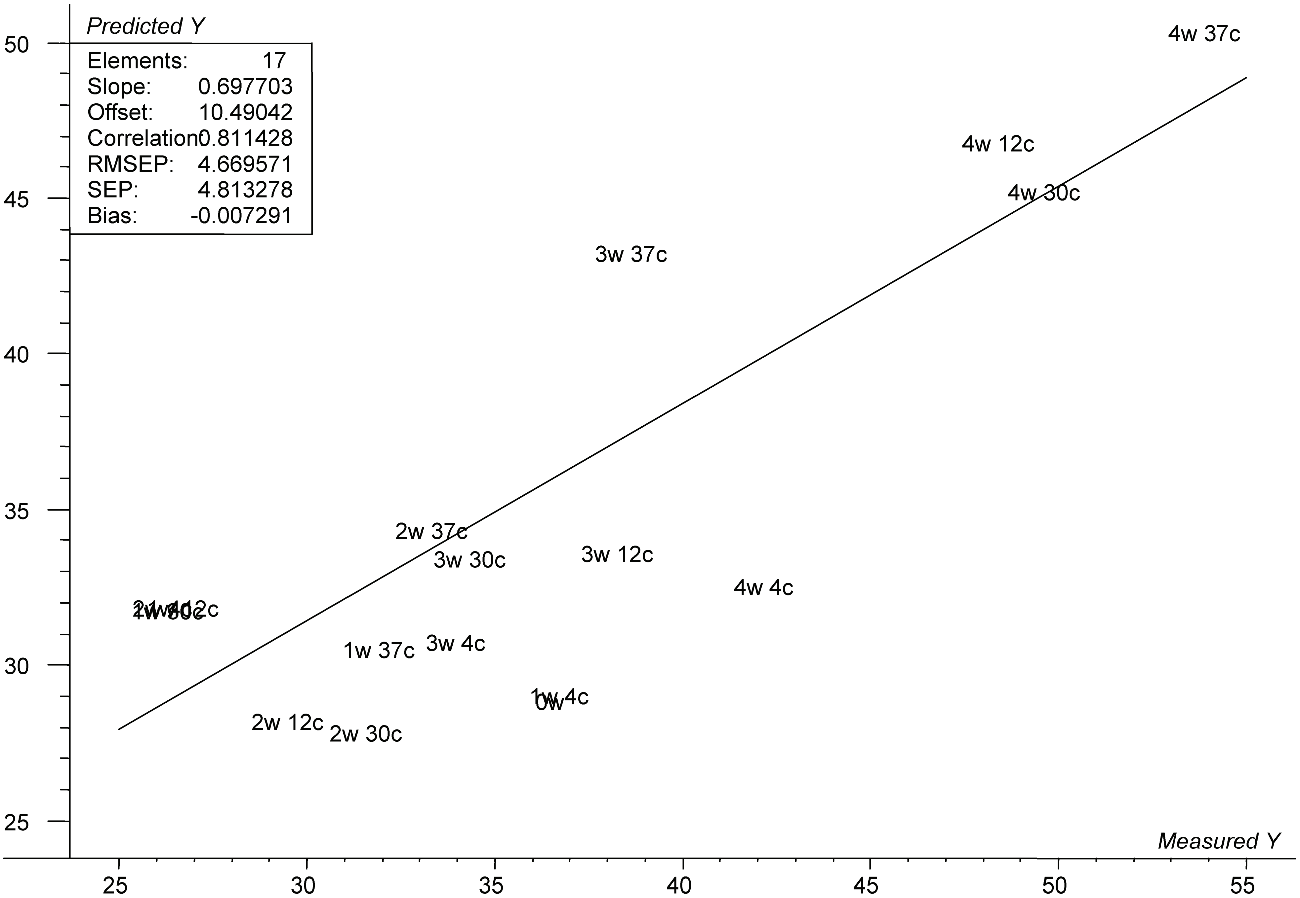

Fig. 1. Prediction of overall scoring for sensory staleness in lager A from SPME data on four aldehydes using partial least square regression (PLS1).

were quantified using an ion trap (Finnegan MAT IT S40) operating at 70 eV in ion-scan mode.

Score of overall stale character

Sensory data was taken from experimental work reported elsewhere[15] using assessors (10) trained with 9-month old lagers to understand stale characters. Aliquots (25 mL) were scored for intensity of stale overall character[15] and data was averaged after two-way ANOVA to check assessor performance.

Data analyses

ANOVA of chromatographic data employed Minitab v.13.1 and partial least square regression used Unscrambler v7.6 (CAMO A/S, Oslo, Norway); and artificial neural network prediction Neuralyst v. 1.4 (Cheshire Engineering Corporation, USA)[16].

