Using a combined temporal approach to evaluate the influence of ethanol concentration
 on liking and sensory attributes of Lager beer

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20 Abstract

21 A low alcohol beer evoking similar sensory enjoyment as its higher alcohol counterpart is potentially an attractive proposition to breweries for increased sales volumes, as well as 22 23 consumers due to health and societal reasons. This study aimed to determine the influence of ethanol on the temporal sensory characteristics and liking of beer as perceived by beer 24 consumers. A commercial 0% ethanol concentration lager was spiked with ethanol to different 25 concentrations (0.5%, 2.8%, 5% ethanol). Consumers (n=101) indicated their liking using 26 temporal liking (TL) methodology (rated throughout consumption) and overall liking (rated at 27 28 the end of consumption). Consumers also denoted the sensory properties perceived using temporal Check-All-That-Apply (TCATA). Overall, liking data divided consumers into 3 29 30 clusters with different patterns of liking. As ethanol concentration increased from 0 to 5%, the 31 TL time that best predicted overall liking shifted from 60 sec to 10-20 sec indicating that liking of higher alcohol products was decided earlier on in consumption. Data suggested that in a 32 lower ethanol beer, a liking judgement may not be stabilized until later in the evaluation, while 33 34 in high ethanol beers, a liking judgement, either positive or negative, stabilised more rapidly. TCATA results revealed different temporal sensory profiles among the different ethanol 35 concentrations. As ethanol concentration increased, the citation of sweetness, fullness/body 36 and alcohol warming sensation increased. However, the relationship between TCATA citations 37 38 and TL varied among the three clusters highlighting that, in relation to ethanol concentration, 39 different negative and positive sensory drivers of preference exist for different segments of consumers. 40

41 **1. Introduction**

42 Beer consumers are accustomed to a product that offers a well-defined and complex taste (Blanco, Andres-Iglesias, & Montero, 2016). In addition to these sensory considerations, 43 44 the increasing interest of consumers regarding health and societal issues has motivated breweries to expand their portfolio of beers with low or no alcohol content products (Rehm, 45 Lachenmeier, Llopis, et al., 2016; SeekingAlpha, 2016). As beer consumers are accustomed to 46 47 particular attributes, the development of a low alcohol beer that displays a similar sensory profile to its higher alcohol counterpart is an attractive proposition. This would allow 48 49 consumers to still enjoy the sensory properties of a beer while making responsible drinking choices (Missbach, Majchrzak, Sulzner, et al., 2017). 50

The challenge remains that sensory attributes in alcohol-free and alcohol-reduced beers 51 52 differ from those in regular beer. Beers vary in their alcohol content but the majority of beers consumed contain between 3-8% ethanol (Preedy, 2011). Ethanol is an effective olfactory and 53 trigeminal stimulus, contributing to the warming/burning perception of beer (Clark, Hewson, 54 55 Bealin-Kelly, et al., 2011a; Green, 1987). Ethanol also contributes to the perception of different tastes, predominantly sweetness, bitterness and sourness (Hellekant, Danilova, Roberts, et al., 56 57 1997; Martin & Pangborn, 1970; Scinska, Koros, Habrat, et al., 2000). Consuming beer is a multimodal experience and the influence of ethanol on sensory perception and its interactions 58 59 with the other components in beer has been documented (Clark, et al., 2011a). For example, 60 ethanol interacts with hop acids to suppress a warming sensation at 4.5%, but also interacts with low levels of CO₂ to yield an increased alcohol warming sensation (Clark, et al., 2011a). 61 Furthermore, ethanol has been found to physically influence aroma release in beer during 62 consumption (Clark, Linforth, Bealin-Kelly, et al., 2011b). The influence of ethanol 63 concentration on dynamic headspace recovery of different volatile compounds in ethanol/water 64 solutions using proton transfer reaction mass spectrometry (PTR-MS) with concentrations 65

66 similar to those found in beer (0, 2.5 and 6.2% v/v) showed that increased ethanol concentration decreased volatile release (Aprea, Biasioli, Mark, et al., 2007). This reported similar findings 67 to Clark, et al. (2011b), again with dynamic headspace, with the change being attributed to an 68 69 increase in the solubility of aroma compounds (Aprea, et al., 2007; Conner, Birkmyre, Paterson, et al., 1998; Perpete & Collin, 2000). Ethanol clearly has the capability to impact 70 sensory perception of beer. Therefore, an understanding of how ethanol reduction in beer 71 affects consumer perception and acceptance is important (Kaneda, Kobayashi, Watari, et al., 72 2002; Porretta & Donadini, 2008). Previous studies have reported that consumers can 73 74 distinguish among beers containing different ethanol concentrations. For example, in one triangle test, consumers could distinguish between an alcohol free (0.5% ethanol) and regular 75 76 (5% ethanol) beer but interestingly were not able to identify which was of a higher alcoholic 77 strength, suggesting consumers are not necessarily aware of the characteristics associated with ethanol (Lachenmeier, 2014). In another study, consumers were able to distinguish between an 78 alcohol-reduced (3.8% ethanol) and regular beer (5.3% ethanol), with the standard strength 79 80 beer having more overall appeal than the lower strength (Segal & Stockwell, 2009). However, these studies did not report consumer liking of the products, which is an important piece of 81 information for innovating a commercially successful product. 82

83 Beer possesses a highly complex sensory profile (Clark, et al., 2011a) and as with other 84 beverages including wine (Baker, Castura, & Ross, 2016), displays a temporal aspect. In short, 85 beer perception changes over the consumption period, from the moment the beer is placed in the mouth to when the final sensations of that beer, including aftertaste, abate. Particularly, the 86 sensory attributes of beer arising from the presence of ethanol (ethanol warming) and iso-alpha 87 88 acids (bitter taste) are well documented to have a temporal quality in beer (Arrieta, Rodriguez-Mendez, de Saja, et al., 2010). Thus to better understand consumer perception of a low-alcohol 89 beer, the application of temporal methods is important. Previous testing of the temporal sensory 90

aspects of beer has relied upon the use of time intensity or dominance testing using Temporal
Dominance of Sensation (TDS) (Missbach, et al., 2017), and usually with trained panels. Using
TDS, differences among three beers based on their ethanol concentration with trained panellists
were identified. Beer samples containing <0.5%, 3.4% and 5% ethanol displayed differences
in the dominance of astringency and other fermentation-related flavours, with the higher
ethanol concentrations showing increased bitterness and astringency (Missbach, et al., 2017).
However, it is unclear what impact this might have had on consumer liking.

Understanding the sensory attributes that drive consumer liking of food and beverage 98 99 products is critical to both the food and beverage industry. In the present study, the impact of ethanol concentration on the perception of beer was investigated with consumers using a 100 101 combination of methods to evaluate temporal and overall liking and the temporal perception of 102 key sensory attributes. Temporal Check-All-That-Apply (TCATA) methodology (Baker, et al., 2016; Castura, Antúnez, Giménez, et al., 2016a) was chosen over TDS as it does not limit 103 evaluation to just dominant attributes. Previous studies have successfully employed similar 104 105 methods to determine drivers of liking (Ares, Alcaire, Antúnez, et al., 2017; Thomas, Visalli, Cordelle, et al., 2015); however, no studies have yet examined temporal liking in beer. 106

107 The objectives of this study were therefore to i) evaluate the influence of ethanol on 108 consumer liking of lager and perception of its sensory characteristics; ii) determine if particular 109 time points during temporal liking related to overall liking; and iii) investigate the relationship 110 between the temporal sensory profile of beer and temporal liking data identifying critical 111 attributes driving consumer acceptance of beer in relation to ethanol concentration.

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113 **2. Methods**

114 **2.1 Participants**

115 Consumers (n=101: 53 men, 48 women; aged 19-70 (mean age 32)), who self-reported 116 consumption of beer at least once every two months, participated in this study. Data concerning 117 frequency of consumption and the types of beer consumed was also obtained. Approval from 118 the University of Nottingham Medical Ethics Committee was granted before the study 119 commenced and the subjects were offered an inconvenience allowance to participate.

120 **2.2 Beer samples**

121 A 0% ABV lager style beer (Carlsberg, Northampton, UK) was used as a base beer from which four experimental beer samples (0, 0.5, 2.8 and 5% ethanol) were prepared. These 122 123 ethanol concentrations were selected to reflect a full ethanol beer (5%), an intermediate ethanol concentration (2.8%), a low ethanol beer (0.5%), and an alcohol free beer (0%). In the United 124 125 States, an alcohol-free beer is described as having 0% ethanol concentration, a non-alcoholic 126 beer corresponds to a beer containing 0.5% ethanol or less and a lower alcohol beer contains less than 3.5% ethanol. In the United Kingdom, alcohol duty rates are increased when a beer 127 exceeds 2.8% ethanol concentration and so some brewers try to satisfy this target for their lower 128 alcohol beers (Branyik, Silva, Baszczynski, et al., 2012). The above points were considered 129 when selecting the specific concentrations to represent ethanol concentrations of beer 130 131 commercially available in each of these categories.

To create the 0.5, 2.8 and 5% ethanol samples, 1.7, 9.6 and 17.5 mL of 99.5% food grade ethanol (VWR International, Lutterworth, UK) and 28.3, 20.4 and 12.5mL of still water (Danone, Paris, France) were added, respectively, to 300ml of beer. The 0% ethanol beer also had 30mL of water added to ensure that all samples were treated the same. Commercial bottles of beer (330ml) stored at $4\pm1^{\circ}$ C, were opened as close to sample testing as possible, 30ml was poured out of the bottle, and the relevant ethanol/water solution was added back in after which the bottle was inverted to ensure adequate mixing. Beer samples (30ml) were poured into plastic serving cups and were used within 20 mins of opening. This approach was used tominimise sample handling and limit the decarbonation and volatilisation of the samples.

141 2.3 Sensory Attributes

Attributes and definitions for beer evaluation were developed in reference to published
literature (Langstaff & Lewis, 1993; Martin & Pangborn, 1970; McMahon, Culver, Castura, et
al., 2017; Meilgaard, Dalgliesh, & Clapperton, 1979) as well as through the use of a naïve panel
of six beer consumers.

146 **2.4 Procedure**

147 All consumers participated in two evaluation sessions over two weeks at the Sensory Science Centre, Sutton Bonington campus, University of Nottingham. Both sessions began 148 with a familiarisation session (15 min) after which consumers evaluated samples in isolated 149 150 sensory booths (45 min). Consumers evaluated temporal liking (TL) first and overall liking (OL) second to gain an understanding of consumer liking of the product during specific periods 151 of consumption (before swallow and aftertaste) and then an overall score. TL and OL were 152 evaluated in session one and sensory attributes using TCATA in session two. Although not 153 always shown to cause bias (Jaeger, Giacalone, Roigard, et al., 2013) this order was chosen to 154 avoid analysis of sensory attributes influencing liking results as reported in other studies 155 (Earthy, MacFie, & Hedderley, 1997; Popper, Rosenstock, Schraidt, et al., 2004). 156

157 2.4.1 Familiarisation Sessions

Previous research has shown that a short familiarisation session (7-10 mins) can result in a small increase in consumer ability to discriminate among samples (Jaeger, Beresford, Hunter, et al., 2017). In session one familiarisation involved the explanation and practice of the evaluation protocol for TL and OL. In session two, the TCATA method was described to the consumers as a relatively new technique, and the importance of checking and unchecking

perceived attributes during evaluation was discussed (Castura, Baker, & Ross, 2016b). The
attributes (Table 1) were also reviewed to ensure consumers understood them all.

For all in-mouth evaluations, the in-mouth protocol remained the same: consumers 165 were asked to place the sample in the mouth and press the green start button immediately, move 166 the sample around in the mouth and then swallow at 10s when a prompt appeared on-screen. 167 Although not necessarily normal drinking behaviour, this enabled the protocol to be controlled 168 and facilitated comparison between TL and TCATA data. Consumers continued the evaluation 169 up to 60s, at which point it ceased. If nothing was perceived before reaching the end of the 170 171 evaluation time consumers were told to deselect attributes. Consumers were given a handheld tablet (Apple, Cupertino, California, USA) and practice sample at the end of each 172 familiarisation session so that they could interact with the method and software prior to formal 173 174 evaluations.

In each session all samples (n=4) were presented monadically under Northern
hemisphere lighting using a randomised balanced design according to a Williams Latin Square
(Meyners, Castura, & Carr, 2013). Data were captured using Compusense© Cloud software
(Guelph, Ontario, Canada). To minimise fatigue and carryover, consumers were given a forced
2 min break between each sample, and were told to take at least 2 sips of water (Evian, Danone,
France) during this break to cleanse the palate.

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2.4.2 Temporal Liking Measurement

During the first session, consumers used a 15-cm semi-structured line scale, anchored with dislike extremely and like extremely to continuously quantify their current liking. During the 60s evaluation time, consumers were instructed to click on the scale at any point that their perceived liking changed. The total duration of evaluation (60s) was established through preliminary investigations as a duration that was adequate to capture relevant changes in

187 aftertaste perception while minimising fatigue to the consumers. Data was recorded at one data188 point per second.

189 2.4.3 Overall Liking Measurement

Within 30s of completing the TL measurement, consumers assessed their overall likingof the sample using a 9-pt hedonic scale ranging from 'dislike extremely' to 'like extremely'.

192 2.4.4 Temporal evaluation of sensory attributes in mouth using Temporal Check-All-

193 That-Apply (TCATA)

In the second session, consumers assessed the presence of 10 attributes within each sample. Prior to the test, consumers were instructed to familiarise themselves with the position of the attributes on screen, which were presented in a three-column format. The attribute order was randomised across subjects to balance bias associated with list order but was retained for a given panellist (Meyners & Castura, 2016).

199 2.5 Instrumental Analyses

Instrumental analyses were conducted to record the impact of ethanol concentration on 200 key chemical characteristics. The ethanol content, density and specific gravity were all 201 measured in triplicate across sample bottles prepared as described in section 2.2, using an 202 203 Anton Paar Alcolyzer and DMA4500 (Graz, Austria). The pH of all samples was determined 204 using a Metler Toledo FiveGo pH meter (Columbus, Ohio, USA) and the titratable acidity (TA) 205 measurements were made using a Metrohm 702 SM Titrino potentiometric titrator (Metrohm 206 UK Ltd, Cheshire, UK) after calibration with pH 4.0 and 7.0 standards. To determine if 207 differences existed between samples, an ANOVA was performed followed by a comparison of means calculated by Tukey's Honest Significant Difference (HSD) post-hoc test (XLStat 208 209 19.01, Addinsoft, New York, USA).

210 **2.6 Data Analyses**

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An α risk of 0.05 was set as the level of significance in all data analyses.

212 **2.6.1** Overall Liking

To determine if differences existed between samples in terms of overall liking a mixed 213 model two-factor ANOVA (sample, panellist), with panellist as a random effect was performed 214 followed by a comparison of means calculated by Tukey's HSD post-hoc test (XLStat 19.01, 215 Addinsoft, New York, USA). To ascertain if liking patterns varied across consumers a cluster 216 analysis (XLStat 19.01, Addinsoft, New York, USA) on overall liking data was performed 217 218 using agglomerative hierarchical clustering employing a dissimilarity matrix with Euclidean distance and Ward's method in the agglomeration (Desai, Shepard, & Drake, 2013). Further 219 220 analysis was then performed, with a two-factor ANOVA (as above) to examine differences between samples within each cluster. Cluster membership was further explored according to 221 the demographic variables collected in this study using a Chi square analysis and Fishers exact 222 223 test (Gellynck, Kühne, Van Bockstaele, et al., 2009).

224 2.6.2 Temporal Liking

For each product and consumer, six liking scores were extracted from the temporal data i.e. every 10s until 60s. As the cluster analysis discovered 3 different patterns of liking the temporal liking data was assessed taking different clusters into account. For each cluster, a twofactor ANOVA (sample and time point) with liking as the dependent variable was then performed (XLStat 19.01, Addinsoft, New York, USA). Tukey's HSD tests were subsequently used to identify where significant differences occurred between time points and clusters.