RESULTS

Calibration

Calibration curves (Correlation coefficients – R^2) were constructed for each aldehyde: 0.1–50 ppb for furfural (0.997) and hexanal (0.989); 10–1000 ppb for 5-HMF (0.948); and 0.01–5 ppb for *trans*-2-nonenal (1.00). Such values for R^2 indicated this strategy was suitable for quantifying aldehydes at concentration observed.

Congener and sensory data on staling

In lager A ANOVA of scores for the overall sensory stale character and concentrations of the four staling aldehydes (Table I) showed significant differences in the concentrations of all 4 aldehydes ($p < 0.001$) with a direct relationship with time over the 28 days and temperature. The exception was hexanal where at day 7 its concentration was significantly increased only at 30 and 37°C. Each of the four aldehydes was most abundant after 28 days at 37°C, when the beers also scored highest for stale character. Scoring of *stale* character ($p = 0.054$) was more correlated with time than temperature

For lager B (Table II), the *trans*-2-nonenal concentrations were markedly increased by day 14 at 12, 30 and 37°C, but not at 4°C. The sensory score for *stale* character was significantly different for the samples stored at the four temperatures. Congener concentrations differed significantly with storage condition with $p < 0.001$ for furfural, hexanal, and *trans*-2-nonenal; and $p = 0.036$ for 5-HMF. Moreover, the sensory *stale* score was highest on days 14 and 28 ($p = 0.077$) and lower at day 21.

Partial least square (PLS1) modelling

Modelling sensory stale scoring and aldehyde concentrations in lager A was initially performed with PLS1 using 2 components. Fig. 1 shows the prediction with correlation coefficients of 0.89 for calibration and 0.81 for