231 **2.6.3 Relating Temporal liking to Overall Liking**

Liking data were extracted for all time points, however only data relating to 10, 20, 40 and 60s were subsequently further analysed as no differences in liking were found at 30 and 50s. These liking data were modelled against overall liking which had been determined after the 60s evaluation period had ceased (Table 5). In order to determine if particular time points during TL related to overall liking, an ordered probit model was employed (Stata 14.0 (Stata Corp, College Station, TX, USA)). This model was selected because the dependent variable
was an ordered scale, ranging from 1 to 9 (Long, 1997). A separate model was estimated for
each consumer cluster at temporal liking times of 10 (swallow), 20, 40 and 60s (end of test) to
identify which time point best related to the overall liking.

241 **2.6.4 Analysis of TCATA data**

242 2.6.4.1 Analysis of Average Proportions of Citations

The analysis of the average proportion of citations followed a similar method as McMahon, et al. (2017), with each attribute being assessed as the proportion of the 60s time period in which it was selected (XLStat 19.01, Addinsoft, New York, USA). For example, if malty was checked for a duration of 15s and hoppy for 25s, the average proportion of citations would be 15/60 = 0.25 for malty and 25/60 = 0.42 for hoppy.

248 **2.6.4.2 TCATA Curves**

Following a similar procedure as described in Castura, et al. (2016a); and McMahon, 249 et al. (2017), data were exported for each attribute at 0.1s intervals in the form of either '1' or 250 251 '0' to show presence or absence of this attribute. Proportions of citations were calculated as the percentage of panellists who perceived (or checked) an attribute at any given moment during 252 the evaluation period. For each attribute, TCATA curves (smoothed using the cubic spline 253 function in R (The R Foundation, Vienna, Austria) to reduce noise in the data (McMahon et 254 al., 2017)) were calculated per treatment at each time point (each 0.1 s during the evaluation 255 256 period). Thicker sections of an attribute line were used to represent segments where the proportion of citations was significantly different in contrast to the other samples. The average 257 proportion of citation of the attribute for the other samples was plotted on the same figure, 258 259 when significant, using a dotted line enabling visualisation of the direction of the difference i.e. higher or lower citation, and the time periods during which significant differences were 260 observed. 261

262 **2.6.4.3 Multivariate Analysis of TCATA Attributes**

The relationship between beer samples and TCATA attributes was investigated using principal component analysis (PCA) on unfolded data, to create a two-way matrix with sensory attributes in columns and rows corresponding to sample (ethanol concentration) by time point (Castura, et al., 2016a; Castura, et al., 2016b) (Stata 14.0 (Stata Corp, College Station, TX, USA)). PCA plots were constructed to show how attributes were perceived and evolved in relation to treatments (McMahon, et al., 2017).

269 2.6.5 Relationships between temporal sensory attributes (TCATA) and temporal liking 270 (TL)

To evaluate the contribution of each TCATA attribute to temporal liking, a random 271 effects regression model was used (Stata 14.0 (Stata Corp, College Station, TX, USA)). This 272 273 analysis was selected so as to compare, by panellist, the evaluation of the same attribute at different points in time. Because the same panellist is evaluating the same attribute at various 274 points in time, the evaluations of that panellist are correlated with each other. A random effects 275 276 model takes into account this non-independence among the observations. For this model, TL was the dependent variable whilst the TCATA attribute (i.e. astringent, malty, etc.) was used 277 as the independent variable, with z-values showing whether this was a positive or negative 278 association. 279

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281 **3. Results**

282 **3.1 Instrumental Analyses**

The instrumental analyses confirmed that the planned concentrations of ethanol were achieved. The ANOVA showed that the effect of ethanol concentration was significant (F (3, 11) = 897, p=<0.0001) as were associated specific gravity (F (3, 11) =67.8, p=<0.0001) and density values (F (3, 11) = 69.1, p=<0.0001) (Table 2). Analysis of the pH values of the samples, although close, were significantly affected (F(3, 87) = 2.83 p = 0.043) with the Tukey test indicating the 0% and 0.5% having a significantly higher pH compared to the 5% ethanol sample (<0.05). The analysis of variance showed that the effect of TA was significant (F(3,11)= 35.8, p = <0.0001), whereby the Tukey test indicated 0% and 5% were significantly different (p < 0.05), although still quite close in absolute value (differential= 0.703g/L). Theoretically, this increase in acidity might have increased the citation of the sour attribute in the TCATA for the 5% sample, however this was not found.

294 **3.2 Overall Liking**

ANOVA revealed no significant differences (F(3, 403) = 0.426, p = 0.735) among the four beer samples in terms of overall liking. However, agglomerative hierarchical clustering analysis was subsequently performed and three clusters of consumers were identified.

298 Table 3 shows the average overall liking scores of the three consumer clusters. The ANOVA yielded significant differences for the interaction between sample identity and cluster 299 (F(2, 6) = 15.2, p = <0.0001), indicating that the overall liking of the samples varied with the 300 301 consumer cluster. Statistically, scores for cluster 1 (C1, n=23) showed significant differences for consumers liking (F(3,91) = 15.7, p = < 0.0001) with Tukey test indicating that the overall 302 liking was significantly higher for the 5% beer compared to the 0%, 0.5% and 2.8% samples, 303 which were 'disliked slightly' (p<0.05). Cluster 2 (C2, n=50) showed no significant difference 304 in overall liking among the samples (F(3, 199) = 0.913, p=0.436), but rated all samples higher 305 than the other clusters as either 'like slightly' or 'like moderately'. The ANOVA for cluster 3 306 (C3, n=28) yielded significant differences for consumer liking (F(3,111) = 14.5, p = <0.0001) 307 with the Tukey test revealing that the overall liking for the 0%, 0.5% and 2.8% was 308 significantly higher than for the 5% beer, which was rated as 'dislike very much' (p<0.05). 309 Interestingly consumers in this cluster disliked all beer samples. 310

Cluster membership was further explored according to the demographic variables collected in this study which included beer consumption patterns, gender, age and types of beer consumed (e.g. ale and non-alcoholic beer) but low cell numbers meant no inference could be made regarding their effect on cluster membership. In addition to this, the familiarity of beer styles (more specifically non-alcoholic beer) over all consumers was studied, but no significant differences were found to suggest that non-alcoholic beer drinkers rated the 0% sample higher, as might be expected.

318 **3.3 Temporal Liking**

319 Because of the different patterns of liking found among consumers in overall liking, subsequent analyses looked at each cluster separately. Figure 1 shows the average temporal 320 321 liking curves for each sample by cluster. In general, they show that temporal liking of the beer 322 samples in each cluster reflected those results seen in the overall liking (Table 3). The ANOVA showed that the effect of ethanol concentration on liking was significant (F(3, 91) = 15.7,323 p = < 0.0001) for C1, and the Tukey test showed a significantly higher and constant level of 324 325 liking for 5% ethanol sample over the entire 60s evaluation period (p<0.05). Some reduction in liking for the other three samples was evident around and after swallowing. No significant 326 differences were found in liking scores between samples for C2 (F(3, 199) = 0.913, p=0.436) 327 and, visually, the level of liking was generally consistent throughout the evaluation. C3 328 generally showed consistent dislike for most of the samples throughout the temporal 329 330 evaluation, as seen with the overall liking data. Again ANOVA showed that there was a significant difference in terms of liking between samples (F (3, 111) = 14.5, p=<0.0001), with 331 the tukey test indicating the 0% sample scoring significantly higher for the duration. This 332 cluster also clearly disliked the 5% sample the most, particularly after swallowing (p<0.05). 333 The ANOVA performed to compare liking for each sample within a given cluster at each 334 increasing 10s of the evaluation time highlighted some of these differences between the 335

samples. For C1 and C2, no significant differences were found. However, for C3, a difference was found for the 5% ethanol beer (F(5, 143) = 4.31, p=0.001), with the Tukey test showing a significant decrease in liking when assessed at latter time points (40, 50 and 60s), during the aftertaste, compared to the first point which was in mouth, at 10s (p<0.05).

340 **3.4 Relating Temporal Liking to Overall Liking**

The relationship between liking at a given time point (determined using TL) and overall liking determined at the end of the test (using a 9-pt hedonic scale) was assessed and although clusters showed similar trends there were differences and hence the data was interrogated by cluster (Table 5).

The ordered probit estimates revealed that the time point from the TL data that best 345 predicted overall liking varied with beer sample and cluster. For 0% ethanol, TL at 60s (the 346 347 end of the evaluation) best predicted overall liking in both C1 (p=0.015) and C2 (p=0.006). None of the TL evaluations significant predicted overall liking in C3. No significant time point 348 was found for C3. For 0.5% ethanol, TL at 60s again best predicted overall liking in C1 349 350 (p=0.049). For C2, overall liking was significantly predicted by liking at both 40 (p=0.001) and 60s (p=0.001). Again, evaluations at none of the time points was a significant predictor of 351 overall liking for C3. For 2.8% ethanol, overall liking for both C1 (p=0.014) and C2 (p=0.009) 352 was significantly predicted by TL at 40s. No significant time point was found for C3. Finally, 353 354 for 5% ethanol, overall liking for C1 was significantly predicted by evaluations at 10 (p=0.005)355 and 60s (p=0.041). For C2 (p=0.005) and C3 (p=0.002), overall liking was significantly related to liking at 20s. 356

To a certain extent, as ethanol content decreased, overall liking was better predicted by temporal liking increasingly later in the consumption process. For cluster 3, who did not really like any beers, it was more difficult to find a temporal point relating to OL except for the 5% beer. In this beer, evaluations early in the consumption process better predictedoverall liking.

362 **3.5 Impact of ethanol concentration on temporal perception of sensory attributes** 363 (TCATA)

364 **3.5.1** Analysis of Average Citation Rates for Temporal Data

The average proportion of citations of various attributes varied among the beer samples as analysed using Cochran's Q analysis (Table 4). The citation of the mouthfeel attributes of fullness/body and alcohol warming were higher in the 5% ethanol sample compared to the 0, 0.5 and 2.8% ethanol samples (p<0.05). In the citation of the sweet attribute, the 5% ethanol sample was higher than the other three samples, with significant differences also observed between the 0 and 2.8% ethanol samples.

371 **3.5.2 TCATA Curves**

Differences were observed among the samples in the citation of sensory attributes over 372 time (Figure 2). For the 0% ethanol sample, in general, fewer attributes were cited compared 373 374 to the other three samples. Between ~14 and 60s, fullness/body was cited significantly less frequently (p<0.05) compared to the three other ethanol concentrations, as well as sweet taste 375 and fruity flavour from ~4 to 60s. The warming attribute was cited significantly less often 376 (p<0.05) compared to the three other ethanol concentrations at ~26s and ~30s, within the 0% 377 378 ethanol sample, however, interestingly it was not at zero which may have been expected 379 suggesting other attributes may contribute to its perception in beer.

For the 0.5% ethanol sample, several significant differences in the citations of attributes were found. Compared to the other 3 beer samples, sweetness was cited significantly less frequently (p<0.05) from ~4 to 60s and malty flavour from ~20 to 60s. Alcohol warming sensation was also cited significantly less often from ~21 to 60s and bitter taste from ~16 to 20s (p<0.05). For the 2.8% ethanol sample, bitter taste was cited significantly less frequently

from ~15 to 23s and ~27 to 44s. From ~16 to 24s, malty flavour was perceived less often (p<0.05).

For the 5% ethanol sample, attributes were cited more frequently compared to the 0 and 0.5% ethanol samples. Malty flavour was cited less often (p<0.05) from ~15 to 60s and bitter from ~16 to 60s. Sour was highlighted as an attribute being cited significantly less (p<0.05) from ~30 to 40s and hoppy flavour from ~25 to 37s. Alcohol warming sensation was cited significantly more often (p<0.05) in the 5% beer between ~55 and 60s.

As ethanol concentration increased attributes were cited more frequently. The lower ethanol concentration samples were cited significantly less compared to the other samples for sweetness, fullness/body and alcohol warming sensation. For the higher ethanol concentration sample, alcohol warming sensation was cited significantly more often compared to all other samples.

397 **3.5.3 Multivariate Analysis of TCATA Attributes**

The ethanol content in the beer clearly influenced the temporal citation of flavour, taste 398 and mouthfeel sensory attributes. The influence of ethanol content described above is clearly 399 visualised through the use of a PCA (Figure 3), showing the multivariate space and the 400 temporal evolution of attributes in the beer samples over the 60s evaluation period. Ethanol 401 concentration is labelled at the 40s evaluation point. The two components accounted for 402 403 83.05% variation in the data. PC1 is strongly correlated to bitter (0.934), malty (0.918), hoppy 404 (0.866) and fruity (0.858), whereas, PC2 is strongly correlated with tingly sensation (0.902) and fullness/body (0.758) and negatively correlated with astringent (-0.568). The trajectories 405 for each beer sample start at the top left (t=0) where the citation rate for all attributes is 0. As 406 407 this biplot is not a continuous loop, it shows that consumers were still perceiving attributes up until the end of the evaluation at 60s. As evaluated by citation frequency, the early onset 408 attributes in the beer samples were tingly, fullness and sweet occurring around~10s. The 409

410 delayed onset attributes, appearing at ~45s, were identified as astringent and malty and they
411 were more associated with the beer aftertaste.

When comparing the beer samples in their temporal evolution, the 0 and 0.5% ethanol 412 413 samples displayed similar profiles, as the trajectories show these samples initially described as tingly, evolving to become more sour and ending with being described as having malty and 414 astringent aftertastes. The 2.8% ethanol sample again was initially described as tingly, however 415 416 there was a more delayed onset of alcohol warming sensation and fruity, finishing with bitter and hoppy aftertastes. The 5% ethanol sample was initially described as tingly, but also 417 418 displayed delayed onset attributes of fullness, sweet, fruity and warming, with a sour and hoppy aftertaste. 419

420 **3.6 Relationships between temporal sensory attributes (TCATA) and temporal liking**

421 (TL)

422 The random effects regression analyses highlighted the influence of the TCATA attributes on liking in each cluster. For C1, presence of tingly sensations exerted a significant 423 424 positive influence on liking for all four samples (Table 6). For 0, 2.8 and 5% ethanol samples, having body also positively influenced liking. A sour note was a significant negative driver of 425 liking (p<0.001) for all samples except for the 2.8% ethanol. Alcohol warming sensation was 426 a negative driver of liking for both the 0 (p=0.033) and 0.5% (p<0.0001), becoming non-427 significant as the ethanol concentration increased. Presence of a fruity note was a negative 428 429 driver of liking for the 0 (p<0.0001) and 2.8% (p=0.047), but positive for the 0.5 (p<0.0001) and 5% (p<0.0001) ethanol samples. Sweet was a significant negative driver of liking for the 430 0% (p<0.0001), yet when the ethanol concentration increased to 0.5% (p=0.002) and 5% 431 432 (p<0.0001), this attribute became a positive driver of liking. Interestingly, bitter was a negative driver of liking for all samples (p=0.048 for 0% ethanol; p<0.0001 for 0.5% and 2.8% ethanol); 433 434 however, at 5%, it became a significant positive driver of liking (p=0.011).

435 For C2 (Table 6), the significant positive drivers of liking for samples other than 5% ethanol were the presence of the attributes of malty (p<0.0001) and sweet for 0% ethanol 436 (p=0.003) and 0.5 and 2.8% ethanol (p<0.0001). Other significant positive drivers of liking 437 438 were presence of alcohol warming sensation for 0% and 5% (p<0.001), as well as 0.5% ethanol (p=0.039). The citation of the fruity attribute positively influenced liking in the 0% ethanol 439 (p=0.004), 2.8% and 5% ethanol samples (p<0.0001). Astringent (p<0.0001) and tingly 440 (p=0.034) sensations were identified as significant positive drivers of liking for the 0% ethanol 441 sample, but then significant negative drivers of liking for all the higher ethanol concentration 442 443 samples (p<0.0001).