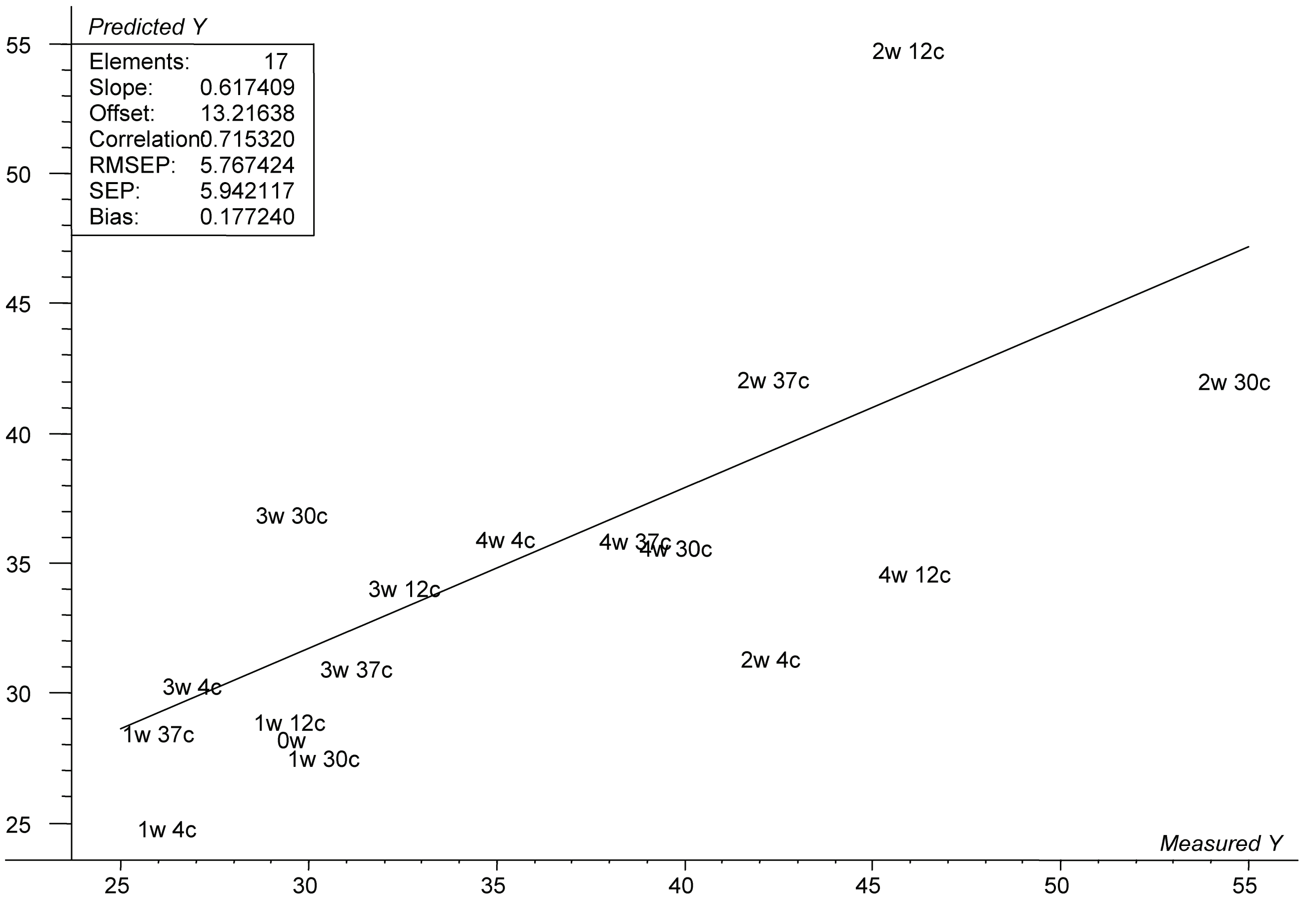

Fig. 2. Prediction of overall scoring for sensory staleness in lager B from SPME data on four aldehydes using partial least square regression (PLS1).

validation. In the PLS product space (Factor 1 vs Factor 2) the control lager was directly opposed to the samples stored longest at higher temperature (28 d, 30 and 37°C). The four aldehydes were scored similarly on Factor 1, but *trans*-2-nonenal and furfural scored positively on factor 2, linked to 28-day samples at 12, 30 and 37 and the 21-day sample at 37°C. In contrast hexanal and 5-HMF were scored negatively on this factor, linked to intermediate samples (shorter time and/or lower temperatures). Modelling regression coefficients for the four congeners were positive for *trans*-2-nonenal (0.793) and furfural (0.275) and negative for hexanal (–0.142) and 5-HMF (–0.138).

Modelling of the relationship for staling in lager B was again effected using PLS1 (Fig. 2), which gave correlation coefficients of prediction of 0.85 and 0.72 for calibration and validation, respectively using 2 components. Factor 1 showed staleness positively scored and linked to all four aldehydes. On Factor 2, *trans*-2-nonenal was positively scored, linked to 14-day samples (12, 30, and 37°C) polar to furfural and 5-HMF with hexanal intermediate. Positive regression coefficients for this model were *trans*-2-nonenal (0.764) and hexanal (0.429), and negative were 5-HMF (–0.233) and furfural (–0.228).

Artificial neural network (ANN) modelling

A three-layer feed forward ANN model was used in prediction of staleness intensity in both lagers to determine if predictions could be improved by introducing a non-linear function. The premium lager prediction, with full cross validation, was optimal at 1.54×10^5 training epochs, which reduced the root mean square error (RMS error) to 0.181 with a correlation coefficient of 0.84 (Fig. 3).

When ANN modelling was repeated for the standard lager, training was optimal at 2.16×10^5 training epochs, which reduced RMS error to 0.322 with a correlation coefficient of 0.86 (Fig. 4).

DISCUSSION

Staling characters in lagers remain unclear – firstly in relation to canned beer shelf-life and secondly as to whether assessors provide information relevant to consumer perceptions. The latter is discussed for these lagers elsewhere[15].

From the literature[2–5,7,8,12,13,17,18], it is clear that the aldehydes produced as a result of storage oxidation processes are central to lager staling. Aldehydes quantified in this storage experiment were: furfural and 5-hydroxymethyl furfural, heterocyclic compounds (product of browning reactions), *trans*-2-nonenal (from browning reactions) and hexanal (from lipid oxidation). In this study furfural concentrations reached maxima of 132.3 and 59.3 ppb in lager A and lager B respectively, with corresponding maxima for 5-HMF of 19.6 and 36.6 ppm. Thus the average A:B ratios for furfural and 5-HMF, were respectively 2:1 and 1:2. Maximal values for hexanal were not significantly different (3.33, 3.34 ppb). In contrast, concentrations of *trans*-2-nonenal were 30.9 and 9.2 ppt for lager A and lager B, respectively. All values were similar to those using SPME reported for other lagers: furfural up to 458 ppb, *E*-2-nonenal at 30 ppt, and hexanal at 2.5 ppb after 12 weeks at 30°C[18]; 5-HMF at 7 ppm after 13 days at 37°C[13]. In contrast, values for flavour thresholds[2,9,18] are as follows: 150 ppm for furfural; 1000 ppm for 5-HMF; 300 ppb for hexanal; and 0.11 ppb for *trans*-2-nonenal. There could be two possible explanations for this. Flavour thresholds were not determined in a solution that provided information relevant to lagers; these compounds act in concert in evoking stale character at sub-threshold concentrations[9].