For C3 (Table 6), a sour note exerted a significant positive influence on liking for all 444 beer samples (0% ethanol (p=0.007), 0.5% ethanol (p<0.0001), 2.8% ethanol (p=0.014) and 445 446 5% ethanol (p<0.00001). The citation of tingly positively influenced liking for all samples except the 2.8 % ethanol (p<0.0001). Sweet had a positive influence on liking for the 0.5% 447 sample (p<0.0001); however, as the ethanol concentration increased to 5%, this negatively 448 449 influenced liking (p<0.0001). A similar trend was observed with bitterness, exerting a positive influence on liking for the 0% ethanol (p=0.002) but the liking of 2.8 and 5% ethanol samples 450 was negatively influenced by the presence of bitterness (p < 0.0001). 451

452 Overall each cluster showed differences in terms of attributes which drove liking and 453 disliking for all samples. C1 seemed to enjoy the mouthfeel attributes of tingly and 454 fullness/body sensations at all ethanol concentrations, with the tastes of sweetness and 455 bitterness seeming to be negative drivers of liking. C2 enjoyed malty and sweet attributes and 456 disliked astringent and tingly sensations when ethanol concentration increased. C3 liked sour 457 and tingly sensations and disliked bitterness as the ethanol concentration increased.

458

459 **4. Discussion**

The market for low alcohol beer is increasing rapidly and so an understanding of the sensory properties that ethanol contributes to a beer is important. Here the impact of ethanol on the temporal sensory signature and temporal, as well as overall liking was investigated. Furthermore, whether a particular time point related to overall liking was explored, as were the temporal sensory drivers of liking.

The instrumental analysis confirmed ethanol concentrations of the beer samples to be 465 in the regions of 0, 0.5, 2.8 and 5%, and showed significant differences among samples in terms 466 of their pH and titratable acidity. As the ethanol concentration in the beer sample increased, 467 468 the pH decreased and titratable acidity increased. The ranges in values measured were in accordance with typical values expected in beer (pH 4.0 ± 0.2) (Taylor, 1990). Despite ethanol 469 concentration affecting changes in pH and TA, the differences were below the thresholds 470 471 previously identified for sensory detection in wine (Amerine, 1976) (0.02-0.05% for TA and 472 0.05 for pH). It is noted that the medium in this latter study was wine and not beer and so these results cannot be applied directly, however no research has been done for beer. Therefore, it 473 474 can be concluded that these parameters were unlikely to have contributed to a sensory difference across the beer samples. 475

476 **4.1 The influence of ethanol concentration on liking**

In the initial analysis of overall liking of the four beer samples, no significant differences were found. However, with the application of cluster analysis, three consumer clusters were identified and so understanding that there are individual differences within a population for beer liking in relation to ethanol content is key for the brewing industry in the development of new products (Guinard, Uotani, & Schlich, 2001).

While differences in overall liking were found among clusters, no demographic predictors of cluster membership could be identified due to insufficient cell counts for the statistical analysis. The clusters were therefore likely to be a result of the differences in liking

of the sensory profile of the samples brought about by the variation in ethanol concentration.
C1 consumers preferred the high ethanol beer whilst C3 consumers preferred the low or no
ethanol beer samples. C2 was composed of consumers who did not show any preference for
the samples. Consumers within this cluster could be described as 'enthusiasts' as their overall
liking for all samples was considerably higher than other clusters; a similar group was found
in other products such as bread (Gellynck, et al., 2009) and quinoa (Wu, Ross, Morris, et al.,
2017).

It is important to note that the number of consumers for C1 and C3 were too low to draw strong conclusions from and so the results for these clusters can only be viewed as trends in the consumer data. Suggestions for future work would be to increase the number of consumers participating, to ensure stronger conclusions can be drawn from the data.

496 Previous studies have shown that liking is not a static measurement but rather a 497 temporal event (Delarue & Loescher, 2004; Lee & Pangborn, 1986; Taylor & Pangborn, 1990; Veldhuizen, Wuister, & Kroeze, 2006). Consumers were able to perform the task of evaluating 498 499 their liking over time, supporting previous research (Sudre, Pineau, Loret, et al., 2012; Thomas, et al., 2015). The three consumer clusters created from the overall liking measurements 500 reflected similar patterns of preference as the liking curves generated through TL. It should be 501 noted that measuring OL straight after TL may have introduced some bias and could explain 502 503 why the clusters followed similar patterns of liking for both liking measurements. Other 504 research has shown similar results in orange lemonades, displaying relatively flat hedonic curves for temporal liking for the whole assessment procedure from ~2.5s to 30s (Veldhuizen, 505 Wuister, et al., 2006). However, in a temporal study of liking of cheese, the most liked products 506 507 overall were found to be liked significantly less at the beginning of evaluation, but this may be due to the change in product matrix through mastication (Thomas, et al., 2015). Therefore a 508 recommendation for further work would be to investigate the effects of multiple sips of beer 509

510 on temporal liking as suggested in other literature (Guinard, Pangborn, & Lewis, 1986; Jamieson & Wantling, 2017). 511

In the current study, the liking of all clusters was shown to be significantly stable 512 throughout the 60s evaluation period. Although the figures show some variability in liking for 513 all products between 0-15s, further analysis at earlier time points (5s and 8s) showed no 514 significant differences in liking between time points (p>0.05). This may have been because 515 liking by some consumers was registered as late as 26s into the evaluation period which may 516 not reflect the normal experience for a consumer. Generally, temporal liking was found to be 517 518 more discriminating than overall liking, with changes seen over the 60s consumption period. In C1, the temporal liking of the most liked sample (5% ethanol concentration) is maintained 519 throughout evaluation, however for the least liked products the liking diminishes after 520 521 swallowing. This is similar for C3, where the liking of the least liked sample (5% ethanol concentration) diminishes rapidly after swallowing. 522

523

4.2 Relating overall liking to temporal liking

The relationship between OL and TL was assessed to see at which time point consumers 524 might base their overall liking. One of the main findings from this study was that OL and TL 525 526 results gave consistent sample rankings for each cluster. In addition to this, TL evaluations were found to be fairly stable over time for all clusters, although they did highlight a drop in 527 528 liking for some samples after swallowing. Only two studies to our knowledge (Sudre, et al., 529 2012; Thomas, et al., 2015) have linked time intensity of liking data or continuous liking with overall liking. In both of these studies, consumers registered their overall liking responses early 530 in the consumption experience. In a study by Thomas, et al. (2015) overall liking was recorded 531 532 at 17s, with the total consumption experience being 36s, thus describing more of the first impression of the product rather than after swallowing/aftertaste of the product (Sudre, et al., 533 2012; Thomas, et al., 2015). Interestingly, in the current study, there was not a particular time 534

that best related to liking. It appeared to be dependent on ethanol concentration. As ethanol 535 concentration increased in the beer samples, the time during the temporal evaluation that best 536 related to overall liking shifted. For C1, as ethanol concentration increased from 0% to 5%, the 537 time point that significantly related to overall liking decreased from 60s to 10s. The liking of 538 the most liked sample (5%) in C1 was maintained throughout evaluation, with the lower ethanol 539 concentration products diminishing in liking after swallowing. For C3 the overall liking did 540 541 not significantly relate to temporal liking for any samples, apart from the 5% sample (at 20s), which was the most disliked product. This suggests that the highly liked and disliked products 542 543 within each cluster related best to overall liking earlier on into evaluation. It could also have been due to familiarity of the beer, as the 5% sample is assumed to be closer to the consumers' 544 expectations and so could be easier for them to evaluate. In addition, as consumers followed a 545 546 strict procedure to drink the beer, this likely influenced their overall liking. Looking deeper into the data C1 (who preferred the 5% sample) and C3 (who disliked the 5% sample most) 547 were found to perceive the ethanol related attribute of sweetness at 10s significantly more than 548 C2 and so it could be deduced that these consumers either liked or disliked this respectively, 549 which formed their overall liking score. Finally, the use of TL should be discussed based on 550 the results of this study. TL for consumers appeared to be an easy task, but, not surprisingly, 551 was longer and more cumbersome compared to OL. It gave stable results over time. TL 552 evaluation may be well suited to foods where clear consumption periods can be defined (e.g. 553 554 mastication, swallow, aftertaste) or for drinks with strong aftertastes (e.g bitter tea, coffee, wine) to understand the change in liking over these periods of consumption. 555

556 **4.3 Influence of ethanol on sensory attributes of beer**

557 **4.3.1 TCATA**

558 Overall, the TCATA curves showed a difference in temporal sensory profiles among 559 all beer samples over time. As ethanol concentration in the beer sample increased, the citation

of alcohol warming sensation increased, following results from other research in beer (Clark, et al., 2011a). However, interestingly in the current study, alcohol warming sensation was only significantly cited more often during the ~55 to 60s time period in the 5% ethanol beer sample, reflecting its later presentation. This later presentation may have been due to the interaction effect of other factors within the beer, including the presence of carbon dioxide and hop acids, which have both been found to suppress warming sensation (Clark, et al., 2011a).

 CO_2 has also been found to interact with ethanol at lower ethanol concentrations (0, 2.25 and 4.5%) to modify warming sensation; this may explain why alcohol warming sensation was still cited at the 0% and 0.5% ethanol levels in the beer samples (Clark, et al., 2011a). It has also been speculated that this could have been due to the irritation from the carbonic acid from the CO_2 (Dessirier, Simons, Carstens, et al., 2000; Simons, Dessirier, Carstens, et al., 1999).

The increase in ethanol concentration was also accompanied by the increased citation 572 of other sensory attributes such as sweetness and fullness/body. Previous studies have found 573 that ethanol enhances the perception of sweetness at ethanol concentrations between 0 and 24% 574 (Clark, et al., 2011a; Martin & Pangborn, 1970). Ethanol (0.3-10%) stimulates sweet-best 575 fibres due to taste-taste mechanisms, as well as activates nerve fibres sensitive to sugar which 576 can be used to explain these differences among samples (Hellekant, et al., 1997; Scinska, et al., 577 2000). In terms of fullness/body, Langstaff, Guinard, and Lewis (1991) reported that the 578 579 fullness of commercial beers was moderately correlated with alcohol content with correlation coefficients of 0.41 for density and 0.50 for viscosity. 580

581 No significant differences were found in the overall citation rates of flavour attributes 582 malty, hoppy and fruity. Instrumental results using in-vivo atmospheric pressure chemical 583 ionisation mass spectrometry (APCI-MS) by Clark, et al. (2011b) found that as ethanol 584 concentration increased from 0 to 4.5% the in-breath release of ethyl acetate, isoamyl alcohol

and phenylethyl alcohol increased. This may suggest an expected increase in citation of related
sensory attributes, however this was not the case here, and hence if volatile release was higher
in the higher ethanol samples this was not perceivable. The differing results between this study
and Clark, et al. (2011b) could have been due to the volatile compounds measured and their
correlated sensory attributes (Conner, et al., 1998).

590 No significant differences were found in the current study in the overall citation rates 591 of astringency, but when looking at the temporal evaluation of this attribute the lower alcohol 592 samples were found to be significantly more astringent towards the end of consumption time, 593 with this attribute being temporally negatively correlated with PC2.

The onset of attributes also differed in that some attributes were cited more frequently 594 earlier in the evaluation time, while others were delayed and thus were cited later in the 595 596 evaluation time. For all beer samples, tingly sensation was one of the first attributes to appear. Delayed onset attributes which appeared after swallowing included malty flavour, bitterness 597 and hoppy flavour. Work by Missbach, et al. (2017) showed similar results with worty off-598 flavour being most pronounced between 0 and 30s, with the dominance of malty flavour 599 increasing after swallowing. Bitterness was also found to dominate the flavour profile after 600 swallowing. A study by Vázquez-Araújo, Parker, and Woods (2013) showed a similar time to 601 maximum intensity of both hoppy flavour and bitter taste in commercial lagers. Bitterness was 602 603 also found to be the attribute which lingered longer, and estery/fruity notes were found to abate 604 first (Vázquez-Araújo, et al., 2013).

605 **4.3.2 Influence of temporal sensory attributes on TL**

Acceptance of the beer samples was also contextualized by an examination of the TCATA attributes. Thomas, et al. (2015) found that the dominance of attributes plays a role in consumer liking, however the drivers of liking are mainly through the synergy of several components. The present study supported this earlier finding, showing that all attributes (and not just dominant attributes) were related to ethanol concentration and liking within the threedifferent clusters of consumers.

C1 (who preferred the 5% sample) were found to like tingly and fullness/body 612 attributes, which are both linked to a higher ethanol concentration. In addition, alcohol 613 warming sensation was a significant driver of disliking at the lower concentrations, with the 614 consumers also disliking sourness mostly in the 0% beer. Alcohol has been reported to suppress 615 616 sourness due to the decrease in the physiological response of the chorda tympani nerve in the presence of a sour stimulus (Martin & Pangborn, 1970). The consumers in C1 in the present 617 618 study also disliked bitterness until the ethanol concentration reached 5%, when it became a positive driver of liking. Ethanol concentration has been found to have an additive effect on 619 bitter sensation as it intensifies flavour perception (Martin & Pangborn, 1970; Meillon, Viala, 620 621 Medel, et al., 2010; Missbach, et al., 2017) thus the consumers within this cluster may have perceived this at the higher concentration. 622

623 C2 (who liked all samples) liked malty flavour, sweet taste and alcohol warming 624 sensation. Interestingly a study by Porretta and Donadini (2008) showed similar results, with 625 conclusions being drawn that overall flavour preference was highest for a malty flavour beer, 626 which reflects the fact that this was the largest beer consumer cluster. Consumers within C2 627 disliked astringent and tingly sensations when the ethanol concentration was increased to 0.5%, 628 and ethanol has been found to enhance both of these sensations.

629 C3 (who disliked the 5% sample most) enjoyed sourness and tingly sensations and 630 disliked alcohol, bitter and sweet attributes perceived within the 5% sample. All these attributes 631 can be related to the added ethanol within the beer and the interactions between the components 632 impacting sensory perception (Clark, et al., 2011a). Conclusions can be drawn from this study 633 that attributes are not only drivers of liking or disliking depending on the ethanol concentrations 634 of beer samples, but that these vary depending on the consumers, as was evident from the

635 clustering. One hypothesis for this is that at different concentrations of ethanol different attributes are enhanced or masked which drive liking/dislike in the different clusters 636 differentially. It is important to note that the balance of the overall profile of attributes is just 637 638 as important as the particular attributes themselves and so this needs to be considered when developing a new low alcohol beer, to form a favourable product; although this may only be a 639 favourable product to some consumers within a population. It is recognised that one limitation 640 in this study is that the beers were not fully optimised as would happen commercially when 641 changing the ethanol concentration. This may also have had a difference in the integration of 642 643 the flavour compared to when the beer is brewed to a certain alcohol percentage. The use of dealcoholisation apparatus to develop a base non-alcohol beer which can be adjusted for its 644 chemical composition and to produce samples only varying in ethanol content, may offer 645 646 improved insights into the effects of ethanol concentration. In addition to this, this study only looked into the effect of ethanol concentration in the context of lager and therefore this does 647 not necessarily apply to other beer styles, which would be an interesting area for future 648 649 research.

Many papers have looked at combining overall liking data with TCATA, TDS and 650 CATA results (Ares, et al., 2017; Thomas, Chambault, Dreyfuss, et al., 2017; Thomas, et al., 651 2015), however to the authors' knowledge this is the first paper to combine TCATA data with 652 653 temporal liking. However the fact that only ten attributes were included could be seen as a 654 limitation as others characteristics may be important but were not included on the list. Using a temporal measure of liking enabled additional insights into which aspect of the product drove 655 liking via the combination of TL and TCATA results and/or at what time of the consumption 656 657 process.

658 **5. Conclusions**

This study evaluated the influence of ethanol on the temporal perception of beer including both the perception of liking and sensory attributes, as well as identified critical attributes that drive consumer acceptance. Overall, it showed that consumers can be clustered to show their liking and disliking of beer samples containing different ethanol levels, including a cluster that liked low/no alcohol beer products similarly to standard beers. A study with larger numbers of consumers would help confirm this.