In the two lagers studied, furfural was a driver of staling in lager A as judged from both quantity and the regression coefficient in the PLS1 prediction. In contrast, in the standard product, hexanal was a driver of the prediction model based on the PLS1 regression coefficient. In both models *E*-2-nonenal had high regression coefficients, suggesting a major contribution, and 5-HMF low coefficients. The role of furfural in *stale* character has been reported previously[2,3,17,18] whereas that of hexanal has not. It is clear that the contribution from each should be explored when considering staling.

Although the concentration of the aldehydes increased in a direct relationship with time and also storage temperature in lager A, this was not observed with lager B. In the sensory data from this study[15], the score for sensory stale character for B showed a second minimum. This suggested that differences between the beers related to style, original gravity, ethanol content, composition and

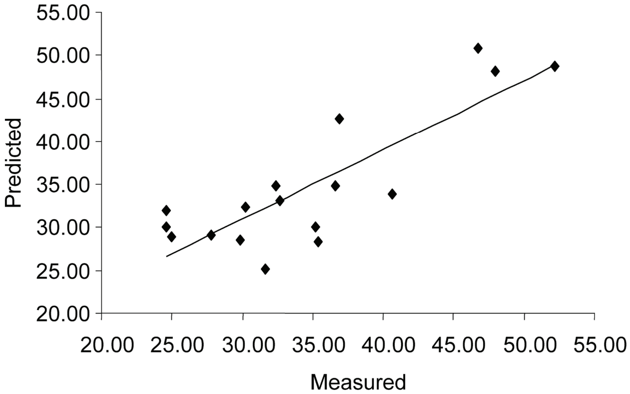

Fig. 3. Prediction of overall scoring for sensory staleness in lager A from SPME data on four aldehydes using an artificial neural network.

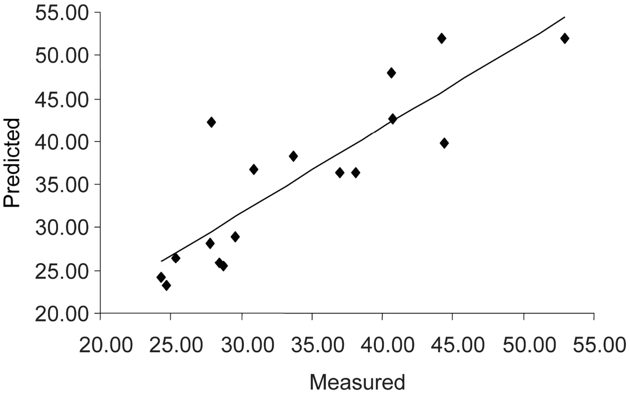

Fig. 4. Prediction of overall scoring for sensory staleness in lager B from SPME data on four aldehydes using an artificial neural network.

hop acid concentration, all reflected in the sensory character, which included the flavour profile.

In this study, PLS1 and ANN modelling with limited data on aldehydes, showed a clear prediction of scoring of overall stale character. The PLS regression coefficients contribution of *E*-2-nonenal suggests that the use of this compound, as a quality control marker for *stale* character, is justified[7,8,11,12,18]. From this model the suggestion is that 5-HMF is also a staling marker[13] although it may also contribute to character, as in European lagers where 5-HMF concentrations of 0.5–4.0 ppm are typical, with 71.5 ppm reported in a German dark beer[2]. A previous PLS study[3] that suggested 5-HMF has a higher regression coefficient in staleness prediction than *trans*-2-nonenal, cannot from this study be regarded as a general model, but may be valid for the specific products.

In this study ANN and PLS modelling gave fairly clear predictions, but the latter provides more information. The training function of ANN showed improvements in RMS error – for PLS RMS error values were 4.67 for premium and 5.77 for standard lagers The ANN model may be more suited to routine quality control application as modelling parameters can be established with the calibration set and predictions of stale character made without analyst intervention. The similarity in prediction suggests limited nonlinearity in sensory response to staling aldehydes. In other studies improved predictions from ANN models have been reported for milk flavours[6], overall blackcurrant character[1], and food additives[10].

CONCLUSIONS

Concentrations of furfural, 5-HMF, hexanal, and *trans*-2-nonenal in two lagers stored for up to 28 days at two sub-ambient and two supra-ambient temperatures, increased in parallel with scoring of *stale* character by trained assessors. In modelling sensory scoring of staleness from aldehydes data, *E*-2-nonenal made the largest contribution to flavor prediction and furfural and hexanal made different contributions in lager A and lager B. In neither model did 5-HMF contribute to the prediction of staleness.

The two lagers differed in wort composition, brewing process and style. The staling characteristics of the two lagers also differed, although the underlying processes appeared similar. Staling is not considered to be a controllable flavour, but dominating mechanisms should be considered in new product development[14] and establishment of shelf life. In this study, canned storage at 4°C gave the least staling in terms of sensory scoring and congener production.

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