This study also reported the relationship between temporal liking and overall liking to 665 understand particular time points in products where consumers judge their overall liking, with 666 667 results showing this was dependent upon the consumer, as well as the ethanol content of the beer sample. In the higher ethanol samples, liking was determined more rapidly compared to 668 the lower alcohol samples. In addition, differences in sensory attributes among beer samples 669 670 with different ethanol concentrations were described, with a 5% beer having significantly more sweetness, fullness/body and alcohol warming sensation, highlighting the importance and role 671 of ethanol within beer. 672

This research is important for the brewing industry as it shows the overall sensory experience during consumption of a beer. It provides insight into a broad range of sensory attributes which are altered when ethanol is modified in beer, and highlights which attributes should be targeted by manufacturers when developing new low alcohol products. A new technique giving greater insight into liking was also described to link temporal liking with TCATA results to understand the drivers of liking at certain time points across different products.

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Table 1. TCATA attributes and definitions provided to consumers during familiarisation

815 session.

Flavour and Taste Attributes	Definition
Malty Flavour	Smell and taste of malty cereals. Can be related to smell of Ovaltine drink.
Hoppy Flavour	Smell and taste of hops which can be flowery and herbal.
Fruity Flavour	The aroma and taste of fruit characteristics – including banana, apple, pineapple, peach, lemon, orange.
Bitter Taste	Taste stimulated by strong black coffee, beer, red wine or tonic water.
Sweet Taste	Taste stimulated by sugar when experienced in mouth.
Sour Taste	Taste stimulated by acids when experienced in mouth.
Fullness/Body	Feeling of thickness/fullness as beer is moved around in the mouth.
Alcohol Warming Sensation	The feeling of warming which is characteristic of ethanol throughout the mouth.
Tingly Sensation	Perception of irritation such as prickling, stinging and bubbles bursting in mouth from carbonation. The feeling of pins and needles.
Astringent Mouthfeel	The feeling in mouth of roughing, puckering and drying.

Table 2. Mean (3 replicates) chemical profile of the beer samples.

Beer Sample	Alcohol by volume (ABV%)	рН	Density (g/cm ³)	Specific Gravity (SG)	Titratable Acidity (g/L)
0% Ethanol	0.06 ^d	4.209 ^a	1.019 ^a	1.021 ^a	0.848 ^c
0.5% Ethanol	0.64 ^c	4.202 ^a	1.018 ^b	1.020 ^b	1.130 ^b
2.8%	2.85 ^b	4.185 ^{ab}	1.015 °	1.017 °	1.260 ^b
Ethanol					
5% Ethanol	5.25 ^a	4.175 ^b	1.012 ^d	1.014 ^d	1.551 ^a

818 abcd different letters within a column represent a significant difference at p<0.05 (Tukey's 819 HSD)

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Table 3. Overall mean liking scores for beer samples by cluster.

Beer Sample	Cluster 1 (n=23)	Cluster 2 (n=50)	Cluster 3 (n=28)
0% Ethanol	4.04 ^{bB}	6.78 ^{aA}	4.04 ^{aB}
0.5% Ethanol	4.57 ^{bB}	6.44 ^{aA}	4.29 ^{aB}
2.8% Ethanol	4.00 ^{bB}	6.72 ^{aA}	4.96 ^{aB}
5% Ethanol	6.65 ^{aA}	6.32 ^{aA}	2.32 ^{bB}

822 Different letters within a cluster^{ab} or beer sample^{AB} represent a significant difference in

823 *liking* (*Tukey's* HSD (p < 0.05))

	Flavour Attributes			Taste Attributes			Mouthfeel Attributes			
Beer Sample	Beer Sample Malty Hoppy Fruity		Bitter	Sweet	Sour	Fullness/	Alcohol	Tingly	Astringent	
						Body	Warming			
0% Ethanol	0.39	0.18	0.18	0.32	0.23 ^c	0.17	0.08 ^b	0.06 ^b	0.22	0.20
0.5% Ethanol	0.35	0.22	0.17	0.31	0.29 ^{bc}	0.18	0.13 ^b	0.04 ^b	0.21	0.16
2.8% Ethanol	0.37	0.16	0.19	0.31	0.36 ^b	0.13	0.13 ^b	0.09 ^b	0.22	0.17
5% Ethanol	0.31	0.17	0.25	0.27	0.48 ^a	0.14	0.19 ^a	0.17 ^a	0.25	0.15

Table 4. Average proportion of consumer panel citations of TCATA sensory attributes.

^{abc}Different letters within a column represent significant differences among samples (Fisher's
 Exact Test (p<0.05)).

Table 5. Ordered probit coefficients and associated p values illustrating the relationship
between overall liking (9-pt hedonic scale) and temporal liking (15-cm line scale) for all

consumer clusters and beer samples at 10, 20, 40 and 60 seconds of evaluation. Bold font indicates significant relationships (p<0.05).

0% Ethanol Beer											
	Cluster 1				Cluster 3						
Evaluation	coefficient	p-	coefficient	p-	coefficient	p-					
time (s)		value		value		value					
10	0.161	0.191	-0.105	0.114	0.011	0.949					
20	0.214	0.130	0.165	0.081	0.155	0.716					
40	-0.183	0.426	0.156	0.076	0.648	0.468					
60	0.528	0.015	0.260	0.006	0.553	0.331					
	0.5% Ethanol Beer										
	Cluster 1		Cluster 2		Cluster 3						
Evaluation	coefficient	p-	coefficient	p-	coefficient	p-					
time (s)		value		value		value					
10	-0.056	0.663	-0.054	0.519	0.05	0.842					
20	0.243	0.1	0.029	0.801	-0.189	0.708					
40	0.100	0.681	0.446	0.001	0.979	0.319					
60	0.392	0.049	0.321	0.001	0.801	0.328					
	2.8% Ethanol Beer										
	Cluster 1		Cluster 2		Cluster 3						
Evaluation	coefficient	p-	coefficient	p-	coefficient	p-					
time (s)		value		value		value					
10	-3.3x10 ⁻⁶	1	0.857	0.289	0.281	0.809					
20	-0.13	0.4	0.109	0.272	0.471	0.151					
40	0.80	0.014	0.336	0.009	-0.363	0.569					
60	-0.589	0.841	0.119	0.282	0.636	0.192					
		5% I	Ethanol Beer								
	Cluster 1		Cluster 2 Cluster 3								
Evaluation	valuation coefficient p-		coefficient	p-	coefficient	p-					
time (s)		value		value		value					
10	0.528	0.005	-0.28	0.676	0.051	0.622					
20	0.526	0.066	0.253	0.005	0.672	0.002					
40	-0.763	0.114	0.117	0.379	-0.261	0.638					
60	0.780	0.041	0.258	0.032	0.821	0.163					

Table 6. z and associated p values from regression analysis denoting influence of TCATA attributes on temporal liking by cluster over consumption time. Black shading shows a significant negative driver of liking; grey shading shows a significant positive driver of

liking.

Cluster 1										
	0% Ethanol		0.5% Eth	nanol	2.8% Ethan		5% Ethanol			
Attribute	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value		
Malty	-5.30	<0.0001	1.77	0.077	4.51	<0.0001	-4.40	<0.0001		
Astringent	-6.20	<0.0001	0.47	0.636	-6.13	<0.0001	0.55	0.580		
Alcohol	-2.13	0.033	-4.14	<0.0001	0.48	0.634	0.35	0.728		
Bitter	-1.98	0.048	-8.34	<0.0001	-6.33	<0.0001	2.55	0.011		
Fruity	-4.77	<0.0001	5.10	<0.0001	-1.99	0.047	6.54	<0.0001		
Body	3.15	0.002	-5.63	<0.0001	5.06	<0.0001	8.24	<0.0001		
Sour	-11.00	<0.0001	-4.17	<0.0001	0.48	0.633	-6.57	<0.0001		
Sweet	-4.89	<0.0001	3.15	0.002	1.51	0.131	5.20	<0.0001		
Tingly	2.08	0.037	6.31	<0.0001	4.31	<0.0001	4.06	<0.0001		
				Cluster 2	•					
	0% Etha	nol			2.8% Ethanol		5% Ethanol			
Attribute	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value		
Malty	6.37	<0.0001	5.17	0.000	8.91	<0.0001	0.90	0.369		
Astringent	9.45	<0.0001	-2.47	0.013	-6.06	0.000	-7.17	<0.0001		
Alcohol	6.38	<0.0001	2.06	0.039	-0.50	0.616	3.97	<0.0001		
Bitter	0.14	0.892	1.50	0.134	3.76	<0.0001	0.16	0.871		
Fruity	2.86	0.004	0.61	0.543	4.64	0.000	14.32	<0.0001		
Body	0.09	0.926	-1.78	0.076	0.02	0.984	-4.93	<0.0001		
Sour	-2.88	0.004	1.22	0.223	1.00	0.318	1.03	0.304		
Sweet	2.94	0.003	7.92	<0.0001	4.59	<0.0001	-0.17	0.861		
Tingly	2.12	0.034	-2.44	0.015	-5.57	<0.0001	-3.81	<0.0001		
				Cluster 3						
	0% Etha	nol	0.5% Eth	nanol	anol 2.8% Ethanol			5% Ethanol		
Attribute	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value		
Malty	-5.18	<0.0001	-4.30	<0.0001	0.95	0.342	-0.79	0.428		
Astringent	-1.88	0.061	-2.61	0.009	3.88	<0.0001	-4.67	<0.0001		
Alcohol	-0.32	0.749	-1.30	0.194	-0.88	0.380	-3.73	<0.0001		
Bitter	3.13	0.002	1.44	0.150	-6.24	<0.0001	-5.17	0.000		
Fruity	1.82	0.069	-1.69	0.091	3.97	<0.0001	0.31	0.760		
Body	0.33	0.742	-0.02	0.986	9.24	<0.0001	1.18	0.239		
Sour	2.69	0.007	3.52	<0.0001	2.46	0.014	4.31	<0.0001		
Sweet	1.38	0.168	4.57	<0.0001	-5.15	0.000	-3.68	<0.0001		
Tingly	15.88	<0.0001	5.28	<0.0001	1.12	0.261	7.36	<0.0001		

- 840 Figure Legends:
- Figure 1. Temporal liking curves for Cluster 1 (A), Cluster 2 (B) and Cluster 3 (C) showingthe mean liking of each beer sample by cluster.
- **Figure 2.** Smoothed TCATA attribute curves (continuous lines) for A: 0% ethanol; B: 0.5%
- Ethanol; C: 2.8% ethanol and D: 5% ethanol. Thicker segments represent time period where
- proportion of citation is significantly different to the other 3 samples. In contrast, dotted lines
- represent pooled average proportion of citations for the other 3 samples, where significantly
- 847 different (p<0.05). Each attribute is represented by a different colour.
- **Figure 3.** Principal components analysis (PCA) biplot of the TCATA citation of attribute data over the 60s period for all beer samples. The arrow head > indicates swallow time (at 10s) and
- shows the development of these attributes over the 60 sec evaluation period. Beer sample
- trajectories are labelled with the ethanol concentration at the first 40 sec of evaluation time.
- 852 Time markers (dots) \bullet are positioned along the remainder of each of the trajectories at 5 sec
- 853 intervals to show progression of evaluation time

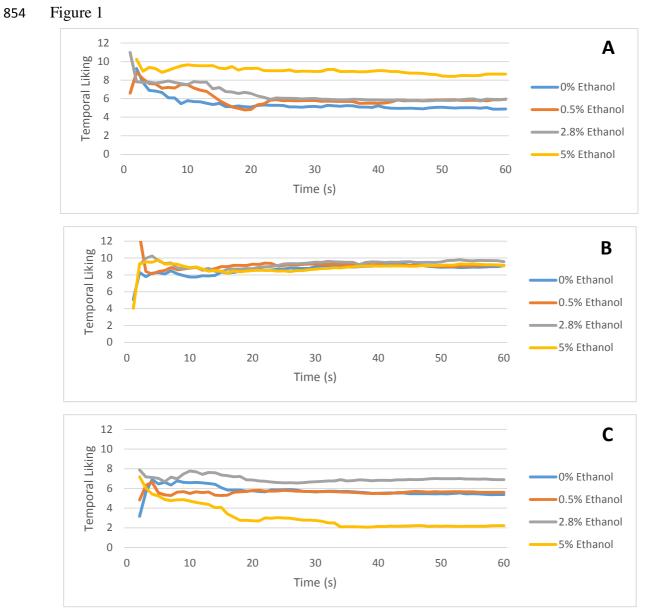


Figure 2

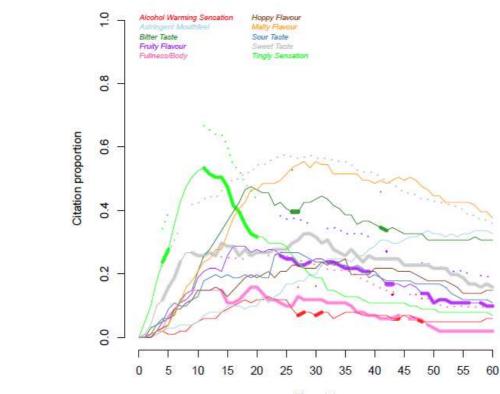
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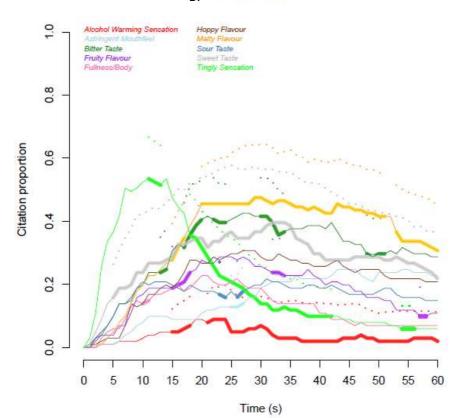
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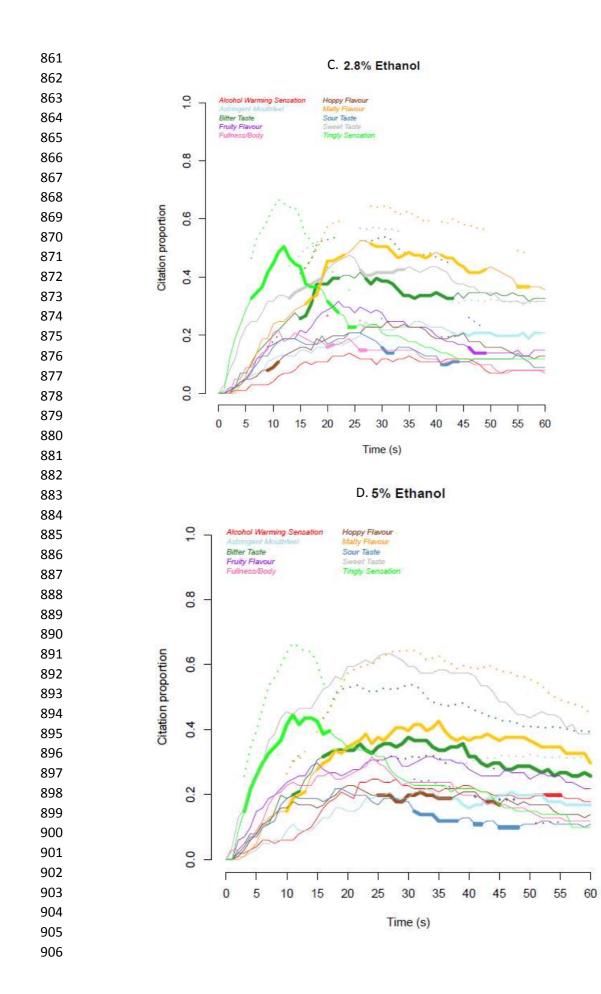
A. 0% Ethanol



Time (s)

B. 0.5% Ethanol





907 Figure 3

